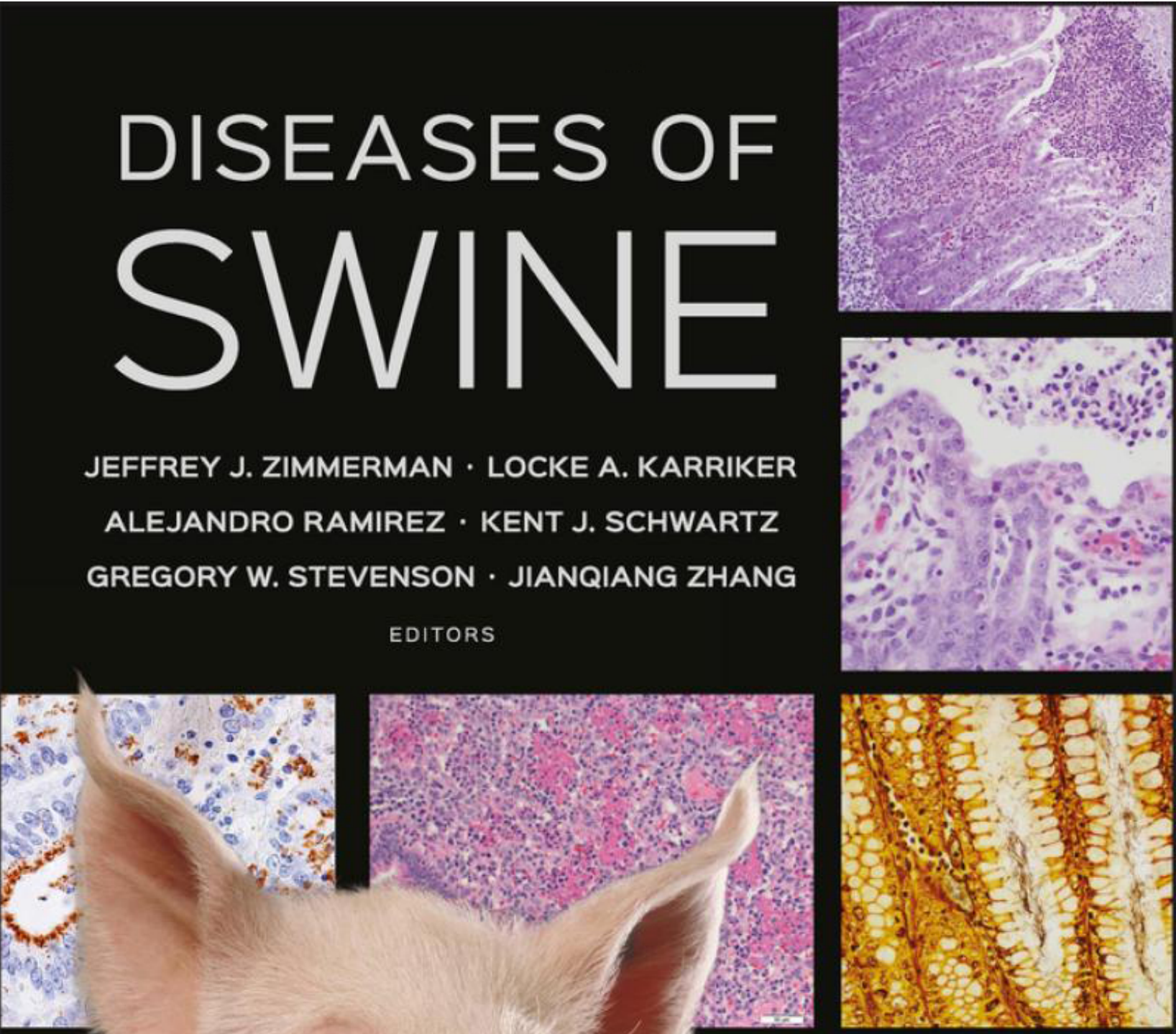
DISEASES OF SWINE

JEFFREY J. ZIMMERMAN · LOCKE A. KARRIKER
ALEJANDRO RAMIREZ · KENT J. SCHWARTZ
GREGORY W. STEVENSON · JIANQIANG ZHANG
EDITORS



11th
ELEVENTH EDITION

AMERICAN ASSOCIATION OF SWINE VETERINARIANS

WILEY Blackwell

Diseases of Swine

Diseases of Swine

Edited By

Jeffrey J. Zimmerman
Locke A. Karriker
Alejandro Ramirez
Kent J. Schwartz
Gregory W. Stevenson
Jianqiang Zhang

Eleventh Edition

WILEY Blackwell

This edition first published 2019

Edition History
Iowa State Press (1e, 1958; 2e, 1964; 3e, 1970; 4e, 1975; 5e, 1981, 6e, 1986; 7e, 1992; 8e, 1999). Blackwell Publishing (9e, 2006). John Wiley and Sons, Inc. (10e, 2012).

Registered Office
John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA

Editorial Office
111 River Street, Hoboken, NJ 07030, USA

For details of our global editorial offices, customer services, and more information about Wiley products visit us at www.wiley.com.

Wiley also publishes its books in a variety of electronic formats and by print-on-demand. Some content that appears in standard print versions of this book may not be available in other formats.

Library of Congress Cataloging-in-Publication Data

Names: Zimmerman, Jeffrey J., editor.
Title: Diseases of swine / edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, Jianqiang Zhang.
Description: 11th edition. | Hoboken, NJ : Wiley-Blackwell, 2019. | Includes bibliographical references and index. |
Identifiers: LCCN 2018042674 (print) | LCCN 2018043009 (ebook) | ISBN 9781119350903 (Adobe PDF) | ISBN 9781119350897 (ePub) | ISBN 9781119350859 (hardcover)
Subjects: LCSH: Swine–Diseases. | MESH: Swine Diseases
Classification: LCC SF971 (ebook) | LCC SF971 .D57 2019 (print) | NLM SF 971 | DDC 636.4/0896–dc23
LC record available at https://lccn.loc.gov/2018042674

Cover image: © ChristianLohman / iStock.com,© Dr. Eric R. Burrough, © Dr. Darin Madson,© Dr. Gregory W. Stevenson, © Dr. Michael
Cover design by Wiley

Set in 10/12pt Warnock by SPi Global, Pondicherry, India

10 9 8 7 6 5 4 3 2 1

Contents

Contributors *xi*
Editors' Note *xxi*
Acknowledgments *xxii*

Section I Veterinary Practice *1*

1 Herd Evaluation *3*
Records, benchmarks, four circles approach, diagnostic approaches, prioritizing interventions, reporting, blood sample collection, oral fluid collection.

2 Behavior and Welfare *17*
Definitions of welfare, normal vs. abnormal behaviors, maternal behaviors, minimizing welfare impact of invasive procedures, feeding and drinking behaviors, human interactions, behavior responses due to disease, recognizing pain, vices, objective measures of pain.

3 Genetics and Health *42*
Genetic influences on mortality, disease resistance, immune response and sow productive lifetime, with a summary of recent genetic advances.

4 Effect of Environment on Health *50*
Evaluation of the environment, recommended air temperatures, minimum ventilation rates, space recommendations, water requirements, feeder space recommendations. Investigation of death due to ventilation failure. Impact of noise levels and stray voltage.

5 Differential Diagnosis of Diseases *59*
Diarrhea, vomiting, rectal prolapses, respiratory distress, anemia, sneezing, skin, neurologic, lameness, reproductive, congenital, zoonotic, vesicular lesions, urinary tract.

6 Diagnostic Tests, Test Performance, and Considerations for Interpretation *75*
How diagnostic tests are performed, advantages, and disadvantages. PCR testing considerations including quantitative interpretation. Appropriate uses of genetic sequencing. Descriptions of metagenomics technology.

7 Optimizing Sample Selection, Collection, and Submission to Optimize Diagnostic Value *98*
Developing the diagnostic plan, diagnostic sample selection, pig necropsy, necropsy safety, knife sharpening.

8 Collecting Evidence and Establishing Causality *112*
Sources of variation in test results, sensitivity and specificity, testing in series or parallel, selecting test cutoff values, selecting appropriate sample size, screening and confirmatory tests. Evidence-based medicine, establishing baselines, evaluating interventions.

9 Disease Control, Prevention, and Elimination *123*
Routes of transmission, ecology of disease, pathogen cycles, biological risk management, principles of biosecurity, biocontainment, bioexclusion.

10 **Drug Pharmacology, Therapy, and Prophylaxis** *158*
Antimicrobial drug classes, considerations for treatment, residue avoidance, establishing treatment regimens, limiting the development of resistance, parasiticides, impact of drug treatment on immunity, bacteriophages, probiotics, hormones, anti-inflammatory drugs.

11 **Anesthesia and Surgical Procedures in Swine** *171*
Injectable anesthetic agents, sedation, catheterization techniques, epidural injection, anesthetic drug combinations, reversal agents and pain management. Surgical procedures including castration, correction of prolapses, cystostomy procedures, cesarean section, fracture repair, tusk removal, abdominal and musculoskeletal procedures.

12 **Preharvest Food Safety, Zoonotic Diseases, and the Human Health Interface** *197*
Physical, chemical, and biologic hazards, drug residues, MRSA, feed safety, certification programs.

13 **Special Considerations for Show and Pet Pigs** *211*
Dynamics of the show pig industry, biosecurity, zoonotic implications, vaccination protocols, behavior and training, ethics, teeth trimming, hoof trimming, obesity, and parasites.

Section II Body Systems *221*

14 **Cardiovascular and Hematopoietic Systems** *223*
Anatomy, pathophysiology, mulberry heart disease, anemia, shock, clinical pathology.

15 **Digestive System** *234*
Interactions of flora, nutrition, immune system, anatomy, pathophysiology, gastric ulcers, hemorrhagic bowel syndrome, prolapses, hernias.

16 **Immune System** *264*
Innate and adaptive immunity. Cellular, humoral, mucosal and passive immune mechanisms. Stress, nutrition, immunosuppression, vaccination. Lactogenic immunity.

17 **Integumentary System: Skin, Hoof, and Claw** *292*
Pathophysiology of skin, infectious conditions, vesicles, ear necrosis, porcine dermatopathy and nephropathy syndrome. Pathophysiology of coronary band, foot and claw. Traumatic and nutritional contributors to foot and claw lesions.

18 **Mammary System** *313*
Structure and development, physiology of lactation, physiology of maternal immunity, pathophysiology of lactation dysfunction, mastitis, dysgalactia, risk factors.

19 **Nervous and Locomotor System** *339*
Pathophysiology of nervous system, muscle, bone, joint, and eye. Congenital abnormalities, splayleg, congenital tremor, myopathy, porcine stress syndrome, arthritis, metabolic bone disease, rickets, osteochondrosis.

20 **Diseases of the Reproductive System** *373*
Control of estrus, pregnancy, and parturition. Pregnancy diagnosis, dystocia, prolapse, discharge, male reproductive function, semen quality. Laboratory investigation of abortion and reproductive failure.

21 **Respiratory System** *393*
Anatomy, structure, function, pathophysiology.

22 **Urinary System** *408*
Anatomy, structure, function, pathophysiology.

Section III Viral Diseases *425*

23 Overview of Viruses *427*
General characteristics of viruses, virus taxonomy, detection, characterization of viruses.

24 Adenoviruses *438*

25 African Swine Fever Virus *443*

26 Anelloviruses *453*
Torque teno sus viruses.

27 Astroviruses *457*

28 Bunyaviruses *461*
Akabane virus, Lumbo virus, Oya virus, Tahyna virus.

29 Caliciviruses *464*
Porcine noroviruses, porcine sapoviruses, St-Valérien virus, vesicular exanthema of swine virus.

30 Circoviruses *473*

31 Coronaviruses *488*
Hemagglutinating encephalomyelitis virus, porcine deltacoronavirus, porcine epidemic diarrhea virus, porcine respiratory coronavirus, porcine torovirus, transmissible gastroenteritis virus.

32 Filoviruses *524*
Reston ebolavirus, Zaire ebolavirus.

33 Flaviviruses *530*
Japanese encephalitis virus, Murray Valley encephalitis virus, West Nile virus, other flaviviruses.

34 Hepatitis E Virus *544*

35 Herpesviruses *548*
Malignant catarrhal fever (ovine herpesvirus 2), porcine cytomegalovirus, porcine lymphotropic herpesviruses, pseudorabies (Aujeszky's disease) virus.

36 Influenza Viruses *576*

37 Paramyxoviruses *594*
Menangle virus, Nipah virus, porcine parainfluenza virus 1, porcine rubulavirus (blue eye paramyxovirus).

38 Parvoviruses *611*

39 Pestiviruses *622*
Atypical porcine pestivirus, border disease virus, bovine viral diarrhea virus, Bungowannah virus, classical swine fever virus.

40 Picornaviruses *641*
Encephalomyocarditis virus, foot-and-mouth disease virus, porcine enteroviruses, porcine kobuvirus, porcine picornavirus Japan, porcine sapelovirus, porcine teschovirus, Seneca Valley virus, swine pasivirus, swine vesicular disease virus.

41 **Porcine Reproductive and Respiratory Syndrome Viruses (Porcine Arteriviruses)** *685*

42 **Swinepox Virus** *709*

43 **Reoviruses (Rotaviruses and Reoviruses)** *715*

44 **Retroviruses** *728*

45 **Rhabdoviruses** *733*
Rabies virus, vesicular stomatitis viruses.

46 **Togaviruses** *740*
Eastern equine encephalitis virus, Getah virus, Sagiyama virus, Ross River virus.

Section IV Bacterial Diseases *743*

47 **Overview of Bacteria** *745*
Characteristics of genera, disease mechanisms, table of bacterial diseases. Issues in the development of bacterial disease (normal flora, biofilms, dysbacteriosis).

48 **Actinobacillosis** *749*
Actinobacillus pleuropneumoniae – pleuropneumonia; *Actinobacillus suis* – septicemia, pleuropneumonia; *Actinobacillus equuli* – septicemia.

49 **Bordetellosis** *767*
Bordetella bronchiseptica – nonprogressive atrophic rhinitis, bronchopneumonia.

50 **Brucellosis** *778*
Brucella suis – infertility, abortion, perinatal mortality.

51 **Clostridial Diseases** *792*
Clostridium perfringens type C – necrohemorrhagic enteritis; *Clostridium perfringens* type A – enteritis; *Clostridium difficile* – necrotizing colitis; *Clostridium septicum, perfringens* type A, *novyi, chauvoei – cellulitis* and gas gangrene; *Clostridium tetani* – tetanus; *Clostridium botulinum* – botulism.

52 **Colibacillosis** *807*
Neonatal *E. coli* diarrhea, post weaning *E. coli* diarrhea and edema disease, *E. coli* causing fatal shock, systemic *E. coli* infections, coliform mastitis, nonspecific urinary tract infection.

53 **Erysipelas** *835*
Erysipelothrix rhusiopathiae – septicemia, arthritis, endocarditis; *Erysipelothrix tonsillarum* – arthritis, endocarditis.

54 **Glässer's Disease** *844*
Haemophilus parasuis – fibrinous polyserositis and arthritis.

55 **Leptospirosis** *854*
Leptospira spp. – abortion and stillbirths.

56 **Mycoplasmosis** *863*
Mycoplasma hyopneumoniae – enzootic pneumonia; *Mycoplasma hyorhinis* – polyserositis, arthritis; *Mycoplasma hyosynoviae* – arthritis; *Mycoplasma suis (Eperythrozoon suis)* – anemia.

57 Pasteurellosis *884*
Pasteurella multocida – progressive atrophic rhinitis, pneumonia, septicemia.

58 Proliferative Enteropathy *898*
Lawsonia intracellularis – acute proliferative hemorrhagic enteropathy (PHE), chronic porcine proliferative enteropathy (PPE).

59 Salmonellosis *912*
Salmonella typhimurium, *typhimurium* I 1,4,[5],12:i:-variant, *heidelberg, typhisuis* – enterocolitis; *Salmonella choleraesuis* var. *kunzendorf* – septicemia, enterocolitis; *Salmonella dublin, enteriditis* – meningitis.

60 Staphylococcosis *926*
Staphylococcus hyicus – exudative epidermitis; *Staphylococcus aureus* – skin infections, mastitis, others.

61 Streptococcosis *934*
Streptococcus suis – septicemia, meningitis, others; *Streptococcus porcinus* – cervical lymphadenitis; *Streptococcus dysgalactiae* subsp. equisimilis – septicemia, arthritis, others. Other streptococci – various conditions. *Enterococcus durans* and *hirae* – diarrhea.

62 Swine Dysentery and Brachyspiral Colitis *951*
Brachyspira hyodysenteriae, hampsonii, suanatina – swine dysentery; *Brachyspira pilosicoli* – intestinal (colonic) spirochetosis; *Brachyspira intermedia, murdochii* – brachyspiral (mild) colitis.

63 Tuberculosis *971*
Mycobacterium avium subsps. *avium* and *hominissuis*, *M. bovis, M. caprae, M. tuberculosis* – localized alimentary lymphadenitis, rare disseminated tuberculosis.

64 Miscellaneous Bacterial Infections *981*
Actinobaculum suis (Eubacterium suis) – cystitis, pyelonephritis; *Actinomyces hyovaginalis* – abortion, lung abscesses; *Bacillus anthracis* – anthrax; *Burkholderia pseudomallei* – melioidosis; *Campylobacter* spp. – enterocolitis; *Chlamydia* – enteritis, pneumonia, abortion, etc.; *Enterococci* – neonatal diarrhea; *Klebsiella pneumoniae* – septicemia; *Listeria monocytogenes* – septicemia, encephalitis, abortion; *Rhodococcus equi* – granulomatous lymphadenitis; *Treponema pedis* – ear necrosis, other skin lesions; *Trueperella abortisuis* – *abortion; Trueperella pyogenes* – pyogenic sepsis; *Yersinia* spp. – enterocolitis.

Section V Parasitic Diseases *1003*

65 External Parasites *1005*
Mange *(Sarcoptes, Demodex)*, lice, fleas, mosquitoes, flies (myiasis), ticks.

66 Coccidia and Other Protozoa *1015*
Coccidia *(Cystoisospora, Eimeria)*, *Toxoplasma*, *Sarcocystis*, *Cryptosporidium*, *Giardia*, microsporidia *(Entercytozoon, Encephalitozoon)*, *Balantidium coli, Entamoeba.*

67 Internal Parasites: Helminths *1028*
Nematodes – *Gongylonema, Hyostrongylus, Strongyloides, Ascaris, Trichinella, Trichuris, Oesophagostomum, Metastrongylus, Paragonimus, Stephanurus*, and others; Cestodes – *Echinococcus, Taenia*, and others; parasiticides.

Section VI Noninfectious Diseases *1041*

68 Nutrient Deficiencies and Excesses *1043*
Investigation of nutrient deficiencies or excesses, critical control points, inclusion rates, feed analysis, common nutrient concerns including amino acids, vitamins, and minerals.

69 Mycotoxins in Grains and Feeds *1055*
Aflatoxin, ochratoxin, citrinin, trichothecenes (T2 toxin, DON), zearalenone, fumonisins.

70 Toxic Minerals, Chemicals, Plants, and Gases *1072*
Minerals, feed additives, pesticides, toxic plants, nitrite, effects of water quality, toxic gases, ventilation failure.

Index *1088*

Contributors

Soren Alexandersen
Geelong Centre for Emerging Infectious Diseases (GCEID)
School of Medicine
Deakin University
Geelong, VIC
Australia

Gordon M. Allan
School of Biological Sciences
Queen's University Belfast
Belfast, Northern Ireland
United Kingdom

Gary C. Althouse
New Bolton Center
School of Veterinary Medicine
University of Pennsylvania
Kennett Square, Pennsylvania
United States of America

David E. Anderson
Department of Large Animal Clinical Sciences
University of Tennessee
Knoxville, Tennessee
United States of America

Michael D. Apley
College of Veterinary Medicine
Kansas State University
Manhattan, Kansas
United States of America

Virginia Aragon
Centre de Recerca en Sanitat Animal (CReSA)
Institut de Recerca i Tecnologia Agroalimentària (IRTA)
Universitat Autònoma de Barcelona
Barcelona
Spain

Zbigniew J. Arent
University Centre of Veterinary Medicine UJ-UR
University of Agriculture in Krakow
Krakow
Poland

Marisa L. Arias
Centro de Investigación en Sanidad Animal (CISA)
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria
Valdeolmos, Madrid
Spain

Bailey L. Arruda
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Paulo H. E. Arruda
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

David H. Baum
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Graham J. Belsham
National Veterinary Institute
Technical University of Denmark
Lindholm, Kalvehave
Denmark

David A. Benfield
Ohio Agricultural Research and Development Center (OARDC)
College of Food, Agricultural, and Environmental Sciences
The Ohio State University
Wooster, Ohio
United States of America

John Bingham
Commonwealth Scientific and Industrial Research Organization (CSIRO)
Australian Animal Health Laboratory (AAHL)
Geelong, VIC
Australia

Paola Boggiatto
National Animal Disease Center
Agricultural Research Service
United States Department of Agriculture
Ames, Iowa
United States of America

Matthew T. Brewer
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Susan L. Brockmeier
National Animal Disease Center
Agricultural Research Service
United States Department of Agriculture
Ames, Iowa
United States of America

André Broes
870 Avenue Madeleine de Vercheres
Quebec, QC
Canada

Michael C. Brumm
Brumm Swine Consultancy, Inc.
North Mankato, Minnesota
United States of America

Caitlyn E. Bruns
DNA Genetics
Columbus, Nebraska
United States of America

Eric R. Burrough
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Michelle Calvo-Lorenzo
Elanco Animal Health
Greenfield, Indiana
United States of America

Ranald Cameron
St. Lucia, QLD
Australia

Steven A. Carlson
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Chih-Cheng Chang
Department of Veterinary Medicine
National Chiayi University
Chiayi City
Taiwan

Chia-Yi Chang
Animal Health Research Institute
Council of Agriculture, Executive Yuan
Tansui, New Taipei City
Taiwan

Christopher Chase
Department of Veterinary and Biomedical Sciences
South Dakota State University
Brookings, South Dakota
United States of America

Jane Christopher-Hennings
Department of Veterinary and Biomedical Sciences
South Dakota State University
Brookings, South Dakota
United States of America

Johann F. Coetzee
College of Veterinary Medicine
Kansas State University
Manhattan, Kansas
United States of America

Jessica D. Colpoys
Agricultural Science Department
Truman State University
Kirksville, Missouri
United States of America

Tania A. Coutinho
Universidade Federal Rural de Pernambuco
Recife, Pernambuco
Brazil

Marie R. Culhane
College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota
United States of America

Scott A. Dee
Pipestone Applied Research
Pipestone Veterinary Services
Pipestone, Minnesota
United States of America

Aldo Dekker
Central Veterinary Institute of Wageningen UR
Lelystad
The Netherlands

Joachim Denner
Robert Koch Institute
Nordufer, Berlin
Germany

Joel M. DeRouchey
Department of Animal Sciences and Industry
Kansas State University
Manhattan, Kansas
United States of America

Mariano Domingo
Centre de Recerca en Sanitat Animal
(CReSA)
Departament de Sanitat i d'Anatomia Animals
Facultat de Veterinària
Universitat Autonòma de Barcelona
Barcelona
Spain

Steve S. Dritz
College of Veterinary Medicine
Kansas State University
Manhattan, Kansas
United States of America

Richard Drolet
Faculty of Veterinary Medicine
University of Montreal
Saint-Hyacinthe, Quebec
Canada

Jitender P. Dubey
Animal and Natural Resources Institute
Agricultural Research Service
United States Department of
Agriculture
Beltsville, Maryland
United States of America

Lily N. Edwards-Callaway
Department of Animal Sciences
Colorado State University
Fort Collins, Colorado
United States of America

Bernhard Ehlers
Robert Koch Institute
Nordufer, Berlin
Germany

William A. Ellis
Killinchy, Newtownards
Northern Ireland
United Kingdom

Steve M. Ensley
College of Veterinary Medicine
Kansas State University
Manhattan, Kansas
United States of America

Gene A. Erickson
Raleigh, North Carolina
United States of America

John M. Fairbrother
Faculté de Médecine Vétérinaire
Université de Montréal
Saint-Hyacinthe, Quebec
Canada

Chantal Farmer
AAFC, Dairy and Swine R and D Centre
Sherbrooke, Québec
Canada

Deborah Finlaison
Virology Laboratory
Elizabeth Macarthur Agriculture Institute
New South Wales Department of Primary
Industries
Menangle, New South Wales
Australia

Timothy S. Frana
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Matthew J. Freeman
College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota
United States of America

Tânia Rosária Pereira Freitas
Laboratorio Nacional Agropecuário - Minas Gerais
Pedro Leopoldo, Minas Gerais
Brazil

Robert M. Friendship
Department of Population Medicine
University of Guelph
Guelph, Ontario
Canada

Julie Funk
College of Veterinary Medicine
Michigan State University
East Lansing Michigan
United States of America

Phil Gauger
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Connie J. Gebhart
College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota
United States of America

Robert D. Goodband
Department of Animal Sciences and Industry
Kansas State University
Manhattan, Kansas
United States of America

Marcelo Gottschalk
Faculté de Médecine Vétérinaire
Université de Montréal
Saint-Hyacinthe, Quebec
Canada

John H. Greve
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Ronald W. Griffith
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Patrick G. Halbur
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

William F. Hall
Googong, New South Wales
Australia

David J. Hampson
School of Veterinary and Life Sciences
School of Veterinary and Biomedical Sciences
Murdoch University
Murdoch, Western Australia
Australia

Samantha J. Hau
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Richard A. Hesse
College of Veterinary Medicine
Kansas State University
Manhattan, Kansas
United States of America

Derald J. Holtkamp
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Chin-Cheng Huang
Council of Agriculture, Executive Yuan
Taipei
Taiwan

Anna K. Johnson
College of Agriculture and Life Sciences
Iowa State University
Ames, Iowa
United States of America

Kwonil Jung
Ohio Agricultural Research and Development Center
Department of Veterinary
Preventive Medicine
The Ohio State University
Wooster, Ohio
United States of America

Locke A. Karriker
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Johannes Kauffold
Faculty of Veterinary Medicine
University of Leipzig
Leipzig
Germany

Tuija Kekarainen
Kuopio Center for Gene and
Cell Therapy
Kuopio
Finland

David R. Kinker
National Veterinary Services Laboratories
Animal and Plant Health Inspection Service
United States Department of Agriculture
Ames, Iowa
United States of America

Peter D. Kirkland
Virology Laboratory
Elizabeth Macarthur Agriculture Institute
New South Wales Department of Primary Industries
Menangle, New South Wales
Australia

Nick J. Knowles
Vesicular Disease Reference Laboratory Group
The Pirbright Institute
Pirbright, Woking, Surrey
United Kingdom

Frank Koenen
Veterinary and Agrochemical Research Centre
(CODA-CERVA)
Groeselenberg 99, Ukkel
Belgium

Adam C. Krull
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Alberto Laddomada
Istituto Zooprofilattico Sperimentale
della Sardegna
Sassari
Italy

Marie-Frédérique Le Potier
Agence Nationale de Sécurité
Sanitaire (ANSèS)
Laboratoire de Ploufragan-Plouzané
Laboratory
Zoopôle Les Croix
Ploufragan
France

David S. Lindsay
College of Veterinary Medicine
Virginia Polytechnic Institute and
State University
Blacksburg, Virginia
United States of America

Crystal L. Loving
National Animal Disease Center
Agricultural Research Service
United States Department of Agriculture
Ames, Iowa
United States of America

Alan T. Loynachan
Veterinary Diagnostic Laboratory
University of Kentucky
Lexington, Kentucky
United States of America

Joan K. Lunney
Animal Parasitic Diseases Laboratory
Agricultural Research Service
United States Department of
Agriculture
Beltsville, Maryland
United States of America

John S. MacKenzie
Faculty of Health Sciences
Curtin University
Perth, Western Australia
Australia

Darin M. Madson
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Dominiek Maes
Faculty of Veterinary Medicine
Ghent University
Merelbeke
Belgium

Glenn A. Marsh
Commonwealth Scientific and Industrial Research
Organization (CSIRO)
Australian Animal Health
Laboratory (AAHL)
Geelong, VIC
Australia

Douglas G. Marthaler
College of Veterinary Medicine
Kansas State University
Manhattan, Kansas
United States of America

Guy-Pierre Martineau
Department of Animal Production
École Nationale Vétérinaire
de Toulouse
Toulouse
France

John J. McGlone
Department of Animal and
Food Sciences
Texas Tech University
Lubbock, Texas
United States of America

Steven McOrist
299 Queens Rd. Central
Hong Kong

Daniel G. Mead
College of Veterinary Medicine
University of Georgia
Athens
Georgia

Xiang-Jin Meng
College of Veterinary Medicine
Virginia Polytechnic Institute and State University
Blacksburg, Virginia
United States of America

Thomas C. Mettenleiter
Friedrich-Loeffler-Institut
Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health
Greifswald-Insel Riems
Germany

Suzanne T. Millman
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Kristie Mozzachio
Mozzachio Mobile Veterinary Services
Hillsborough, North Carolina
United States of America

Thomas Müller
Friedrich-Loeffler-Institut
Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health
Greifswald-Insel Riems
Germany

Pierre Yves Mulon
College of Veterinary Medicine
University of Tennessee
Knoxville, Tennessee
United States of America

Michael P. Murtaugh
College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota
United States of America

Éric Nadeau
Saint-Hyacinthe, Quebec
Canada

Eric A. Nelson
Department of Veterinary and Biomedical
Sciences
South Dakota State University
Brookings, South Dakota
United States of America

Eric J. Neumann
East Taieri
Dunedin
New Zealand

Charles Nfon
National Centre for Foreign Animal Disease
Canadian Food Inspection Agency
Winnipeg, Manitoba
Canada

Tracy L. Nicholson
National Animal Disease Center
Agricultural Research Service
United States Department of Agriculture
Ames, Iowa
United States of America

Pauline Nol
Wildlife Livestock Disease Investigations Team
Animal and Plant Health
Inspection Service
United States Department of
Agriculture
Fort Collins, Colorado
United States of America

Steven C. Olsen
National Animal Disease Center
Agricultural Research Service
United States Department of Agriculture
Ames, Iowa
United States of America

Tanja Opriessnig
The Roslin Institute
University of Edinburgh
Easter Bush Campus
Midlothian, Scotland
United Kingdom

Olli Peltoniemi
Department of Production Animal Medicine
Faculty of Veterinary Medicine
University of Helsinki
Saarentaus
Finland

Christina E. Phillips
Smithfield Hog Production Division
Rose Hill, North Carolina

Maria G. Pieters
College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota
United States of America

Karen W. Post
Clayton, North Carolina
United States of America

Scott L. Radke
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Alejandro Ramirez
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Karen B. Register
National Animal Disease Center
Agricultural Research Service
United States Department of Agriculture
Ames, Iowa
United States of America

Gábor Reuter
Department of Medical Microbiology
and Immunology
University of Pécs
Pécs
Hungary

Matthew J. Ritter
Elanco Animal Health
Greenfield, Indiana
United States of America

Suelee Robbe-Austerman
National Veterinary Services Laboratories
Animal and Plant Health Inspection Service
United States Department of Agriculture
Ames, Iowa
United States of America

Nicholas A. Robinson
Department of Biomedical Sciences
Cummings School of Veterinary Medicine
Tufts University
North Grafton, Massachusetts
United States of America

Stephanie Rossow
College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota
United States of America

Linda J. Saif
Ohio Agricultural Research and
Development Center
Department of Veterinary Preventive Medicine
The Ohio State University
Wooster, Ohio
United States of America

Luis Samartino
Instituto de Patobiología
Centro de Investigaciones en Ciencias Veterinarias y
Agronómicas
Instituto Nacional de Tecnologia
Agropecuaria (INTA)
Buenos Aires
Argentina

José Manuel Sánchez-Vizcaíno
Universidad Complutense de Madrid
Facultad de Veterinaria
Madrid
Spain

Mónica Santín-Durán
Animal and Natural Resources Institute
Agricultural Research Service
United States Department of Agriculture
Beltsville, Maryland
United States of America

Joy Scaria
Department of Veterinary and Biomedical Sciences
South Dakota State University
Brookings, South Dakota
United States of America

Kent J. Schwartz
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Joaquim Segalés
Centre de Recerca en Sanitat Animal (CReSA)
Departament de Sanitat i d'Anatomia Animals
Facultat de Veterinària
Universitat Autonòma de Barcelona
Barcelona
Spain

Mariela Segura
Faculté de Médecine Vétérinaire
Université de Montréal
Saint-Hyacinthe, Quebec
Canada

Frances K. Shepherd
College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota
United States of America

Durda Slavic
Animal Health Laboratory
University of Guelph
Guelph, Ontario
Canada

J. Glenn Songer
Emeritus Professor
University of Arizona
Tucson, Arizona
United States of America

Tomasz Stadejek
Department of Pathology and Veterinary Diagnostics
Faculty of Veterinary Medicine
Warsaw University of Life Sciences
Warsaw
Poland

Kenneth J. Stalder
College of Agriculture and Life Sciences
Iowa State University
Ames, Iowa
United States of America

Alberto Stephano
Stephano Consultores SC
Leon, Guanajuato
Mexico

Gregory W. Stevenson
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

André Felipe Streck
Universidade de Caxias do Sul
Caxias do Sul
Brazil

Mhairi A. Sutherland
Massey Innovative Farm Systems
AgResearch Ltd
Hamilton
New Zealand

Sabrina L. Swenson
National Veterinary Services
Laboratories
Animal and Plant Health
Inspection Service
United States Department of Agriculture
Ames, Iowa
United States of America

David J. Taylor
Emeritus Professor
University of Glasgow
Lennoxtown, Glasgow
United Kingdom

Jens Peter Teifke
Friedrich-Loeffler-Institut
Federal Research Institute for Animal Health
Greifswald-Insel Riems
Germany

Charles O. Thoen (deceased)
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Jill R. Thomson
Scottish Agricultural College Veterinary
Services
Bush Estate, Penicuik
Midlothian
Scotland

Mike D. Tokach
Department of Animal Sciences and Industry
Kansas State University
Manhattan, Kansas
United States of America

Montserrat Torremorell
College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota
United States of America

Jerry Torrison
College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota
United States of America

Uwe Truyen
Institute for Animal Hygiene and
Veterinary Public Health
University of Leipzig
Leipzig
Germany

Anita L. Tucker
Department of Population Medicine
University of Guelph
Guelph, Ontario
Canada

A.W. (Dan) Tucker
Department of Veterinary Medicine
University of Cambridge
Cambridge
United Kingdom

Valarie V. Tynes
Premier Veterinary Behavior Consulting
Sweetwater, Texas
United States of America

Francisco A. Uzal
School of Veterinary Medicine
University of California, Davis
San Bernardino, California
United States of America

William G. Van Alstine
College of Veterinary Medicine
Purdue University
West Lafayette, Indiana
United States of America

Kristien Van Reeth
Faculty of Veterinary Medicine
Ghent University
Merelbeke
Belgium

Fabio A. Vannucci
College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota
United States of America

Amy L. Vincent
National Animal Disease Center
Agricultural Research Service
United States Department of Agriculture
Ames, Iowa
United States of America

Anastasia N. Vlasova
Ohio Agricultural Research and Development
Center
Department of Veterinary Preventive Medicine
The Ohio State University
Wooster, Ohio
United States of America

Elizabeth Wagstrom
National Pork Producers Council
Des Moines, Iowa
United States of America

Fun-In Wang
School of Veterinary Medicine
National Taiwan University
Taipei
Taiwan

Qiuhong Wang
Ohio Agricultural Research and Development Center
Department of Veterinary Preventive Medicine
The Ohio State University
Wooster, Ohio
United States of America

Sherrie R. Webb
American Association of Swine
Veterinarians
Perry, Iowa
United States of America

Hana M. Weingartl
Special Pathogens Unit
National Centre for Foreign Animal Disease
Canadian Food Inspection Agency
Winnipeg, Mannitoba
Canada

David T. Williams
Commonwealth Scientific and Industrial Research Organization (CSIRO)
Australian Animal Health Laboratory (AAHL)
Geelong, VIC
Australia

Amy L. Woods
Heartland Veterinary Services, LLC
Oxford, Indiana
United States of America

Jason C. Woodworth
Department of Animal Sciences and Industry
Kansas State University
Manhattan, Kansas
United States of America

Shaobo Xiao
College of Veterinary Medicine
Huazhong Agricultural University
Wuhan
China

Michael J. Yaeger
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Hanchun Yang
College of Veterinary Medicine
China Agricultural University
Beijing
China

Kyoung-Jin Yoon
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Jianqiang Zhang
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Zhidong Zhang
Lanzhou Veterinary Research Institute
Chinese Academy of Agricultural Sciences
Gansu
China

Jeffrey J. Zimmerman
College of Veterinary Medicine
Iowa State University
Ames, Iowa
United States of America

Editors' Note

Dr. Howard Dunne and Iowa State University Press released the first edition of *Diseases of Swine* in 1958. Our goal for the 11th edition is to provide swine health specialists the knowledge needed for effective responses to pig diseases on farms and at local, regional, and global levels. In this we have endeavored to follow the standards of excellence initially established by Dr. Dunne.

As a sojourner of a slower time, Dr. Dunne could not have foreseen either the extent or the accelerated pace at which innovations in engineering, genetics, management, molecular biology, and nutrition have revolutionized pig production. Biologically, economically, and ecologically, the successful application of new technologies to pig production has produced unprecedented advances that benefit society and provide healthful, wholesome pork products to the consumer.

Successes in disease control and pig health assurance are to be celebrated but tempered with the reality that control (much less elimination) of both emerging and historic swine health adversaries has faltered. Endemic viral and bacterial pathogens remain a pernicious burden on pig health. More sobering, the interconnectivity and interdependence of the contemporary world have accelerated the speed and inevitability with which emergent pathogens are dispersed to distant locations. Despite the considerable efforts of the animal health community, African swine fever virus, classical swine fever virus, foot-and-mouth disease virus, porcine circoviruses, porcine coronaviruses, porcine reproductive and respiratory syndrome viruses, and other major pathogens circulate widely in many parts of the world and threaten those that remain free.

Ideally, recognition of our shared vulnerabilities should spur the search for more effective solutions to animal and public health disease threats: there is much to be learned and applied. Thus, we respectfully dedicate this edition of *Diseases of Swine* to our readership as a tool in their search for solutions to swine and public health challenges.

Locke A. Karriker
Alejandro Ramirez
Kent J. Schwartz
Gregory W. Stevenson
Jianqiang Zhang
Jeffrey J. Zimmerman

Acknowledgments

With our deepest appreciation, we thank Ella Akin, Cassandra Fitzgerald, Victoria Monahan, and Kristin J. Skoland for their excellent work on behalf of the 11th edition of *Diseases of Swine*. Without their dedication and herculean efforts, the outcome would have been in doubt.

Section I

Veterinary Practice

1

Herd Evaluation

Alejandro Ramirez and Locke A. Karriker

Introduction

With changes in the structure of the swine industry, there have also been changes in the roles of swine veterinarians. Swine veterinarians today focus more on preventive medicine and improving overall herd health rather than responding after disease occurs, the latter common in traditional "fire engine" practices of 20+ years ago. Swine veterinarians now have a proactive role in anticipating problems and preventing disease with a concurrent responsibility to provide care to each pig. This is a challenge as resources (money, labor, and time) are always limited. Consequently, swine veterinarians are highly motivated to be innovative. The use of modern technology, applied research, epidemiologic principles, biostatistics, and improved diagnostic methods guides them through the diagnosis as well as the prioritization and allocation of resources to improve the health and welfare of pigs. The successful veterinarian is not only the one that solves a problem but also creates opportunities and promotes the financial success of their clients.

Before starting any evaluation of a farm, it is important to understand the objectives and goals of each individual involved in the farm operation. This is critical as ultimately the success of any intervention requires actions by the client or those working for the client. Better understanding of the client's goals and constraints will ensure that recommendations on herd health are made in that context. The context often requires swine veterinarians to innovate because recommendations will often vary between clients and may change for a particular client over time. For example, a client may be focused on improving average daily gain for a period but may transition to reducing cost of gain as their facts, business inputs, or understanding changes. The most important question for an owner or manager who is requesting veterinary services to answer is: "What is my goal?"

Investigation of health or production issues is best approached by site visits – that is, inspection of pigs in their environment. As seen in the following discussion, there are many factors that contribute to compromised health and well-being of pigs. Many of the assumptions made by clients or swine veterinarians can only be validated by a well-designed, systematic on-farm site visit.

Preparing for a site visit

History and records

If possible, history and record evaluation should occur prior to any herd evaluation or investigation. Looking at the operation's medical records and past diagnostic laboratory reports helps provide a picture of previous areas of concern and guidance on the expected health status of the herd. It is important to see the actual past reports rather than rely on client's interpretation of results, particularly when serving a new client or as a second opinion. Experience dictates that even with the best intentions, managers and owners are more likely to recall some results while downplaying or neglecting to mention others based on their particular biases.

Production records, usually computerized, are common in modern swine operations. The value of computerized records lies in the ability to instantly query the data and summarize it in meaningful ways. Morris (1982) is reported to be one of the first to suggest the concept of "performance-related diagnosis." This capability to evaluate herd performance and then determine the need for interventions has created a dilemma in regard to the term "subclinical" (Polson et al. 1998). The true definition of subclinical implies not measurable, but today's modern records allow for measuring slight differences in productivity (clinical manifestation),

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

which without records would have gone unnoticed (subclinical). All information gathered on a farm, including records, should be evaluated objectively from a perspective of "trust yet verify." Inaccurate or misinterpreted information and records will often lead to misdiagnosis and inappropriate recommendations.

Benchmarks

Benchmarking is a unique tool that allows operations to identify areas of concern or areas where improvements can be made. Many studies have reported different benchmarks to use as targets (see review by Polson et al. 1998). Others have suggested that the best production benchmarks are those set by the herd's own records (Lloyd et al. 1987). Over time, productivity and processes change such that older benchmarks may no longer be relevant. Depending on the objectives and changing constraints of a specific operation, a particular benchmark may not have the utility or impact that it did under previous conditions. As benchmark information has becomes more available in the age of the Internet, it is increasingly important to determine the characteristics of the operations from which these benchmarks were derived. Experienced swine veterinarians are able to decipher the intricate methods of data reporting and have insight for which circumstances certain parameters are achievable. For those just starting to learn about swine production medicine, it is best to use benchmarks as means to understand the appropriate magnitudes of different parameters rather than using them as specific goals per se.

From the veterinary and diagnostic perspectives, it is better then to focus on understanding the relationship of different production parameters rather than memorizing specific values. A good example of this conceptual thinking can be seen in Figure 1.1. This figure helps show the interrelationship of several different parameters and their impact on a breeding herd's wean pig output. Basically, throughput (i.e. pigs weaned) is determined by multiplying capacity (female inventory or facility space) by efficiency (how many pigs are produced per female inventory or facility space). The advantage of understanding this productivity tree is that all factors influencing throughput can be evaluated at the same time and interventions can be implemented in different areas of the tree. Extending this example to the evaluation of number of pigs weaned, issues like preweaning mortality are obvious, while others such as female removal and replacement rates or lactation length may not initially come to mind. In the case of a producer with a target of >28-day weaning age, the number of litters weaned/female/year will automatically be impacted (fewer) by the system design.

Reporting structure

Reporting structure refers to the organization of workers, management, and owners as it occurs in larger production systems. It also refers to whom a veterinarian is to report findings and recommendations. It is important for swine veterinarians to ask and understand the proper reporting structure for any new client. This is true for operations of all sizes. For the small or family farm, it is important to know what information the owner wants to share with workers. In a larger corporate setting (corporate ownership or part of a producer cooperative),

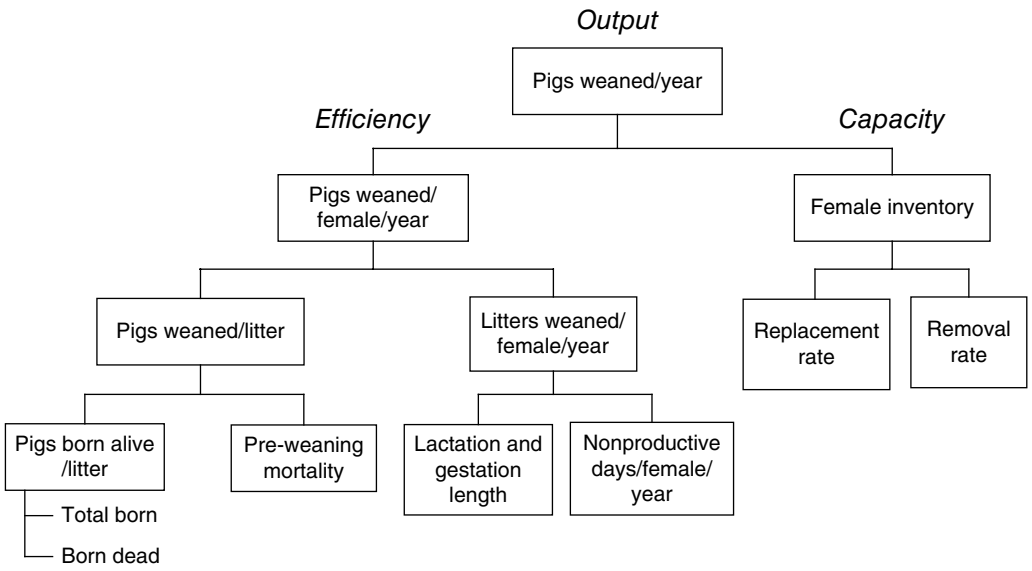


Figure 1.1 Weaned pig output productivity tree for investigating variables that impact the number of pigs weaned per year.
Source: Adapted from Gary Dial.

it is even more important to understand how decisions are made, who makes decisions, and who should get veterinary reports. Understanding reporting structures is critical in ensuring that the veterinarian and managing team are working together and a single consistent message is being delivered to workers. Providing information to the wrong person may actually hinder progress, as many times those closer to the pigs and daily processes may not be fully aware of all considerations influencing a business decision.

Frequently in the United States, the owner of the pigs is different than the caretaker. The caretaker may be focused on minimizing his/her labor efforts, while the owner may be more focused on the cost of a particular treatment or prevention option. The veterinarian is focused on food safety, maximizing pig health and welfare, operational sustainability, and owner profitability. Ultimately, the owner decides what is to be implemented.

Biosecurity

Biosecurity has been a major topic of concern for the swine industry from many years. This topic is covered in greater detail in Chapter 9.

Protocols to prevent disease transmission into the farm, within the farm, or to neighboring farms are now commonplace. Swine veterinarians and personnel need to proactively follow proper biosecurity protocols to ensure the safety and security of our food supply. The key point when performing a herd examination is for the veterinarian to be fully aware, and fully comply, with all biosecurity guidelines for the operation. To do this, the veterinarian has to be proactive and always ask for biosecurity requirements *before* visiting the site. Being informed ahead of time will help ensure that the veterinarian is prepared and able to meet the required downtime and follow proper biosecurity protocols once on-site.

Site visit

Introduction to the four circles

One of the most important concepts of a proper herd evaluation is to be consistent! It is critical to ensure that herd examinations are performed in a consistent manner so as to be thorough and efficient and to minimize the opportunity for missing something important. Checklist may be helpful for specific routine evaluations, but often not practical for a complete and thorough investigation. Checklist approaches limit the problem-solving ability of the veterinarian and are especially poor approaches to new problems. There are too many areas of interest as well as too many differences in facility type and design to make a single valid checklist across all farms.

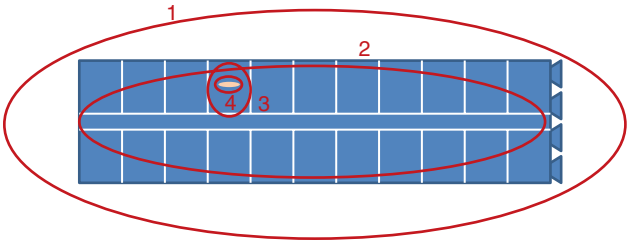


Figure 1.2 Diagram showing the concept of the four-circle approach to herd evaluation. (1) Complete circle evaluation of the "outside" of the building/site. (2) Complete circle evaluation of the "inside" of the building. (3) Complete circle evaluation of individual "pens." (4) Complete circle evaluation of individual "animals."

Farm-specific checklists or checklists for particular aspects of an operation can, however, be useful.

One systematic approach involves the concept of the four circles (Figure 1.2). The overall objective is to be systematic in the evaluation of an operation to make sure that all relevant information is evaluated when looking after pigs' health and welfare. Each successive circle becomes more focused, culminating in the evaluation of individual pigs. The most important question the veterinarian must be able to answer after going through the four-circle process is: "Is there currently a disease or welfare issue or is one imminent?"

Circle 1: evaluation of outside of the building/site

The first circle involves walking around the outside of buildings to assess the overall site. This first circle is especially important when visiting a new site. Evaluation of the outside of a building has value both clinically for the pigs and practically with respect to informing the veterinarian about the caretakers' attention to maintenance and facility management.

As one walks around the site, biosecurity risks for the operation will be better understood. Are there any other hog sites in close proximity? Is the health status of these other operations known? How close are public roads from hog buildings? What appears to be the traffic pattern for this particular site (feed delivery, removal of dead carcasses, employee parking)? How well maintained is the site? If the site is not well maintained, could it be due to lack of attention to details, insufficient staffing, or tight budgets? Either of these reasons would suggest that the veterinarian's recommendations should be tailored to accommodate these realities. For example, a manager who is very attentive to detail is more likely to follow a complex or detailed treatment protocol.

Circle 2: evaluation of inside of the building

The second circle involves walking through the inside of the building. In this case the objective is to get a better

feel for the overall environment of the pigs covering all regions of the building. One must walk from one end of the building all the way though the other side. If one takes too long to walk from one end to the other, it becomes more difficult to identify ventilation differences as one starts to become adapted to the new environment.

Stocking density is also evaluated at this time. It is important to note differences in stocking densities between pens as well as between barns. Lower stocking densities may indicate high mortalities in a particular pen or barn. Recommended stocking densities are listed in Table 1.1. Pig sizes are also assessed using the guidelines in Table 1.2 on expected pig weights based on age.

The general health of all pigs in the barn is evaluated at this time. Is there coughing, sneezing, or signs of diarrhea? The magnitude of the problem should be quantified. This is easily done by estimating the number of affected pigs in a pen as well as the total number of pigs in the pen. For example, if there are approximately 5 pigs coughing in every pen and there are around 25 pigs per pen, then it would suggest that approximately 20% of the pigs are affected. On the other hand, if it is found that only 1 or 2 pigs are affected in every other pen, then it would suggest the prevalence to be approximately 2–4% of the barn. The quantification of prevalence does not have to be exact, as usually we are more concerned on the size of the magnitude of the problem (60 vs. 10%) rather than knowing the exact prevalence of the clinical sign (8 vs. 12%). Determining general prevalence has three main goals: It allows for the correct perspective on the extent of the problems (i.e. is there currently a disease or welfare issue or is one imminent?). It helps to differentiate herd problems from individual pig issues, thus helping to determine the correct level of treatment (i.e. whole herd treatment or individual pig treatments). Finally, it provides a baseline for determining the effect of any intervention. This is especially important as although coughing may still be present after 5 days of treatment, the change in prevalence from 25 to 4% is a good indicator of improvement, suggesting that further intervention may not be warranted.

Table 1.1 Recommended space per pig by phase of production.

	Indoor		
	Solid	**Slatted**	**Outdoor**
Phase		**Area per pig in m^2 (ft^2)**	
Gilts	1.86 (20)	1.49 (16)	2.32 (25)
Sows	2.2 (24)	1.86 (20)	2.32 (25)
Farrow pen	8 (88)	NA	NA
Farrow crate	4.4 (48)	4.4 (48)	NA
Boars	NA	1.86 (20)	NA
Nursing	NA	2.0 (22)	NA
Nursery 20 kg	0.37 (4)	0.28 (3)	0.74 (8)
Nursery 40 kg	0.37 (4)	0.40 (4.4)	0.74 (8)
Grower 60 kg	0.56 (6)	0.53 (5.8)	1.86 (20)
Finisher 80 kg	0.74 (8)	0.67 (7.2)	1.86 (20)
Finisher 110 kg	0.75 (8)	0.75 (8)	1.86 (20)

Source: Dewey and Straw (2006). Adapted from English et al. (1982), Baxter (1984a,b,c), Patience and Thacker (1989a,b), and Gonyou and Stricklin (1998).
NA, not applicable.

Circle 3: evaluation of individual pens

The third circle is performed by doing an evaluation of individual pens. Based on the second circle, pens identified in the evaluation of the room are selected for further evaluation of the extent of the problem. Veterinarians must get in the pens with pigs. One cannot make a full assessment of the problem by simply walking the alleyway of the barn as many pig issues will be missed. This is the point in time that feeders and waterers are also checked for proper function (Table 1.3). Also see Chapter 4 for the effect of the environment on swine health.

The overall behavior/attitude within the pen is evaluated, identifying individual pig concerns as well as pen concerns. Differences in sizes of pigs in a pen are again noted at this time (Table 1.2). It is very important to always ask if any type of size sorting (regrouping by size) has occurred as well as knowing the expected age difference for the barn. This is a good time to look closely for evidence of diarrhea. Many times the diarrhea is first noted by the fecal character that may be present on the floor or walls of the facility, and extra observational time is needed to identify the individual pigs that may be affected.

There are no specific recommendations on how many individual pens need to be evaluated. A key point is to make sure several pens from different parts of the building are evaluated to have a true representation of the potential herd issues recognized by the second circle evaluation. Individual pig issues of concern, especially those related to welfare (severe, chronic, or moribund individuals), should also be identified at this time.

Circle 4: evaluation of individual pigs

The fourth and final circle involves a complete evaluation of individual pigs. Pigs are evaluated from head to tail. Anomalies are noted as well as suspected chronicity of issue. Rectal temperatures are taken at this time as a measure of presence of infectious disease processes and

Table 1.2 Weights and daily gain by age and relative growth rate.

	Slow				Moderate				Ideal			
Age	Weight		Daily gain in the previous 20 days		Weight		Daily gain in the previous 20 days		Weight		Daily gain in the previous 20 days	
Days	lb	kg	lb	g	lb	kg	lb	g	lb	kg	lb	g
20	8–10	3.6–4.5			10–12	4.5–5.5			12–14	5.5–6.4		
40	18–22	8.2–10.0	0.50–0.60	227–273	22–26	10.0–11.8	0.60–0.70	273–318	26–30	11.8–13.6	0.70–0.80	318–364
60	33–40	15.0–18.2	0.75–0.90	341–409	40–47	18.2–21.4	0.90–1.05	409–477	47–54	21.4–24.5	1.05–1.20	477–545
80	54–64	24.5–29.1	1.05–1.20	477–545	64–74	29.1–33.6	1.20–1.35	545–614	74–84	33.6–38.2	1.35–1.50	614–682
100	82–95	37.3–43.2	1.40–1.55	636–705	95–108	43.2–49.1	1.55–1.70	705–773	108–122	49.1–55.5	1.70–1.90	773–864
120	110–126	50.0–57.3	1.40–1.55	636–705	126–142	57.3–64.5	1.55–1.70	705–773	142–160	64.5–72.7	1.70–1.90	773–864
140	138–157	62.7–71.4	1.40–1.55	636–705	157–176	71.4–80.0	1.55–1.70	705–773	176–198	80.0–90.0	1.70–1.90	773–864
160	165–187	75.0–85.0	1.35–1.50	614–682	187–209	85.0–95.0	1.50–1.65	682–750	209–235	95.0–106.8	1.65–1.85	750–841
180	191–216	86.8–98.2	1.30–1.45	591–659	216–241	98.2–109.5	1.45–1.60	659–727	241–271	109.5–123.2	1.60–1.80	727–818
20–60			0.63–0.75	284–341			0.75–0.88	341–398			0.88–1.00	398–455
60–180			1.32–1.47	598–667			1.47–1.62	667–735			1.62–1.81	735–822
0–180			1.06–1.20	482–545			1.20–1.34	545–609			1.34–151	609–684

Source: Dewey and Straw (2006). Reproduced with permission of John Wiley and Sons.

Table 1.3 Recommended water requirements, water flow rates, and feeder space per pig by phase of production.

	Water requirements		Feeder space/pig
	l/day	l/minute	mm (in.)
Restricted feed			
Gestating sows	12–25	2	457–610 (18–24)
Lactating sow	10–30	2	
Boar	20	2	
Nursing	1	0.3	
Nursery	2.8	1	254 (10)
Grower	7–20	1.4	260 (10)
Finisher	10–20	1.7	330 (13)
Ad libitum			
Nursery	2.8	1	60 (2.3)
Grower	7–20	1.4	65 (2.5)
Finisher	10–20	1.7	76 (3)

Source: Dewey and Straw (2006). Adapted from Baxter(1984a,b,c), Patience and Thacker (1989a,b), Swine Care Handbook (2003), and Muirhead and Alexander (1997a,b).

stage of infection (e.g. fever tends to suggest an acute infection). Table 1.4 provides a summary of the expected normal temperature, respiratory, and heart rates of pigs based on size. A key point to remember is that as the environmental temperature increases, so will the average respiratory rates and body temperatures for healthy pigs.

For breeding herd examinations, the body condition of females should be evaluated periodically (Table 1.5). When making recommendations for feed or feeding changes, the stage in the reproductive cycle must be considered. Females entering the farrowing house should be in their best body condition (target body condition score [BCS] of 3), while gilts exiting the farrowing house (end of lactation) will have lower BCS. Feed changes are best executed by making small changes (0.5–1.0 kg) in the daily feed allotments.

This is also a good time to identify individual pigs requiring treatment as well as acutely infected animals that would be useful for diagnostic sample collection. Animals appropriate for euthanasia, necropsy, and tissue collection are also identified at this time. When selecting pigs for diagnostic tissue sample collection

Table 1.4 Temperature, respiration, and heart rate of pigs of different ages.

	Rectal temperature (range ± 0.30 °C, 0.5 °F)			
Age of pig	°C	°F	Respiratory rate (breaths/min)	Heart rate (beats/min)
Newborn	39.0	102.2	50–60	200–250
1 hour	36.8	98.3		
12 hours	38.0	100.4		
24 hours	38.6	101.5		
Unweaned piglet	39.2	102.6		
Weaned piglet (20–40 lb) (9–18 kg)	39.3	102.7	25–40	90–100
Growing pig (60–100 lb) (27–45 kg)	39.0	102.3	30–40	80–90
Finishing pig (100–200 lb) (45–90 kg)	38.8	101.8	25–35	75–85
Sow in gestation	38.7	101.7	13–18	70–80
Sow				
24 hours' prepartum	38.7	101.7	35–45	
12 hours' prepartum	38.9	102.0	75–85	
6 hours' prepartum	39.0	102.2	95–105	
Birth of first pig	39.4	102.9	35–45	
12 hours' postpartum	39.7	103.5	20–30	
24 hours' postpartum	40.0	104.0	15–22	
1 week postpartum until weaning	39.3	102.7		
1 day post weaning	38.6	101.5		
Boar	38.4	101.1	13–18	70–80

Source: Dewey and Straw (2006). Reproduced with permission of John Wiley and Sons.

Table 1.5 Sow body condition scoring.

Body condition score (BCS)	Condition	Back fat mm (in.)	Description	Comments
BCS 1	Excessively thin	<10 (<0.39)	Ribs, hips, and backbone are easily visible and palpable	Sow is in poor condition and needs large amounts of muscle and fat gain to maintain productivity. Needs a significant increase in feed
BCS 2	Moderately thin	10–15 (0.39–0.58)	Ribs, hips, and backbone can be palpated with slight pressure	A moderate increase in feed is required
BCS 3	Ideal condition	15–22 (0.59–0.89)	Ribs, hips, and backbone can be palpated with firm pressure, but cannot be observed visually	Monitor feeding to maintain this body condition
BCS 4	Moderately fat	23–29 (0.90–1.13)	Ribs, hips, and backbone cannot be palpated	May be appropriate to cut back slightly on feeding
BCS 5	Excessively fat	≥30 (≥1.14)	Ribs, hips, and backbone cannot be palpated	Sow has excessive amounts of fat tissue. Reduce feeding to bring her back to a BCS 3

Source: Adapted from Ken Stalder.

(also see Chapter 7), there are several important points to consider:

1) An animal's life will be sacrificed for the good of the herd, and due consideration should be placed into selecting the appropriate pigs.
2) Animals must be selected that truly represent the major clinical signs of concern in the herd.
3) Animals should be in the early stages of the disease process. The selection of acute cases will increase the probability that the primary causative agent and compatible lesion are identified.
4) An animal that has received no antimicrobials or therapy is usually preferred.

The number of animals selected for necropsy and tissue sample collection depends on the objective. As a general rule, animals that are found dead are necropsied first. Mortalities are necropsied until a pattern of disease process is apparent, which suggests the primary herd disease issue rather than unrelated individual animal afflictions. Based on necropsy findings and clinical evaluation, representative live animals are euthanized for fresh tissue sample collection. The number of animals euthanized depends on the individual case presentation and necropsy findings in the euthanized pig. When considering multifactorial etiologies, it is important to remember that not all animals in the herd will have all pathogens present at any one time point. This suggests that in a large herd, it may be necessary to euthanize sufficient animals to completely represent the full range of clinical and pathological findings and to identify the multiple interacting disease agents. In other cases where there may be only one primary pathogen of concern, 1 or 2 euthanized pigs may be sufficient to answer the diagnostic question. The goal is to sacrifice the least number of animals yet maximize the diagnostic value for the benefit of the rest of the herd, thereby benefiting the current group as well as future groups. Live animal (antemortem) sampling is commonly done. For some pathogens (e.g. influenza A virus via nasal swabs or oral fluids), simply finding the agent in the herd is all that may be necessary. In other cases, finding a common endemic potential pathogen of interest (e.g. porcine circovirus type 2) must be in association with compatible lesions to support the role of such agent in the current clinical presentation.

Summary of four circles

The concept of the four circles is to obtain a systematic and complete picture of the clinical status of the site. It provides a systematic view that is important in deciding what interventions need to be implemented to mitigate the effects of the current disease. It starts with a big-picture overview and then narrows the focus to individual pigs. It helps separate unrelated individual pig afflictions from whole herd disease problems, both of which need to be addressed, but priorities and recommendations will be different depending on context and the client's goals and objectives. The role of the veterinarian is to help guide the client to maximize the impact of any intervention. Information obtained from this systematic approach will also help differentiate what issues are primarily due to pathogens and which ones are being confounded or even caused by management practices or management failures. It will help veterinarians formulate a more complete assessment of the prognosis and expected outcomes of the current health situation. Once mastered, the process can be quick and very efficient.

Asking Questions

The process of data collection should not be restricted to the veterinarian's observations. It is very helpful to ask others working on the farm or within the operation for their perspectives. This should be done not only from upper management individuals (i.e. managers or owners) but also from the workers themselves. Often, the managers make many assumptions as to what they believe is being done on the farm, but the actual workers have a different perspective. This may be due to lack of training, poor communication of protocols, or inadvertent deviations in protocols of which participants are unaware. This is why it is useful to ask the same questions to different people in the same production system for confirmation and to assess consistency. Questions should be formulated as open ended rather than seeking a simple yes or no answer. It is also helpful to have employees demonstrate how to perform a task ("show me how") rather than providing an explanation ("tell me how"). This ensures that the actual process and technique are observed and allow evaluation of significantly more details than are apparent in a verbal description. This has been especially useful in troubleshooting intensive, high impact procedures such as heat detection and artificial insemination.

As a site visit is performed, it is also important to examine storage and utility areas and investigate refrigerators or medicine cabinets. This process should help support and validate the different worker's answers to questions regarding processes and protocols. For example, an operation that claims routine vaccination of sows pre-farrowing and yet has no vaccine on-site may need further evaluation and discussion to ascertain vaccine management and handling procedures. A second example may be a protocol describing a temperature to store semen but no thermometer in the semen storage unit.

On-site records

Production sites should have treatment and mortality records on-site. The minimum requirement for treatment records include date, animal ID, product name, dose, route, person administering, and product withdrawal time. Mortality records are helpful in determining the total number of pigs in the original lot, number of mortalities, and the chronology of mortalities to date. Caretakers should be instructed to record euthanized animals in a different manner. A good practice is to also record a presumed "death reason" and educate clients on how to properly evaluate mortalities and record such. However, research has shown that there are significant differences between recorded and actual death reasons (Lower et al. 2007). To facilitate this process, the focus should be on the actual observations that can be accurately made by caretakers. For example, it is difficult for a caretaker to diagnose *Escherichia coli*-associated diarrhea as cause of death. Instead, the mortality should be recorded as due to diarrhea. There should also be a second code to identify whether the animal died on its own or was euthanized. Practical and more valid mortality records can be collected by simply narrowing down the options provided, focusing on general clinical signs rather than a specific disease etiology, and training all individuals on how to properly categorize mortalities.

Records for farrowing, nursery, and finishing sites may include daily water consumption and daily high and low barn temperatures. This information is easy to collect in today's modern facilities and can be helpful (especially the water) in predicting a possible respiratory outbreak (Brumm 2006). The high and low barn temperature recording is helpful in identifying possible concerns with the ventilation system. It is best to utilize an independent high–low thermometer to record temperature fluctuations rather than using the barn's electronic control system in order to validate the proper function of the controller. Finally, these records can be used to confirm each group of pigs is being checked at least daily.

For breeding herds there are many other records that are kept on-site. These records can vary in form and content from hand notes to an actual computer on-site. Log sheets are very helpful in ensuring jobs are routinely done. For example, a simple semen log can track the date, time, current temperature of the semen storage unit, and initials of the individual who rotated the semen (e.g. manually resuspended semen in extender by gently rocking the semen bags/bottles back and forth). The advantage of having this type of manual record is that it ensures this important job is done routinely and having individuals write down their initials facilitates accountability. It is a reality that in operations with multiple workers, duties are sometimes not performed because a worker believes that someone else was doing the job.

Computer records can be accessed either through daily/weekly reports provided to the farm or through direct access to a computer. The number and variety of reports that are available from computerized sow record systems precludes discussion here. It is important for the swine veterinarian to understand and objectively evaluate different herd performance parameters. The greatest advantage of computerized record systems is their ability to summarize relevant data in many different ways and, as previously mentioned, compare to relevant internal or external benchmarks to help identify those performance parameters in need of improvement.

When looking at reports, it is important to remember that data is usually summarized based on time or by cohort. In a time-based report, data is simply attributed to a particular time period. For example, January breeding and farrowing number summarizes data for all the

sows that were bred in January as well as the sows that farrowed in January, which are two distinct groups of animals. This information is helpful in monitoring the overall herd's performance, but it is not helpful in evaluating cause and effect within a particular group. To better evaluate a particular group, a cohort-based report must be used. In this case all parameters reported are specific to a common group of animals so the breeding and farrowing data pertain to the same group of animals although accumulated at different dates. This cohort-based report is very useful in evaluating the effects of different interventions.

The most important part of any data collection is the desire to take action when an abnormality is detected. When a veterinarian requests data to be collected by workers or caretakers, effective communication should outline the importance of the data, how it will be used, at what threshold they are expected to take action, and the consequences of failing to act. For example, simply recording the daily temperature of the semen storage unit has no value unless action is taken when temperature is outside of the desired range.

Diagnosis

Once a site's evaluation has been performed (four circles) and data has been collected, it is then necessary to interpret all the findings in the context of the veterinarian's clinical observations. The Greek word "diagnosis" literally means "through thinking" (Morley 1991). The process of arriving at a diagnosis can vary among individuals and clinical presentations. What is important is to be systematic, once again, to ensure that decisions are focused and objective. Figure 1.3 summarizes the field investigation and case management process. The following brief summaries are a few examples of different approaches/aspects that can be considered.

Soap

One of the traditional means for summarizing data in the medical profession is to utilize a process in which subjective observations, objective data, an assessment, and the resulting plan (SOAP) are all specified. Four senses (sight, hear, smell, and touch) are generally used when gathering data. Subjective data is focused on identifying issues reported by the owner, manager, or other workers as well as any other qualitative observations. The objective section is focused on quantitative data. The assessment is an evaluation or interpretation of both subjective and objective data. Finally, a plan of action is provided in response to the assessment. Using this SOAP approach allows for a complete and thorough thought process to occur before any diagnosis is made. It is a systematic way to ensure completeness. Consistency is king!

Grouping observations

Many times it is helpful to group observations based on commonalities. It is especially helpful to categorize based on organ system relationships. Grouping observations helps apply Occam's razor (the simplest explanations are more probable). In other words, it is more likely that pulmonary edema, ascites, and respiratory dyspnea in a pig are caused by circulatory system failure rather than the pig having three completely different pathogens, each independently causing one of the clinical findings noted. After grouping observations, a possible differential list can then be compiled.

Damnit

This approach focuses on coming up with a complete differential list to ensure all possibilities, so as to avoid too narrow a focus on infectious diseases. The following list helps identify the terms associated with each letter of the acronym:

D = Degenerative
A = Anomaly
M = Metabolic
N = Nutritional or neoplasia
I = Inflammatory, infectious, or immune mediated
T = Trauma or toxicity

One of the disadvantages of this particular acronym is that it does not help prioritize the list. It also encourages veterinarians, especially those in their early career, to generate a very long list of possible, yet not probable, differentials.

Five production inputs model

One other approach in thinking of differential diagnosis and risk factor list is to think more holistically and ensure that all aspects of production are considered. The five production inputs model of integrating cause and risk factors includes consideration of nutrition, environment, disease, genetics, and management. This model is very useful as it helps ensure multifactorial causes contributing to the clinical issue of concern. The nutritional aspect of veterinary medicine has become more important in recent years as feed prices have dramatically increased. High feed prices have promoted the use of alternative feedstuffs including the use of dry distillers grains (DDGs). The effects of these changes in diets and variability in quality of ingredients on the health of pigs have not been fully investigated. Environment also plays a key role in the health and welfare of pigs as is mentioned throughout this book but especially

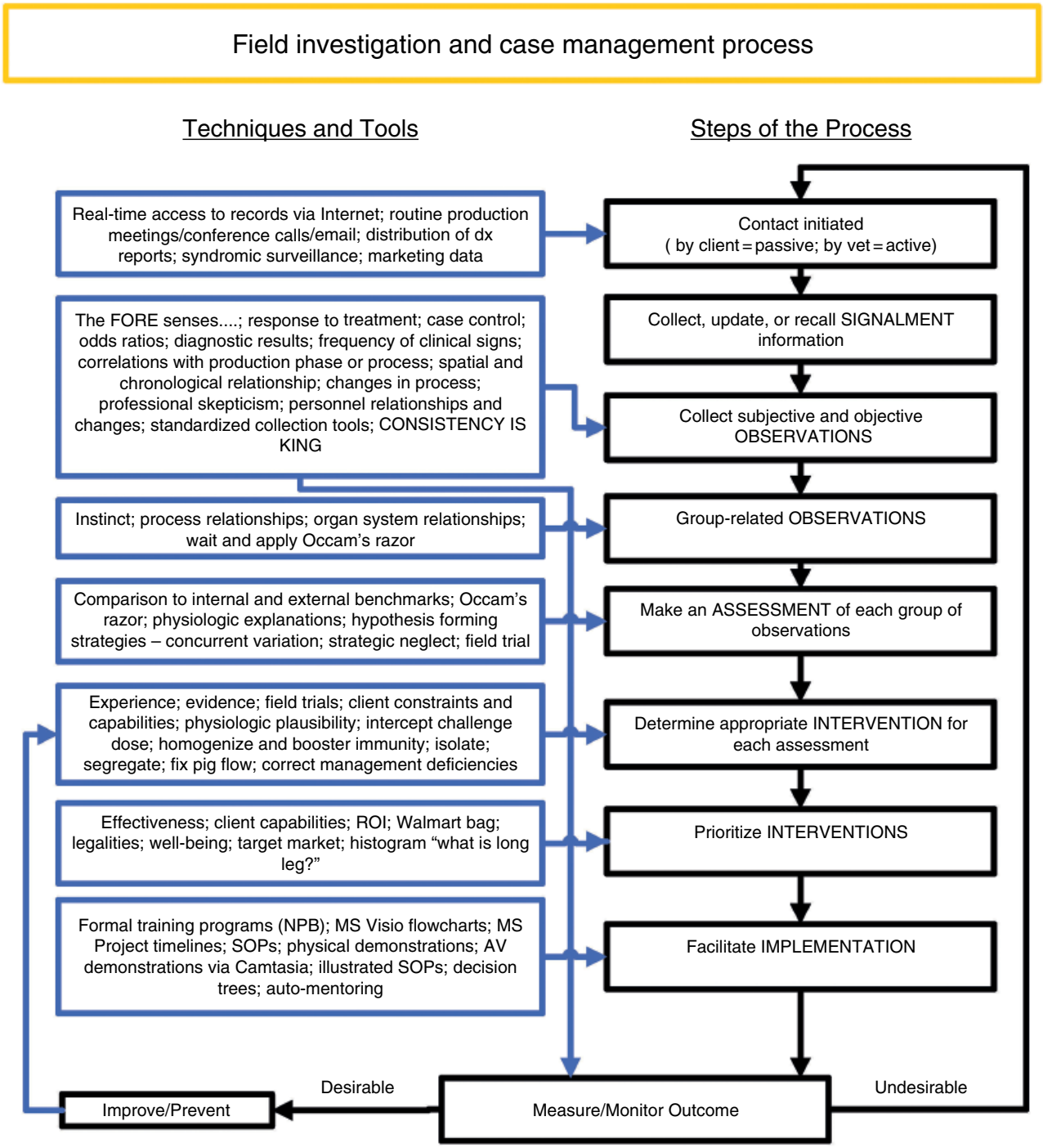


Figure 1.3 Chart depicting the flow of the field investigation and case management process. *Source:* Locke A. Karriker.

in Chapters 2 (behavior and welfare) and 4 (effect of the environment on swine health). The disease component is typically the first focus of veterinarians and is the focus of many chapters in this book. Genetics (Chapter 3) is an input that many times can be confusing as genotype and phenotype expressions are very complex especially when focused on clinical significance. Finally, management, especially all the people involved, is a very integral part of livestock production and can have a tremendous influence on the health, welfare, and success of raising animals. With the urbanization of the world and increasingly fewer people with an agricultural background, training workers on basic husbandry practices is becoming an integral part of any successful operation. New entry-level workers generally have very limited, if any, experience and knowledge on how to raise pigs.

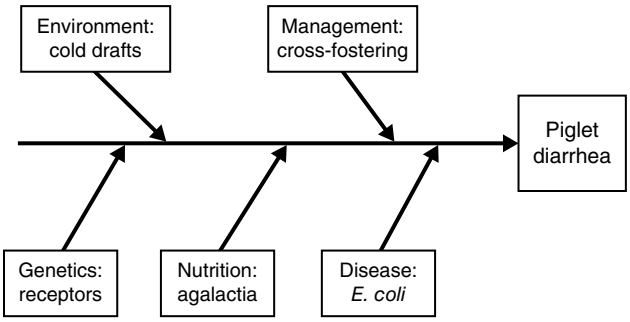


Figure 1.4 Fishbone diagram for piglet diarrhea incorporating the five production inputs model. *Source:* Kent Schwartz.

The five production inputs model works to integrate the interactions of different factors that may be working together at the same time and are influencing the health of a pig. The diagram in Figure 1.4 demonstrates the interaction of possible contributing factors associated with a simple example case of piglet diarrhea.

Determining interventions and prioritization

After observations are made and a list of differentials has been created, the next step is to identify appropriate interventions and prioritize their implementation. This step of the process becomes easier with experience. Personal experiences, client constraints and capabilities, ease, likelihood of success, and impact of intervention all play an important role in helping guide prioritization. It is important to always keep in mind the client's goals and objectives.

From the pigs' point of view, the priorities for survival and health are (fresh) air, (clean) water, (wholesome) food, and appropriate vaccination or treatment as needed. A producer's expectation and a veterinarian's training sometimes place therapeutic intervention as first priority. Vaccines will not be successful unless the pig is placed in an environment that allows the vaccine to work to its full potential. From the pig's perspective, the last area of need is vaccination or treatment as compared to having good quality air as the top priority, with access to good quality feed and water of similar priority.

Many times a diagnostic workup may be necessary to rule different differentials either in or out. Necropsies have been mentioned above, and sample collecting (blood, oral fluids, etc.) will be discussed at the end of this chapter. Chapter 5 will cover some lists for differential diagnosis. Further general information on diagnostics and their interpretation are covered in Chapters 6–8.

Usually priority is given to interventions that will have the greatest impact on the greatest number of animals. Because resources (time and money) are always limited, priorities need to be evaluated based on their cost and benefit as well as overall welfare of pigs and sustainability of operations. The benefit does not always have to be financial. Priorities that require substantial investment in resources usually will require a justification on the expected return.

Reporting

Once interventions have been identified and prioritized, it is critical to provide this information to the client in a concise and clear manner. A farm report or client letter is a very helpful tool in making sure the correct information is being communicated. Written reports and instructions will minimize miscommunications. Reports should be concise and should include a prioritized list (bullet points) with only two or three top interventions. Personal experience suggests that providing too many recommendations allows for the client to lose focus. They may select only recommendations that are desired or easiest to implement. The client may feel as though the veterinarian's recommendations are being followed but in reality have a false sense of security and may be neglecting the most important recommendations. The report should be short (preferable up to 1 page long and definitely no more than 2 pages), which helps ensure the client will actually read it. Very long reports are conducive for a quick skimming by the client, and thus many important points can be missed. Certainly there are times when a comprehensive report is needed, but for routine investigations, simpler is better. Client letters need to be provided back to the client in a timely manner (usually within a few days) in order to maximize implementation of recommendations. Integrated or complex production systems also require knowledge and understanding of the farm or company reporting structure. Veterinarians must understand and follow the proper reporting structure in order to meet client's expectations. The structure serves as means for the central entity and decision-maker(s) to have an understanding on the issues of the entire system. Following proper reporting structures ensures everyone is working together as a team.

Client reports are no substitutes for medical records. Veterinarians should keep detailed records on clinical observations and diagnosis. These complete medical records will serve as an excellent reference for future visits and have legal implications, including the justification for the use of any antibiotic per label or in an extra-label manner.

Monitoring outcomes

It is important for the client to be able to measure outcomes that can help determine the effectiveness of the intervention plans (Figure 1.3). Veterinarians must demonstrate the value they bring in order to be viewed as an asset rather than just a liability (expense).

Sample collection

Blood sampling

Blood sampling is one of the most common sample collecting techniques practiced today. There are several different techniques used in blood sample collecting in swine. Blood sample collecting requires a good understanding of pig's anatomy as all major blood vessels are non-visible, and thus a blind stick is performed. Mastery is achieved though practice. Much of this blood sampling information has been summarized from Dewey and Straw (2006).

Pig restraint

It is important to properly restrain pigs for safe sample collecting both from the perspective of the pig and from that of the person. The size of the pig and the comfort level of the restrainer will dictate the desired method. Figures 1.5 and 1.6 depict two approaches commonly used for restraint. In both cases, the person doing the restraining is just as important as the person collecting the blood sample. Pigs need to be immobilized and held in the correct position to facilitate access to the target veins. In the standing pig, it should have all four feet squarely placed on the ground. Its neck should not be stretched too much; otherwise access to the veins will be much more difficult.

Anterior vena cava

The pig's right jugular groove is identified, and the needle is inserted just cranial to the thoracic inlet. The needle is inserted aiming to the top of the opposite shoulder. This is approximately at a 30° angle from the median and 90° angle from the neckline (line from thoracic inlet to the head). Figure 1.7 depicts the approximate location of major veins. The pig's right side is used for sample collection as the right vagus nerve provides less innervation to the heart and diaphragm than the left vagus nerve. Vagus nerve puncture can cause the pig to start showing signs of dyspnea, cyanosis, and convulsions (Dewey and Straw 2006).

Jugular vein

To reach the jugular vein, the procedure is similar to that of the anterior vena cava with the needle being inserted about 5 cm cranially from the thoracic inlet (Figure 1.5). The right side of the pig is still preferred. The jugular vein is located more superficial than the anterior vena cava but cannot be visualized as in many other species. The process still requires a blind stick.

Figure 1.5 Method of restraining pigs weighing less than 20 kg for blood collection from the anterior vena cava (circle). Location of the cephalic vein is indicated by the dashed line. *Source:* Dewey and Straw (2006). Reproduced with permission of John Wiley and Sons.

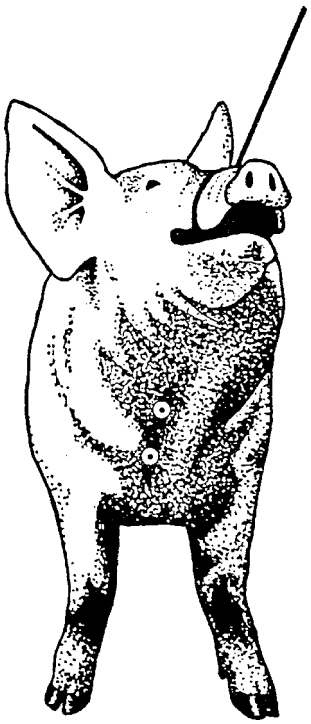


Figure 1.6 Pig restraint for blood sampling from a standing pig. The lower circle indicates the site for sampling from the anterior vena cava; the upper circle indicates the site for sampling from the jugular vein. *Source:* Dewey and Straw (2006). Reproduced with permission of John Wiley and Sons.

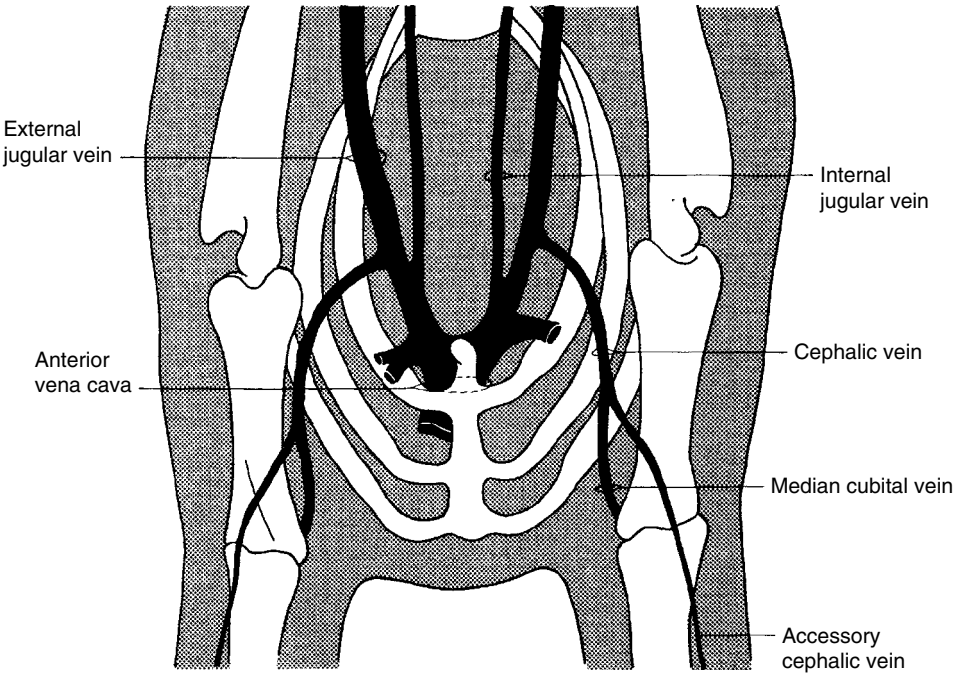


Figure 1.7 Location of some of the major veins in the pig in relation to the skeleton. *Source:* Dewey and Straw (2006). Reproduced with permission of John Wiley and Sons.

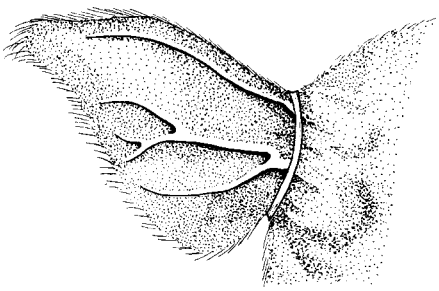


Figure 1.8 Ear veins of a pig raised by a rubber band placed on the base of the ear. *Source:* Dewey and Straw (2006). Reproduced with permission of John Wiley and Sons.

Ear veins

Ear veins can be raised by using a slight tourniquet (usually a rubber band around the ear or pressure with one's thumb) as seen in Figure 1.8. Slight slapping of the back of the ear with one's back of the fingers can help stimulate the raising of the veins. Veins in pigs with colored ears are more difficult to visualize. Venipuncture is done starting at the most distal point (toward the ear tip) of the largest vein so if a hematoma is formed, a more cranial point can still be used for sample collection. A butterfly catheter and syringe should be used. For PCR testing a simple prick of an ear vein with the tip of a 20 g needle can provide enough blood for collection with a Dacron swab.

Miscellaneous Methods

Tail bleeding (Muirhead 1981), femoral vein (Brown et al. 1978), cephalic vein (Sankari 1983; Tumbleson et al. 1968), cardiac puncture (Calvert et al. 1977), and orbital venus sinus bleeding (Huhn et al. 1969) have all been described.

Oral fluid collection

Oral fluid collection for veterinary testing is becoming a more common practice in swine medicine. Oral fluid is a mixture of saliva and oral mucosal transudates. Oral fluids can contain both organisms and antibodies of interest (Prickett et al. 2008).

The process of oral fluid collection is simple and practical. Its use and diagnostic value is described in Chapters 7 and 8.

Sample needs to be identified as an oral fluid sample when submitting for testing as special testing protocols need to be used by the diagnostic laboratory. The variety of PCR and antibody assays validated for oral fluids continue to increase.

Acknowledgments

A special thank you goes to Dr. Anna Forseth for her input in improving this chapter.

References

Baxter S. 1984a. The pig's response to the thermal environment. In *Intensive Pig Production. Environmental Management and Design*, pp. 35–50. London: Granada Publishing.

Baxter S. 1984b. The pig's influence on its climatic environment. In *Intensive Pig Production. Environmental Management and Design*, pp. 55–62. London: Granada Publishing.

Baxter S. 1984c. Space and place. In *Intensive Pig Production. Environmental Management and Design*, pp. 216–248. London:Granada Publishing.

Brown JR, Tyeryar EA, Harrington DG, et al. 1978. *Lab Anim Sci* 28:339–342.

Brumm MC. 2006. Patterns of drinking water use in pork production facilities. Nebraska Swine Report Publication EC 06–219. University of Nebraska, Lincoln, NE, pp. 10–13.

Calvert GD, Scott PJ, Sharpe DN. 1977. *Aust Vet J* 53:337–339.

Dewey CE, Straw BE. 2006. Herd examination. In Straw BE, Zimmerman JJ, D'Alliare S, et al., eds. *Diseases of Swine*, 9th ed. Blackwell Publishing, pp. 3–14.

English PR, Smith WJ, MacLean A. 1982. Weaning, mating and pregnancy maintenance. In *The Sow—Improving Her Efficiency*, p. 240. Ipswich: Farming Press Ltd.

Gonyou HW, Stricklin WR. 1998. Effects of floor area allowance and group size on the productivity of growing/finishing pigs. *J Anim Sci* 76:1326–1330.

Huhn RG, Osweiler GD, Switzer WP. 1969. *Lab Anim Care* 19:403–405.

Lloyd JW, Kaneene JB, Harsh SB. 1987. *J Am Vet Med Assoc* 191:195–199.

Lower A, Johnson C, Waddell J, et al. 2007. Improving the quality of mortality data through necropsy. In Proceedings of the American Association of Swine Veterinarians, Orlando, Florida, pp. 51–52.

Morley PS. 1991. *Comp Cont Ed Pract Vet* 13:1615–1621.

Morris RS. 1982. New techniques in veterinary epidemiology: Providing workable answers to complex problems. In Proceedings of the British Veterinary Association Centenary Congress, Reading, England, pp. 1–30.

Muirhead MR. 1981. *Practice* 3:16–20.

Muirhead MR, Alexander TJL. 1997a. Managing health and disease. In Managing Pig Health and the Treatment of Disease, p. 103. Sheffield: 5 M Enterprises Ltd.

Muirhead MR, Alexander TJL. 1997b. Managing and treating disease in the weaner, grower and finishing periods. In Managing Pig Health and the Treatment of Disease, p. 294. Sheffield: 5 M Enterprises Ltd.

Patience JF, Thacker PA. 1989a. Feeding the weaned pig. In *Swine Nutrition Guide*, pp. 186–187. University of Saskatchewan, Saskatoon: Prairie Swine Centre.

Patience JF, Thacker PA. 1989b. Feeding management of market hogs. In *Swine Nutrition Guide*, pp. 206–207. University of Saskatchewan, Saskatoon: Prairie Swine Centre.

Polson DD, Marsh WE, Dial GD. 1998. *J Swine Health Prod* 6:267–272.

Prickett J, Simer R, Yoon K-J, et al. 2008. *J Swine Health Prod* 16:86–91.

Swine Care Handbook. 2003. National Pork Board. http://porkcdn.s3.amazonaws.com/sites/all/files/documents/Resources/04010.pdf.

Sankari S. 1983. *Acta Vet Scand* 24:133–134.

Tumbleson ME, Dommert AR, Middleton CC. 1968. *Lab Anim Care* 18:584–587.

2

Behavior and Welfare

Anna K. Johnson, Jessica D. Colpoys, Lily N. Edwards-Callaway, Michelle Calvo-Lorenzo, John J. McGlone, Suzanne T. Millman, Christina E. Phillips, Matthew J. Ritter, Mhairi A. Sutherland, Anita L. Tucker, and Sherrie R. Webb

Defining animal welfare and animal cruelty

Animal welfare

Understanding, maintaining, and promoting animal welfare are an integral component of all livestock production systems. Animal welfare is also a topic that in the past several decades has garnered significant attention from the public as the desire to know where and how food is raised intensifies. Over time, many animal welfare definitions have emerged within and between producers, researchers, veterinarians, consumers, packers, and retailers. However, all of them usually include some combination of the following areas of focus: biological function (immune function, growth, etc.), affective states (fear, pain, hunger, etc.), and living in an animal's natural environment (Duncan and Fraser 1997; Fraser et al. 1997).

Disagreements sometimes arise in what constitutes good animal welfare because stakeholders place different levels of importance on biological function, affective states, and natural living based on their personal values. Initially Tannenbaum (1991) and later Fraser (1995) have argued that there exists an "inextricable connection" between animal welfare and values, thus determining that animal welfare cannot be only assessed as a technical issue but must additionally include ethical consideration.

Some focus on the biological functioning of animals as a key welfare indicator, which includes parameters such as reproductive success, immune function, disease presence, and injury (Barnett et al. 1991; Broom 1986, 1991; Mormède 1990; Warnier and Zayan 1985). Assessing affective states such as fear, distress, and pain but also positive states such as pleasure is used in part to measure and understand an animal's welfare state. Duncan and Fraser (1997) has argued that using the term "welfare" in and of itself requires the inclusion of subjective feelings per its definition; if something impacts an animal's welfare, it must impact how an animal feels. The public usually voices concern regarding the inclusion of an animal's ability to act naturally as a significant impact to its welfare state. Although expressing natural behavior is important, some have argued that it actually may decrease overall animal welfare status to express all natural behaviors as some represent states of distress or fear, such as distress calls or behavioral responses to extreme temperatures (Hughes and Duncan 1988).

In 1965 the Brambell Commission was formed to determine what components are essential to ensuring animal welfare in livestock species (Brambell Commission 1965), and this was a poignant event in the realm of livestock species animal welfare. Based on a review of the scientific literature available at the time, the commission proposed several conditions deemed necessary to ensure livestock welfare. In 1979, the Farm Animal Welfare Council revised the Brambell Commission recommendations and created the "five freedoms." The "five freedoms" serve as the basis for many of the livestock welfare educational, assessment, and third-party auditing programs and regulations globally. The five freedoms include the critical aspects of biological functioning (health and nutrition), nature-based measures (expression of normal behavior), and affective states (fear and distress) as discussed previously.

These freedoms are:

1) Freedom from hunger and thirst by ready access to freshwater and a diet to maintain full health and vigor.
2) Freedom from discomfort by providing an appropriate environment including shelter and a comfortable resting area.
3) Freedom from pain, injury, and disease by prevention or rapid diagnosis and treatment.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

4) Freedom to express normal behavior by providing sufficient space, proper facilities, and company of the animal's own kind.
5) Freedom from fear and distress by ensuring conditions and treatment, which avoid mental suffering.

A widely recognized animal welfare definition developed by Broom (1986) notes that the welfare of an individual animal is based on "its state as regards its attempts to cope with its environment." More recently, the World Organization for Animal Health (OIE) has defined animal welfare as "How an animal is coping with the conditions in which it lives" and provides examples that contribute to good animal welfare that include a combination of biological function, affective state, and concepts of natural living (OIE 2010). The OIE is recognized by the World Trade Organization as the international standard setting body for animal health and welfare; therefore, this definition is often referenced in international animal welfare discussions, including those regarding international trade. As retailers and food chains become more focused on animal welfare within their supply chain, many companies have been adopting the "five freedoms" as part of their animal welfare policies for suppliers.

Public, legal, and technical definitions of animal welfare

Over time, three types of animal welfare definitions have been identified: public, legal, and technical (Gonyou 1993). Public definitions of animal welfare reflect society's view of animals and are constructed from the public's previous knowledge of and experience with animals, which can be highly variable. The public definition is constantly changing as societal views evolve. Legal definitions, crafted by legislators, must satisfy and be accepted by the general public as well as be clear and concise for interpretation by the judicial system. Technical definitions of animal welfare are based on measures of welfare and influence how scientific data is interpreted. Different sectors of the population have emphasized one type of measure over another when interpreting animal welfare. Producers and large-animal veterinarians tend to focus on the biological function of the animal, whereas consumers tend to focus on what they perceive to be natural living. There is a fundamental need for a multidisciplinary approach to measuring animal welfare that includes evaluation of biological function (immune function, growth, etc.), affective states (fear, pain, hunger, etc.), and living in an animal's natural environment (Fraser et al. 1997).

Defining animal cruelty

Animal cruelty can be classified as animal abuse or animal neglect. Animal abuse is an intentional act by an individual to purposely inflict physical harm or injury to an animal (USLegal 2010) whereas animal neglect is a failure to act by the animal caretaker. Simple neglect, or failure to provide basic sustenance needs, could potentially be committed due to a lack of knowledge or ability of the owner and can be corrected through education and training. In the swine industry, animal abuse and neglect are defined as acts outside of normally accepted production practices that intentionally cause pain and suffering including but not limited to malicious hitting or beating an animal; applying electric prods to sensitive areas of the animal; driving pigs off high ledges, platforms, or steps while moving, loading, or unloading; dragging of conscious animals by any part of their body; purposeful dropping or throwing animals; causing physical damage to the snout or tusks of a boar as a means to reduce aggression (excludes nose ringing and tusk trimming); and purposeful failure to provide food, water, or minimal care that results in serious harm or death (NPB 2017).

While there are currently no federal laws in the United States that govern livestock care on farm, there are animal cruelty laws in all 50 states. The language, enforcement, and penalties of these laws vary state to state, and it is important for all veterinarians and livestock producers to be familiar with these state laws. They should be familiar with how livestock are defined and classified within the state, what acts constitute abuse, what penalties are associated with violations, and any mandatory reporting requirements that may exist specifically for veterinarians.

Due to the implications of animal cruelty on the health and welfare of animals and people, the American Veterinary Medical Association and the American Animal Hospital Association have policy statements that support veterinarians reporting cases of animal cruelty to the appropriate authorities when education of the caretaker is inappropriate or has failed, even if animal cruelty reporting is not legally mandated in a state (AAHA 2009; AVMA 2009). Anyone involved in animal care should be aware that accurate recordkeeping and documentation of these cases are essential. All of the major audit tools that have been created to monitor animal welfare within the livestock supply chain include some measure of animal abuse and neglect. The North American Meat Institute Animal Handling Audit (NAMI 2013) and the National Pork Board Common Swine Industry Audit (NPB 2017) include observation of animal abuse or neglect as an immediate audit failure. Additionally, pig farms are expected to have a zero-tolerance policy for animal abuse and neglect. All caretakers should be trained on the policy, understand how to report abuse and neglect, and understand the disciplinary steps that are associated with abuse and neglect (NPB 2017).

Comparisons between the domesticated and wild pig

When a veterinarian is assessing animal welfare, they will seek to determine if it is exhibiting normal behaviors. It is necessary to have a concept of what behaviors a feral or wild pig may choose to engage in and how this may be relevant to the domesticated pig. Comparisons between a variety of species when domesticated and wild indicate that the behavioral repertoire of a species remains relatively static during domestication, whereas the quantity of or threshold at which individual behaviors are performed may change (Price 1997). For example, the domesticated pig may perform the same behaviors as its wild ancestor but may not perform those behaviors as frequently, or more frequently. Stolba and Woodgush (1989) observed adult pig behavior in a semi-natural environment and found that although raised in confinement, adult pigs exhibited many behaviors performed by the European wild boar such as rooting, grazing, and nesting. Because domesticated pigs raised in confinement do have similar behavioral needs to their wild counterparts, the environments in which pigs are raised should be designed with the opportunity to express positive behaviors that they are highly motivated to perform.

Deviations in behavior for domesticated pigs compared with their wild/feral counterparts may indicate impaired animal welfare. For example, the presence of stereotypies (behavior(s) performed repeatedly without an obvious function) can be indicative of impaired welfare. Pig stereotypical behaviors include bar biting, sham chewing, and belly nosing. It has been hypothesized that these behaviors develop when a pig is unable to perform highly motivated behaviors, such as foraging, nest building, or suckling (Fraser 1975). However, not all behavioral deviations result in impaired welfare; anti-predator behavior, for example, is a useful behavioral sequence to have in a wild/feral setting for survival but is less important in a controlled and protected housing environment.

Scientific approaches to animal welfare

Biological function: production, health, and animal welfare

Stress can be defined as the nonspecific response of the body to any demand (Selye 1973). In commercial swine production, stressors, defined as stress-producing factors (Selye 1973), can include handling by humans, novel environments (Gray 1979), disease prevalence, high or low temperature, and aggressive pig temperament (Black et al. 2001). While the stress response is essential for animal survival and biological function, it can antagonize swine production goals such as feed efficiency, growth, carcass quality, and welfare.

Stress can occur during both positive and negative situations. Moberg (2000) defines eustress as a nonthreatening stress response and distress as a stress response with deleterious effect on the individual's welfare. Stress is often closely related to pig welfare and hence is often measured. The short-acting stress response, also referred to as the "fight or flight" response (Cannon 1929), is controlled by the sympathetic–adrenal medullary system and is typically measured through epinephrine and norepinephrine. The longer-acting, sustained stress response is controlled by the hypothalamic–pituitary–adrenal axis and is typically measured through adrenocorticotropic hormone (ACTH) and cortisol. Other measures that are commonly used to evaluate the pigs' stress response include endorphin, lactate and glucose concentrations in the blood, heart rate, respiration rate, electroencephalography, and behavior.

Responses to stress influence key metabolic, immunological, and reproductive processes governing disease resistance and production performance. Therefore, health and production performance are also used as animal welfare indicators. Stress can have negative consequences on swine performance as it results in catabolism of body tissues through lipolysis, proteolysis, and glycogenolysis (Weissman 1990). Additionally, behavioral stress responses of decreased feed intake and altered activity level also alter swine performance (Elsasser et al. 2000). In breeding animals, physiological stress responses also influence the hypothalamic–pituitary–ovarian axis. The effects of psychological stressors on performance have been well established in numerous experiments by Hemsworth et al. (1986, 1987, 1996). Unpleasant handling, in comparison with sympathetic handling, resulted in pigs that were more fearful and had chronically elevated corticosteroid levels, slower growth rates, lower pregnancy rates in gilts, and delayed reproductive development in young boars.

Stressful environmental conditions can increase the susceptibility of pigs to infectious diseases through alteration of the immune system (Kelley 1980). Sustained high levels of corticosteroid hormones in the blood can reduce proliferation of lymphocytes and decrease antibody production, impairing the ability of the pig to resist infection. Immune challenge techniques provide another potential set of measures that have been used to assess animal welfare. Morrow-Tesch et al. (1994) demonstrated that social status of pigs had an impact on lymphocyte proliferation in response to a pokeweed mitogen. The pigs that were both dominant and subordinate had lower proliferation than the intermediate pigs in the social hierarchy.

Although production parameters have been considered as appropriate measures of animal welfare (Curtis 1987) and poor productivity can be a useful indicator of a welfare problem, high levels of productivity alone are not always indicative of a high standard of welfare. It is a paradigm that a physically healthy animal is "faring well" but a healthy pig with high productivity could be mentally compromised.

Affective states of animals

Affective states, also referred to as emotional or psychological states, are an integral component of an animal's overall welfare. Although some areas of the scientific community find it hard to accept that animals can experience emotions, neuroscience has indicated that brain structures and neurotransmitters in humans and animals have similar functions and structures (Butler and Hodos 2005; Jerison 1997; Panksepp et al. 2002). Thus, pigs are considered sentient.

The "triune brain," a concept described by MacLean (1990), provides a simple illustration of the adaptations between reptilian, mammalian, and human brain regions. The centermost brain region, shared by all groups, is the limbic brain region. The limbic system is at the top of the spinal cord deep within the cortex and includes structures such as the amygdala, hippocampus, and parts of the diencephalon. It is the emotional center of the brain for both humans and animals (Panksepp 1998). Emotional circuits controlling anger and fear have been mapped in the limbic system (Panksepp 1990; Siegel 2005).

Emotions motivate an animal's behavior. When studying how certain management and production systems impact animal affective states, researchers, veterinarians, and producers usually focus on the negative emotions. For example, scientists have tried to mitigate weaning stress by studying different weaning methods (Colson et al. 2012). Additionally, caretakers try to ameliorate practices that cause stress and fear in pigs such as mixing, transport, and handling. Frustration is another emotion that is studied and often manifests itself in the expression of abnormal behaviors. For example, pigs are highly motivated to perform certain behaviors such as rooting, and when they are prevented from doing so, they may begin to develop oral stereotypies.

Modern animal welfare studies are shifting toward evaluating positive in addition to negative affective states. In a study evaluating pig behavior in anticipation of a reward, Reimert et al. (2013) identified play, playbark vocalizations, and tail movements to be indicators of positive affective states. Lay et al. (1999) assessed both behaviors expressing positive (play) and negative (aggression and stereotypies) affective states in pigs housed in hoop structures as compared with an environmentally controlled slatted floor building. They observed a lower incidence of abnormal behaviors and a higher incidence of play behaviors in the pigs housed in the outdoor hoop structures.

Animal affective states are not only characterized by changes in behavior but also by changes in certain physiological parameters such as activation of the hypothalamic–pituitary–adrenal axis and the sympathetic–adrenal medullary system (i.e. a "stress response"). These changes occur to prepare the animal for the stressor with which they are confronted. It is important to note that many of the physiological changes associated with a stress response are found in response to both negative and positive stressors, and therefore caution needs to be taken when interpreting physiological parameters (Dawkins 1998). Ethologists have designed a variety of experiments that can be used to determine how animals feel about various housing conditions and management systems. Preference tests can also be used to measure an animal's motivation for resources or environments with the underlying assumption that animals approach what they find positive and avoid what they find aversive. When given a choice between different circumstances, pigs can express their relative preference on matters such as diet, floor type, thermal environment, and degree of social contact. Refer to Elmore (2010) for studies detailing how provision of various resources can impact sow motivation and behavior.

Welfare monitoring and assessment

Monitoring and assessing animal welfare provide the producer with benchmarks. These benchmarks can then be used for decision-making regarding best management practices and provide a way for producers to demonstrate that their pigs are receiving care. On-farm measures of animal welfare typically fall into two categories: resource-based or animal-based measures.

Resource-based measures are also called input-, management-, or design-based measures. Examples include space allowance, stocking density, feed and water quantity and quality, frequency of inspections, and stockperson training and other caretaker characteristics such as attitudes, knowledge, and competency. The disadvantage of resource-based measures is that they are indirect indicators of animal welfare and therefore do not provide a true evaluation of how the animal is coping with its environment (Barnett and Hemsworth 2009). However, the advantage of resourced-based measures is that they can identify potential causes of poor animal welfare prior to the welfare of an animal being negatively impacted. Therefore, resource-based measures can be considered "lead" indicators because corrective and preventative actions can be taken for the pigs being evaluated (Manning et al. 2007).

Animal-based measures are also called output- or outcome-based measures. Examples include mortality,

morbidity, culling rates, lameness, injuries, body condition, stereotypic behaviors, aggressive behaviors, and fear behaviors. The advantages to using animal-based measures are that they serve as a direct indicator of animal welfare and they allow for variation in system design and management (Blockhuis et al. 2003). The disadvantage of these measures is that they tend to "lag" indicators, meaning that any existing welfare issues have already occurred for the pigs being evaluated and changes can only be made for future production cycles (Manning et al. 2007).

A robust animal welfare assessment program should include both animal-based measures to identify and fully understand the actual welfare of the animal and resource-based measures to identify potential causes of poor welfare. An animal's welfare state is dynamic and can be influenced by subtle changes in its health or the environment. Therefore, monitoring animal welfare must be an ongoing process.

Several science-based programs have been developed to assess on-farm swine welfare through a combination of first-, second-, and third-party evaluations. Through live observation, the observer evaluates the animals, caretakers, facilities, and records. The objective for first- and second-party evaluations is to benchmark performance and educate on good production practices. The objective of third-party evaluations is independent verification of compliance with a set standard of care. The value of these on-farm evaluations to an animal's welfare, regardless of the observer's relationship with the farm, is found in the feedback of strengths and opportunities for improvement. The producer can use this information to make informed decisions about production practices and procedures and ultimately protect and promote good animal welfare.

Recent technology advancements have introduced the concept of remote video auditing as a tool for animal welfare assessment and monitoring. Video auditing technology can help achieve good biosecurity because new people or materials are not entering the farm to conduct an audit. The technology also provides opportunity for continuous monitoring and spontaneous audits. However, remote video auditing may be difficult to implement on farm due to some facility designs. Video auditing protocols require further development to assure animal- and resource-based measures can be properly evaluated. Wearable video technology may hold merit to resolve this limitation.

Maternal behaviors

Pre-farrowing behaviors of the Sow

Gilts and sows exhibit a specific pattern of behaviors prior to farrowing (Widowski and Curtis 1989, 1990). In non-confined sows (i.e. outdoor arks, indoor huts, or pens), nest building occurs during the last 24 hours' pre-parturition and is most intense 6–12 hours before farrowing (Jensen 1986). During the same time period, sows housed in farrowing stalls have an increased number of posture changes, indicating restlessness, and nest-building behavior is redirected at pen fixtures with the absence of suitable material (Haskell and Hutson 1996).

Preweaning mortality, overlay, and trauma

Preweaning mortality is a welfare and economic problem in all swine housing systems. Piglet survival is due to a variety of complex interactions involving the sow, the piglet, and the environment (Edwards 2002). The causes of piglet mortality, including crushing, starvation, disease, and savaging, can be affected by nutrition, experience, age, health, and injury status (Barnett et al. 2001). Crushing of the piglet by the sow is the predominant cause of preweaning mortality, accounting for 70–80% of total deaths (English and Morrison 1984). Historically, crushing has been viewed as involuntary, mainly caused by the physical environment (Andersen et al. 2005). Recently, it has been hypothesized that differences in maternal behavior play a role in the variation of piglet mortality (Johnson et al. 2007). Crushing can be viewed as a sow's failure to protect her offspring. Among sows, there is a large variation in piglet mortality, even within one farrowing environment. Andersen et al. (2005) found that sows that did not crush any of their piglets ("non-crushers") showed a more protective mothering style than those that crushed several piglets ("crushers"). Non-crushers performed more nest-building activity, responded sooner to piglet distress calls, initiated nose contacts sooner after distress calls, and nosed more piglets during a posture change. These studies suggest it may be possible to decrease preweaning mortality by focusing on maternal behavior.

Housing design heavily influences preweaning mortality. For sows housed in farrowing stalls, most crushing is reported when the sow lies down, and almost none when she rolls over (Weary et al. 1996). The design of the stall can reduce these types of crushing events. In loose farrowing systems, piglets are crushed when the sow lies down and when she rolls over (Damn et al. 2005).

Considering the reciprocal relationship between sow and litter, newborn piglets are dependent on the sow for nutrition, but at the same time, the sow is the greatest threat to piglet welfare due to the possibility of crushing (Grandinson et al. 2003; Lay et al. 1999). Malnourished or starved piglets are more vulnerable to crushing for two possible reasons. First, persistent suckling attempts force them to stay close to the sow for long periods of time (Alonso-Spilsbury et al. 2007), and second, they have poor mobility due to decreased milk intake, and

they are often too weak to respond in a timely fashion to move out of the way of a sow changing postures (Marchant et al. 2001).

Savaging

Aggression directed to newborn piglets by a sow (referred to as savaging) can be defined as an attack using the jaws that results in serious or fatal bite wounds (Chen et al. 2008). Although the cause is poorly understood, the incidence of savaging has been reported to range from 5 to 12% (Harris and Gonyou 2003; Knap and Merks 1987; van der Steen et al. 1988). The cause of savaging in sows is poorly understood. Sows do not exhibit clear behavioral cues, indicating that they will savage in advance, although it has been found that sows that savage had a greater frequency of posture changes beginning before parturition and through the expulsion phase (Chen et al. 2008). Pain and fear are hypothesized to predispose gilts to savaging (Pomeroy 1960). Other possible suggestions for causation include the inability of sows to isolate themselves and perform nesting behavior, climatic stress, and human interference during parturition (Luescher et al. 1989). Savaging almost always occurs during farrowing or directly afterward (Chen et al. 2008) and has been found to be more common in primiparous sows (Harris and Gonyou 2003). Spicer et al. (1985) found that sows who savage often direct their aggression to only the firstborn piglet and are more likely to have been mated at a low body weight.

Harris and Gonyou (2003) suggested that the savaging of piglets born outside of working hours could be reduced by keeping farrowing rooms continuously lit. If savaging occurs and a caretaker is on hand, there are a few steps that can be taken in order to calm the sow: massage the udder, inject a tranquilizer (English and Morrison 1984), and remove the piglets from the sow until farrowing is complete. However, Chen et al. (2008) point out that sedation cannot prevent the behavior before it is administered or guarantee no return of the behavior after recovery from sedation.

Invasive procedures

How can we recognize pain in swine?

Pain is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." The IASP adds, "The inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment." This is an important point, especially when discussing animal pain as they use auditory, physiological, and physical signs to communicate pain rather than verbal language. Pain is a complex phenomenon, and it involves multiple nerve cells, types of nerve chemicals, and different nerve cell receptors to which the nerve chemicals bind in order to propagate a pain signal to the spinal cord and brain (Coetzee et al. 2008). Not only is pain complex from the standpoint of transmission, processing, and control, but it is also complex in that there are different types of pain that have been identified based on cause or pathophysiology, the most important of which are acute and chronic pain. Because of the complexity of pain, it is understandable that pain management and pain control are complicated and difficult.

Acute pain is a protective mechanism that makes one notice an injury, move away from the danger that caused the injury, and then take care of the injury; thus, it is generally short-lived. Pain associated with more severe trauma, like surgery, begins as acute pain but can become chronic with prolonged inflammation. Chronic pain is a persistent kind of pain that may or may not be associated with injury, but is generally associated with inflammation, changes to nerve cells, and hyperexcitability of the nerve cells in the spinal cord and brain (Gudin 2004). This hyperexcitability phenomenon, or "wind-up," is a physiologic increase in sensitization of excitable nerve cells. Because the brain and spinal cord are wound up to detect pain, they are hypersensitive to future painful stimuli; thus, normally mild pain becomes intense pain after repeated physical insults. Prolonged inflammation caused by damaged tissue helps perpetuate the wind-up phenomenon and plays a large role in chronic pain. In addition, the changes in the spinal cord and brain associated with wind-up make pain resistant to treatment with analgesics (Coetzee et al. 2008). Preventing the wind-up phenomenon is an important human presurgery consideration; studies have shown that if analgesic or anti-inflammatory drugs are given to a patient prior to surgery, less analgesic or anti-inflammatory drugs are needed to control pain after surgery. Pigs are commonly teeth clipped, tail docked, and castrated without analgesia or anesthesia on commercial pig farms in the United States (FDA 2010). Scientific information describing effective pain management for these procedures is limited.

Tail docking

In North America, the majority of pigs are tail docked (Marchant-Forde et al. 2009) to prevent tail biting (refer to tail biting in the "ORAL AND LOCOMOTOR BEHAVIORS" section). Tail docking in pigs is usually performed within the first week of life and can be performed with teeth clippers, cutting pliers, scissors, scalpel blade, and gas or electrical cautery iron. The length of

the tail stump varies depending on the producers' standard operating procedures, although generally the remaining stump needs to be at least 2 cm (1 in.) long so that the tail stump covers the vulva in females.

Tail docking using side cutting pliers caused an increase in cortisol concentrations compared with non-docked controls up to 60 minutes after docking (Sutherland et al. 2008, 2011). The behavioral response to tail docking can include tail jamming (clamping of tail stump between the hind limbs without side-to-side movement) (Torrey et al. 2009), tail wagging (Noonan et al. 1994), and posterior scooting (Sutherland et al. 2008). Furthermore, tail-docked piglets produced more grunts (Noonan et al. 1994) and peak vocal frequencies during the procedure (Marchant-Forde et al. 2009; Torrey et al. 2009) compared with control piglets.

There is relatively little research comparing various methods of tail docking or methods of pain relief for tail docking in pigs. Tail docking using a heated cautery iron did not affect ACTH, cortisol, or lactate concentrations in young pigs (Prunier et al. 2005; Sutherland et al. 2008), and cortisol concentrations were lower in pigs tail docked using a cautery iron 60 minutes after docking compared with pigs tail docked using side cutting pliers (Sutherland et al. 2008). In contrast, Marchant-Forde and others (2009) found that docking using cautery iron had a tendency to increase the number of squeals during docking compared with docking using cutting pliers. Administering local anesthetic prior to tail docking or inducing general anesthesia using carbon dioxide gas reduced the percentage of stress vocalizations performed by pigs during tail docking (Herskin et al. 2016; Sutherland et al. 2011). In addition, pigs administered a nonsteroidal anti-inflammatory drug (NSAID) 30 minutes prior to tail docking were less likely to spend time isolated from other pigs than docked pigs given a placebo (Tenbergen et al. 2014). However, administering anesthetic locally or topically to the wound, inducing general anesthesia using carbon dioxide gas, or administering an NSAID did not reduce the cortisol response to tail docking in pigs (Sutherland et al. 2011; Tenbergen et al. 2014). Tail docking is routinely conducted to help prevent tail biting in pigs, and currently there is no alternative to tail docking except to not tail dock. However, strategies can be put in place to help prevent tail biting behavior such as providing enrichment in pens (refer to tail biting in the "ORAL AND LOCOMOTOR BEHAVIORS" section).

Teeth clipping

Born precocious, pigs have their deciduous canines and third incisors fully erupted at birth. These eight sharp "milk" or "needle" teeth function as weapons during sibling rivalries for preferred teats during the first 2–3 days after birth (Fraser and Thompson 1991). As the incidence of facial injuries and udder wounds is higher when needle teeth are left intact, some or all of the teeth may be clipped or ground within a day of birth (Fraser 1975).

When clipping is carried out, different techniques may be used with regard to the portion of the tooth being removed and the instruments used to do so (electric grinder vs. side cutting pliers). To prevent exposure of the vascularized and innervated pulp chamber to infection, it is preferable to remove only the tip of the tooth as opposed to the entire tooth (Heinritzi et al. 1994). Maintaining the appropriate equipment and utilizing good technique also help prevent sharp fragmentation or shattering of the tooth, two conditions that can lead to tongue and gingival lacerations and possible mouth infections (Brown et al. 1996; Meunier-Salaün et al. 2002). As a litter will establish a consistent teat order within 72 hours of birth, removal of the needle teeth beyond this time period is unnecessary and may in fact increase the chances of infection.

Teeth clipping of young pigs did not affect ACTH, cortisol, or lactate concentrations (Prunier et al. 2005); however, β-endorphin concentrations were greater in pigs after teeth grinding compared with clipping (Marchant-Forde et al. 2009). The behavioral response to teeth clipping or grinding includes increased grunting, escape attempts, and squeals (Marchant-Forde et al. 2009; Noonan et al. 1994).

No research has identified a chemical intervention to reduce or eliminate the behavioral or physiological reactions associated with teeth clipping in piglets. As the procedure is commonly performed within a day of birth when piglets are still immature, giving any pain medication that impedes piglet motor skills could increase the risk of crushing when in the presence of the sow. The extra costs and time associated with administering drugs to individual piglets are also considered to be unreasonable for most producers. This once routine procedure has become less practiced by producers in North America as the labor costs and possible risk of oral injury and infection associated with clipping are weighed against the often superficial and limited injuries resulting from piglet fighting.

Castration

Surgical castration of male piglets is a common management practice carried out on commercial swine farms to reduce the performance of aggressive and sexual behaviors and to prevent the development of boar taint. Boar taint is used to describe the unpleasant smell and flavor that can occur in pork from intact mature male pigs. Castration is usually performed surgically by making one or two incisions on either side of the scrotum using a scalpel and then removing the testes. The spermatic

cords are severed by cutting or pulling. Pigs are usually castrated within the first week of life. Reduced suckling behavior was observed in pigs in the 6 hour period following castration (McGlone et al. 1993); therefore, it may be preferable not to castrate pigs within the first 24 hours of life so as not to affect colostrum intake or establishment of teat order.

Pigs surgically castrated without pain relief have increased cortisol (Carroll et al. 2006; Prunier et al. 2005), ACTH and lactate concentrations (Prunier et al. 2005), mean arterial blood pressure (Haga and Ranheim 2005), heart rate (Haga and Ranheim 2005; White et al. 1995), and respiration rates (Axiak et al. 2007) compared with non-castrated control animals. Behavioral changes include reduced nursing, walking, and lying and increased pain-related behaviors (Carroll et al. 2006; Hay et al. 2003; McGlone and Hellman 1988; Moya et al. 2007; Taylor et al. 2001). Castration has also been shown to increase the duration and percentage of stress vocalizations (Puppe et al. 2005) and performance of defense behaviors (Leidig et al. 2009) in pigs.

Orally administered aspirin and butorphanol have been reported ineffective at reducing the behavioral response to surgical castration in pigs (McGlone et al. 1993). However, administering an NSAID (meloxicam) prior to castration reduced postsurgical pain-related behaviors (Hansson et al. 2011). General anesthetics including an injectable anesthetic consisting of xylazine, ketamine hydrochloride, and glyceryl guaiacolate administered intravenously (McGlone et al. 1993); ketamine, climazolam, and azaperone administered intramuscularly or intranasally (Axiak et al. 2007); and gaseous anesthetics including isoflurane (Hodgson 2006, 2007; Walker et al. 2004), sevoflurane (Hodgson 2007), carbon dioxide gas (Gerritzen et al. 2008; Sutherland et al. 2012; Van Beirendonck et al. 2011), and nitrous oxide (Rault and Lay 2011) have been used to reduce the pain caused by castration in pigs with varying levels of success. McGlone et al. (1993) observed an increase in mortality in piglets anesthetized using a general anesthetic and piglets that survived showed suppressed nursing behavior. The sedentary effects of an injectable or inhaled anesthetic (i.e. ketamine, climazolam, and azaperone; isoflurane) can last from 2 to 50 minutes (Axiak et al. 2007; Hodgson 2006, 2007; Walker et al. 2004). A prolonged recovery period from anesthesia could increase the risk of crushing of the piglet by the sow and reduce feeding opportunities. Pigs given local anesthetic prior to surgical castration had reduced mean arterial blood pressure (Haga and Ranheim 2005), slower heart rate (White et al. 1995), and behavioral changes (Kluivers-Poodt et al. 2012; McGlone and Hellman 1988; White et al. 1995) compared with pigs surgically castrated without pain relief. Administration of local anesthetic subcutaneously into the scrotal sac (Haga and Ranheim 2005; White et al. 1995) or intratesticularly (Haga and Ranheim 2005; Leidig et al. 2009) has been shown to reduce the behavioral and physiological response to castration in piglets. Furthermore, Haga and Ranheim (2005) demonstrated that injecting local anesthetic intratesticularly or intrafunicularly equally reduced indications of nociception. Ranheim et al. (2005) recommended injecting local anesthetic into the testes as the local anesthetic is then rapidly transported up the spermatic cords and the animal receives the benefit of analgesia at two anatomical sites, but only one injection is required. Ranheim et al. (2005) demonstrated that the highest concentration of local anesthetic is available in the testicular tissues 3 minutes after injection into the testes. Pigs given local anesthetic 2, 3, or 5 minutes prior to castration showed a reduction in frequency, duration, or number of vocalizations as compared with piglets castrated without any pain relief (Leidig et al. 2009; White et al. 1995). Local anesthetic administered to the testes at least 3 minutes prior to surgical castration appears to provide the most effective pain relief. However, topical administration of a short- or long-acting anesthetic to the castration wound was not effective in reducing the pain associated with surgical castration in pigs (Sutherland et al. 2010).

Alternatives to castration include slaughtering pigs before they reach sexual maturity, using immunocastration techniques, sperm sexing for selection of female offspring, and genetic selection for pigs with low levels of boar taint (Rault et al. 2011). Slaughtering pigs before they reach sexual maturity means harvesting pigs at a lower body weight. However, at a body weight of 80–90 kg (176–198 lbs), 5% of carcasses still exhibit boar taint (Bonneau 1998). Furthermore, the average weight of pigs at slaughter is increasing, and light carcasses are less profitable for commercial swine processors (EFSA 2004). Immunocastration involves immunizing boars against gonadotropin-releasing hormone (GnRH), which uses the boars' own immune system to suppress GnRH, consequently shutting down the stimulus to the testes, resulting in a temporary inhibition of testicular function (Thun et al. 2006). Currently, alternatives such as sperm sexing are still in the experimental stages and are not ready for implementation on farms (EFSA 2004; von Borell et al. 2009).

Tusk trimming

Boars with tusks pose a potential risk to both human handlers and other pigs. Current codes of practice for Western Australia recommend that trimming be carried out in situations where injury is likely to occur, and legislation within Canada prohibits the transportation of tusked boars in the presence of other animals (Health of Animals Act 1990). Current research by

Paetkau and Whiting (2008), however, suggests that injuries are not reduced with tusk trimming, either for boars in transit or when being held in lairage. Though aggression is common among newly mixed boars, neither the length of time assembled in pens, stocking density, size of boar, nor the presence of tusks was found to be influential in the skin injuries sustained in fighting.

Removal of the distal end of boar tusks is often carried out twice per year or prior to transport using one of two methods: clipping (using hoof trimmers or bolt cutters) or sawing (with hacksaw or orthopedic/embryotomy wire). Although more restraint is required, sawing is the preferred method as it provides more precision and less chance of pulp exposure or fracturing. Based on research by Bovey et al. (2008), the length of pulp chamber extending into the tusk beyond the gingiva varies greatly and is not related to boar age. Recommendations for the length at which tusks should be trimmed are approximately 1.5 cm (0.59 in.), as this was found to be slightly beyond the longest extending pulp chamber within their sample group.

Immunohistochemical and histological evaluation of commercial boar tusks by Bovey et al. (2008) indicates that the pulp chamber of boar tusks frequently extends into the tusk beyond the gum line and is innervated in both vascularized and non-vascularized areas. Such innervation suggests the possibility of pain; however, more research is required to determine the type of innervation present (autonomic or sensory). Exposure of the pulp cavity to bacterial infection was also a common condition associated with trimming too close to the gingiva and can progress into gingivitis and pulpitis, two conditions presumed to be painful. Little work has been scientifically conducted to understand the pain associated with tusk trimming. Housing or transporting boars singly reduces the need to trim tusks.

Pain management in the neonatal piglet during routine management: grading the quality of evidence and strength of recommendations

In 2014 global pain experts were invited by the National Pork Board to review the current published work on pain management in the neonatal piglet and were charged to provide recommendations related to pain mitigation. The Dzikamunhenga et al. (2014) systematic review's aim was to synthesize the existing primary scientific literature regarding the effectiveness of pain management interventions used for routine procedures on piglets. The review question was, "In piglets under twenty-eight days old, undergoing castration, tail docking, teeth clipping, and/or methods of identification that involve cutting of the ear tissue, what is the effect of pain mitigation compared with no pain mitigation on behavioral and non-behavioral outcomes that indicate procedural pain and post-procedural pain?" A review protocol was designed a priori. Data sources used were AGRICOLA (EBSCO), CAB Abstracts (Thomson Reuters), PubMed, Web of Science (Thomson Reuters), BIOSIS Previews (Thomson Reuters), and ProQuest Dissertations and Theses Full Text. No restrictions on year of publication or language were placed on the search. Eligible studies assessed an intervention designed to mitigate the pain of the procedures of interest and included a comparison group that did not receive an intervention. Eligible non-English studies were translated using a translation service. Two reviewers independently screened titles and abstracts for relevance using predefined questions. Data were extracted from relevant articles onto predefined forms. From the 2203 retrieved citations, forty publications containing 52 studies met the eligibility criteria. In 40 studies, piglets underwent castration only. In seven studies, piglets underwent tail docking only. In one study, piglets underwent teeth clipping only, and in one study piglets underwent ear notching only. Three studies used multiple procedures. Thirty-two trial arms assessed general anesthesia protocols, 30 trial arms assessed local anesthetic protocols, and 28 trial arms assessed NSAID protocols. Forty-one trial arms were controls where piglets received either placebo or no treatment. Forty-five outcomes were extracted from the studies; however only the results from studies that assessed cortisol (six studies), β-endorphins (one study), vocalizations (nine studies), and pain-related behaviors (nine studies) are reported. Other outcomes were reported in only one or two studies. The authors concluded that confident decision-making would likely be difficult based on this body of work because lack of comprehensive reporting precludes calculation of the magnitude of pain mitigation for most outcomes. In a companion paper, O'Connor et al.'s (2014) objectives were to develop recommendations for pain mitigation in 1- to 28-day-old piglets undergoing castration. Recommendation development followed a defined multistep process that included an evidence summary and estimates of the efficacies of interventions. Recommendations were developed for three interventions: CO_2/O_2 general anesthesia, NSAIDs, and lidocaine for use during castration. The ability to make strong recommendations was limited by low-quality evidence and strong uncertainty about variation in stakeholder values and preferences. The panel strongly recommended against the use of a CO_2/O_2 general anesthesia mixture, weakly recommended for the use of NSAIDs, and weakly recommended against the use of lidocaine for pain mitigation during castration of 1- to 28-day-old piglets.

Feeding and drinking behaviors

Dental development in the pig

The beginning of initial food mastication is highly dependent on the level of cheek teeth development (Langenbach and Van Eijden 2001), and this is one physical feature often overlooked in the ontogeny of the pig. Having a diphyodont dentition, pigs have all deciduous teeth replaced by their permanent counterparts by approximately 2 years of age (Tonge and McCance 1973). Their deciduous dentition numbers 28 ($2 \times$ incisors $^{3\ \text{upper}}/_{3\ \text{lower}}$, canines $^{1}/_{1}$, premolars $^{3}/_{3}$, molars $^{0}/_{0}$) with their permanent set increasing to 44 ($2 \times i^{3}/_{3}$, $c^{1}/_{1}$, $p^{4}/_{4}$, $m^{3}/_{3}$), the most comprehensive for any eutherian mammal (Tonge and McCance 1973). The majority of teeth used for masticating feed (i.e. the deciduous premolars) erupt between the first and fifth week of life (Tucker and Widowski 2009) and influence preweaning feeding behavior in an age-dependent fashion. Initial premolar eruption often induces bleeding and localized inflammation of the surrounding gingiva and is associated with lower feed-oriented behavior prior to 18 days of age (Tucker et al. 2010). By 21 days of age, piglets engage in more feeding behavior as premolars continue to erupt and occlude (make contact in opposing jaws). Increased levels of occlusion result in more efficient feeding (Huang et al. 1994). At weaning, piglets having both their p^{3} and p_{4} erupted (i.e. the two premolars required for initial occlusion) and have higher weight gains in the following 3 weeks (Tucker et al. 2010). In addition to eruption, growth of the masticatory muscles and learning of the motor patterns involved with chewing are also essential for feeding development. Factors influential to the timing of premolar eruption include piglet birth weight and average daily gain (ADG) in the first 2 weeks of life (Tucker and Widowski 2009).

Development of feeding and drinking behaviors

The development of independent ingestive behaviors (i.e. feeding and drinking) follows different trajectories and is controlled by different motivational systems in the young pig (Widowski et al. 2008b). Drinking has been defined as voluntary oral ingestion of liquids (Hurnik et al. 1995) and refers to the total consumption of water, which includes water that is often contained in feed (Fraser and Broom 1997). The discovery and consumption of water after birth can be facilitated by supplying bowl versus nipple or press lever drinkers and is significantly more effective if auditory bubbling cues are present. Drinking behavior can develop within several hours of birth when piglets require supplemental nutrition or hydration, particularly in response to high environmental temperatures (Fujii et al. 1990; Phillips et al. 2001). Between birth and 4 weeks, water intake increases as a function of age, but consumption per kg body weight remains constant at about 50–65 mL/kg (Phillips and Fraser 1990).

Immediately after weaning, piglets often increase their time at the drinker, possibly to alleviate feelings of hunger (by increasing gastric fill) that develop in response to low feed intakes or to relieve gastrointestinal discomfort associated with a sudden shift in feed composition and form (i.e. from a high fat and lactose-rich liquid diet to a high protein and starch solid diet). As feeding becomes established after weaning and consistent meal patterns develop, drinking becomes prandial, and most water is consumed around meal times.

The transition from suckling to independent feeding requires development in both the piglet's peripheral features (i.e. eruption of teeth, gastrointestinal maturation) and central features (i.e. shifting of motivational systems; Huang et al. 1994). The time course of this development often varies greatly between individuals and litters but is always gradual in nature. Abrupt artificial weaning in modern intensive systems therefore presents one of the most difficult periods to manage as piglets experience nutritional, emotional, and environmental challenges simultaneously. Exploration and social facilitation help piglets during the earlier stages of feeding development, with nutritional benefits becoming increasingly important as maternal inputs decline (Appleby et al. 1992; Delumeau and Meunier-Salaün 1995; Morgan et al. 2001). Overall physical maturity is the best indicator for when piglets develop independent feeding, with larger, more robust individuals ingesting more feed at earlier ages relative to their smaller, less mature littermates (Appleby et al. 1991).

Providing creep diets prior to weaning can familiarize piglets with solid feed and entice earlier consumption, particularly if those diets are complex or offered in gruel form (Fraser et al. 1994; Toplis et al. 1999). Another creep feed attribute that improves feed intake following weaning is pellet diameter. van den Brand et al. (2014) found that piglets prefer larger versus small pellet diameter (12 mm vs. 2 mm) in the preweaning period, and this led to increased feed intake and body weight gain after weaning.

Ingestion of creep feed can help prepare the gastrointestinal system for the post weaning diet by stimulating the production of certain digestive enzymes (de Passillé et al. 1989), a necessary step in the complex digestive transition accompanying weaning. Because there is significant variation in feeding, both within and across litters, with the majority of piglets not consuming significant quantities until after 19 days of age (Fraser et al. 1994), the effectiveness of using creep feeding as a management practice in preparation for weaning needs to be carefully considered with weaning age in mind.

Troubleshooting to enhance feeding and drinking behaviors

Farm animals form a social hierarchy or rank order that can affect accessibility to key resources within their pen (Bouissou 1965). In competitive situations, higher ranked animals might have more access to feed and water. If the producer considers the placement of drinkers within a pen and/or the ratio of drinkers to pigs, then lower ranking animals might have more success in obtaining water. Likewise, by increasing feeder space and feeding times (e.g. by using multiple trickle feeders for group-housed sows), aggression surrounding this limiting resource will be reduced.

Oral and locomotor behaviors

Tail biting

Tail biting behavior occurs when one pig takes the tail of another pig into its mouth and causes damage to the appendage (Schrøder-Petersen and Simonsen 2001). The behavior is often described as beginning with non-damaging exploratory behavior by one pig, termed "tail-in-mouth" behavior, which leaves no visible trauma but which then escalates to a damaging stage and the development of lesions (Fraser and Broom 1997; Schrøder-Petersen and Simonsen 2001). Once damage occurs, other pigs may quickly join in performing tail biting behavior, which can result in pen-wide or even barn-wide outbreaks (Fraser 1987). In addition to causing pain and distress, tail biting is associated with reduced feed intake and weight gain (Sutherland et al. 2009; Wallenbeck and Keeling 2013). The behavior can also lead to infection, spinal abscess, disease transmission, carcass damage, and, in some cases, cannibalism and death (Kritas and Morrison 2007; Schrøder-Petersen and Simonsen 2001).

Recommended as an animal-based measure for on-farm welfare audits (Goossens et al. 2008), the frequency of tail biting is a serious welfare and production issue. Although tail docking may reduce tail biting, it does not eliminate it, with upward of 2% of tail-docked pigs exhibiting signs of having been tail bitten by the time they arrive at the packing plant (Moinard et al. 2003; Smulders et al. 2008).

While there appears to be no single factor that results in tail biting (Goossens et al. 2008; Schrøder-Petersen and Simonsen 2001), numerous management, environmental, and individual factors have been implicated (D'Eath et al. 2014; Sutherland et al. 2009; Wallenbeck and Keeling 2013). For example, a barren growing environment has been shown to result in an increased percentage of pigs with a bitten tail (Bolhuis et al. 2005; Moinard et al. 2003). There is increasing evidence that crowded environments lead to tail biting (Moinard et al. 2003; Randolph et al. 1981), although it may be space allowance in the post weaning period that is most critical (Bovey et al. 2010; Smulders et al. 2008). Therefore, adequate space allowance in the nursery and grower barns can help reduce the behavior, as can the installation of chains or other chew toys, or the provision of straw (Day et al. 2008; Zonderland et al. 2008). These enrichments may work to draw the pigs' oral attention away from one another.

Tail dock length may also play a role in tail biting; however, the ideal length is still undetermined. Recently Bovey et al. (2010) found that a longer (4.5 cm [1.8 in.]) docked tail led to more tail biting than a shorter (1.2 cm [0.5 in.]) docked tail. It has been proposed that longer tails are more easily damaged, as pigs are able to bite them with their check teeth (Paoli et al. 2016).

Not all pigs tail bite, and it appears that some pigs may be predisposed to performing injurious oral behaviors, while others are predisposed to receiving it (Brunberg et al. 2013). Pigs with a predisposition to tail bite may be lighter at weaning (Beattie et al. 2005), be more active, and perform more nosing behavior (Keeling et al. 2004) compared with other pigs. There also appears to be a genetic component to tail biting, with the behavior correlated with lean tissue growth and backfat thickness (Breuer et al. 2003). In addition, there appear to be "neutral" pigs that have a genetic and behavioral profile that contributes to them being resistant to performing or receiving pig-directed abnormal behaviors (Brunberg et al. 2013).

Recent studies have also indicated physiological differences associated with the tail biting behavioral phenotype. Both tail biter pigs and bitten pigs were found to have lower peripheral serotonin levels compared with neutral pigs (Ursinus et al. 2014). Differences between neurotransmitters in the brain regions of pigs that tail bite and those that are bitten have also been identified. Those pigs that tail bite show higher serotonin metabolism in the prefrontal cortex, while bitten pigs show changes in both dopamine and serotonin metabolism in their limbic cortex and striatum (Valros et al. 2015).

Regardless of the cause of the behavior, the removal of both the tail biter and tail bitten pigs is an important management strategy to both reduce the probability of social facilitation of tail biting and stem any increased harm to the pigs being injured.

Belly nosing

Belly nosing was first described over 30 years ago as the distinctive, rhythmic up-and-down movement of one piglet rubbing the belly of another with its snout (Fraser 1978). This behavior, when performed persistently, can result in skin lesions on the belly and flank of the receiver

and may ultimately lead to ulceration (Straw and Bartlett 2001). Although most piglets perform some belly nosing, not all do, and there is a wide variation in the amount of belly nosing individual piglets perform.

Although belly nosing is most often associated with weaning at an early age (Fraser 1978; Worobec et al. 1999), the motivation behind the behavior is yet undetermined. It has been suggested that this behavior is the result of discomfort and stress in newly weaned piglets (Dybkjær 1992). However, since the motor patterns performed during belly nosing appear similar to those used in suckling, many researchers have hypothesized that belly nosing is redirected suckling behavior (Fraser 1978; Metz and Gonyou 1990; Widowski et al. 2008a). Other factors associated with higher frequencies of belly nosing include the presence of certain forms of enrichment (Funbar) (Bulens et al. 2015), rearing entire male pigs (Tallet et al. 2013), and rearing pigs in artificial rearing systems (Rzezniczek et al. 2015).

There appears to be a link between age and weight-for-age (Gardner et al. 2001; Torrey and Widowski 2006), but it is unclear whether there is an optimum age or weight at which to wean piglets. There also appears to be some genetic component to belly nosing, with Landrace pigs performing the behavior more than Duroc pigs (Bench and Gonyou 2007; Breuer et al. 2003). Provision of environmental enrichment (EE) (Oostindjer et al. 2011; Rodarte et al. 2004; Waran and Broom 1993), suckling devices (Rau 2002; Widowski et al. 2005), alternative drinkers (Torrey and Widowski 2004), and rearing pigs in loose housing systems during lactation (Oostindjer et al. 2011) have been successful in reducing, but not eliminating, the behavior.

Lameness

Swine lameness on farm can result in negative affective states (i.e. pain) to individual animals (Jensen et al. 2012). Veterinarians and caretakers can use on-farm scoring methods to determine lameness level within their herd. The implemented scoring system needs to be quick and affordable yet accurate. Two subjective scoring systems, the numerical rating scale and visual analogue scale, have been applied to characterize lameness in animals (Quinn et al. 2007). The numerical rating scale uses 4–6 ordinal categories to score lameness (1 being a sound animal and 6 being an animal that is unable to rise). Alternatively, the visual analogue system utilizes an observer's perception of lameness. An observer is asked to place a mark on a 100 mm (4 in.) line between two endpoints of normal and "could not be more lame" for an individual's level of lameness (Quinn et al. 2007).

There has been interest in testing other lameness tools. Tools include the embedded microcomputer-based force plate system (Sun et al. 2011), the GAITFour pressure mat gait analysis walkway system (Karriker et al. 2013; Mohling et al. 2014a; Pairis-Garcia et al. 2015a), nociceptive threshold tests (Mohling et al. 2014b; Tapper et al. 2013), classification lameness tress (Abell et al. 2014), and behavior (Pairis-Garcia et al. 2015b; Parsons et al. 2015, 2016).

When considering behavior in more detail, Stienezen (1996) observed sows prior to farrowing and through lactation for overgrown hooves. The authors reported no behavioral (percentage standing, dog sitting, or lying) differences between normal sows and sows with overgrown hooves in the 6 hours leading up to the first piglet being born but found some differences when observing the sows immediately before, during, and after their morning feed. Phenotypically normal (control) sows spent more time feeding and more time standing than sows with overgrown hooves. There were also some differences in the number of rear leg slips and rising attempts between control and overgrown hoof sows. In addition, sows with overgrown hooves tended to produce smaller-sized litters compared with control sows. In another study, Leonard et al. (1997) found that time spent feeding and standing decreased and weight shifts and slipping increased in sows with overgrown rear hooves. These results indicate that sows with overgrown rear hooves exhibited discomfort and thus decreased the amount of weight-bearing time spent on the overgrown hooves.

Pairis-Garcia et al. (2015a) noted that caretakers and veterinarians can use husbandry and management tools to provide supportive care for pigs experiencing lameness. Supportive care may include providing additional bedding or a rubber mat to create a more comfortable area for lying and resting (Elmore et al. 2010; Pluym et al. 2013). Campler et al. (2016) provided a practical case study for the use of mats in the farrowing/lactation house. This case study covered the cost, implementation, and longevity of mats. The authors concluded that (1) perforated rubber mats may provide an easy and inexpensive way to improve sow comfort in the farrowing stall; (2) mat size, cleanliness, cost, durability, and management are important factors to consider; and (3) rubber mats need to be placed properly under the sow and fastened properly to ensure maximum sow benefit.

Another approach for on-farm pain management is pharmacological techniques such as analgesics. NSAIDs are common analgesic medications used in livestock as they are easy to administer, long lasting, and cost effective. The pharmacokinetic profile of meloxicam (Pairis-Garcia et al. 2014) and flunixin (Pairis-Garcia et al. 2013) in mature sows has been determined. Paris-Garcia et al. suggested that meloxicam and flunixin meglumine are effective pharmaceutical interventions for alleviating pain associated with a chemically induced synovitis lameness model. Although analgesic drugs may be a key

tool to manage negative pain affective states associated with lameness at the time of writing, meloxicam and flunixin meglumine are not approved pain management treatments in swine in the United States.

Human and animal interactions

The role of the caretaker and the interaction of people and pigs

There is a prevalent and long-held belief that the caretaker has a more important influence on pig welfare than the choice of production system (Brambell 1965). This is likely because humans play a number of important roles for the pig. Humans act indirectly through their responsibility for the design of the environment and development of husbandry and management regimens. Caretakers also act directly by providing the day-to-day care of the animals. The human caretaker is the critical factor in the success or failure of a housing system and can impact pig welfare (Hemsworth et al. 1989, 1993, 1994). There are three important factors that will determine whether or not an individual will be a successful caretaker: (1) the caretaker's knowledge and expertise; (2) the caretaker's personality, attitude, and beliefs (Broom and Johnson 1993); and (3) the caretaker's situational variables (personnel details; Spoolder and Waiblinger 2009), all of which may be interrelated.

Fear of humans

Animals are "neophobic," that is, they are fearful of novel or unfamiliar things (Rushen 1996), and excessive fear is of concern to animal producers. Fear is defined as the general susceptibility of an individual to react to potentially threatening situations (Boissy et al. 2007), and fearfulness has been posited as a personality trait in a variety of animal species (Gosling 2001). Fearful animals are likely to grow more slowly and less efficiently than nonfearful animals and to have reduced reproductive output (Hemsworth et al. 1987, 1989, 1993).

Human exposure is one of the most frightening events that farm animals are likely to experience (Boissy 1995). In swine production, humans may have little interaction with pigs other than situations that might be perceived as negative by the pig. These situations can include medically treating (Weimer 2012), castration, tail docking, restraining, and sorting (Waiblinger et al. 2006). With little opportunity to habituate, it is suggested that even domesticated animals may often perceive humans as predators (Suarez and Gallup 1982). However, previous positive experiences with humans such as gentle tactile interaction, talking, and food provision may decrease pigs' fear of humans (Brajon et al. 2015; Muns et al. 2015; Tallet et al. 2014). Additionally, genetic selection (Colpoys et al. 2014), pig sex (Colpoys et al. 2015; Reimert et al. 2014), and housing system (Reimert et al. 2014) have been shown to alter pig–human interactions.

Fatigued pigs

Transport losses due to injury, fatigue, or death represent significant animal welfare, regulatory, and economic concerns and are estimated to cost the US swine industry $46M annually (Ritter et al. 2009a). These dead and nonambulatory pigs are most commonly observed during unloading at the packing plant, but these losses can occur at any stage of the marketing process from loading at the farm to stunning at the plant. Transport losses at US packing plants include:

- Dead on arrival (DOA): A pig that died during transportation.
- Dead in yard (DIY) or dead in pen (DIP): A pig that died after unloading at the plant.
- Nonambulatory pig: A pig unable to move or keep up with the rest of the group at the plant.

There are two types of nonambulatory pigs observed under US commercial conditions: Fatigued pigs are pigs without obvious injury, trauma, or disease that refuse to walk at any stage of the marketing process from loading at the farm to stunning at the plant. Meanwhile, injured pigs have a compromised ability to move due to structural unsoundness or due to an injury sustained during the marketing process (Ritter et al. 2009a).

Incidence of dead and nonambulatory pigs at packing plants

According to national statistics reported by the Food Safety and Inspection Service (FSIS), the percentage of dead market swine at USDA-inspected packing plants has averaged 0.20% over the last 25 years (Ritter et al. 2017). Although national statistics are not available for nonambulatory pigs at the plant, a recent summary of 23 US commercial field trials involving 6.6 million pigs reported the following rates for transport losses at the plant: 0.25% for dead pigs and 0.44% for nonambulatory pigs. It is important to note that the vast majority of nonambulatory pigs in these studies were classified as fatigued (Ritter et al. 2009a).

The fatigued pig syndrome

Ivers et al. (2002) evaluated acute stress signs and metabolic parameters in 35 normal and 35 fatigued pigs during unloading at the packing plant. Fatigued pigs showed more clinical signs of acute stress including open-mouth breathing (44 vs. 0%, respectively), skin discoloration (77 vs. 0%, respectively), muscle tremors (83 vs. 3%,

respectively), and abnormal vocalizations (30 vs. 0%, respectively). Furthermore, fatigued pigs had higher stress hormone concentrations (cortisol, epinephrine, norepinephrine), higher creatine kinase values, and blood parameters consistent with metabolic acidosis. Controlled studies have demonstrated that the vast majority of fatigued pigs will metabolically recover if the stressors are removed and pigs are allowed to rest for 2–3 hours (Ritter et al. 2009a).

Porcine stress syndromes

It is interesting to note the striking similarities between the symptoms and metabolic characteristics of fatigued pigs to those of pigs with porcine stress syndrome (PSS) (see Chapter 3), which is caused by a C to T mutation at nucleotide 1843 of the RYR1 gene and is referred to as HAL-1843. Therefore, a commercial survey involving 2109 pigs was conducted at four Midwestern US packing plants to determine the impact of the HAL-1843 mutation on the incidence of dead and fatigued pigs at US packing plants. This study demonstrated that 98% of the normal pigs, 95% of the dead pigs, and 98% of the fatigued pigs evaluated were free of the HAL-1843 mutation (Ritter et al. 2008), suggesting that the HAL-1843 mutation has minimal effects on dead and fatigued pigs at the packing plant.

It is possible that other genes or mutations may be responsible for the fatigued pig syndrome. Recently, Nonneman et al. (2012) reported that a mutation in the dystrophin gene (DMD) was associated with death in pigs during routine handling and transportation. Additional research is necessary to understand if this new stress syndrome contributes to the fatigue pig syndrome.

Predisposing factors for transport losses

Transport losses are a multifactorial problem consisting of people (handling tools and handling intensity), pig (genetics, diet, ractopamine, gut fill, live weight, health status, and previous handling experiences), facility design (pen size, pre-sorting strategies, aisle width, distance moved, and loading ramp angle), transportation (trailer design, mixing of unfamiliar pigs, loading density, and length of journey), packing plant (waiting time at the plant, unloading procedures, distance moved, facility design, and lairage time), and environmental factors (season, temperature, relative humidity, and trailer settings for bedding, boarding, and misting), which have been reviewed by Ritter et al. (2012), Johnson et al. (2013), and Zurbrigg et al. (2017). A review by Ritter et al. (2012) concluded that transport losses are impacted by (1) the HAL-1843 mutation, (2) aggressive handling, (3) group size during handling, (4) facility design, (5) crowding pigs during transport, and (6) extreme hot and cold weather conditions.

Management strategies to reduce transport losses

Preslaughter stressors have additive effects on the stress responses (rectal temperature, blood lactate, and blood pH values) of market weight pigs (Ritter et al. 2009b). Therefore, removing or spacing out the stressors to allow the pig to return to homeostasis during the marketing process can improve the pig's well-being and can potentially reduce the risk of transport losses at the plant. Management strategies to reduce transport losses under US commercial conditions include better preparing pigs for transport, improving facility design, minimizing stress during handling, and optimizing transport conditions (reviewed by Ritter et al. 2012).

Aggression

The domestic pig is largely a social animal. In the wild or feral state, pigs are found either in a matriarchal group of one to five adult females and two or more ages of offspring from successive pregnancies (Barrett 1978). When males reach puberty, they leave the herd and travel either alone or in small groups (ex. 2 boars). Females and young males are clearly social, while adult males are often (but not always) solitary. As piglets from different mothers in a herd are born, they interact with other piglets with minimal aggression. They may play fight or have small skirmishes, but among prepubertal pigs, there are infrequent injuries from fighting. Boars do fight and can injure each other in the wild; however, injury is uncommon. And sows, generally, are dominant to boars most of the time. When sows are in estrus, they will allow boars into the group for mating.

When pigs moved from outdoor pastures (where litters were socialized from birth) to indoors, litters were often kept apart from birth through weaning. We have known for decades that aggression is common on commercial farms (Signoret 1962). This aggression is a function of the production system in that unfamiliar pigs are abruptly introduced without a preweaning socialization period.

For growing pigs, mixing-induced aggression can occur when young pigs are mixed after birth to equalize litter size, after weaning, during transport, and at the packing plant. Sows are mixed when returning from farrowing to the breeding herd – and if they were previously housed individually or in small groups, they will fight. Post mixing aggression establishes a social hierarchy. This fighting is stressful but is reduced over time. McGlone (1986) showed that aggressive interactions

last for 19 hours after mixing pigs. He further reported that access to feed (or not) did not change the amount of fighting observed. Water access is more closely related to social stress. Aggressive behavior and the development of a dominance hierarchy will occur regardless of resources (feed, water, space, etc.). However, chronic or sporadic aggression may be due to limited resources. For example, even with established dominance hierarchies, growing pigs and sows that are limit fed (less than ad libitum) will show aggression. These challenges to the dominance order will occur, for example, when feed is given in limited amounts (Graves et al. 1978). Hunger makes pigs more aggressive, even if it is a few hours between meals (Kelley et al. 1980). Pigs may fight over limited resources such as feed, water, breeding mates, and/or nesting sites (Barnett et al. 1994; Csermely and Wood-Gush 1987; Edwards et al. 1994; Séguin et al. 2006). Interestingly, the social hierarchy is established without the need for all pigs to fight with each other. Mendl and Erhard (1997) mixed 4 pigs from 1 established group with 4 pigs from another group 11 times and in no single case did all 16 possible unacquainted pairs fight before stability was reached. There is a mechanism by which domestic pigs are able to assess their relative fighting ability or relative place in the hierarchy based upon information gained from their own interactions and probably from interactions of other pairs. Preexposure that permits a mixture of visual (e.g. physical size), auditory (e.g. frequency or duration of vocalizations), and olfactory (McGlone 1985) cues could reduce fighting post mixing (Durrell et al. 2003).

Persistent aggression can decrease welfare as indicated by increased stress hormone concentrations (Otten et al. 1999), increased heart rates (Marchant et al. 1995), increased injuries, and restricted access to resources (O'Connell et al. 2003) in animals that are aggressive or ones that are being attacked. Aggression can also increase costs by slowing growth and decreasing productivity (Mendl et al. 1992). During an aggressive act, a pig focuses its bites on its opponent's head and ears (Kelley 1980). When one pig submits to another pig in close quarters, it tries to protect its head and ears (McGlone and Curtis 1985). McGlone and Curtis (1985) also first reported the strong relationship between duration of aggression and the presence of wounds. Wound scores can be used in practice to determine the relative amount of aggression in groups of pigs.

A number of options have been explored to manage and reduce aggression in pigs. Pen shape has been reported to affect aggression in the short term. For example, pigs often use corners to "hide" (McGlone and Curtis 1985), and circular pens resulted in higher levels of aggression than square or rectangular pens. A solid barrier within the pen reduces the total number of aggressive interactions over a 12 hour post mixing period in sows (Edwards et al. 1993) and has longer-term benefits in sows. Barnett et al. (1993) compared adult pigs at mixing when placed into small rectangular pens (1.4 m^2/pig [15.07 ft^2]) for aggressive interactions and the consequent retaliations. The authors reported during the period of 15–90 minutes after grouping lower aggressive interactions, but the presence of stalls had no effects at this time. On the day following grouping, lying alone and standing were reduced, and concurrent lying and use of stalls (when present) were increased. In dynamic systems for sows, where subgroups are mixed into a larger resident group, dividing the pen into distinct lying bays, with one assigned to each subgroup on introduction, may have long-term advantages in reducing aggression by giving each subgroup its own "territory" (Bünger and Kallweit 1999).

Aggression can also be managed by adjusting group size. There are two hypotheses with regard to optimum group size: (1) that the number of fights will increase with the number of hierarchy positions to settle (Anderson et al. 2000; Schmolke et al. 2003) and (2) that pigs become less aggressive and may shift to a low aggressive social strategy in large social groups that may in turn provide potential benefits for the welfare of pigs under commercial production situations (Samarakone and Gonyou 2009). For a review on the impact of large groups on productivity, see Turner et al. (2003). However, to date, the optimum group size, parity balance within a group, body weight allocation, and space allowance remain relatively undecided in the United States. Probably very large groups of pigs (e.g. 1000 pigs in a large pen or building) never allow all pigs to establish a dominance order, so a small amount of fighting might be observed, or alternatively, pigs learn to not initiate aggressive interactions due to the large number of individuals in the group.

Chemical and nutritional interventions can be utilized to reduce aggression. Gonyou et al. (1988) compared levels of aggression when injected with amperozide (1.0 mg/kg IM), azaperone (2.2 mg/kg IM), or saline (0.1 mL/kg IM) immediately prior to mixing. Both drugs reduced total fighting. Amperozide resulted in fewer fights involving two pigs than azaperone or saline. Injuries to the ears and total injuries were less severe in amperozide-treated pigs than in pigs on the other treatments. Amperozide-treated pigs spent less time eating on day 1 than saline- or azaperone-treated pigs but compensated on day 2 such that total eating time in 2 days did not differ. Both drugs reduced agonistic behavior but had no effect on performance. Similar effects have been found using anti-aggression (amperozide) (Barnett et al. 1993, 1996) and sedative (azaperone) (Luescher et al. 1990) drugs. With both of these, aggression appears to be

reduced, while the effects of the drug last, but once the effects have worn off, aggression rebounds to that seen with untreated animals.

Pigs have a well-developed sense of smell. Removal of the olfactory bulb significantly reduced pig aggression (Meese and Baldwin 1975), and pigs can tell one another apart by olfaction alone (Meese et al. 1975). McGlone (1985) and McGlone et al. (1987) found evidence that biological fluids change pig behavior including aggression. They proposed several pheromones that increase or decrease pig aggressive or submissive behaviors. In addition, they proposed that male odors might reduce aggression of prepubertal pigs.

McGlone and Morrow (1988) compared prepubertal crossbred pigs to determine the minimum dose of androstenone (5α-androst-16-en-3-one) that would reduce the level of agonistic behavior among dyads of newly regrouped pigs. The authors concluded that a single application of as little as 0.5 μg/pig androstenone reduced aggressive behavior among prepubertal pigs and, therefore, may be a way of reducing fighting among newly regrouped prepubertal pigs.

Boar presence can also impact aggression. Grandin and Bruning (1993) compared barrows and gilts at the packing plant with or without a mature boar in their lairage pens (via a pheromone effect similar to that reported by McGlone and Morrow [1988]). The authors reported that boar presence reduced both the incidence and the intensity of fighting. Docking et al. (2001) found that aggressive interactions, skin damage, and flight distance for sows were all reduced by at least 28% over a 28 hour post mixing period by boar presence. However, Séguin et al. (2006) found that mixing sows in the presence of a boar following the breeding period was minimally effective at reducing fighting and scratches compared with controls and that sows showed a greater stress response in the presence of a boar.

Morrow-Tesch and McGlone (1990) identified skin secretions from sows that piglets could recognize. Pageat (1998) isolated maternal skin secretions (a mixture of fatty acids) that could be maternal–neonatal pheromones. McGlone and Anderson (2002) later showed that application of these putative maternal–neonatal pheromones reduced aggression and stimulated post weaning weight gain in pigs. Recently, Plush et al. (2016) showed that this maternal–neonatal pheromone slightly reduced aggression among group-housed adult sows; however, skin lesions were not reduced by this putative pheromone compared with a control group.

Early social experience may also play a role as a longer-term solution to reduce aggression at mixing (Pitts et al. 2000). Mixing piglets prior to weaning has been shown to benefit social skills in the longer term. Socialized piglets are able to form stable dominance hierarchies during future encounters with unfamiliar pigs quicker than piglets mixed after weaning (D'Eath 2005). Early socialization also increases consistency of behavior during social encounters (D'Eath 2004). However, the amount of aggression at mixing can still be reduced later in life by practicing repeated mixing, premixing, or preexposure with and to other pigs. With repeated mixing, pigs that are remixed three or four times post weaning subsequently show reduced aggression when mixed at 5 months of age, compared with pigs mixed just once or twice (Durrell et al. 2003; van Putten and Buré 1997). Lastly, and with largely untested potential, is the practice of preexposing pigs prior to mixing. Kennedy and Broom (1996) placed groups of five gilts in a small pen within a large pen and let the resident sows have olfactory, auditory, visual, and limited physical contact with them for 5 days before mixing. Once mixed, aggression was reduced by 60% over the course of the mixing day and the following 2-week period compared with gilts that were mixed into the resident group without preexposure. Jensen and Yngvesson (1998) have also reported this preexposure effect on aggression in nursery pigs and a reduction in interaction nosing phase.

EE for the pig may also be considered to redirect aggression onto "another" item rather than a pig within that pen (Jensen and Pedersen 2010). Elmore (2010) proposed that EE be defined as biologically relevant (i.e. have meaning for the animal in terms of its natural biology). Additions or modifications to the environment that allow coping with stressors (Moberg 2000) by promoting species-specific (i.e. "natural") coping behavior may be linked to the experience of positive affective states in animals (Boissy et al. 2007). Schaefer et al. (1990) compared EE on aggressive behavior in newly weaned pigs. Six-week-old gilts were divided into two treatment groups, and each pen either had a car tire suspended on a chain or no device. Pigs offered the tire and chain device displayed a lower frequency of total aggressive acts. Most notable was the reduced frequency of head-to-head knocks. In a further experiment, the authors compared approximately 28-day-old barrows and gilts that were assigned nothing, a pacifier (sugar–mineral block suspended in a metal basket), or a teeter-totter (metal bar with rubber belts on the ends). Pigs offered a play device committed fewer total aggressive acts (compared with the control pigs). The authors concluded that enriching their environment with play objects could modify aggression frequency in intensively raised pigs.

In a review of literature, Johnson and McGlone (2011) showed that aggressive and submissive behaviors were lowly (but significantly) correlated. Aggression of sows toward piglets (a maladaptive behavior) is highly correlated (Knap and Merks 1987). Aggression may also be

managed through selection of pigs that display low levels of aggression; although still in its infancy for application, selection of pigs based on levels of aggression is being considered (Erhard et al. 1997). Turner et al. (2000) rediscovered the correlation between wounding and aggressive behavior (McGlone and Curtis 1985) and showed that part of this relationship is genetically determined. Post mixing aggressiveness of pigs was assessed to have a heritability of 0.22. The response to selection, when all selection pressure was placed on the lesion score (LS) trait, was a 25% reduction in LS per generation. Further work by Turner et al. (2000) used a Bayesian approach to estimate the heritability of three traits associated with aggressiveness in pigs during the 24 hour post mixing: duration of reciprocal aggression and whether in receipt of or delivery of nonreciprocal aggression (NRA). The authors concluded that based on the estimated genetic parameters, the selection of breeding values for reduced LS (especially LS for the central region of the body) is expected to reduce reciprocal aggression and the delivery of NRA, but will not change the receipt of NRA directly. In pigs (and other species), selection for increased ADG can cause an increase in aggressiveness (ADG and aggressiveness are correlated phenotypically; Vargas 1987). However, selecting groups of pigs for increased ADG and reduced aggression can improve ADG (Camerlink et al. 2013). One strategy to improve the welfare of pigs is to include behavioral measures such as aggression in selection programs (Rodenburg and Turner 2012). We know that feral pigs are more aggressive than most domestic pigs. Therefore, we do understand that behaviors can be selected that work better in a commercial setting. Consumers and retailers may prefer pork from pigs that do not show damaging behaviors toward each other. We should expect genetic improvement in animal welfare in the future, including selection for less stressful and damaging behaviors.

Influence of disease on behavior

As a result of behavioral changes, ill pigs are frequently subjects of investigation and bullying by their pen mates. Consequently, diseased and injured individuals comprise vulnerable populations within the swine operation, presenting unique behavioral and welfare needs (Millman 2007). Behaviors such as decreased feeding and drinking, decreased exploration, increased sleep, heat seeking, and lethargy are often the first clinical signs of disease that are observed by caretakers and veterinarians. These "sickness behaviors" are displayed by a wide range of vertebrate species in response to bacterial, viral, and protozoan pathogens and appear to be an evolved behavioral strategy that complements the innate immune system (Hart 1988). Sickness behaviors result when proinflammatory cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor cross the blood–brain barrier or are produced by glial cells in the central nervous system (CNS) (Dantzer and Kelley 2007). These cytokines act as neurotransmitters, producing characteristic changes in physiology (e.g. fever) and behavior (e.g. anorexia).

Sickness behavior is organized as a motivational state and as such competes with other motivational states such as escape or vigilance for expression (Aubert 1999). Individual pig behavioral responses to particular pathogens will differ according to the pig's previous experiences and perception of its current environment. Understanding of the motivational factors that influence expression of sickness behavior can inform observation protocol and detection of ill individuals within a herd. Pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV) spend more time lying relative to their uninfected pen mates (Escobar et al. 2007; Sutherland et al. 2007). Furthermore, PRRSV-infected pigs display lying postures that confer heat conservation (lying ventrally, lying in contact with other pigs) when housed in neutral thermal environments (24 °C), but not when housed at warmer (32 °C) temperatures (Sutherland et al. 2007). Although intake of water (Swinkels et al. 1994) and feed (Escobar et al. 2007) decrease during illness, affected pigs do not consistently reduce the amount of time spent at the feeder, perhaps due to social facilitation. In the absence of attentive observation, this behavior may provide the illusion of a healthy appetite and negatively impact detection of ill individuals in the pen.

Husbandry, handling, and housing of diseased pigs have important implications for animal welfare and pathogen transmission. Moving an ill or injured pig into a hospital can provide benefits in terms of minimizing the risk of bullying by healthy pen mates, reduced competition for resources, and ease of monitoring and medical intervention. However, social stress associated with mixing of unfamiliar pigs' results has been shown to increase shedding and transmission of *Salmonella typhimurium* (Calloway et al. 2006) and should be avoided when feasible. Furthermore, activation of the peripheral immune system has been shown to negatively impact spatial cognitive processing in piglets (Dilger and Johnson 2010), which could affect how compromised pigs respond to handling, and to navigate and find resources in novel environments. Hence, hospital pens need to be closely monitored to ensure ill and injured pigs are responding to treatment and for decision-making about humane endpoints when euthanasia is warranted (Millman 2015).

Euthanasia

Timely euthanasia is required for severely injured, non-ambulatory, and emaciated pigs and for compromised pigs that are in pain or that have little possibility of recovery. A standardized euthanasia protocol can improve overall well-being of the herd and reduce the economic costs of providing continued care for compromised pigs (Morrow et al. 2006). Euthanasia of low birth weight piglets (<0.9 kg [2 lb]), for example, may be recommended because they have higher mortality preweaning and in the nursery (Smith et al. 2007) and have higher likelihood of being of poor quality at weaning, in the nursery, and into the finishing period (Fix et al. 2010). Additionally, an euthanasia protocol for pigs entering the nursery that are identified as weak, lame, and suffering from prolapse or from two or more concurrent conditions (e.g. injury, damaged digits, hernia) has been shown to significantly improve herd welfare scores (Morrow et al. 2006). For pigs of all sizes, euthanasia should be carried out in a manner that minimizes pain, anxiety, and distress while rendering the animal rapidly insensible. The euthanasia process should result in rapid loss of consciousness, followed by cardiac or respiratory arrest and subsequent loss of brain function. Loss of consciousness should precede loss of muscle movement (AVMA 2013). Many euthanasia techniques are effective at rendering the animal insensible and causing death in one step, whereas two-step methods effectively stun the animal but require a secondary step such as exsanguination to achieve death.

There are three primary means of achieving death: chemical depression of the CNS, hypoxia, and physical disruption of brain activity (AVMA 2013). Direct depression of the CNS by anesthetic overdose initiates unconsciousness by induction into a deep state of anesthesia followed by cardiac and respiratory failure. Hypoxia limits oxygen delivery to the brain, ultimately shutting down vital centers for cardiac and respiratory function. Physical disruption of the brain, whether by concussion, depolarization by electrocution, or direct injury, targets the cerebral cortex and brain stem to damage critical pathways and regions of the brain essential for life. The prefrontal cortex and brain stem along with their connections in the thalamus are the brain regions associated with consciousness and arousal (Seth et al. 2005). If signs of sensibility are present subsequent to performing the euthanasia procedure, and it is safely possible, corrective action should be immediate. In order to ensure that death occurs without the perception of pain, signs of insensibility in the animal should be monitored throughout the process until death is confirmed.

Observations of brain stem and spinal or nociceptive reflexes, similar to those used to determine effective stunning prior to slaughter or depth of anesthesia during surgery, are the most practical measures for determining insensibility during euthanasia of animals on farms (Erasmus et al. 2010). Key brain stem reflexes include the corneal, palpebral, and pupillary light reflexes; when insensible, the animal does not exhibit a blink response when either the eyelid or the cornea is touched and the pupil remains fixed and dilated in the presence of light (Gregory 2008). Any natural blinking without stimulus indicates that the animal is sensible (Grandin 2010). However, ocular reflexes are not reliable indicators of anesthetic depth in pigs (Smith and Swindle 2008), and weak corneal reflexes can be observed during unconsciousness following damage to the cerebral cortex when the brain stem remains intact (e.g. head-only electrical stunning; Grandin 2010). Therefore, spinal or nociceptive reflexes, such as the pedal reflex, response to nose prick, or anal reflex, are important for assessing insensibility (Kaiser et al. 2006). Absence of withdrawal responses to painful stimuli indicates that the animal no longer perceives pain.

In addition to the sensory reflexes, several behavioral observations are important for assessing effectiveness of the euthanasia technique. These include the absence of rhythmic breathing, absence of vocalizations, and loss of muscle tone (Gregory 2008). The return of rhythmic breathing is one of the earliest signs of return to consciousness (Anil 1991). Vocalizations are a sign of pain or distress and should not be present at any time during the euthanasia process (Warriss et al. 1994), though unconscious pigs may exhibit involuntary noises and movements. Loss of posture and muscle tone occurs with the onset of unconsciousness, and a limp jaw or tongue is a reliable indicator of insensibility in pigs. Clonic muscle spasms, characterized by kicking or paddling movements, and tonic spasms, characterized by rigid extension of the limbs, are associated with some euthanasia techniques. These are involuntary muscle spasms and should not be confused with voluntary movements or deliberate escape attempts (Grandin 2010).

Veterinarians should work with the caretaker to design a euthanasia protocol using the most up-to-date guidelines. Human safety, costs, the technical skill required, and personnel preferences should also be taken into account. The recommended methods of euthanasia currently include veterinary-administered anesthetic overdose, carbon dioxide, gunshot, penetrating and non-penetrating captive bolt, and electrocution (NPB 2016). Manually applied blunt force trauma for small pigs (<5.44 kg [12 lb]) is accepted (NPB 2016), but due to limitations of the method and aesthetics, active pursuit of alternatives is recommended (AVMA 2013, NPB 2016).

Carbon dioxide induces unconsciousness and anesthesia by altering the pH of cerebrospinal fluid and ultimately causes death by respiratory arrest (NPB 2016).

Specifically designed equipment is necessary to ensure pain and distress is minimized during the CO_2 euthanasia procedure, including an airtight container, with non-slip flooring, and gas regulator. Compressed gas in cylinders is the recommended source of CO_2. Other sources of CO_2, such as dry ice, fire extinguishers, or chemical reactions, are unacceptable (NPB 2016). Stocking density in the container should allow all pigs to lie down without being required to lie on top of another pig. Solitary pigs may display more distress behaviors than those euthanized in groups (Fiedler et al. 2016); however, practical considerations such as the size of the pigs relative to container and discrepancy in condition of the pigs may make euthanasia of a solitary pig preferable. Though important to consider stocking density as well as other factors such as ill health that impacts the respiratory system (e.g. swine respiratory disease), these factors have minimal impact on the efficacy of CO_2 euthanasia (Sadler et al. 2014a,b).

Although CO_2 is effective for causing death, this method is controversial (Mota-Rojas et al. 2012); loss of consciousness is not immediate (Chevillon et al. 2004), and vocalizations, signs of breathlessness, and active avoidance are sometimes observed during the inhalation phase (Raj and Gregory 1996; Rault et al. 2015). Although other gases such as nitrous oxide and argon have been successfully utilized in trials (Fiedler et al. 2016; Rault et al. 2015), there are practical considerations that make these alternatives prohibitive to implement, including attainability and difficulty in maintaining an efficacious gas concentration.

Gunshot, penetrating captive bolt, non-penetrating captive bolt, and blunt force trauma should inflict sufficient physical damage to both the cerebral cortex and the brain stem to cause immediate and irreversible brain damage leading to death. For all methods, restraint allowing proper application is necessary. A gunshot to the head can be effective for pigs (AVMA 2013; NPB 2016). The trajectory of the bullet should follow the angle of the spine ideally passing through the brain and lodging in the brain stem (Woods et al. 2010). An alternative shot location is behind the ear, aiming toward the opposite eye (NPB 2016). It is imperative that the gun and load be sufficient to be effective with one shot. Neck and heart shots are not acceptable.

Captive bolt guns rely on concussion and destruction of brain tissue to render the animal immediately insensible. Sufficient transfer of energy from the gun to the head is required, and effectiveness depends on both the diameter and velocity of the bolt (Gregory 2007). Penetrating captive bolts can either stun or kill the pig. With the proper configuration, including gun design and charge, a penetrating captive bolt can be successful as a single step. Non-penetrating captive bolt guns do not cause sufficient brain damage to effectively stun or kill larger pigs and are only approved for pigs up to 31.57 kg (70 lb; NPB 2016). Similar to penetrating captive bolt, successful application of this procedure is depended on gun and charge configuration. For suckling piglets, physical methods of euthanasia may be the most practical for the caretaker. A non-penetrating captive bolt can be effective without a secondary step for suckling piglets, but the shape of the percussive bolt head and depth of depression of the bolt head into the cranium may be important. Widowski et al. (2008b) found that some piglets showed signs of return of sensibility and had a variable time to cardiac arrest when a round-headed percussive bolt was used for neonatal piglets. When the bolt head was modified to a conical shape, which resulted in a greater depth of depression, the method proved highly effective (Casey-Trott et al. 2013, 2014). Clonic convulsions (kicking and paddling) should be expected following application of blunt trauma and percussive bolt; brain stem reflexes as well as behavioral indicators should be checked to ensure insensibility.

Electrocution is another physical method of euthanasia. Only commercially available electric stunners should be used. It is important to note that both head-to-heart and head-only methods are acceptable. However, head-only electrocution causes loss of consciousness but not cardiac arrest; therefore, it is reversible and must be followed by a secondary step within 15 seconds (Anil 1991; Blackmore and Newhook 1981). Tonic and clonic neuromuscular spasms should be present in the head-only method (McKinstry and Anil 2004), but these are not seen in association with head-to-heart electrocution, which also causes cardiac fibrillation (Gregory 2008; Wotton and Gregory 1986). Electric methods should only be used on pigs over 3 days of age.

References

Abell CE, Johnson AK, Karriker LA, et al. 2014. *Animal* 8:1000–1009.

Alonso-Spilsbury M, Ramirez-Necoechea R, Gonzalez-Lozano M, et al. 2007. *J Anim Vet Adv* 6:76–86.

American Animal Hospital Association (AAHA). 2009. Animal Abuse Reporting Statement American Animal Hospital Association. https://www.aaha.org/professional/resources/animal_abuse_reporting.aspx. Accessed January 31, 2017.

American Veterinary Medical Association (AVMA). 2009. http://www.avma.org. Accessed August 31, 2010.

American Veterinary Medical Association (AVMA). 2013. https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx. Accessed May 30, 2013.

Andersen IL, Berg S, Bøe KE. 2005. *Appl Anim Behav Sci* 93:229–243.
Anderson IL, Andenas H, Boe K, et al. 2000. *Appl Anim Behav Sci* 68:107–120.
Anil MH. 1991. *Meat Sci* 30:13–21.
Appleby MC, Pajor EA, Fraser D. 1991. *Anim Prod* 53:361–366.
Appleby MC, Pajor EA, Fraser D. 1992. *Anim Prod* 55:147–152.
Aubert A. 1999. *Neurosci Biobehav Rev* 23:1029–1036.
Axiak SM, Jäggin N, Wenger S, et al. 2007. *Schweiz Arch Tierheilkd* 149:395–402.
Barnett JL, Hemsworth PH. 2009. *J Appl Anim Welf Sci* 12:114–131.
Barnett JL, Hemsworth PH, Cronin GM, et al. 1991. *Appl Anim Behav Sci* 32:23–33.
Barnett JL, Cronin GM, McCallum TH, et al. 1993. *Appl Anim Behav Sci* 36:135–148.
Barnett JL, Cronin GM, McCallum TH, et al. 1994. *Appl Anim Behav Sci* 39:339–347.
Barnett JL, Cronin GM, McCallum TH, et al. 1996. *Appl Anim Behav Sci* 50:121–133.
Barnett JL, Hemsworth PH, Cronin GM, et al. 2001. *Aust J Agric Res* 52:1–28.
Barrett RH. 1978. *Hilgardia* 46:283–355.
Beattie VE, Breuer K, O'Connell NE, et al. 2005. *Anim Sci* 80:307–312.
Bench CJ, Gonyou HW. 2007. *Appl Anim Behav Sci* 105:26–41.
Black JL, Giles LR, Wynn PC, et al. 2001. In 8th Biennial Conference of the Australasian Pig Science Association, Adelaide, Australia, pp. 9–36.
Blackmore DK, Newhook JC. 1981. *N Z Vet J* 29:219–222.
Blockhuis HJ, Jones RB, Geers R, et al. 2003. *Anim Welf* 12:445–455.
Boissy A. 1995. *Q Rev Biol* 70:165–191.
Boissy A, Manteuffel G, Jensen MB, et al. 2007. *Physiol Behav* 92:375–397.
Bolhuis JE, Schouten WGP, Schrama JW, et al. 2005. *Appl Anim Behav Sci* 93:213–228.
Bonneau M. 1998. *Meat Sci* 49S1:S257–S272.
von Borell E, Baumgartner J, Giersing M, et al. 2009. *Animal* 3:1488–1496.
Bouissou MF. 1965. *Ann Biol Anim Biochim Biophys* 5:327–339.
Bovey K, Lawlis P, DeLay J, et al. 2008. Department of Animal and Poultry Science, University of Guelph, ON, Canada.
Bovey K, Laplante B, Correa J, et al. 2010. The effects of docked tail length and nursery space allowance. In Proceedings of the 7th Annual Dr. Mike Wilson University of Guelph Swine Research Day, University of Guelph, ON, Canada, p. 5.
Brajon S, Laforest JP, Bergeron R, et al. 2015. *Appl Anim Behav Sci* 162:9–19.
Brambell FWR. 1965. Report of the Technical Committee to Enquire into the Welfare of Animals Kept Under Intensive Livestock Husbandry Systems. Cmnd. 2836. Her Majesty's Stationery Office, London.
van den Brand H, Wamsteeker D, Oostindjer M, et al. 2014. *J Anim Sci* 92:4145–4153.
Breuer K, Sutcliffe MEM, Mercer JT, et al. 2003. *Appl Anim Behav Sci* 84:58–74.
Broom DM. 1986. *Br Vet J* 142:524–526.
Broom DM. 1991. *J Anim Sci* 69:4167–4175.
Broom D, Johnson KG. 1993. *Stress and Animal Welfare*. Oxford, UK: Chapman and Hall.
Brown JME, Edwards SA, Smith WJ, et al. 1996. *Prev Vet Med* 27:95–105.
Brunberg E, Jensen P, Isaksson A, et al. 2013. *PLoS One* 8:e66513.
Bulens A, Van Beirendonck S, Van Thielen J, et al. 2015. *Appl Anim Behav Sci* 169:26–32.
Bünger B, Kallweit E. 1999. *Landbauforschung Volkenrode* 44:151–166.
Butler AB, Hodos W. 2005. *Comparative Vertebrate Neuroanatomy: Evolution and Adaptation*, 2nd ed. Hoboken, NJ: John Wiley & Sons.
Calloway TR, Morrow JL, Edrington TS, et al. 2006. *Curr Issues Intest Microbiol* 7:65–72.
Camerlink ISP, Turner P. Bijma and Bolhuis JE. 2013. Indirect Genetic Effects and housing conditions in relation to aggressive behaviour in pigs. *PLoS One* 8:e6516. https://doi.org/10.1371/journal.pone.0065136.
Campler M, Pairis-Garcia M, Stalder KJ, et al. 2016. *J Swine Health Prod* 24:142–146.
Cannon WB. 1929. *Arch Neurol Psychiatry* 22:282–294.
Carroll JA, Berg EL, Strauch TA, et al. 2006. *J Anim Sci* 84:1271–1278.
Casey-Trott TM, Millman ST, Turner PV, et al. 2013. *J Anim Sci* 91:5477–5484.
Casey-Trott TM, Millman ST, Turner PV, et al. 2014. *J Anim Sci* 92:5166–5174.
Chen C, Gilbert CL, Yang G, et al. 2008. *Appl Anim Behav Sci* 109:238–248.
Chevillon P, Mircovich C, Dubroca S, et al. 2004. In Proceedings of the International Society for Animal Hygiene, pp. 45–46.
Coetzee J, Lubbers B, Toerber S, et al. 2008. *Am J Vet Res* 69:751–762.
Colpoys JD, Abell CE, Young JM, et al. 2014. *Appl Anim Behav Sci* 159:34–40.
Colpoys JD, Abell CE, Gabler NK, et al. 2015. *J Anim Sci* 93:1267–1275.
Colson V, Martin E, Orgeur P, et al. 2012. *Physiol Behav* 107:59–64.
Csermely D, Wood–Gush DGM. 1987. *Appl Anim Behav Sci* 18:389.
Curtis SE. 1987. *Vet Clin North Am Food Anim Pract* 3:369.
D'Eath RB. 2004. *Aggress Behav* 30:435–448.

D'Eath RB. 2005. *Appl Anim Behav Sci* 55:21–35.
D'Eath R, Arnott G, Turner S, et al. 2014. *Animal* 8:1479–1497.
Damn BI, Forkman B, Pedersen LJ. 2005. *Appl Anim Behav Sci* 90:3–20.
Dantzer R, Kelley KW. 2007. *Brain Behav Immun* 21:153–160.
Dawkins, MS. 1998. *Q Rev Biol* 73:305–328.
Day JEL, Van de Weerd HA, Edwards SA. 2008. *Appl Anim Behav Sci* 109:249–260.
Delumeau O, Meunier-Salaün MC. 1995. *Behav Processes* 34:185–196.
Dilger RN, Johnson RW, 2010. *Brain Behav Immun* 24:1156–1165.
Docking C, Kay R, Day J, et al. 2001. The effect of stocking density, group size and boar presence on the behaviour, aggression and skin damage of sows in a specialized mixing pen at weaning. In Proceeding of the British Society of Animal Science, p. 46.
Duncan IJH, Fraser D. 1997. In Appleby MC and Hughes B, eds. *Animal Welfare*. CAB International: Wallingford, UK, pp 19–31.
Durrell JL, Beattie VE, Sneddon IA, et al. 2003. *Appl Anim Behav Sci* 84:88–99.
Dybkjær L. 1992. *Appl Anim Behav Sci* 35:135–147.
Dzikamunhenga RS, Anthony R, Coetzee J, et al. 2014. *Anim Health Res Rev* 15:14–38.
Edwards SA. 2002. *Livest Prod Sci* 78:3–12.
Edwards SA, Mauchline S, Stewart AH. 1993. *Farm Building Prog* 113:20–23.
Edwards SA, Mauchline S, Marston GC, et al. 1994. *Appl Anim Behav Sci* 41:272.
Elmore MR. 2010. The impact of environmentally enriched housing on sow motivation, behavior and welfare. PhD dissertation, Purdue University.
Elmore, M, J Garner, A Johnson, et al. 2010. *Appl Anim Behav Sci* 123:7–15.
Elsasser TH, Klasing KC, Filipov N, et al. 2000. The metabolic consequences of stress: Targets for stress and priorities of nutrient use. In Moberg GP, Mench JA, eds. *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*. Oxon, UK: CAB International, pp. 77–110.
English PR, Morrison V. 1984. *Pig News Info* 5:369–376.
Erasmus MA, Turner PV, Widowski TM. 2010. *J Appl Poult Res* 19:288–298.
Erhard HW, Mendl M, Ashley DD. 1997. *Appl Anim Behav Sci* 54:137–151.
Escobar, J, Van Alstine WG, Baker DH, et al. 2007. *Appl Anim Behav Sci* 105:42–50.
European Food and Safety Association (EFSA). 2004. *The EFSA J* 91:1–18.
Fiedler KJ, Parsons RL, Sadler LJ, et al. 2016. *Anim Welf* 25:83–89.
Fix JS, Cassady JP, Holl JW, et al. 2010. *Livest Sci* 132:98–106.
Food and Drug Administration (FDA). 2010. Guidance for Industry development of target animal safety and effectiveness data to support approval of non-steroidal anti-inflammatory drugs (NSAIDS) for use in animals. http://www.fda.gov. Accessed June 24, 2010.
Fraser D. 1975. *Anim Prod* 21:59–68.
Fraser D. 1978. *Anim Behav* 26:22–30.
Fraser D. 1987. Attraction to blood as a factor in tail-biting in pigs. *Appl Anim Behav Sci* 17:61–68.
Fraser D. 1995. *Animal Welf* 4:103–117.
Fraser AF, Broom DM. 1997. *Farm Animal Behaviour and Welfare*. UK: CAB International.
Fraser D, Thompson BK. 1991. *Behav Ecol Sociobiol* 29:9–15.
Fraser D, Feddes JJR, Pajor EA. 1994. *Can J Anim Sci* 74:1–6.
Fraser D, Weary DM, Pajor EA, et al. 1997. *Anim Welf* 6:187–205.
Fujii J, Fraser D, Patience JF, et al. 1990. Water for piglets and lactating sows; quantity, quality and quandaries. In Haresign W, Cole DJA, eds. *Recent Advances in Animal Nutrition*. London: Butterworths, pp. 137–160.
Gardner JM, Duncan IJH, Widowski TM. 2001. *Appl Anim Behav Sci* 74:135–142.
Gerritzen MA, Kluivers-Poodt M, Reimert GM, et al. 2008. *Animal* 2:1666–1673.
Gonyou HW. 1993. *J Agric Environ Ethics* 2:37.
Gonyou HW, Parfet KAR, Anderson DB, et al. 1988. *J Anim Sci* 66:2856–2864.
Goossens X, Sobry L, Ödberg F, et al. 2008. *Anim Welf* 17:35–41.
Gosling SD. 2001. *Psychol Bull* 127:45–86.
Grandin T. 2010. In Grandin T, ed. *Improving Animal Welfare: A Practical Approach*. Wallingford, UK: CAB International, pp. 160–185.
Grandin T, Bruning J. 1993. *Appl Anim Behav Sci* 33:273–276.
Grandinson K, Rydhmer L, Strandberg E, et al. 2003. *Livest Prod Sci* 83:141–151.
Graves, HB, Graves KL, Sherritt GW. 1978. Social behavior and growth of pigs following mixing during the growing-finishing period. *Appl Anim Ethol* 4:169–180.
Gray JA. 1979. *Br J Psychol* 70:425–440.
Gregory NG. 2007. *Animal Welfare and Meat Production*, 2nd ed. Wallingford, UK: CABI, p. 299.
Gregory NG. 2008. *Meat Sci* 80:2–11.
Gudin JA. 2004. www.medscape.com, article #481798. Accessed June 28, 2014.
Haga HA, Ranheim B. 2005. *Vet Anaesth Analg* 32:1–9.
Hansson M, Lundheim N, Nyman G, et al. 2011. *Acta Vet Scand* 53:34–42.
Harris MJ, Gonyou HW. 2003. *Can J Anim Sci* 83:435–444.
Hart BL, 1988. *Biobehav Rev* 12:123–137.
Haskell MJ, Hutson GD. 1996. *Appl Anim Behav Sci* 49:375–387.

Hay M, Vulin A, Genin S, et al. 2003. *Appl Anim Behav Sci* 82:201–218.
Health of Animals Act. 1990. Health of Animals Regulation. Part XII. Transportation of Animals. http://laws.justice.gc.ca. Accessed March 5, 2011.
Heinritzi K, Hutter ST, Reich E. 1994. In Proceedings of the 13th International Pig Veterinary Society Congress, Bangkok, p. 489.
Hemsworth PH, Barnett JL, Hansen C. 1986. *Appl Anim Behav Sci* 15:303–314.
Hemsworth PH, Barnett JL, Hansen C. 1987. *Appl Anim Behav Sci* 17:245–252.
Hemsworth PH, Barnett JL, Coleman GJ, et al. 1989. *Appl Anim Behav Sci* 23:301–314.
Hemsworth PH, Barnett JL, Coleman GJ. 1993. *Anim Welf* 2:33–51.
Hemsworth PH, Coleman GJ, Barnett JL. 1994. *Appl Anim Behav Sci* 39:349–362.
Hemsworth PH, Barnett JL, Campbell RG. 1996. *Appl Anim Behav Sci* 49:389–401.
Herskin MS, Di Giminiani P, Thodberg K. 2016. *Res Vet Sci* 108:60–67.
Hodgson DS. 2006. *Vet Anaesth Analg* 33:207–213.
Hodgson DS. 2007. *Vet Anaesth Analg* 34:117–124.
Huang X, Zhang G, Herring SW. 1994. *Comp Biochem Physiol* 107A:647–654.
Hughes BO, Duncan IJH. 1988. *Anim Behav* 36:1696–1707.
Hurnik JF, Webster AB, Siegel P. 1995. *Dictionary of Farm Animal Behavior*, 2nd ed. Ames, IA: Iowa State University Press.
Ivers DJ, Richardson LF, Jones DJ, et al. 2002. *J Anim Sci* 80:39.
Jensen P. 1986. *Appl Anim Behav Sci* 16:131–142.
Jensen MB, Pedersen LJ. 2010. *Appl Anim Behav Sci* 123:1–6.
Jensen P, Yngvesson J. 1998. *Appl Anim Behav Sci* 58:49–61.
Jensen, TB, Kristensen HH, Toft N. 2012. *Livest Sci* 149:209–214.
Jerison HJ. 1997. Evolution of the prefrontal cortex. In Kragnegor NA, Lyon GR, Goldman-Rakic P, eds. *Development of the Prefrontal Cortex: Evolution, Neurobiology and Behavior*. Baltimore, MD: Paul H Brooks Publishing Co., pp. 9–27.
Johnson AK McGlone JJ. 2011. Behaviour genetics of the domestic pig. In Rothschild MF, Ruvinsky A, eds. *Genetics of the Pig*. CAB International
Johnson AK, Morrow JL, Dailey JW, et al. 2007. *Appl Anim Behav Sci* 105:59–74.
Johnson A, Gesing L, Ellis M, et al. 2013. *J Anim Sci* 91(6):2481–91.
Kaiser G, Heuer M, Fruhauk N, et al. 2006. *J Surg Res* 130:73–79.
Karriker LA, Abell CE, Pairis MD. et al. 2013. *J Anim Sci* 91:130–136.
Keeling LJ, Bracke MBM, Larsen A. 2004. In Proceedings of the 38th Congress of ISAE, Helsinki, Finland, p. 70.
Kelley KW. 1980. *Ann Rech Vet* 11:445–478.
Kelley KW, McGlone JM, Gaskins, CT. 1980. Porcine aggression: Measurement and effects of crowding and fasting. *J Anim Sci* 50:336–341.
Kennedy MJ, Broom DM. 1996. Factors modulating aggression received by pigs mixed individually into groups. In Duncan IJH, Widowski TM, Haley DN, eds. *Proceedings of the 30th International Congress of the International Society for Applied Ethology*. Guelph: Center for Study of Animal Welfare, p. 52 (Abstract).
Kluivers-Poodt M, Houx BB, Robben SRM, et al. 2012. *Animal* 6:1469–1475.
Knap PW, Merks JWM. 1987. *Livest Prod Sci* 17:161–167.
Kritas SK, Morrison RB. 2007. *Vet Rec* 160:149–152.
Langenbach GEJ, Van Eijden TMGJ. 2001. *Am Zool* 41:1338–1351.
Lay DC, Haussmann MF, Buchanan HS, et al. 1999. *J Anim Sci* 77:2060–2064.
Leidig MS, Hertrampf B, Failing K, et al. 2009. *Appl Anim Behav Sci* 116:174–178.
Leonard FC, Stienezen I, Lynch PB. 1997. In Agriculture Research Forum, Dublin.
Luescher UA, Friendship RM, Lissemore KD, et al. 1989. *Appl Anim Behav Sci* 22:191–214.
Luescher UA, Friendship RM, McKeown DB. 1990. *Can J Anim Sci* 70:363–370.
MacLean PD. 1990. *The Triune Brain in Evolution*. New York: Plenum Press.
Manning L, Chadd SA, Baines RN. 2007. *Worlds Poult Sci J* 63:46–62.
Marchant JN, Mendl MT, Rudd AR, et al. 1995. *Appl Anim Behav Sci* 46:49–56.
Marchant JN, Broom DM, Corning S. 2001. *Anim Sci* 72:19–28
Marchant-Forde JN, Lay DC Jr, McMunn KA, et al. 2009. *J Anim Sci* 87:1479–1492.
McGlone JJ. 1985. *Physiol Behav* 34:195–198.
McGlone JJ. 1986. *Behav Processes* 12:135–144.
McGlone JJ, Anderson D. 2002. *J Anim Sci* 80:2043–2050.
McGlone JJ, Curtis SE. 1985. *J Anim Sci* 60:20–24.
McGlone JJ, Hellman JM. 1988. *J Anim Sci* 66:3049–3058.
McGlone JJ, Morrow JL. 1988. *J Anim Sci* 66:880–884.
McGlone JJ, Curtis SE, Banks EM. 1987. *Behav Neural Biol* 47:27–39.
McGlone JJ, Nicholson RI, Hellman JM, et al. 1993. *J Anim Sci* 71:1441–1446.
McKinstry JL, Anil MH. 2004. *Meat Sci* 67:121–128.
Meese GB Baldwin BA. 1975. *Appl Anim Behav Sci* 1:251–262.
Meese GB, Conner DJ, Baldwin BA. 1975. *Physiol Behav* 15:121–125.
Mendl MT, Erhard HW. 1997. Social choices in farm animals: To fight or not to fight? In Animal

Choices. BSAS Occasional Publication 20, pp. 45–53.
Mendl MT, Zanella AJ, Broom DM. 1992. *Anim Behav* 44:1107–1121.
Metz JHM, Gonyou HW. 1990. *Appl Anim Behav Sci* 27:299–309.
Meunier-Salaün MC, Bataille G, Rugraff Y, et al. 2002. *J Anim Sci* 85:371.
Millman ST. 2007. *Anim Welf* 16:123–125.
Millman ST. 2015. In Proc of the American Association of Swine Veterinarians Annual Meeting, Orlando, FL, March 1–3, 2015.
Moberg GP. 2000. Biological response to stress: Implications for animal welfare. In Moberg GP, Mench JA eds. *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*. Oxon, UK: CAB International, pp. 1–21.
Mohling CM, Johnson AK, Coetzee JF, et al. 2014a. *Livest Sci* 165:120–128.
Mohling CM, Johnson AK, Coetzee JF, et al. 2014b. *J Anim Sci* 92:3073–3081.
Moinard C, Mendl M, Nicol CJ, et al. 2003. *Appl Anim Behav Sci* 81:333–355.
Morgan CA, Lawrence AB, Chirnside J, et al. 2001. *Anim Sci* 73:471–478.
Mormède P. 1990. In Zayen R, Dantzer R, eds. *Social Stress in Domestic Animals*. Dordrecht, the Netherlands: Kluwer, pp. 203–211.
Morrow WEM, Meyer RE, Roberts J, et al. 2006. *J Swine Health Prod* 4:25–34.
Morrow-Tesch J, McGlone J. 1990. *J Anim Sci* 68(11):3563–3571.
Morrow-Tesch JL, McGlone JJ, Salak-Johnson JL. 1994. *J Anim Sci* 72:2599–2609.
Mota-Rojas D, Bolanos-Lopez D, Concepcion-Mendez J, et al. 2012. *Int J Pharm* 8:141–151.
Moya SL, Boyle LA, Lynch PB, et al. 2007. *Appl Anim Behav Sci* 111:133–145.
Muns R, Rault JL, Hemsworth P. 2015. *Physiol Behav* 151:162–167.
National Pork Board (NPB). 2016. On farm euthanasia of swine: Recommendations for the producer. Pub. 04970-11/16, Des Moines, IA.
National Pork Board (NPB). 2017. Common Swine Industry Audit. http://www.pork.org/common-industry-audit/. Accessed January 2017.
Nonneman DJ, Brown-Brandl T, Jones SA, et al. 2012. *BMC Genomics* 13:233–241.
Noonan GJ, Rand JS, Priest J, et al. 1994. *Appl Anim Behav Sci* 39:203–213.
North American Meat Institute (NAMI). 2013. Recommended Animal Handling Guidelines and Audit Guide: A Systematic Approach to Humane Handling. http://www.animalhandling.org/ht/d/sp/i/26752/pid/26752. Accessed January 2017.
O'Connell NE, Beattie VE, Moss BW. 2003. *Anim Welf* 12:239–249.
O'Connor A, Anthony R, Bergamasco L, et al. 2014. *Anim Health Res Rev* 15:39–62.
Oostindjer M, van den Brand H, Kemp B, et al. 2011. *Appl Anim Behav Sci* 134:31–41.
Otten W, Puppe B, Kanitz E, et al. 1999. *J Vet Med* 46:277–292.
Paetkau L, Whiting T. 2008. *Can Vet J* 49:489–493.
Pageat P. 1998. Patent US 6169113 B1.
Pairis-Garcia MD, Karriker LA, Johnson AK, et al. 2013. *BMC Vet Res* 9:165–172.
Pairis-Garcia MD, Johnson AK, Kukanich B, et al. 2014. *J Vet Pharm Therapy*. doi: 10.111?jvp.12170.
Pairis-Garcia MD, Johnson AK, Abell CA, et al. 2015a. *J Anim Sci* 93:2100–2110.
Pairis-Garcia MD, Johnson AK, Stalder KJ, et al. 2015b. *Anim Welf* 24:93–99.
Panksepp J. 1990. The psychoneurology of fear: Evolutionary perspectives and the role of animal models in understanding human anxiety. In Burrows GD, Roth M, Noyes JR, eds. *Handbook of Anxiety No. 3, The Neurobiology of Anxiety*. Elsevier Science Publishers BV.
Panksepp J. 1998. *Affective Neuroscience: The Foundations of Human and Animal Emotions*. New York: Oxford University Press.
Panksepp J, Knutson B, Burgdorf J. 2002. *Addiction* 97:459–469.
Paoli MA, Lahrmann HP, Jensen T, et al. 2016. *Anim Welf* 25:287–296.
Parsons RL, Johnson AK, Coetzee JF, et al. 2015. *Acta Agric Scan A Anim Sci*. doi: https://doi.org/10.1080/09064702.2015.1110617.
Parsons RL, Johnson AK, Coetzee JF, et al. 2016. *Acta Anim A* 66:115–118.
de Passillé AMB, Pelletier G, Menard J. 1989. *J Anim Sci* 67:2921–2929.
Phillips PA, Fraser D 1990. *Am Soc Agric Eng* 6:79–81.
Phillips PA, Fraser D, Pawluczuk B. 2001. *Am Soc Agric Eng* 17:845–847.
Pitts AD, Weary DM, Pajor EA, et al. 2000. *Appl Anim Behav Sci* 68:191–197.
Plush K, Hughes P, Herde P, et al. 2016. *Appl Anim Behav Sci* 185:45–51.
Pluym LM, Van Nuffel A, Van Weyenberg S, et al. 2013. *Animal* 7:1174–1181.
Pomeroy RW. 1960. *J Agric Sci* 54:31–56.
Price EO. 1997. In Grandin T, ed. *Genetics and the Behaviour of Domestic Animals*. Academic Press, pp. 31–65.
Prunier A, Mounier AM, Hay M. 2005. *J Anim Sci* 83:216–222.
Puppe B, Schön PC, Tuchscherer A, et al. 2005. *Appl Anim Behav Sci* 95:67–78.

van Putten G, Buré RG. 1997. *Appl Anim Behav Sci* 54:173–183.
Quinn MM, Keuler NS, Lu Y, et al. 2007. *Vet Surg* 36:360–367.
Raj A, Gregory N. 1996. *Anim Welf* 5:71–78.
Randolph JH, Cromwell GL, Stahly TS, et al. 1981. *J Anim Sci* 53:922–927.
Ranheim B, Haga HA, Ingebrigtsen K. 2005. *J Vet Pharmacol Ther* 28:481–483.
Rau JA. 2002. Behavior and performance of early-weaned pigs: Effects of trough-anchored blind teats and liquid food. MSc thesis, University of Guelph, Canada, pp. 81–85.
Rault J-L, Lay DC Jr. 2011. *J Anim Sci* 10:3318–3325.
Rault J-L, Lay DC Jr, Marchant-Forde JN. 2011. *Appl Anim Behav Sci* 135:214–225.
Rault J, Kells N, Johnson C, et al. 2015. *Physiol Behav* 151:29–37.
Reimert I, Bolhuis JE, Kemp B, et al. 2013. *Physiol Behav* 109:42–50.
Reimert I, Rodenburg TB, Ursinus WW, et al. 2014. *Appl Anim Behav Sci* 151:24–35.
Ritter M, Ellis M, Hollis G, et al. 2008. *J Anim Sci* 86(3):511–514.
Ritter MJ, Ellis M, Berry NL, et al. 2009a. *Prof Anim Sci* 25:404–414.
Ritter MJ, Ellis M, Anderson DB, et al. 2009b. *J Anim Sci* 87:351–362.
Ritter M, Rincker P, Carr S. 2012. In Proceedings of the 2012 London Swine Conference, pp. 109–120.
Ritter MJ, Johnson AK, Benjamin ME, et al. 2017. Review: Effects of Ractopamine Hydrochloride (Paylean) on welfare indicators for market weight pigs. *Transl Anim Sci* 1(4):533–558.
Rodarte LF, Ducoing A, Galindo F, et al. 2004. *J Appl Anim Welf Sci* 7:171–179.
Rodenburg TB, Turner SP. 2012. *Anim Front* 2: doi: https://doi.org/10.2527/af.2012-0044.
Rushen J. 1996. *J Anim Sci* 74:1990–1995.
Rzezniczek M, Gygaxa L, Wechslera B, et al. 2015. *Appl Anim Behav Sci* 165:57–65.
Sadler LJ, Karriker LA, Schwartz KJ, et al. 2014a. *Anim Welf* 23:145–155.
Sadler LJ, Karriker LA, Johnson AK, et al. 2014b. *J Swine Health Prod* 22:125–133.
Samarakone TS, Gonyou HW. 2009. *Appl Anim Behav Sci* 121:8–15.
Schaefer A, Salomons M, Tong A, et al. 1990. *Appl Anim Behav Sci* 27:41–52.
Schmolke S, Li Y, Gonyou H. 2003. *J Anim Sci* 81:874–878.
Schrøder-Petersen D, Simonsen H. 2001. *Vet J* 162:196–210.
Séguin M, Friendship R, Kirkwood R, et al. 2006. *J Anim Sci* 84:1227–1237.
Selye H. 1973. *Am Sci* 61:692–699.
Seth AKB, Baars BJ, Edelman DB. 2005. *Conscious Cogn* 14:119–139.
Siegel A. 2005. *The Neurobiology of Aggression and Rage*. Boca Raton, FL: CRC Press.
Signoret JP. 1962. In Hafez ESE. *Domestic Animal Behavior*. London: Baillière Tindall.
Smith AC, Swindle MM. 2008. Anesthesia and analgesia in swine. In *Anesthesia and Analgesia in Laboratory Animals*, 2nd ed. Elsevier Press.
Smith A, Stalder K, Serenius T, et al. 2007. *J Swine Health Prod* 15:213–218.
Smulders D, Hautekiet V, Verbeke G, et al. 2008. *Anim Welf* 17:61–69.
Spicer EM, Driesen SJ, Fahy VA, et al. 1985. Trauma overlay and savaging of baby pigs. In Outteridge PM, ed. *Australian Advances in Veterinary Science*. Artarmon, Australia: The Australian Veterinary Association, p. 122 (Abstr.).
Spoolder HA, Waiblinger MS. 2009. Chapter 7: Pigs and humans. In Forde JM, ed. *The Welfare of Pigs*. Springer Press, pp. 211–236.
van der Steen H, Schaeffer L, de Jong H, et al. 1988. *J Anim Sci* 66:271–279.
Stienezen I. 1996. Welfare of confined sows with overgrown hooves in the farrowing house. MS thesis, Moorepark Dairy Production Research Institute, Fermoy, Ireland.
Stolba A, Woodgush DGM. 1989. *Anim Prod* 48:419–425.
Straw BE, Bartlett P. 2001. *J Swine Health Prod* 9:19–23.
Suarez SD, Gallup GG. 1982. *J Comp Physiol Psychol* 96:432–439.
Sun G, Fitzgerald RF, Stalder LA, et al. 2011. Development of an embedded microcomputer – Based force plate system for measuring sow weight distribution and detection of lameness. *Appl Eng Agric* 27:475–482.
Sutherland MA, Niekamp SR, Johnson RW, et al. 2007. *Physiol Behav* 90:73–81.
Sutherland M, Bryer P, Krebs N, et al. 2008. *Animal* 2:292–297.
Sutherland M, Bryer P, Krebs N, et al. 2009. *Anim Welf* 18:561–570.
Sutherland MA, Davis BL, Brooks TA, et al. 2010. *Animal* 4:2071–2079.
Sutherland MA, Davis BL, McGlone JJ. 2011. *Animal* 5:1237–1246.
Sutherland MA, Davis BL, Brooks TA, et al. 2012. *J Anim Sci* 90:2211–2221.
Swinkels JM, Pijpers A, Vernooy SJCM, et al. 1994. *J Vet Pharmacol Ther* 17:299–303.
Tallet C, Brilloüet A, Meunier-Salaün M-C, et al. 2013. *Appl Anim Behav Sci* 145:70–83.
Tallet C, Sy K, Prunier A, et al. 2014. *Livest Sci* 167:331–341.

Tannenbaum J. 1991. Ethics and animal welfare: The inextricable connection. *J Am Vet Med Assoc* 198:1660–1676.
Tapper KR, Johnson AK, Karriker LA, et al. 2013. *Livest Sci* 157:245–253.
Taylor A, Weary D, Lessard M, et al. 2001. *Appl Anim Behav Sci* 73:35–43.
Tenbergen R, Friendship R, Cassar G, et al. 2014. *J Swine Health Prod* 22:64–70.
Thun R, Gajewski F, Janett F. 2006. *J Physiol Pharmacol* 57:189–194.
Tonge CH, McCance RA. 1973. *J Anat* 115:1–22.
Toplis P, Blanchard PJ, Miller HM. 1999. Creep feed offered as a gruel prior to weaning enhances performance of weaned piglets.In Proceedings of the 7th Conference of Australasian Pig Science Association, p. 129.
Torrey S, Widowski TM. 2004. *J Anim Sci* 82:2105–2114.
Torrey S, Widowski TM. 2006. *Appl Anim Behav Sci* 101:288–304.
Torrey S, Devillers N, Lessard M, et al. 2009. *J Anim Sci* 87:1778–1786.
Tucker A, Widowski T. 2009. *J Anim Sci* 87:2274–2281.
Tucker A, Friendship R, Widowski TM. 2010. *J Swine Health Prod* 18(2):68–74.
Turner SP, Sinclair AG, Edwards SA. 2000. *Appl Anim Behav Sci* 67:321–334.
Turner S, Allcroft D, Edwards SA. 2003. *Livest Prod Sci* 82:39–51.
Ursinus WW, Van Reenen CG, Reimert I, et al. 2014. *PLoS One* 9(9):e107040.
USLegal. 2010. Animal Cruelty Law and Legal Definition http://definitions.uslegal.com/a/animal-cruelty/. Accessed August 31, 2010.
Valros A, Palander P, Heinonen M et al. 2015. *Physiol Behav* 143:151–157.
Van Beirendonck S, Driessen B, Verbeke G, et al. 2011. *J Anim Sci* 89:3310–3317.
Vargas J. 1987. *J Anim Sci* 65:463–474.
Waiblinger S, Boivin X, Pedersen V, et al. 2006. *Appl Anim Behav Sci* 101:185–242.
Walker B, Jäggin N, Doherr M, et al. 2004. *J Vet Med* 51:150–154.
Wallenbeck A, Keeling LJ 2013. *J Anim Sci* 91, 2879–2884.
Waran N, Broom D. 1993. *Anim Prod* 56:115–119.
Warnier A, Zayan R. 1985. In Zayan R, ed. *Social Space for Domestic Animals*. Dordrecht, the Netherlands: Martinus Nijhoff, p. 128
Warriss P, Brown S, Adams S. 1994. *Meat Sci* 38:329–340.
Weary D, Pajor E, Thompson B, et al. 1996. *Anim Behav* 51:619–624.
Weimer SL. 2012. Animal-human interaction comparing live human observation and digital image evaluation methodologies. MS thesis, Iowa State University.
Weissman C. 1990. *Anesthesiology* 73:308–327.
White R, DeShazer J, Tressler C, et al. 1995. *J Anim Sci* 73:381–386.
Widowski T, Curtis S. 1989. *J Anim Sci* 67:3266–3276.
Widowski T, Curtis S. 1990. *Appl Anim Behav Sci* 27:53–71.
Widowski T, Yuan Y, Gardner J. 2005. *Lab Anim* 39:240–250.
Widowski T, Elgie R, Lawlis P. 2008a. In Proceedings of the AD Leman Swine Conference, pp. 107–111.
Widowski T, Torrey S, Bench C, et al. 2008b. *Appl Anim Behav Sci* 110:109–127.
Woods J, Shearer J, Hill J. 2010. Recommended on-farm euthanasia practices. In Grandin T, ed. *Improving Animal Welfare: A Practical Approach*. Wallingford, UK: CAB International, pp. 186–213.
World Organisation for Animal Health (OIE). 2010. Glossary of the Terrestrial Animal Health Code. http://www.oie.int/standard-setting/terrestrial-code/. Accessed August 25, 2010.
Worobec E, Duncan I, Widowski T. 1999. *Appl Anim Behav Sci* 62:173–182.
Wotton S, Gregory N. 1986. *Res Vet Sci* 40:148–151.
Zonderland J, Wolthuis-Fillerup M, van Reenen C, et al. 2008. *Appl Anim Behav Sci* 110:269–281.
Zurbrigg K, van Dreumel T, Rothschild M, et al. 2017. *Can J Anim Sci*. doi: https://doi.org/10.1139/CJAS-2016-0193.

3

Genetics and Health

Caitlyn E. Bruns and Kenneth J. Stalder

Survival is a major economically relevant trait in the swine industry (Bergsma et al. 2008; Hermesch et al. 2014). Decreasing mortality at all production phases would increase profitability for the operation. Preweaning, nursery, and finisher livability has been trending downward over the last 6 years (Stalder 2016). Sow mortality has been increasing from 8 to 9% between 2010 and 2015 (Porth 2016). Given the mortality levels, there is opportunity for improvement. Genetic selection methods along with best management practices can be utilized to improve mortality throughout the swine industry.

Preweaning mortality

Preweaning mortality has increased by almost 1% per year for the last 6 years increasing to 17.4% in 2015 (Stalder 2016). The reason for this is not clear but may be attributed to the increase in litter size. Number weaned remained relatively flat not following the trend for increasing total born (Stalder 2016). Larger litters tend to have lower individual pig birth weights (Omtvedt et al. 1966; Roehe 1999). Lower pig birth weights observed in larger litters could result in higher preweaning mortality.

Genetic correlation estimates between total born and preweaning survival are varied. Nielsen et al. (2013) reported genetic correlations (SE) between total born and piglet mortality (including stillborns) to be 0.28 (0.06) and 0.22 (0.07) for Landrace and Yorkshire breeds, respectively. The genetic correlations reported by Putz et al. (2015) between total born and mortality to day 30 were not significantly different from 0. Additionally, Su et al. (2007) reported genetic correlations (SE) of −0.28 (0.12), −0.26 (0.12), and −0.43 (0.20) between total born and survival rate at birth, survival rate from birth to 5 days' post farrowing, and survival rate from 5 days' post farrowing to weaning for Landrace, respectively. The genetic correlation estimates for Yorkshire were −0.38 (0.16), −0.07 (0.23), and −0.527 (0.44) for total born and the survival traits (Su et al. 2007). The genetic correlations (SE) between the direct genetic effects of total born and proportion alive at birth and weaning were estimated to be 0.03 (0.01) and −0.26 (0.11) for Landrace, respectively, while the estimates for Yorkshire were not significantly different from 0 (Lund et al. 2002). The variation in genetic correlation estimates between studies suggests that the relationship between total born and preweaning survival may be highly dependent on the population. Differences in selection pressure over time may explain the varying relationship.

Selection for litter size and pig quality can be accomplished simultaneously. Using an alternative definition of litter size (e.g. number of pigs alive 5 days' post farrowing) can take advantage of desirable correlations with total born and preweaning mortality (Nielsen et al. 2013; Putz et al. 2015; Su et al. 2007). Genetic correlation estimates between total born and live pigs at 5 days ranged from 0.34 to 0.85, while the genetic correlation estimates between preweaning mortality and live pigs at 5 days ranged from −0.35 and −0.57 (Nielsen et al. 2013; Putz et al. 2015; Su et al. 2007).

Survival heritability estimates are low. Reported heritabilities for preweaning mortality ranged between 0.03 and 0.13 (Grandinson et al. 2002; Hellbrügge et al. 2008; Holl and Long 2006; Nielsen et al. 2013; Putz et al. 2015; Su et al. 2007). Selection for birth weight may be preferable when compared to preweaning survival. The heritability for the maternal genetic component of birth weight is greater than the heritability for survival. Reported heritability estimates for birth weight range from 0.04 to 0.10 for the direct genetic effect and from 0.15 to 0.26 for the maternal genetic effect (Grandinson et al. 2002; Holl and Long 2006; Hellbrügge et al. 2008; Putz et al. 2015). Since the birth weight maternal genetic effect has a higher heritability compared to preweaning survival, genetic progress can be made more rapidly in birth weight.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Selection for increased birth weight could indirectly improve preweaning survival. Putz et al. (2015) found the genetic correlation (SE) between pig birth weight and preweaning mortality to be –0.13 (0.11) and –0.14 (0.09) for Landrace and Yorkshire, respectively. These genetic correlations are not significantly different from 0, but the traits were not modeled with a maternal genetic effect. Hellbrügge et al. (2008) reported the genetic correlation (SE) between birth weight and survival rate to be 0.21 (0.16), but, again, the maternal genetic effect was not included in the model.

Based on the heritability estimates for both the direct and maternal genetic components, the maternal effect explains more variation in birth weight compared to the direct effect. It is important to understand the model used for birth weight when comparing genetic parameter estimates. Holl and Long (2006) estimated the genetic correlation (SE not reported) between the birth weight direct effect and the preweaning mortality direct effect to be –0.34, and the genetic correlation between the maternal effects was –0.16. The genetic correlation (SE) between the maternal genetic effects for birth weight and mortality was 0.18 (0.20) (Grandinson et al. 2002). These estimates are in opposite directions and suggest that genetic parameters are population specific and should be evaluated within a population before making decisions to include birth weight in a selection program. Knol et al. (2002) speculated that improving pig survival might not be possible by selecting on birth weight alone given the low genetic correlation estimates reported at that time. With greater estimates being reported in more recent years and the availability of more sophisticated tools like genomic selection, this may be worth reconsidering.

Birth weight is associated with improved performance throughout the pig's life. Pigs with greater birth weights were heavier at off-test and had greater growth rate (Fix et al. 2010a; Holl and Long 2006; Rehfeldt et al. 2008); however, heavier pigs at birth had lower loin depth and intramuscular fat percentage (Holl and Long 2006; Rehfeldt et al. 2008). Fix et al. (2010a) reported a quadratic relationship between birth weight and loin muscle area. Holl and Long (2006) found that pigs with greater birth weights had lower backfat at off-test, but Rehfeldt et al. (2008) and Fix et al. (2010a) reported no significant differences in backfat level for pigs with different birth weights. Fix et al. (2010b) observed a positive relationship between pig birth weight and survival to weaning, through the nursery, and through the finisher. Pigs with greater birth weights were higher quality at weaning and had a greater probability of being full value pigs at marketing when compared with pigs with lower birth weights (Fix et al. 2010b). In a study by Magnabosco et al. (2015), pigs in the lowest birth weight class (410–990 g) had the greatest preweaning, nursery, and finisher mortality compared with pigs in larger birth weight classes. According to Magnabosco et al. (2016), sows that weighed less than 1000 g at birth produced 4.5 fewer total pigs born over parities compared to sows that were heavier at birth. Additionally, the sows with the lowest weight at birth had the shortest productive life compared with heavier pigs (Magnabosco et al. 2016).

Nursery–finish mortality

Combined nursery and finisher mortality or mortality throughout a wean-to-finish system has increased by over 0.4% per year from 2010 to 2015 (Stalder 2016). In 2015, the average nursery, conventional finisher, and wean-to-finish mortality rates were 3.5, 4.5, and 6.7%, respectively (Stalder 2016). The combined nursery and finisher death loss is less than half of the preweaning mortality, suggesting that there is a larger opportunity to improve survival in the farrowing house compared with growing pig survival; however, the economic impact of finishing mortality may be greater depending on when the pig dies. Improving morbidity may provide another means to increase commercial swine producer profitability. Henryon et al. (2001) reported that time to treatment for growing pigs was heritable 0.10 for any disease, which was lower than heritability estimates (0.12–0.19) for time to treatment in individual disease categories. Heritability estimates for performance traits may be improved by accounting for challenge events that occurred during the finishing phase. Zumbach et al. (2008) reported a heritability estimate (SE) for carcass weight when not accounting for heat stress of 0.17 (0.01) and reported heritability (SE) estimates of 0.28 (0.01) and 0.14 (0.01) when grouping the pigs under heat stress and non-heat stress, respectively.

Disease resistance

Pigs that do not respond well to health challenges perform more poorly throughout the grow-finish phase and reduce producer economic efficiency. Additionally, improving disease resistance can improve consumer perception of the pig industry in general (Kanis et al. 2005). There is a genetic component to disease resistance; but oftentimes, this component is underestimated when field data has been used to conduct research. It is hard to guarantee that disease exposure probability was both consistent and sufficient for all pigs in a study; furthermore, clinical diagnoses are not always completely correct (Bishop and Woolliams 2010). Given that heterosis can greatly impact pig survival (Fahmy and Bernard 1972), it is important to understand what population was used to estimate genetic parameters and effects on disease resistance. Most commercial market hogs are

crossbred animals, while nucleus herds are composed of purebred animals. Survival rates are not the same among purebred and crossbred pigs (Fahmy and Bernard 1972), making selection for improved survivability at the commercial level difficult.

When considering incorporating a disease resistance component into a genetic program, it is important to understand the genetic parameters for the specific system in which the pigs will be raised. Many disease resistance studies that have been conducted have only included one line or one environment, meaning the results may not be applicable to other lines or environmental conditions. It is important to consider whether the study was conducted in a research or commercial setting (Guy et al. 2012). Additionally, understanding the selection program's goal is important. Improving disease tolerance may be more desirable than improving disease resistance. Hosts carrying a pathogen load may not experience any negative effects. Selecting for improved health and maintaining productivity when in a challenged environment may be accomplished with an emphasis on disease resistance and disease tolerance (Guy et al. 2012).

Lundeheim (1979) examined the genetic parameters associated with respiratory diseases (pneumonia, pleuritis, and rhinitis) in growing pigs. The heritability estimates ranged from 0.12 to 0.16, while genetic correlation estimates between the predisposition to a respiratory disease and growth and carcass measurements were low and not significantly different from 0 (Lundeheim 1979). The same study reported a negative phenotypic relationship between growth rate and lung lesions, enteric disease, locomotion disorders, and "failure to thrive." This suggests that an animal's health status affects its growth performance. Kadowaki et al. (2012) estimated the heritability for mycoplasma pneumonia score to be 0.07, which is slightly lower than the estimate from Lundeheim (1979); however the genetic correlation estimates between mycoplasma pneumonia score and growth and backfat were low and not significantly different from 0, which is in agreement with previous findings (Lundeheim 1979). Additionally, Kadowaki et al. (2012) demonstrated that pigs could be selected for improved mycoplasma pneumonia score while still increasing growth rate and maintaining backfat.

Immune response

Evaluating and selecting for immune response may be a way to improve the overall pig robustness in different health challenges. Pigs with more desirable immune response levels may perform better in health challenged environments regardless of the pathogens present. Clapperton et al. (2009) examined heritability for different biomarkers (acute-phase proteins and peripheral mononuclear leukocyte subsets) and their relationship with production traits. Heritabilities were estimated separately for pigs in a specific-pathogen-free (SPF) environment and non-SPF environment. The magnitude of the heritabilities was moderate for most biomarkers; however the heritability estimates were different when analyzing SPF versus non-SPF pigs (Clapperton et al. 2009). Alpha-acid glycoprotein was the only biomarker evaluated that was negatively correlated with growth rate in both SPF and non-SPF pigs. Additional negative genetic correlations existed between growth rate and monocytes in SPF pigs and growth rate and CD11R1 in non-SPF pigs. Henryon et al. (2006) estimated the heritabilities for IgG and haptoglobin serum concentration to be moderate, 0.16 and 0.14, respectively. The same study reported extremely high heritabilities for swine leukocyte antigen expression ranging from 0.46 to 1.23. Other studies have similarly reported moderate heritabilities for immune response measurements (Edfors-Lilja et al. 1994; Mallard et al. 1992).

Magnusson et al. (1998) reported that nursery-age pigs selected for high immune response had greater clinical arthritis scores in a challenged environment when compared with pigs selected for low immune response; however, there was no difference in weight gain in a challenged environment between the pigs selected for high and low immune response, and pigs selected for high immune response had higher weight gain in a non-challenged environment. This does not suggest a positive impact on growth performance when selecting for more desirable immune response.

Genetic advancements

Traditional selection for disease resistance and survivability is difficult due to challenges with measuring the traits in a nucleus herd. Nucleus herds have a higher health status when compared with commercial herds; therefore, nucleus animals are not exposed to as many pathogens. The approach to selecting for improved survivability has been to develop a pedigreed commercial herd with different pedigree ties to the nucleus herd. However, there are still challenges with this as well since the disease load is not constant or consistent over time. Disease resistance phenotypes are difficult to measure long term in order to incorporate them into a routine genetic analysis.

A single nucleotide polymorphism (SNP) is a location on the genome where variation exists between animals. These locations of variation can be used to estimate the difference in genetic potential between pigs. A PorcineSNP60 BeadChip with 60,000 SNPs can be utilized to incorporate genomic selection to estimate breeding values in swine genetic evaluations (Ramos et al. 2009). Genomic selection involves combining the estimated allele effects for each SNP analyzed with phenotypes to

calculate the expected breeding value for each pig (Meuwissen et al. 2001). Genomic selection has the most impact on traits that are difficult to measure, sex limited, or measured later in life (Goddard and Hayes 2007). Guo et al. (2015) examined the improvement in reliability when including genomic information when estimating breeding values for mortality up to day 5 post farrowing and were unable to show a significant improvement compared to traditional best linear unbiased prediction (BLUP) selection.

An alternative may be to focus on marker-assisted selection (MAS) to estimate the effect for one quantitative trait loci (QTL) and select to reduce the undesirable allele frequency (Knap 2005). Several markers have been previously identified to impact economically relevant traits like number born alive (estrogen receptor gene [ESR]), growth rate and feed intake (melanocortin-4 receptor [MC4R]), and meat quality (protein kinase adenosine monophosphate-activated gamma(3)-subunit [PRKAG3]) (Kim et al. 2000; Rothschild et al. 2007; Stalder et al. 2005). Porcine stress syndrome has been drastically reduced by incorporating a genetic marker into swine breeding programs (Stalder et al. 1997). The completion of the pig genome sequencing (Archibald et al. 2010) aided in the analysis of potential candidate genes.

Knowing the genome sequence can aid in studies exploring the allelic effects for genes expected to impact animal health. Boddicker et al. (2012) isolated a region on chromosome 4 that could reduce the impact of PRRS in a swine population. The marker was reported to explain 15.7% of the genetic variation in viral load and 11.2% of the genetic variation in weight gain in the populations evaluated. Edfors-Lilja et al. (1998) reported multiple QTLs that were associated with improved leukocyte counts and IgG levels for pigs immunized with an *Escherichia coli* vaccine. Reiner et al. (2002) showed that QTLs can affect the temperature and neurological symptoms when pigs were challenged intranasally with pseudorabies virus. Additionally, genes in the swine leukocyte antigen complex have been shown to influence antigen presentation and immune response (Lunney et al. 2009; Mallard et al. 1989). Utilizing markers found in these studies could aid in improving the pigs' response to disease challenges.

One thing that needs to be considered when determining the effectiveness for any QTL is the population that was studied as well as the pathogen strain used. The QTL effects can differ between swine populations and pathogen strains and may not be beneficial in all selection programs. A more recent approach to disease resistance is gene editing. Gene editing involves replacing a section of the genome with a synthesized sequence. Whitworth et al. (2015) validated the ability to edit the pig genome *in vitro* using clustered regularly interspaced short palindromic repeat (CRISPR) technology. Using the CRISPR technology, Whitworth et al. (2016) demonstrated that pigs edited with a mutation in exon 7 of CD163 displayed no response when infected with PRRS virus. Further investigations examining the long-term effects of the mutation need to be conducted. This may be the biggest discovery to improve PRRS disease resistance found to date. Given that PRRS has the greatest economic impact for any single disease in the swine industry, incorporating the edited gene into the population would likely increase swine industry profitability remarkably.

Sow productive lifetime

Most, if not all, economically important pork production traits including longevity are influenced by genetics and/or breeding system. Genetic effects can impact longevity through other important traits. Rydhmer et al. (1994) and Bidanel et al. (1996) have shown that genetics can impact age, weight, and backfat at puberty, which is associated with sow productive lifetime. Additionally, crossbreeding or heterosis impacts sow productive lifetime (Serenius et al. 2008). Živković et al. (1986) reported that crossbred sows averaged 5.3 litters, while purebred sows averaged 4.4 litters at culling, a significant difference of 0.9 litters per sow or 12%. They also noted that 55.2% of culling in purebred sows occurred in the first three parities. During the first three parities, only 40.4% of the overall culling occurred in the crossbred sows. Jorgensen (2000) reported that mean age and number of litters at removal were lower in purebred Yorkshire sows when compared with crossbred sows. Specifically, the purebred sows had greater culling for locomotion problems and reproductive failure. The difference in sow productive lifetime or parity at culling is particularly important for operations producing replacement gilts using an internal multiplication program. If the internal multiplication program involves purebreds or pure lines, producers must be aware of the expected differential lifetime or number of parities produced in order to maintain adequate replacement parent female and replacement pure line female populations.

Longevity may be influenced by the breed makeup of crossbred breeding females. Hall et al. (2002) noted that sows that were one-quarter Meishan had significantly greater mean productive lifetime days (778 days) when compared with sows that were one-eighth Duroc or one-quarter Duroc 674 and 639 days, respectively. This resulted in a greater mean parity at culling for the one-quarter Meishan sows (4.54) compared with the one-eighth Duroc (3.79) or the one-quarter Duroc sows (3.67) and a greater lifetime number of pigs born alive of 55.0 compared with 42.7 and 42.3, respectively.

Heritability estimates for longevity or sow productive lifetime indicate that selection should be an effective method to improve this trait. However, those experienced at genetic improvement for any trait, but particularly those that are more lowly heritable like fitness and reproduction, recognize that improvement through selection alone will be slow. Sow longevity or sow productive lifetime falls into this type of category. Stayability is a measure that some researchers use to describe longevity. Simply, stayability is the ability of a sow to produce an additional litter after producing the previous litter. Reported longevity or productive lifetime heritability estimates range from 0.05 to 0.25 in swine depending on the trait evaluated (Engblom et al. 2009; Gou et al. 2001; Mészáros et al. 2010; Serenius and Stalder 2004; Serenius et al. 2006; Tholen et al. 1996).

In addition to the direct genetic influence on sow productive lifetime, the genetic relationship between economically important production traits like growth rate, backfat, and feet and leg soundness and sow productive lifetime can impact the phenotypic longevity results observed at the herd level. The relationships or correlations can differ depending on the population evaluated. Length of productive life and lifetime number of pigs born alive were positively associated in Finnish Large White and negatively associated in Finnish Landrace (Serenius and Stalder 2004; Serenius et al. 2008).

Improving sow productive lifetime involves choosing the correct genetic lines. Johnson (2000) reported that results from the National Pork Board's Maternal Line Project demonstrated that traits contributing to longevity and attrition are heritable. The same report noted line differences for percentage of sows producing four litters, live pigs per sow life, and average sow life. Goodwin (2002) extended the analysis for the same maternal line study and found similar differences through the sixth parity. This seems to indicate that the producer's breeding stock source could impact their ability to retain sows in the breeding herd for longer periods of time or parities ultimately producing more piglets per sow lifetime.

Feet and leg soundness or lameness is usually the second largest identifiable reason for sows leaving the breeding herds, particularly the early parities 1, 2, and 3 (Douglas and MacKinnon 1993). Studies have identified a few factors that are significantly associated with improved sow productive lifetime, either positively or negatively, across several studies. Weak pasterns have a positive influence on longevity (Grindflek and Sehested 1996; Serenius et al. 2001), while buck-kneed front legs, swaying hindquarters, and upright pasterns on rear feet were associated unfavorably with longevity (Jorgensen 1996). Numerous studies have shown that feet and leg conformation scores are moderately heritable traits (Bereskin 1979; de Koning 1996; Huang et al. 1995; Lundeheim 1987; Rothschild and Christian 1988a; Serenius et al. 2001) and some of these conformation traits have even been associated with sow longevity (Fernández de Sevilla et al. 2008, 2009; Serenius and Stalder 2004; Tarrés et al. 2006; Tiranti and Morrison 2006). Heritability estimates from these studies for various leg soundness scores range from 0.01 to 0.47, with many values above 0.15.

Rothschild and Christian (1988a) demonstrated that selection for improved front leg structure was quite successful in only five generations. This seems to suggest that lines with poor leg soundness scores, poor structure, or having a substantial number of feet and leg problems could be improved through proper selection. In a related paper, Rothschild and Christian (1988b) indicated that leg weakness problems are antagonistically correlated with backfat. This seems to indicate that some selection for feet and leg soundness is necessary to maintain adequate structure especially if there is a strong selection against backfat. Selection against backfat has been employed by most seedstock suppliers for several years and may help explain some of the feet and leg problems that many commercial producers see in the females in the breeding herd.

Lopez-Serrano et al. (2000) reported unfavorable genetic correlations between stayability from first to second litter and daily gain (–0.28 in Large White, –0.06 in Landrace) and stayability and backfat (0.22 in Large White, 0.24 in Landrace), while a favorable genetic correlation was found between stayability and leg score (0.08 in Large White, 0.19 in Landrace). Similarly, Brandt et al. (1999) and Jorgensen (2000) determined that leg quality had a significant influence on the productive life of sows. In further analyses, Jorgensen (2000) reported that "standing under position" also was negatively associated with sow longevity. Knauer et al. (2011) reported moderate genetic correlations (0.09–0.49) between stayability and sow structure visual scores and a negative genetic correlation (–0.14) between locomotion scores and stayability. Genomic regions with effects on a sow's body composition (e.g. body length, body depth, body width) and the structure (e.g. pasture posture) were identified by Fan et al. (2009). With further investigation into candidate genes, Fan et al. (2011) located specific markers with effects on sow body composition and structure.

Most research results that have been published to date where feet and leg soundness traits have been evaluated were conducted where sows were housed in gestation stalls. It is not clear if the same traits, additional traits, or a completely different set of feet and leg traits would influence productive lifetime if sows were housed in group loose sow housing facilities.

Recent advances in molecular biology may prove useful in improving sow productive lifetime. Mote et al.

(2009) reported that several genes including carnitine O-palmitoyltransferase I (CPT1A) and C–C chemokine receptor 7 (CCR7) were associated with improved survival or sow productive lifetime. That same study identified genes associated with reproductive performance including insulin-like growth factor binding protein (IGFBP1) and angiotensin I converting enzyme (ACE). Two genes were associated with both sow longevity and improved reproductive performance including CPT1A.

Onteru et al. (2011a) conducted a genome-wide association to determine genetic regions with influence on reproduction traits across multiple parities. Regions explaining a significant portion of genetic variation were found for total born, born alive, stillborns, mummies, and gestation length with different regions accounting for variation in litter size traits at different parities. With further investigation, Onteru et al. (2011b) found several gene regions with an effect on sow lifetime production (total number born, born alive, removal parity, and nonproductive days). These results suggest that genotyping information can be used to select for the components of sow longevity. In the future, swine breeders may be able to improve sow longevity using both traditional selection methods and advanced molecular tools to ultimately improve performance observed at the commercial level.

It is important to note that sow reproductive trait heritability estimates can vary when sows experience different health challenges. Additionally, the genetic correlation for the same trait when animals are exposed to different health conditions is not 1, suggesting that selection for increased performance in one condition is not directly correlated with improved performance in another condition. Lewis et al. (2009) reported different heritabilities for litter size and number of matings until conception when using data from a herd during a PRRS outbreak compared with using data from the same herd in a normal health status. Given that a sow's ability to stay in a commercial herd is dependent on her reproductive performance, this suggests that a sow's ability to tolerate a disease may impact her longevity in the breeding herd.

Because genetic line differences exist and the heritability for longevity traits is of sufficient magnitude that selection would be successful in improving sow productive lifetime, pork producers should have opportunities to choose lines that have improved sow longevity. At the same time, genetic suppliers can continue to improve this trait through selection. Many commercial producers have employed internal gilt multiplication systems. Genetic improvement of sow longevity must occur using purchased semen from their genetic supplier.

References

Archibald AL, Bolund L, Churcher C, et al. 2010. *BMC Genomics* 11:438.

Bereskin B. 1979. *J Anim Sci* 48:1322–1328.

Bergsma R, Kanis E, Verstegen WA, et al. 2008. *J Anim Sci* 86:1067–1080.

Bidanel JP, Gruand J, Legault C. 1996. *Genet Sel Evol* 28:103–115.

Bishop SC, Woolliams JA. 2010. *PLoS One* 5(1):e8940.

Boddicker N, Waide EH, Rowland RRR, et al. 2012. *J Anim Sci* 90:1733–1746.

Brandt H, von Brevern N, Glodek P. 1999. *Livest Prod Sci* 57:127–135.

Clapperton M, Diack AB, Matika O, et al. 2009. *Genet Sel Evol* 41:54.

Douglas RGA, Mackinnon JD. 1993. *Pig Vet J* 30: 77–80.

Edfors-Lilja I, Wattrang E, Magnusson U, et al. 1994. *Vet Immunol Immunopathol* 40:1–16.

Edfors-Lilja I, Wattrang E, Marklund L, et al. 1998. *J Immunol* 161:829–835.

Engblom L, Lundeheim N, Schneider M, et al. 2009. *Animal* 3(6):783–790.

Fahmy MH, Bernard CS. 1972. *Can J Anim Sci* 52:440–447.

Fan B, Onteru SK, Mote BE, et al. 2009. *Genet Sel Evol* 41:14.

Fan B, Onteru SK, Du Z-Q, et al. 2011. *PLoS One* 6(2):e14726.

Fernández de Sevilla X, Fábrega E, Tibau J, et al. 2008. *J Anim Sci* 86:2392–2400.

Fernández de Sevilla X, Fàbrega E, Tibau J, et al. 2009. *Animal* 3:446–453.

Fix JS, Cassady JP, Herring WO, et al. 2010a. *Livest Sci* 127:51–59.

Fix JS, Cassady JP, Holl JW, et al. 2010b. *Livest Sci* 132:98–106.

Goddard ME, Hayes BJ. 2007. *J Anim Breed Genet* 124(6):323–330.

Goodwin R. 2002. Sow longevity genetic differences. In Proceedings of the National Swine Improvement Federation Conference Annual Meeting.

Gou SF, Gianola, D, Rekaya R, et al. 2001. Bayesian analysis of lifetime performance and prolificacy in Landrace sows using a linear mixed model with censoring. *Livest Prod Sci* 72:243–252.

Grandinson K, Lund MS, Rydhmer L, et al. 2002. *Acta Agric Scand A Anim Sci* 52(4):167–173.

Grindflek E, Sehested E. 1996. Conformation and longevity in Norwegian pigs. In Danielsen V, ed. *Proceedings of the Nordiska Jordbruksforskares Forening Seminar 265 – Longevity of Sows*. Denmark: Research Centre Foulum, pp. 77–84.

Guo X, Christensen OF, Ostersen T, et al. 2015. *J Anim Sci* 93:503–512.
Guy S, Thomson P, Hermesch S. 2012. *Front Genet* 3:281
Hall AD, Lo S, Rance KA. 2002. *Acta Agric Scand A Anim Sci* 52:183–188.
Hellbrügge B, Tölle K-H, Bennewitz J, et al. 2008. *Animal* 2(9):1273–1280.
Henryon M, Berg P, Jense J, et al. 2001. *Anim Sci* 73:375–387.
Henryon M, Heegaard PMH, Nielsen J, et al. 2006. *Anim Sci* 82:597–606.
Hermesch S, Ludemann CI, Amer PR. 2014. *J Anim Sci* 92:5358–5366.
Holl J, Long T. 2006. Improving weaned pig quality in today's large litters. In Proceedings of the National Swine Improvement Federation Conference Annual Meeting.
Huang SY, Tsou HL, Kan MT, et al. 1995. *Livest Prod Sci* 44:53–59.
Johnson R. 2000. Role of genetics in gilt attrition. In Proceedings of the Allen D Leman Swine Conference, pp. 105–109.
Jorgensen B. 1996. The influence of leg weakness in gilts, on their longevity as sows assessed by survival analysis. *Proc Int Pig Vet Soc* 14:545.
Jorgensen B. 2000. *Acta Vet Scand* 41:105–121.
Kadowaki H, Suzuki E, Kojima-Shibata C, et al. 2012. *Livest Sci* 147:20–26.
Kanis E, De Greef KH, Hiemstra A, et al. 2005. *J Anim Sci* 83:948–957.
Kim KS, Larsen N, Short T, et al. 2000. *Mamm Genome* 11(2):131–135.
Knap PW. 2005. Breeding robust pigs. *Aust J Exp Agric* 45:763–773.
Knauer MT, Cassady JP, Newcom DW, et al. 2011. *J Anim Sci* 89:935–942.
Knol EF, Leenhouwers JI, van der Lende T. 2002. *Livest Prod Sci* 78:47–55.
de Koning, G. 1996. Selection in breeding programmes against leg problems. In: Danielsen V., ed. *Proceedings of the Nordiska Jordbruksforskares Forening Seminar 265 – Longevity of Sows*. Denmark: Research Centre Foulum, pp. 85–87.
Lewis CRG, Torremorell M, Galina-Pantoja L, et al. 2009. *J Anim Sci* 87:876–884.
Lopez-Serrano M, Reinsch R, Looft H, et al. 2000. *Livest Prod Sci* 64:121–131.
Lund MS, Puonti M, Rydhmer L, et al. 2002. *Anim Sci* 74:217–222.
Lundeheim N. 1979. *Acta Agric Scand* 29(3): 209–215.
Lundeheim N. 1987. *Acta Agric Scand* 37:159–173.
Lunney JK, Chak-Sum H, Wysocki M, et al. 2009. *Dev Comp Immunol* 33:362–374.
Magnabosco D, Cunha ECP, Bernardi ML, et al. 2015. *Acta Sci Vet* 43:1274.
Magnabosco D, Bernardi ML, Wentz I, et al. 2016. *Livest Sci* 184:119–125.
Magnusson U, Wilkie B, Mallard B, et al. 1998. *Vet Immunol Immunopathol* 61:83–96.
Mallard BA, Wilkie BN, Kennedy BW. 1989. *Vet Immunol Immunopathol* 21:139–151.
Mallard BA, Wilkie BN, Kennedy BW, et al. 1992. *Anim Biotechnol* 3(2):257–280.
Mészáros G, Pálos J, Ducrocq V, et al. 2010. *Genet Sel Evol* 42:13.
Meuwissen T, Hayes B, Goddard M. 2001. *Genetics* 157:1819–1929.
Mote BE, Koehler KJ, Mabry JW, et al. 2009. *J Anim Sci* 87:2187–2195.
Nielsen B, Su G, Lund MS, et al. 2013. *J Anim Sci* 91:2575–2582.
Omtvedt IT, Whatley JA, Willham RL. 1966. *J Anim Sci* 25:372–376.
Onteru SK, Fan B, Du Z-Q, et al. 2011a. *Anim Genet* 43:18–26.
Onteru SK, Fan B, Nikkilä MT, et al. 2011b. *J Anim Sci* 89:988–995.
Porth M. 2016. Benchmarking summary 2015. In Benchmarking Magazine (Spring Edition) PigCHAMP, pp. 16–17.
Putz AM, Tiezzi F, Maltecca C, et al. 2015. *J Anim Sci* 93:5153–5163.
Ramos A, Crooijmans R, Affara N, et al. 2009. *PLoS One* 4(4):e6524.
Rehfeldt C, Tuchschere A, Hartung M, et al. 2008. *Meat Sci* 78:170–175.
Reiner G, Melchinger E, Kramarova M, et al. 2002. *J Gen Virol* 83:1617–172.
Roehe R. 1999. *J Anim Sci* 77:330–343.
Rothschild MF, Christian LL. 1988a. *Livest Prod Sci* 19:459–471.
Rothschild MF, Christian LL. 1988b. *Livest Prod Sci* 19:473–485.
Rothschild MF, Hu Z, Jiang Z. 2007. *Int J Biol Sci* 3(3):192–197.
Rydhmer L, Eliasson-Selling L, Johansson K, et al. 1994. *J Anim Sci* 72:1964–1970.
Serenius T, Stalder KJ. 2004. *J Anim Sci* 82: 3111–3117.
Serenius T, Sevon-Aimonen AM, Mantysarri EA. 2001. *Livest Prod Sci* 69:101–111.
Serenius T, Stalder KJ, Fernando RL. 2006. Genetic associations of length of productive life with age at first farrowing and leg soundness score in Finnish Landrace population. In Proceedings of the 8th World Congress on Genetics Appl ied to Livestock Production, CD-ROM communication 06-08.
Serenius T, Stalder KJ, Fernando RL. 2008. *J Anim Sci* 86:3324–3329.
Stalder KJ. 2016. *2015 Pork Industry Productivity Analysis*. Des Moines, IA: National Pork Board.

Stalder, KJ, Christian LL, Rothschild MF, et al. 1997. *J Anim Sci* 75:3114–3118.
Stalder KJ, Rothschild MF, Lonergan SM. 2005. *Meat Sci* 69:451–457.
Su G, Lund MS, Sorensen D. 2007. *J Anim Sci* 85:1385–1392.
Tarrés J, Bidanel JP, Hofer A, et al. 2006. *J Anim Sci* 84:2914–2924.
Tholen E, Bunter KL, Hermesch S, et al. 1996. *Aust J Agric Res* 47:1261–1274.
Tiranti KI, Morrison RB. 2006. *Am J Vet Res* 67:505–509.
Whitworth KM, Lee K, Benne JA, et al. 2015. *Biol Reprod* 91(3):1–13.
Whitworth KM, Rowland RRR, Ewen CL, et al. 2016. *Nat Biotechnol* 34(1):20–22.
Živković S, Teodoric M, Kovcin S. 1986. *World Rev Anim Prod* 22(4):11–15.
Zumbach B, Misztal I, Tsuruta S, et al. 2008. *J Anim Sci* 86:2076–2081.

4

Effect of Environment on Health

Michael C. Brumm

From the pig's point of view, the environment it lives in includes temperature, humidity, space, feed and water access, and air quality when housed in confinement facilities. Rather than document the impact of these variables on health, this chapter will discuss the known requirements that allow the healthy pig to grow and perform to its genetic potential.

Temperature

The pig is a homeothermic animal with a deep body temperature of 39 °C (Baxter 1984), meaning it has a constant core body temperature and will adapt behaviors to maintain this temperature. These behaviors are not only postural changes (huddling as an example when cold) but also changes in basal metabolism accomplished through a change in feed intake (more or less metabolic heat production) when forced to live in an environment warmer or colder than the thermal-neutral temperature zone.

At a constant body weight, heat production by growing pigs and lactating females has increased over the years as the rate of lean deposition and milk production has improved in response to improvements in nutrition, health, genetics, housing, etc. For growing pigs, this increase has amounted to about a 10–15% watt (W) increase at the same body weight increase every 10 years (Figure 4.1) (Brown-Brandl et al. 2004). Similar increases have been noted for early weaned pigs (Harmon et al. 1997). In lactation, the large increase in milk production to support larger litters has also resulted in a large increase in metabolic heat output (Pedersen 2002). This increase in metabolic heat production supports the conclusion that today's pigs are more sensitive to air temperature (especially warm or hot temperatures) than previous generations (Renaudeau et al. 2011).

At the same time, pigs have consistently demonstrated a preference for diurnal variation in temperatures (warmer during the days and cooler during nights). While this demonstrated variation is often 5–7 °C (Bench and Gonyou 2006; Morrison et al. 1987), fluctuations of ±4 °C during the first week after weaning have been demonstrated to increase post weaning scours (LeDividich 1981). Similarly, intermittent drafts of 0.99 m/s for 6 weeks after weaning resulted in increased respiratory distress (coughing, sneezing, and more pneumonic lesions; Scheepens et al. 1991). Brumm and Shelton (1988) and Johnston et al. (2013) have demonstrated no negative impact on pig health or pig performance when a reduced nocturnal temperature regimen is begun 1 week after weaning. This regimen often results in air temperatures in the early morning hours that are 4–5 °C lower than daytime (0700–1900 hours) temperatures.

Extended periods of heat stress have been demonstrated to impact intestinal integrity (Pearce et al. 2015), and heat stress *in utero* impacts postnatal body composition and growth potential (Johnson et al. 2015). Heat stress also impacts intestinal barrier function (Pearce et al. 2012), suggesting a negative impact on health.

Table 4.1 lists the recommended controller set points for facilities with mechanical ventilation. Generally this set point is considered to be at the lower end of the thermal-neutral zone. If temperatures in a production facility are lower than this, some type of supplemental heat is usually activated to maintain thermal-neutral conditions in the pig zone. As temperatures warm above this set point, devices such as fans, misters, or evaporative pads are activated to both remove heat and activate evaporative cooling mechanisms to assist the pig in dealing with the warmer conditions.

The low end of the thermo-neutral zone is normally considered the lower critical temperature (LCT), which is defined as the lowest air temperature that a given animal can maintain under homeothermic conditions without increasing metabolism to maintain body temperature (Hillman 2009). The suggested set points in

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

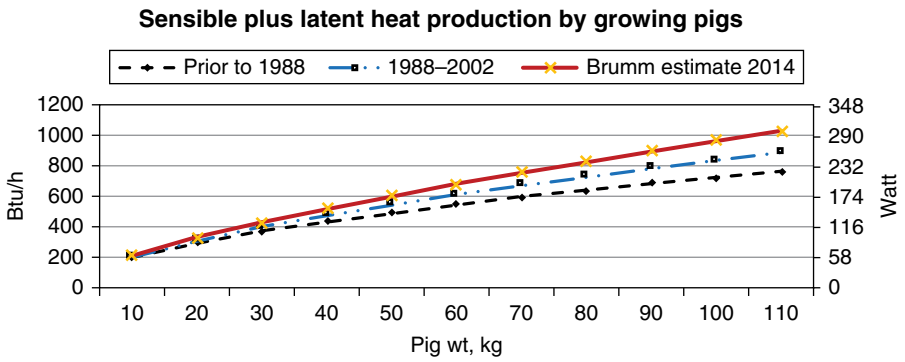


Figure 4.1 Total (sensible plus latent) heat production by growing pigs. *Source:* Adapted from Brown-Brandl et al. (2004).

Table 4.1 Recommended ventilation controller set points for fully slatted facilities.

Pig class or wt	Set point (°C)
Weaning (5–8 kg)	30
25 kg	22
50 kg	19
100 kg+	16
Individually housed gestating females	20
Group-housed gestating females	17
Lactating females week 3	20

Table 4.1 are generally within 1–2 °C of LCT for pigs housed in groups in fully slatted facilities without bedding, hovers, or other facility modifications that allow the pig to modify its LCT. When housed at temperatures in the pig zone at or slightly above the LCT, metabolic heat release to the environment is approximately 2/3 as sensible heat (air temperature) and 1/3 as latent heat (moisture). As temperatures rise, the pig must release a higher percentage of its metabolic heat as latent heat since the difference between skin surface temperature and air temperature lowers, which results in less heat dissipation via convection and conduction modes.

The exact LCT for any given pig or group of pigs will depend upon its feed intake and body size (DeShazer and Yen 2009). Pigs of any given size that consume more feed will have a lower LCT as compared with pigs consuming less feed. As a consequence, when feed intake is reduced during an illness, the general recommendation is to increase air temperature in the pig zone to compensate. The upper end of the thermoneutral zone is normally considered the upper critical temperature (UCT) or the evaporative critical temperature (Hillman 2009). The UCT can be defined as the temperature at which pigs begin to pant or perform other behaviors in order to dissipate heat to the environment in order to maintain body temperature. In general, the goal of swine housing is to provide conditions that result in the least regulatory effort by the growing or adult animal (Hillman 2009).

Humidity

The desired humidity level in swine production facilities is 60–80%. Humidity fluctuates in a diurnal pattern in production facilities, most often being the lowest in morning hours and highest in the midafternoon due to pig activities increasing the amount of moisture that is evaporated from flooring surfaces. During hot weather, high humidity levels are associated with a high total enthalpy in the environment. Because of this high enthalpy, options for using evaporative cooling such as wetting of the pigs or evaporative pads in mechanically ventilated facilities are limited. As a consequence, pig performance is decreased as the pig modifies its behavior by reducing feed intake and activity to decrease total heat production.

The minimum ventilation rates in Table 4.2 are designed to maintain humidity levels at 60–80% in cold weather in full and partially slatted production facilities. Ventilation rates higher than the minimum are generally in response to the need to remove excess heat from a facility as a result of pig growth and/or warming outside temperatures.

Causes of death in ventilation failures

With a majority of the North American pig population housed in facilities with mechanical ventilation, ventilation failures due to equipment malfunction or electrical outages occasionally occur. In many instances these failures are accompanied by pig deaths.

Table 4.2 Recommended ventilation rates for swine production facilities.

Pig class	Weight (kg)	Cold weather minimum (m^3/h)	Mild weather rate (m^3/h)	Hot weather rate (m^3/h)
Weaned pigs	5–15	3.4	17.0	51.0
Growing pigs	15–34	5.1	25.5	76.5
Finishing pigs	34–68	11.9	40.8	130.0
Finishing pigs	68–135	17.0	59.5	200.0
Gestating females		20.4	68.0	425.0
Sow and litter		34.0	85.0	1100.0

Source: Adapted from MWPS (1990)

While it is common for producers and their advisors to talk about "suffocation" as a cause of death, the primary cause of death for all ages other than newly weaned piglets is hyperthermia (Robert et al. 2003; Zulovich and Bundy 1990), not lethal levels of carbon dioxide or other gases. Zulovich and Bundy (1990) demonstrated that at typical stocking densities of North American production facilities, death from hyperthermia can occur as rapidly as 30 minutes after a ventilation failure. In this simulation, only a minimal amount of air infiltration was necessary to keep predicted carbon dioxide and oxygen at reasonable levels. For all conditions simulated, relative humidity in the facility reach 100% within 2 hours of the simulated failure. In many situations where pig death occurs due to ventilation failure, smaller pigs (runts and sick pigs) often survive because their metabolic heat output is lower due to lower levels of feed intake. They do not reach critical core body temperatures before many of the fastest-growing (i.e. highest metabolic heat output) pigs have died and barn temperatures have begun to decline.

Christison and Heidenreich (1968) recorded rectal temperatures of 44.4 °C 5 minutes prior to death when a 25 kg pig was exposed to a 38 °C temperature for 5.25 hours. On-farm rectal temperatures of 41.1 °C have been recorded 4–5 hours after death associated with a ventilation failure event (MC Brumm, personal communication). Robert et al. (2003) conducted a series of experiments in a commercial nursery with 6.8 and 22.7 kg pigs in which the ventilation system was turned off and the rate of change in temperature and carbon dioxide concentration were recorded. For 6.8 kg pigs, they determined that air temperature would never reach a critical level to result in hyperthermia but carbon dioxide levels would become critical within 150 minutes. In the same facility with 22.7 kg pigs and no mechanical ventilation operating, the critical time before temperatures approached levels creating hyperthermia would be about 60 minutes, while the critical time for carbon dioxide levels was 95 minutes.

Pen space

The growing pig's spatial requirement in m^2/pig is well understood for both full and partially slatted facilities (Flohr et al. 2016; Gonyou et al. 2006). When space is defined by the allometric equation $m^2/pig = K * BW(kg)^{0.667}$ where K is a constant and BW is body weight, the generally recognized constant is 0.0336 (Gonyou et al. 2006a). Many welfare codes define the space requirement using a constant (K) of 0.035. In general, as space per pig is restricted, feed intake and daily gain decline with a less predictable response in feed conversion. Table 4.3 details the relationship between pig weight, pen space, and "K." The predicted impact on daily gain versus adequate space (K = 0.0336) is presented in Table 4.4.

There is limited data on the impact of space allocation on pig health. Turner et al. (2000) suggested that pigs in deep straw-bedded facilities given less space had a lower humoral response to an antigen challenge. In contrast, Oh et al. (2010) reported no impact of increasing pig numbers in a fixed dimension weaned pig pen on serum IgA and IgC. Hyun et al. (1998) have suggested that environmental stressors (temperature, space, and social regrouping) have an additive impact on performance.

Feeder space

A common limit to pig performance as sale weights increase is feeder access. Producers and equipment suppliers often size feeders based on the number of "holes" the feeder offers without regard to the issues surrounding the quality of the eating space or "hole."

The width of the feeding space is defined by the shoulder width of the pig. Petherick (1983) defined shoulder width in relation to pig body weight as width (mm) = $64.0 \times (\text{body weight, kg})^{0.33}$.

Table 4.3 Relationship between pig weight, pen space, and "K."

	"K"				
Pig wt (kg)	0.025 m^2/pig	0.028	0.031	0.0336	0.035
20	0.18	0.21	0.23	0.25	0.26
40	0.29	0.33	0.36	0.39	0.41
60	0.38	0.43	0.48	0.52	0.54
80	0.46	0.52	0.58	0.62	0.65
100	0.54	0.60	0.67	0.73	0.76
120	0.61	0.68	0.76	0.82	0.85
140	0.68	0.76	0.84	0.91	0.95

Source: Adapted from Petherick (1983).

Table 4.4 Estimated impact on daily gain when space per pig is restricted in fully slatted facilities.

Wt (kg)	Space/pig (m^2/pig) at $K = 0.0336$	K when space/pig is 0.66 m^2/pig	ADG versus $>K = 0.0335$ from 25 kg to stated wt
20	0.25	0.089	
40	0.39	0.056	No impact
60	0.52	0.043	No impact
80	0.62	0.035	No impact
100	0.73	0.0305	97.5%
120	0.82	0.0270	94.6%
140	0.91	0.0245	92.6%

Source: Adapted from Gonyou et al. (2006). Reproduced with permission of Oxford University Press.

To allow for pig movement at the feeder, feeder spaces should be sized based on 1.1 times the shoulder width. Table 4.5 lists the estimated shoulder width in relation to pig weight and the estimated width of feeder spaces based on these shoulder width dimensions. This table supports the recommendation that feeders for today's production facilities should have a minimum feeder "hole" width of 35.0 cm as slaughter weights in the United States continue to increase (Figure 4.2; NASS, USDA, 2018 and Figure 4.3).

Gonyou and Lou (1998) using pigs with weights up to 95 kg concluded that the best compromise for feeder depth (distance from the front lip of the feeder pan to the feed delivery point) for grow-finish pigs was 20–30 cm. They concluded that the ideal feeder depth for 95 kg pigs was 32 cm. This suggests that a majority of the feeders installed in production facilities in the United States and Canada today, which have depths of 20–25 cm, are limiting to pig performance as sale weights increase.

The problem with increasing feeder depth is that of smaller pigs at time of placement stepping into the feeder to access feed and then tracking feed out of the feed pan. However this has not been an issue with "wean-finish" feeders where pigs are grown from 5.5 kg to slaughter weight on the same feeder. In this author's experience, as sale weights increase, feeders must have at least 25.4 cm of depth from the front lip to the feed delivery device or agitator plate with 28–30.5 cm preferred for dry feeders. If the distance is less than this, pigs end up pushing against the feeder with the crown of their forehead and/or have difficulty accessing the feed agitator plate.

The table also highlights a common feeder problem with swine nursery units. Many nurseries now house pigs until 25 kg or heavier. The vast majority of feeders sold in North America for placement in swine nurseries still have feeder "holes" that are 15.25 cm × 15.25 cm. Based on shoulder dimensions, these feeders most likely limit feed intake or at the minimum limit pig access to feed by limiting the number of usable spaces that pigs can eat from unless the spaces/holes are 20.3 cm wide.

Similar sizing issues arise when drinking water is furnished in trough or cup/bowl drinkers. Pigs grow in three dimensions (length, width, and height), so the need to account for increases in head dimensions for these devices as slaughter weights increase is also a reality.

Table 4.5 Shoulder width of growing pigs in relation to pig weight and estimated feeder "hole" widths necessary to accommodate this dimension.

Pig wt (kg)	Shoulder width (cm)	1.1 × shoulder width (cm)
18.2	16.8	18.3
27.3	19.1	21.1
90.9	28.4	31.2
100.0	29.2	32.3
109.1	30.2	33.0
118.2	31.0	34.0
127.3	31.8	34.8
136.4	32.5	35.6
145.5	33.0	36.3

Source: Adapted from Petherick (1983).

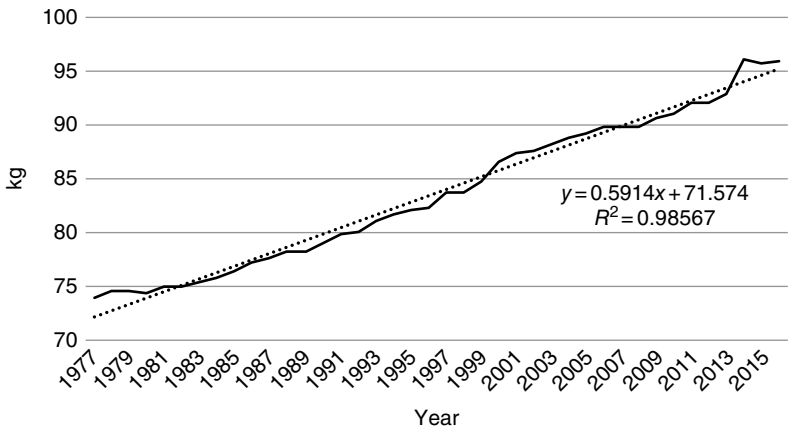


Figure 4.2 USDA-inspected barrow and gilt carcass weights by year. Data *source:* Livestock slaughter, NASS, USDA.

Figure 4.3 Twelve "hole" 2.13 m long double-sided feeder with marked pig (estimated to weigh almost 136 kg) destined for removal and sale immediately after the picture was taken from a pen of 125 finishing pigs.

Water

At birth, water accounts for 82% of the pig's empty body weight. By the time the pig weighs 240 pounds, water comprises only 51% of the empty body weight (Shields et al. 1989). In addition to body tissue and metabolic functions, water is used for (1) the adjustment of body temperature, (2) the maintenance of mineral homeostasis, (3) the excretion of the end products of metabolism (particularly urea), (4) the achievement of satiety (gut fill), and (5) satisfaction of behavioral needs (Brooks et al. 1989). Major sources of water for physiological needs, including growth, reproduction, and lactation, are

water from feedstuffs, water from metabolic processes, and drinking water. As a practical matter, drinking water is the major water source (Thacker 2001) Table 4.6.

Symptoms of water deprivation in swine include reduced feed intake, crowding around drinker devices, dehydration, increased heart rates, increased body temperatures, increased respiration, and death (Thacker 2001).

Water consumption for growing pigs has a distinct periodicity with a peak at the beginning and at the end of the feeding period when nose-operated drinkers are used. Water consumption between feeding periods peaked two hours after the morning feeding and one hour after the afternoon feeding (Olsson and Andersson 1985). Weaned pigs housed under conditions of constant light showed a diurnal pattern for water intake with higher consumption recorded from 0830 to 1700 hours as compared with the 1700 to 0830 hours time period (Brooks et al. 1984). Grow-finish pigs using nipple drinkers showed a large peak from 1500 to 2100 hours and a smaller peak between 500 and 1100 hours (Korthals 1998). The number of pigs in a group (pen) apparently influences water usage. In one study water usage was higher when pigs were housed in groups of 60 versus 20. Total drinking time per pig decreased when group size increased, even though the number of pigs per drinker was the same for both group sizes (Turner et al. 1999).

While there is very good evidence that a majority of water consumption is associated with eating activities in research settings, there is limited data on patterns of water usage in commercial facilities. Brumm (2006) documented drinking water disappearance patterns in production facilities in Minnesota and Nebraska. These facilities varied in the number of pigs per pen, the type of feeder and drinker, the type of ventilation, relative pig health, etc. The similarities between the winter and summer patterns at the sites suggests that two patterns of water usage exist, depending on the temperature in the facility (i.e. time of the year). In thermal-neutral conditions (generally air temperatures in the pig zone <26 °C), grow-finish pigs begin drinking water around 6 am, with a peak in drinking water disappearance in early afternoon and a gradual decline for the remainder of the day. This pattern is in agreement with published literature.

However, when pigs are growing in warm to hot conditions (air temperatures in the pen exceeding 26 °C for 1 or more hours per day), they alter their pattern of drinking water usage. Pigs begin drinking earlier in the day, with a morning peak from 0800 to 0900 hours. There is a decline in drinking water use midday with a second peak in drinking water use from 1700 to 2300 hours. followed by the decline into the night hours.

It is interesting to note that pigs shift to this pattern of drinking water use on the first day of air temperatures in the pig zone (>26 °C or so) and maintain the pattern for 3–5 days, even if these subsequent days have temperatures considered to be thermal neutral. This adaptation is often maintained for several days in anticipation that the heat stress event will be longer than a single day. This suggests that a shift in eating and drinking behavior is one of the first adaptations of the growing pig to heat stress. In the future, it may be possible to use this shift in drinking water usage as a predictor of a performance reduction due to heat stress in grow-finish pigs.

Figure 4.4 shows the typical daily water disappearance curve for pigs in a wean-finish facility equipped with bowl drinkers and offered corn–soybean meal-based diets ad libitum. Based on producer and veterinarian

Table 4.6 Recommended water flow rates from drinking devices.

Pig class	Flow (mL/min)
Nursery pigs	250–500
Grow-finish pigs	750–1000
Breeding animals	1000

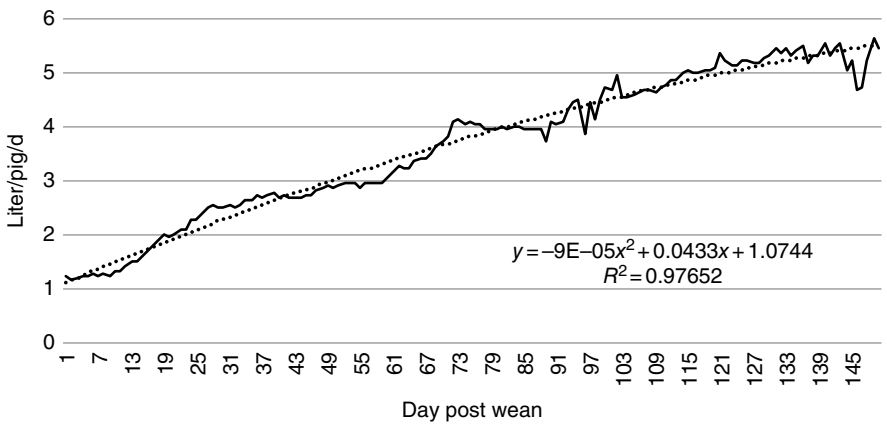


Figure 4.4 Typical drinking water usage for growing pigs. Data based on eight wean-finish groups of pigs from weaning (piglets 17–21 days of age) to day 150 post weaning.

observations, when daily water usage drops for 3 continuous days or drops more than 30% from day to day, this may indicate that a potential health challenge is occurring in the production facility (Brumm 2006).

Water–feed ratios (kg/kg) for liquid feeding systems typically range from 2.5 : 1 to 3.5 : 1 (English et al. 1988). Water–feed ratios ranging from 1.78 : 1 to 2.79 : 1 for pigs weighing from 18 to 114 kg and fed dry feed ad libitum have been reported (Brumm et al. 2000). The lowest reported water–feed ratios were with wet/dry feeders and bowl drinkers, whereas gate-mounted nipple drinkers had the highest ratios. With similar performance, this suggests that the major cause of differences in water–feed ratios between the various drinking devices is due to differences in water wastage, not differences in the amount consumed.

Water–feed ratios decrease as pigs grow. Recent on-farm data (MC Brumm, unpublished data) supports the conclusion that water–feed ratios decline as pigs grow, with a ratio as low as 1.5 : 1 common in facilities that use wet/dry feeders or stainless steel bowl drinkers in late finishing and offered corn–soybean meal-based mash diets ad libitum. Assuming similar water–feed ratios for both barrows and gilts, it follows that barrows drink more water than gilts since barrows eat more feed per day than gilts in mid to late finishing (NRC 2012). Pigs fed meal diets drink more water than pigs fed pelleted diets (Laitat et al. 1999), reflecting similar water–feed ratios and differences in feed conversion efficiency.

General recommendations exist for the number of pigs per drinking device (Midwest Plan Service 1983), but research to support these recommendations is limited. Researchers using 3- to 4-week-old weaned pigs reported a slight reduction in average daily gain and an increase in weight variation within pens of 16 pigs given access to 1 versus 2 nipple drinkers for 5 weeks' post weaning (Brumm and Shelton 1986). Generally, for groups larger than 10 pigs in a nursery and 15–20 pigs in a grow-finish facility, a minimum of 2 delivery devices is recommended (Brumm and Reese 1992).

Grow-finish pigs spent from 3 to 16 minutes per day at nipple drinkers when flow ranged from 1100 down to 100 mL/min (Nienaber and Hahn 1984). This suggests that pigs will exert some extra effort in order to obtain water. But it is not clear at what point having to wait for drinker access or exert extra effort impairs performance.

Lactating sows need considerable amounts of water, both for milk production and to remove the metabolic end products associated with this production (Thacker 2001). Water consumption (measured as disappearance) averaged 18 L/day with a range of 12–40 L/day (Lightfoot 1978). It is expected that as milk output by lactating sows increases due to advances in genetics, nutrition, and housing, this average value will increase, even as improvements in wastage are noted due to newer types of drinking devices.

Noise

The general noise level measured in mechanically ventilated pig buildings was 73 dB with naturally ventilated buildings averaging 10 dB lower levels (Talling et al. 1998). This noise level tends to be monotonous and continuous (Schaffer et al. 2001). Levels averaged 98 dB during transport (Talling et al. 1998). Loud fan noise (85 dB) has been shown to interfere with sow and nursing piglet communication, leading to disrupted nursings (Algers and Jensen 1985).

Kanitz et al. (2005) demonstrated that repeated exposure to 2 hours of 90 dB noise caused considerable alterations to the hypothalamic–pituitary–adrenal axis. They suggested that this alteration would have a substantial impact on the general vulnerability of the pig with respect to health, welfare, and productivity.

Stray voltage

Stray voltage is defined as the voltage between any two animal contact points. Most often these exist as neutral-to-earth voltages (Gustafson and Morgan 2004). These frequently occur in swine housing situations where there are large amounts of metal gating to provide a path for the electric current such as gestation stalls or farrowing crates or where there is metal flooring such as farrowing crates or weaned pig pens. Field reports indicate that when stray voltage is present, there is often a reluctance of pigs to drink from drinkers, a reduction in appetite, restlessness, increases in aggressive encounters, impaired growth, and a variety of health disorders (Robert et al. 1994a).

Current flow through gestating females was always higher on wet versus dry floors (Robert et al. 1994b). However, under controlled conditions, it has been difficult to demonstrate negative impacts on lactating female or growing pig performance or health (Robert et al. 1992, 1996).

References

Algers B, Jensen P. 1985. *Appl Anim Behav Sci* 14:49–61.

Baxter S. 1984. The pig's response to the thermal environment. In *Intensive Pig Production: Environmental Management and Design*. London: Granada Publishing, pp. 35–54.

Bench, CJ, Gonyou HW. 2006. *Can J Anim Sci* 87:299–302.

Brooks PH, Russell RJ, Carpenter JL. 1984. *Vet Rec* 115:513–515.
Brooks PH, Carpenter JL, Barber J, et al. 1989. *Pig Vet J* 23:51–66.
Brown-Brandl TM, Nienaber JA, Xin H, et al. 2004. *Trans ASAE* 47(1):259–270.
Brumm, MC. 2006. Patterns of drinking water use in pork production facilities. Nebraska Swine Report EC06-219. Lincoln: University of Nebraska, pp.10–13.
Brumm MC, Reese DE. 1992. Weaned pig management and nutrition. NebGuide 86-821(Rev 1992). Lincoln: University of Nebraska.
Brumm MC, Shelton DP. 1986. Nursery drinkers – how many? Nebraska Swine Report EC86-219. Lincoln: University of Nebraska, pp. 5–6.
Brumm, MC, Shelton DP. 1988. *J Anim Sci* 66: 1067–1072.
Brumm MC, Dahlquist JM, Heemstra JM. 2000. *J Swine Health Prod* 8:51–57.
Christison GI, Heidenreich CJ. 1968. *Int J Biometeorol* 12(4):365–367.
DeShazer, JA, Yen JT. 2009. Chapter 3: Energetics of biological processes. In DeShazer JA, ed. *Livestock Energetics and Thermal Environmental Management*. St Joseph, MI: American Society of Agricultural and Biological Engineers, pp. 49–71.
English PR, Fowler VP, Baxter S, et al. 1988. *The Growing and Finishing Pig: Improving Efficiency*. Ipswich, UK: Farming Press.
Flohr JR, Tokach MD, DeRouchey JM, et al. 2016. *J Anim Sci* 94:4388–4400.
Gonyou HW, Lou Z. 1998. Grower/Finisher Feeders: Design, Behaviour and Performance. Prairie Swine Centre Monograph 97-01, Saskatoon, SK.
Gonyou HW, Brumm MC, Bush E, et al. 2006. *J Anim Sci* 84:229–235.
Gustafson RJ, Morgan MT. 2004. Chapter 18: Stray voltage problems in agriculture. In: *Fundamentals of Electricity for Agriculture* 4th ed. St Joseph, MI: American Society of Agricultural Engineers, pp 431–451.
Harmon, JD, Xin H, Shao J. 1997. *Trans ASAE* 40(6):1693–1698.
Hillman, PE. 2009. Chapter 2: Thermoregulatory physiology. In DeShazer JA, ed. *Livestock energetics and Thermal Environmental Management*. ASABE #801M0309. American Society of Agricultural and Biological Engineers, pp. 23–48.
Hyun Y, Ellis M, Riskowski G, et al. 1998. *J Anim Sci* 76:721–727.
Johnson JS, Sanz-Fernandez MV, Gutierrez NA, et al. 2015. *J Anim Sci* 93:82–92.
Johnston, LJ, Brumm MC, Moeller SJ, et al. 2013. *J Anim Sci* 91:3429–3435.
Kanitz E, Winfried O, Tuchschere M. 2005. *Livest Prod Sci* 94:213–224.
Korthals RL. 1998. Modeling the drinking behavior of swine. American Society Agricultural Engineers Paper No. 98-4078.
Laitat M, Vandenheede M, Desiron A, et al. 1999. *Anim Sci* 69:491–499.
LeDividich J. 1981. *Livest Prod Sci* 8:75–86.
Lightfoot, AL. 1978. *Anim Prod* 26:386.
Midwest Plan Service. 1983. *Swine Housing and Equipment Handbook*. Publication no. MWPS-8. Ames, IA: Iowa State University.
Midwest Plan Service. 1990. *Mechanical Ventilating Systems for Livestock Housing*. Publication no. MWPS-32. Ames, IA: Iowa State University.
Morrison, WD, Amyot E, McMillan I, et al. 1987. *Can J Anim Sci* 67:903–907.
NASS, USDA. 2018. Livestock slaughter. https://www.ers.usda.gov/data-products/livestock-meat-domestic-data/livestock-meat-domestic-data/#Livestock%20and%20poultry%20live%20and%20dressed%20weights. Accessed November 5, 2018.
Nienaber JA, Hahn GL. 1984. *J Anim Sci* 59:1423–1429.
NRC. 2012. *Nutrient Requirements of Swine*, 11th Rev. ed. Washington, DC: National Academy Press.
Oh HK, Choi HB, Ju WS, et al. 2010. *Livest Prod Sci* 132:113–118.
Olsson O, Andersson T. 1985. *Acta Agric Scand* 35:55–66.
Pearce SC, Mani V, Boddicker RL, et al. 2012. *J Anim Sci* 90:257–259 (Supplement 4).
Pearce SC, Sanz-Fernandez MV, Torrison J, et al. 2015. *J Anim Sci* 93:4702–4713.
Pedersen S. 2002. Heat and moisture production for pigs on animal and house level. American Society Agricultural Engineers Paper No. 02-4178.
Petherick JC. 1983. *Anim Prod* 36:497–500.
Renaudeau D, Gourdine JL, St-Pierre NR. 2011. *J Anim Sci* 89:2220–2230.
Robert S, Matte JJ, Bertin-Mahieux J, et al. 1992. Stray voltage: Its influence on swine production during the fattening period. *Vet Res Commun* 17:41–53.
Robert S, Matte JJ, Martineau GP. 1994a. Stray voltage: Sensitivity of fattening pigs and factors affecting their resistance. *Pig News Information* 15:45N–49N.
Robert S, Matte JJ, Bertin-Mahieux J, et al. 1994b. *Can Agric Eng* 36:37–43.
Robert S, Matte JJ, Martineau GP. 1996. *Am J Vet Res* 57:1245–1249.
Robert MJ, Shaffer CS, Funk TL, et al. 2003. Carbon dioxide and temperature change due to ventilation failure. In Jacobson LD, ed. *Swine Housings II Proceedings*. Publication 701P1303. American Society of Agricultural Engineers, pp 9–15.
Schaffer D, Marquardt V, Marx G, et al. 2001. *Dtsch tieraztl Wschr* 108:60–66.
Scheepens CJM, Tienlen MJM, Hessing MJC. 1991. *Livest Prod Sci* 29:241–254.

Shields Jr. RG, Mahan DC, Graham PL. 1989. *J Anim Sci* 57:43–54.

Talling JC, Lines JA, Wathes CM, et al. 1998. *J Agric Eng Res* 71:1–12.

Thacker PA. Water in swine nutrition. 2001. In Lewis AJ, Southern LL, eds. *Swine Nutrition*. Boca Raton: CRC Press, pp. 381–398.

Turner SP, Edwards SA, Bland VC. 1999. *Anim Sci* 68:617–624.

Turner SP, Ewan M, Rooke JA, et al. 2000. *Livest Prod Sci* 66:47–55.

Zulovich JM, Bundy DS. 1990. Modelling the non steady state conditions in swine facilities during mechanical ventilation failures. American Society Agricultural Engineers Paper No. 90-4002.

5

Differential Diagnosis of Diseases

Alejandro Ramirez

Introduction

The objective of this chapter is to provide a list of differentials to consider under various different clinical presentations. They are organized by system affected. Due to the international scope of this book, these lists are designed to be inclusive rather than exclusive as prevalence is relative to geographic location. The World Organization for Animal Health (OIE) continually updates its list of diseases requiring international reporting (www.oie.int) because of their impact in animal and public health worldwide, including trade concerns. The World Animal Health Information Database (http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home) provides a Web portal for monitoring outbreaks, disease distribution maps, and detailed country diseases for all OIE-listed diseases.

It is envisioned that readers will refer to this section to remind themselves of possible differentials. It is helpful to be open about all possible causes rather than just focus on the common causes especially when dealing with challenging cases and to ensure that new causes to a particular system or region are not missed. Many times clinical disease outbreaks in large populations are multifactorial, and thus focusing on single causes can misguide practitioners. A quick review of the respective body system chapter (Section 2 Chapters 14–22) can help guide the prioritization of the list. Individual causes can then be better researched (etiology, clinical signs, diagnosis, and prevention) in their respective chapters, which are identified in most of the tables.

It is important to remember that because these lists are inclusive rather than exclusive, there are many causes listed for which commercially available diagnostic tests are not available. Chapter 6 reviews many of the different diagnostic tests available including important information on test performance and considerations in interpreting results. Collecting evidence and establishing causality is a critical step for proper diagnosis (see Chapter 8).

Digestive system

Chapter 15 covers valuable information regarding the digestive system including useful tables summarizing the mechanisms of diarrhea (Table 15.1), diagnosis of some common gastrointestinal conditions (Table 15.2), and pathology and diagnostic confirmation of common conditions (Table 15.3).

The approximate age at which certain causes of diarrhea and vomiting are more common is shown in Tables 5.1 and 5.2 respectively. The approximate age is given solely as guidance to help emphasize certain causes based on age of pigs and does not imply the cause to be restricted only to that age group. Table 5.3 provides a general list of possible causes of rectal prolapses including a brief explanation.

Respiratory system

An overview of the respiratory system is provided in Chapter 21. Tables 5.4 and 5.5 summarize differential diagnosis lists for major respiratory clinical presentations.

Integumentary system

The integumentary system is reviewed in Chapter 17. Table 5.6 helps summarize the approximate age when specific skin diseases are more common. Tables 5.7 and 17.1 help narrow down the differential diagnosis of skin diseases based on location and clinical presentation of the lesions.

Hemopoietic system

The cardiovascular and hemopoietic systems are reviewed in Chapter 14. Anemia is a common clinical presentation related to the hemopoietic system. Possible causes of anemia are listed in Tables 5.8 and 14.7.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 5.1 Approximate age at which certain causes of diarrhea in pigs are more common (also see Chapter 15).

1–2 days	3–4 days	5–6 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults	Chapter
Clostridium difficile													51
Clostridium perfringens type A													51
Clostridium perfringens type *C*													51
Bovine viral diarrhea													39
Enterococcus spp.													47
Escherichia coli													52
Porcine reproductive and respiratory syndrome virus													41
Rotavirus													43
Teschovirus													40
African swine fever virus													25
Campylobacter spp.													64
Classical swine fever virus													39
High fever (any condition causing a high fever)													14
Porcine deltacoronavirus													31
Porcine epidemic diarrhea virus													31
Toxoplasma gondii													66
Transmissible gastroenteritis virus													31
	Hypoglycemia (agalactia)												18
	Cryptosporidium spp.												66
		Strongyloides spp.											67
		Eimeria spp.											66
		Cystoisospora suis											66
		Porcine sapovirus											29
			Iron toxicity										68
			Porcine astrovirus										27
				Adenovirus									24
					SBM								15
						Antibiotic-induced colitis							10
						Oesophagostomum dentatum							67
						Salmonella spp.							59
							Brachyspira pilosicoli						62
							Yersinia spp.						64
								Aflatoxin					69
								Arsenic toxicity					70
								Ascaris suum					67
								Bacillus anthracis					64
								Biotin deficiency					68
								Brachyspira hyodysenteriae					62
								Carbamate toxicity					70
								Chromium toxicity					68
								Dimetridazole toxicity					70
								Dipyridal herbicides					70
								Entamoeba spp.					66
								Fluorine toxicity					70

Table 5.1 (Continued)

1–2 days	3–4 days	5–6 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults	Chapter
						Gastric ulcer							15
						Lawsonia intracellularis							58
						Monensin toxicity							10, 70
						Niacin deficiency							19, 68
						Organophosphate toxicity							70
						Porcine circovirus type 2							30
						Salt toxicity							70
						Selenium deficiency							68
						Sulfur toxicity							68
						T-2 toxin							69
						Trichuris suis							67
						Tryptophan toxicity							68
						Vitamin D toxicosis							22
						Vitamin E deficiency							68
						Vomitoxin							69
						Water quality							68
							Ovine herpesvirus 2						35

SBM, soybean meal hypersensitivity.

Table 5.2 Approximate age at which certain causes of vomiting in pigs are more common (also see Chapter 15).

1–2 days	3–4 days	5–6 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults	Chapter
African swine fever virus													25
Classical swine fever virus													39
Porcine deltacoronavirus													31
Porcine epidemic diarrhea virus													31
Pseudorabies virus													35
Transmissible gastroenteritis virus													31
		HEV											31
		EEEV											46
			Yersinia enterocolitica										64
				Adenovirus									24
					Ascaris suum								67
					Diacetoxyscirpenol								69
					Stachybotrys atra toxin								19
					T-2 toxin								69
					Vomitoxin								69
					Strongyloides spp.								67
						Arsenic toxicity							70
						Atresia ani							15
						Bacillus anthracis							64
						Carbamate toxicity							70
						Cocklebur poisoning							70
						Fluorine toxicity							70
						Niacin deficiency							68

(*Continued*)

Table 5.2 (Continued)

1–2 days	3–4 days	5–6 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults	Chapter
						Organophosphate toxicity							70
						Riboflavin deficiency							68
						Salt poisoning							70
						Selenium toxicity							68
						Thiamin deficiency							68
						Vitamin B_6 toxicity							68
						Vitamin D toxicity							68
							Rectal strictures						15
							Gastric ulcers						15
								Hairballs					15
								Foreign body					15

HEV, hemagglutinating encephalomyelitis virus; EEEV, Eastern equine encephalitis virus.

Table 5.3 Causes of rectal prolapses in pigs (also see Chapter 15).

Cause	Comments
Diarrhea	Abnormally acid stool in the rectum causes irritation, tenesmus, and prolapse. Refer to the section on diarrhea for differentiation between causes of diarrhea
Cough	Increased abdominal pressure generated during coughing (especially chronic prolonged bouts) causes displacement of the rectum. Refer to the section on cough for differentiation between causes of cough
Piling	Environmental temperatures too low. Abdominal pressure on the pig at the bottom of the pile produces prolapse
Zearalenone	Estrogens cause swelling of the perineal area, tenesmus, and prolapse
Floor design	Excessively sloped floors for crated sows cause increased pressure on pelvic structures as pregnancy progresses
Antibiotics	Rectal prolapse has been reported in pigs in the first few weeks after lincomycin or tylosin has been added to the feed. Prolapses cease later as pigs apparently become accustomed to the antibiotic
Inherited predisposition	Sporadic reports in the literature of herd outbreaks that occurred in the offspring of certain boars
Postpartum	Complex etiology surrounding farrowing
Prepartum	Constipation and pressure of heavily gravid uterus
Any condition that is associated with tenesmus	Urethritis, vaginitis, rectal or urethral injury post breeding, urethral calculi. Excess salt in the diet

Source: Straw et al. (2006). Reproduced with permission of John Wiley and Sons.

Table 5.4 Approximate age at which certain causes of pneumonia, respiratory distress, or coughing in pigs are more common (also see Chapter 21).

<1 week	1–4 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults	Chapter
PCMV									35
Carbon monoxide toxicity									70
Dermatosis vegetans									17
Porcine reproductive and respiratory syndrome virus									41
Bordetella bronchiseptica									49
Porcine respiratory coronavirus									31
Clostridium tetani									51
Arcanobacterium pyogenes									64
Chlamydia suis									64
Nitrite toxicity									70
Coal tar toxicity									70
Methane toxicity									70
Pseudorabies virus									35
Toxoplasma gondii									66
Strongyloides ransomi									67
Classical swine fever virus									39
African swine fever virus									25
Nipah virus									37
HEV									31
Adenovirus									24
Iron deficiency anemia (or blood loss anemia)									14
Pasteurella multocida									57
Haemophilus parasuis									54
Actinobacillus pleuropneumoniae									48
Actinobacillus suis									48
Streptococcus spp.									61
Influenza A virus									36
Porcine stress syndrome									19
Blue eye paramyxovirus									37
Porcine lymphotropic herpesvirus									35
Salmonella choleraesuis									59
Clostridium botulinum									51
Ascaris suum									67
Metastrongylus spp.									67
Paragonimus kellicotti									67
Vitamin A deficiency									68
Vitamin D toxicity									68
Organophosphate toxicity									70
Carbamate toxicity									70
Chlorinated hydrocarbon toxicity									70
Pentachlorophenol toxicity									70
Dipyridal herbicide toxicity									70
Fumonisin									69

(*Continued*)

Table 5.4 (Continued)

<1 week	1–4 weeks	1 month	2 months	3 months	4 months	5 months	6 months	Adults	Chapter
			Porcine circovirus type 2						30
			Erysipelothrix rhusiopathiae						53
			Mycobacterium spp.						63
			Mycoplasma suis						56
			Mycoplasma hyopneumoniae						56
			Hydrogen sulfide toxicity						70
					Gossypol toxicity				70
								CM	52
								Puffer	21

PCMV, porcine cytomegalovirus; HEV, hemagglutinating encephalomyelitis virus; CM, coliform mastitis; Puffer, puffer sow syndrome.

Table 5.5 Certain causes of sneezing in pigs (also see Chapter 21, especially Table 21.5).

Atrophic rhinitis	Chapter 49
Blue eye paramyxovirus	Chapter 37
Environmental contaminants:	
Ammonia	Chapters 4, 57
Dust, pollen, irritants	Chapter 4
Hemagglutinating encephalomyelitis virus	Chapter 31
Influenza A virus	Chapter 36
Mycoplasma hyorhinis	Chapter 56
Porcine cytomegalovirus	Chapter 35
Porcine reproductive and respiratory syndrome virus	Chapter 41
Pseudorabies virus	Chapter 35

Nervous and locomotor system

Chapter 19 reviews both the nervous and locomotor systems. It is important to note that many times diseases affecting either of these systems have similar general clinical presentations. Table 5.9 lists some causes of neurologic signs. It is important to use the right descriptive terms when describing clinical signs (see Table 19.5) to ensure proper differential diagnosis. Table 19.8 tries to further differentiate clinical presentations. Lameness conditions are summarized in Table 5.10.

Reproductive system

The reproductive system is summarized in Chapter 20. Possible causes of reproductive losses in pigs are summarized in Tables 5.11 and 20.7. Although not directly related to reproductive performance, but rather related to pregnancy, common congenital anomalies are listed in Table 5.12.

Zoonotic

An overview of preharvest food safety and zoonotic diseases is included in Chapter 12. Pig diseases with zoonotic potential are summarized in Table 5.13.

Acknowledgments

The following individuals are acknowledged for their valuable input and guidance provided: Robert Desrosiers, Phil Gauger, Eric Neumann, Kent Schwartz, Ernest Stanford, and Locke Karriker.

Table 5.6 Approximate age at which certain skin diseases in pigs are more frequently seen (also see Chapter 17).

Weeks of age											
1	2	3	4	8	10	14	18	32	50	100	156
Infection of injury caused by trauma, ischemia, or surgical procedures											
Mange and lice											
Ringworm											
Insect bites from fleas, flies, and mosquitoes											
Sunburn or photosensitization											
Abscesses											
Necrobacillosis											
Epitheliogenesis imperfecta											
Teat and knee erosion											
Pustular dermatitis											
Thrombocytopenic purpura											
Dermatosis vegetans											
Staphylococcal acne											
Swinepox											
Acute generalized exudative epidermitis, local exudative epidermitis											
Pityriasis rosea											
Ear necrosis											
Parakeratosis											
Callus of the knee, fetlock, elbow, hock, or tuber ischii											
Porcine dermatitis and nephropathy syndrome											
Bursitis											
Erysipelas											
Dermatosis erythematosa											
Mastitis											
Shoulder ulcer callus											

Source: Straw et al. (2006). Reproduced with permission of John Wiley and Sons.

Table 5.7 Diseases affecting the skin of pigs (also see Chapter 17, especially Tables 17.1).

Location	Normal tissue	Proliferative or nonproliferative	Demarcation of lesions	Possible cause
Face	Elevated		Discrete	Staphylococcal acne
	Flat	Nonproliferative	Discrete	Necrotic stomatitis
Face and feet	Elevated		Discrete	Vesicular diseases[a]
Shoulder	Elevated		Discrete	Hematoma; callus
	Flat	Nonproliferative	Discrete	Ulcer
Knees, elbows, and hocks	Flat	Nonproliferative	Discrete	Knee erosions
	Elevated		Discrete	Callus
	Elevated		Diffuse	Bursitis
Ear	Elevated		Discrete	Hematoma
	Flat	Nonproliferative	Diffuse	Greasy spot behind ear
	Flat	Proliferative	Discrete	Ear necrosis
	Flat	Proliferative	Diffuse	Mange
Ear, eye, and udder	Flat	Nonproliferative	Diffuse	Photosensitization
Extremities	Flat	Nonproliferative	Diffuse	Cyanosis or reddening secondary to disease[b]
Dorsal	Elevated		Discrete	Fleas, flies, mosquitoes
	Elevated	Proliferative	Diffuse	Lumpy skin disease
	Elevated	Proliferative	Diffuse	Hyperkeratinization
	Flat	Nonproliferative	Diffuse	Sunburn
	Flat	Nonproliferative	Discrete	Epitheliogenesis imperfecta
Ventral abdomen	Elevated		Discrete	Pityriasis rosea, eosinophilic dermatitis
	Elevated		Diffuse	Urticarial mange
	Flat	Nonproliferative	Discrete	Transit erythema; teat necrosis
	Flat	Nonproliferative	Diffuse	Mastitis, benign peripartal cyanosis
Ventral cervical area	Elevated		Discrete	Jowl abscess, tuberculosis
	Elevated		Diffuse	Pharyngeal anthrax
Generalized	Elevated		Discrete	Pustular dermatitis, swinepox, infected injuries, neoplasia, abscess
	Elevated		Diffuse	Dermatosis vegetans
	Flat	Proliferative	Diffuse	Parakeratosis, demodectic mange, lice, sarcoptic mange, exudative epidermitis
	Flat	Nonproliferative	Discrete	Ringworm, dermatosis erythematosus, thrombocytopenic purpura, erysipelas
	Flat	Nonproliferative	Diffuse	Carbon monoxide toxicity, porcine stress syndrome, hypotrichosis, cyanosis, or reddening secondary to any bacteremia or viremia
	Flat	Nonproliferative	Discrete	Immune complex disorder possibly associated with circovirus

Source: Straw et al. (2006). Reproduced with permission of John Wiley and Sons.

[a] Foot-and-mouth disease, vesicular exanthema, vesicular stomatitis, swine vesicular disease, Senecavirus A, San Miguel sea lion virus, porcine parvovirus, and drug eruption.

[b] Salmonellosis, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae*, porcine reproductive and respiratory syndrome, colibacillosis, organophosphate toxicity, and hemagglutinating encephalomyelitis.

Table 5.8 Cause of anemia in pigs (also see Chapter 14, especially Table 14.7).

General	
Blood loss (acute or chronic)	Chapter 14
Chronic disease	Chapter 14
Gastric ulcer	Chapter 15
Hemorrhagic bowel syndrome	Chapter 15
Bacterial	
Lawsonia intracellularis	Chapter 58
Mycoplasma suis	Chapter 56
Salmonella spp.	Chapter 59
Deficiencies or toxicities	
Aflatoxin	Chapter 69
Anticoagulant toxicity (warfarin, brodifacoum, etc.)	Chapter 70
Coal tar toxicity (clay pigeons)	Chapter 70
Cobalt toxicity	Chapter 68
Copper deficiency and toxicity	Chapter 68
Folic acid deficiency	Chapter 68
Iron deficiency	Chapter 68
Niacin deficiency	Chapter 68
Trichothecenes	Chapter 69
Vitamin B_{12} deficiency	Chapter 68
Vitamin B_6 deficiency	Chapter 68
Vitamin E deficiency	Chapter 68
Vitamin K deficiency	Chapter 68
Zearalenone	Chapter 69
Parasites	
Fasciola hepatica	Chapter 67
Flea infestation	Chapter 65
Haematopinus suis	Chapter 65
Trichuris suis	Chapter 67
Macracanthorhynchus hirudinaceus	Chapter 67
Strongyloides ransomi	Chapter 67
Viral	
Bovine viral diarrhea virus	Chapter 39
Porcine reproductive and respiratory syndrome virus	Chapter 41

Table 5.9 Cause of neurologic signs in pigs (also see Chapter 19, especially Table 19.8).

General or congenital	
Brain or spinal cord injury	Chapter 19
Congenital malformations	Table 5.12
Congenital tremors	Table 19.9
Hypoglycemia	Chapter 19
Hypoxia/anoxia	Chapter 21
Middle ear infection	Chapter 19
Bacterial or protozoal	
Actinobacillus suis	Chapter 48
Clostridium botulinum	Chapter 51
Clostridium tetani	Chapter 51
Escherichia coli (usually 1–2 weeks post weaning)	Chapter 52
Haemophilus parasuis	Chapter 54
Listeria monocytogenes	Chapter 64
Streptococcus suis	Chapter 61
Toxoplasma gondii	Chapter 66
Other bacterial meningitis	Chapter 19
Deficiencies or toxicities	
Ammonia salt toxicity	Chapter 70
Arsanilic acid toxicity	Chapter 70
Arsenic toxicity	Chapter 70
Calcium deficiency	Chapter 68
Carbamate toxicity	Chapter 70
Carbon dioxide toxicity	Chapter 70
Carbon monoxide toxicity	Chapter 70
Chlorinated hydrocarbon toxicity	Chapter 70
Cocklebur toxicity	Chapter 70
Copper deficiency	Chapter 68
Dichlorvos toxicity	Chapter 70
Hydrogen sulfide toxicity	Chapter 70
Hygromycin toxicity	Chapter 19
Iron toxicity	Chapters 15, 68
Lead toxicity	Chapter 70
Magnesium deficiency or toxicity	Chapter 68
Mercury toxicity	Chapter 70
Niacin deficiency	Chapter 68
Nightshade toxicity	Chapter 70
Nitrate/nitrite toxicity	Chapter 70
Nitrofuran toxicity	Chapter 70

Table 5.9 (Continued)

	Organophosphate toxicity	Chapter 70
	Pantothenic acid deficiency	Chapter 68
	Pentachlorophenol toxicity	Chapter 70
	Phenoxy herbicide toxicity	Chapter 70
	Phosphorus deficiency	Chapter 68
	Pigweed toxicity	Chapter 70
	Riboflavin deficiency	Chapter 68
	Sodium chloride deficiency	Chapter 68
	Sodium fluoroacetate toxicity	Chapter 70
	Strychnine toxicity	Chapter 70
	Streptomycin toxicity	Chapter 19
	Vitamin A deficiency	Chapter 68
	Vitamin B_6 deficiency	Chapter 68
	Vitamin D deficiency	Chapter 68
	Water deprivation (salt poisoning)	Chapter 68
Viral		
	African swine fever	Chapter 25
	Atypical porcine pestivirus	Chapter 39
	Blue eye paramyxovirus	Chapter 37
	Classical swine fever	Chapter 39
	Hemagglutinating encephalomyelitis virus	Chapter 31
	Japanese encephalitis virus	Chapter 33
	Nipah virus	Chapter 37
	Porcine adenovirus	Chapter 24
	Porcine astrovirus type 3	Chapter 27
	Porcine cytomegalovirus	Chapter 35
	Porcine enterovirus	Chapter 40
	Porcine reproductive and respiratory syndrome	Chapter 41
	Porcine sapelovirus	Chapter 40
	Porcine teschovirus	Chapter 40
	Pseudorabies virus	Chapter 35
	Rabies virus	Chapter 45

Table 5.10 Approximate ages at which diseases causing lameness are more common (also see Chapter 19).

Age in months										
1	1.5	2	3	4	5	6	18	30	42	54
Trauma: muscle bruising, sprains, strains, dislocations, fractures										
Clostridium tetani or septicum infection										
Vesicular diseases: foot-and-mouth disease, vesicular exanthema, swine vesicular disease, vesicular stomatitis, San Miguel sea lion virus										
Streptococcus suis infection		Chronic suppurative arthritis due to *S. suis*, *S. equisimilis*, *M. hyorhinis*, *M. hyosynoviae*, *H. parasuis*, *Corynebacterium*, *Staphylococcus*								
Streptococcus equisimilis infection										
	Acute *Mycoplasma hyorhinis* infection									
	Haemophilus parasuis infection									
		Bursitis								
			Rickets							
			Acute erysipelas				Chronic erysipelas			
			Asymmetrical hindquarter syndrome							
			Foot rot							
			Back muscle necrosis							
				Osteochondrosis						
					Osteoarthrosis, degenerative joint disease					
					Epiphysiolysis					
						Brucellosis				
						Laminitis				
						Apophysiolysis				
							Osteomalacia			
								Tarsitis		
								Arthrosis deformans		
								Leg weakness syndrome		

Source: Straw et al. (2006). Reproduced with permission of John Wiley and Sons.

Table 5.11 Causes of reproductive losses in pigs (also see Chapter 20).

	Abortion	Weak births	Stillbirths	Mummification	Small litters	Chapter
General						
Genetics			X		X	3
High environmental temperatures	X	X	X		X	20
Management					X	20
Nutrition					X	20
Bacterial						
Actinobacillus spp.	X					48
Brucella suis	X	X	X			50
Burkholderia pseudomallei	X					64
Chlamydia spp.	X	X	X	X	X	64
Erysipelas rhusiopathiae	X			X	X	53
Lawsonia intracellularis	X					58
Leptospira spp.	X	X	X	X		55
Listeria monocytogenes	X	X	X			64
Mycoplasma suis	X				X	56
Salmonella spp.	X					59
Staphylococcus spp.	X					60
Streptococcus spp.	X					61
Toxicities and deficiencies						
Carbon monoxide toxicity	X	X	X			70
Fumonisin	X					69
Stachybotrys atra	X					19
Vitamin A deficiency	X	X	X			19, 68
Zearalenone					X	69
Parasites						
Toxoplasma gondii	X	X	X			66
Viral						
African swine fever virus	X	X	X		X	25
Blue eye paramyxovirus	X	X	X	X		37
Border disease virus	X	X	X		X	39
Bovine viral diarrhea virus	X	X	X		X	39
Classical swine fever virus	X	X	X	X	X	39
Encephalomyocarditis virus	X	X	X	X		40
Foot-and-mouth disease	X					40
Influenza A virus	X	X	X		X	36
Japanese encephalitis virus	X	X	X	X		33
Menangle virus			X	X	X	37
Nipah virus	X					37
Parvovirus	X[a]			X	X	38
Porcine adenovirus	X					24
Porcine circovirus type 2	X	X	X	X		30
Porcine cytomegalovirus		X	X	X	X	35
Porcine reproductive and respiratory syndrome virus	X	X	X	X	X	41
Pseudorabies virus	X	X	X	X	X	35
Senecavirus A		X				40
Teschovirus		X	X	X	X	40

[a] Parvovirus can cause abortions under rare and unique situations.

Table 5.12 Common congenital anomalies in pigs.

Defect	Prevalence (%)	Etiology	Diagnosis
Microencephaly	0.07	Heat stress midpregnancy	History of heat stress
		Unknown (most cases)	An agent affecting development in early or midpregnancy
Microphthalmia		Vitamin A deficiency	Multiple defects in affected litters; heavy neonatal mortality; history; diet analysis; serum and liver vitamin A analysis
		Hog cholera (HC) infection	HC infection in herd; virus isolation; fluorescent antibody test; serology; congenital tremor AI present in herd
		Heritable	Mode of inheritance uncertain; dominant gene (?)
		Unknown	An agent affecting embryos at 12–16 days of development
Neural tube defects (anencephaly, encephalocele, hydrocephalus, spina bifida)	0.04	Unknown	An agent affecting embryos at 12–16 days of development
		Vitamin A deficiency (hydrocephalus)	Multiple defects in affected litters; heavy neonatal mortality; history; diet analysis; serum and liver vitamin A analysis
Congenital tremor	0.20	HC virus (type AI)	HC infection in herd; virus isolation; fluorescent antibody test; serology; affects piglets of all breeds and both sexes; hypomyelinogenesis; cerebellar hypoplasia; neurochemical analysis of myelin lipids of spinal cord; small cross-sectional area of the spinal cord
		Type All (unidentified virus)	Hypomyelinogenesis of spinal cord; analysis of myelin lipids of spinal cord; small cross-sectional area of the spinal cord
		Type AIII	Monogenic sex-linked gene mutation in Landrace affecting only males and associated with defect in myelin sheath
		Type IV	Autosomal recessive gene in Saddleback affecting both sexes
		Pseudorabies (PR) virus	PR infection in herd; virus isolation; serology
		Neguvon (metrifonate, trichlorfon)	History of dosing sows in midpregnancy; hypoplasia of cerebrum and cerebellum; Purkinje cell loss; changes in neurotransmitters
	0.10	Tobacco stalks, jimsonweed, poison hemlock, wild black cherry	History of exposure to plants in early to midpregnancy
		Vitamin A deficiency	Multiple defects in affected litters; heavy neonatal mortality; history; diet analysis; serum and liver vitamin A analysis
Arthrogryposis		HC attenuated vaccine virus	History of vaccination during early pregnancy
		HC infection	HC infection in herd; virus isolation; fluorescent antibody test; serology; congenital tremor type AI in herd
		Paramyxovirus infection	Menangle virus infection during pregnancy
		Heritable	Recessive gene (?); autosomal recessive in Yorkshire pigs
		Unknown (most cases)	An agent affecting development in early or midpregnancy

Table 5.12 (Continued)

Defect	Prevalence (%)	Etiology	Diagnosis
Micromelia	0.10	Unknown	Possibly caused by limb vascular defects in early pregnancy
Cleft palate/harelip	0.07	Heritable	Possibly a recessive gene; cleft palate in Poland China pigs probably genetic
		Unknown (most cases)	An agent affecting development in early or midpregnancy
Deformed tail	0.08	Possibly heritable	Mode of inheritance uncertain; occasionally urogenital defect associated
		Unknown	Often associated with motor defects in hind limbs; vertebral defects
Myofibrillar hypoplasia	1.05	Heritable	Most common in Landrace, less in Large White; probably polygenic mode of inheritance; incidence modified by maternal stress, slippery floor, birth weight, or maternal nutrition
		Fusarium toxin	Higher mortalities than other forms; feed analysis
Inguinal hernia	0.40	Heritable	Mode of inheritance uncertain; incidence modified by environment
Umbilical hernia	1.00	Unknown	Possibly polygenic mode of inheritance
Anal atresia	0.40	Heritable	Possibly polygenic inheritance or an autosomal recessive or autosomal dominant form of transmission
Hypotrichosis		Heritable in some breeds	Mode of inheritance uncertain
		Iodine deficiency	Stillbirths and high neonatal mortality; enlarged thyroids; skin edematous; feed analysis
Epitheliogenesis imperfecta	0.05	Heritable	Possibly autosomal recessive gene; hydronephrosis associated
Dermatosis vegetans		Heritable	Autosomal recessive; associated with fatal giant cell pneumonia
Pityriasis rosea		Probably heritable	Mode of inheritance uncertain; affects young pigs, especially Landrace; benign and self-limiting
Von Willebrand's disease		Heritable	Recessive gene in Poland China pigs; excess bleeding from minor wounds; decrease in factor VIII and platelet retention time
Navel bleeding	0.14–1.2	Unknown	Cord is edematous; familial linkage
Cardiac defects	0.03	Unknown	Most cases recognized at 4–8 weeks; mostly males
Cryptorchidism	0.39	Probably heritable	Polygenic transmission; left testicle most commonly involved
Female genital hypoplasias, duplications	0.68	Probably a heritable component	Mode of inheritance uncertain; genital tract incomplete or duplicated
	0.06		
Male pseudohermaphroditism	0.2–0.6	Heritable	Mode of transmission uncertain; testicles in abdomen together with female tubular tract
True hermaphroditism		Heritable	Mode of inheritance uncertain; testicular and ovarian tissues usually with female tubular tract

Source: Cutler et al. 2006. Reproduced with permission of John Wiley and Sons.

Table 5.13 Pig diseases with zoonotic potential (also see Chapter 12).

Bacterial	
Bacillus anthracis	Chapter 64
Brucella suis	Chapter 50
Campylobacter jejuni	Chapter 64
Campylobacter coli	Chapter 64
Escherichia coli	Chapter 52
Erysipelothrix rhusiopathiae	Chapter 53
Leptospira interrogans	Chapter 55
Listeria monocytogenes	Chapter 64
Burkholderia pseudomallei	Chapter 64
Salmonella spp.	Chapter 59
Staphylococcus aureus	Chapter 60
Streptococcus suis	Chapter 61
Yersinia pseudotuberculosis	Chapter 64
Yersinia enterocolitica	Chapter 64
Fungal	
Microsporum nanum	Chapter 17
Parasites	
Clonorchis sinensis	
Diphyllobothrium spp.	
Echinococcus granulosus	Chapter 67
Fasciolopsis buski	Chapter 67
Gastrodiscoides hominis	Chapter 67
Gnathostoma doloresi	Chapter 67
Gnathostoma hispidum	Chapter 67
Gongylonema pulchrum	Chapter 67
Macracanthorhynchus spp.	Chapter 67
Opisthorchis felineus	
Paragonimus spp.	Chapter 67
Sarcoptes scabiei	Chapter 65
Schistosoma japonicum	
Strongyloides stercoralis	Chapter 67
Taenia asiatica	Chapter 67
Taenia solium	Chapter 67
Trichinella spiralis	Chapter 67
Trichuris suis	Chapter 67
Protozoal	
Balantidium coli	Chapter 66
Sarcocystis suihominis	Chapter 66
Sarcocystis hominis	Chapter 66
Toxoplasma gondii	Chapter 66
Viral	
Encephalomyocarditis virus	Chapter 40
Ross River virus	Chapter 46
Influenza A virus	Chapter 36
Japanese encephalitis virus	Chapter 33
Kyasanur Forest disease virus	
Nipah virus	Chapter 37
Rabies virus	Chapter 45
Swine vesicular disease virus	Chapter 40
Vesicular stomatitis virus	Chapter 45

Source: Adapted from Glenda Dvorak.

References

Cutler RS, Fahy VA, Cronin GM, et al. 2006. Preweaning mortality. In Straw BE, Zimmerman JJ, D'Alliare S, et al., eds. *Diseases of Swine*, 9th ed. Blackwell Publishing, pp. 1005–1006.

Straw BE, Dewey CE, Wilson MR. 2006. Differential diagnosis of diseases. In Straw BE, Zimmerman JJ, D'Alliare S, et al., eds. *Diseases of Swine*, 9th ed. Blackwell Publishing, pp. 241–283.

6

Diagnostic Tests, Test Performance, and Considerations for Interpretation

Jane Christopher-Hennings, Gene A. Erickson, Richard A. Hesse, Eric A. Nelson, Stephanie Rossow, Joy Scaria, and Durda Slavic

Introduction

Diagnostic testing is used to determine the cause of disease and for surveillance of pathogens that may cause disease. There are many agents that cause disease including viruses, bacteria, protozoa, other parasites, and toxins. However, just detecting the presence of these agents or exposure to them does not necessarily indicate that they are the etiologic agent of the particular clinical disease at hand. Therefore, an accurate diagnosis of each specific case is based on the total picture including the herd history, clinical signs, gross and microscopic pathology (histopathology), and results of diagnostic tests. In addition, some organisms may only cause disease at specific thresholds. Since no single test is 100% sensitive (the test correctly identifies 100% of all infected pigs, indicating there are no "false-negative" results) or 100% specific (the test correctly identifies 100% of all noninfected pigs, indicating there are no "false-positive" results), an incorrect diagnosis could result if only one test is used and the stage and current context of the disease is not taken into consideration. To determine whether a specific test is identifying the cause of disease, multiple tests or repeated testing over time may be required, and when results of diagnostic testing are received, evaluations of the outcomes in the context of history, clinical signs, and pathology (if available) are critical (Figure 6.1).

This chapter describes common tests used for the diagnosis of swine diseases or surveillance of swine pathogens and is intended to help determine the appropriate test and interpretation of results for swine diseases. The tests are described in alphabetical order along with their diagnostic strengths and weaknesses (Table 6.1).

Agar-gel immunodiffusion

Agar-gel immunodiffusion (AGID) is a serological test for measuring the presence of antibodies to a specific antigen. It can be used to detect host exposure to a pathogen or to serotype field isolates. It has been routinely used for influenza A virus (IAV) serological testing and to serotype *Haemophilus parasuis* field isolates (Del Río et al. 2003). Although AGID continues to be used in some laboratories for *H. parasuis* typing due to the ease of use and low cost, the method has largely been replaced by indirect hemagglutination inhibition (IHA) and enzyme-linked immunosorbent assay (ELISA) due to their greater specificity and sensitivity.

The test is performed in petri plates coated with agar and a seven-well pattern (center well surrounded by six equally spaced test wells). Test antigen is used to fill the center well, and positive control serum is placed in alternating test wells around the center well. Sera to be tested are placed in the remaining wells, and the test is incubated for 1–2 days. Test plates are examined with a bright indirect light source to visualize the specific lines of identity (white precipitate) between the antigen well and positive control serum wells after diffusion of the antibody and antigen from their respective wells. A positive result is recorded when a test serum produces a line of identity in the agar between the serum and reference positive control serum. Test specificity is determined by the quality of the antigen used.

Bacterial culture and antimicrobial susceptibility testing (AST)

For detection of the majority of bacterial diseases, bacterial culture is the most common, but not necessarily the most rapid diagnostic method. It is routinely used by veterinary diagnostic laboratories to grow bacteria from clinical samples, providing evidence of their viability, in contrast to molecular diagnostic methods where detection

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

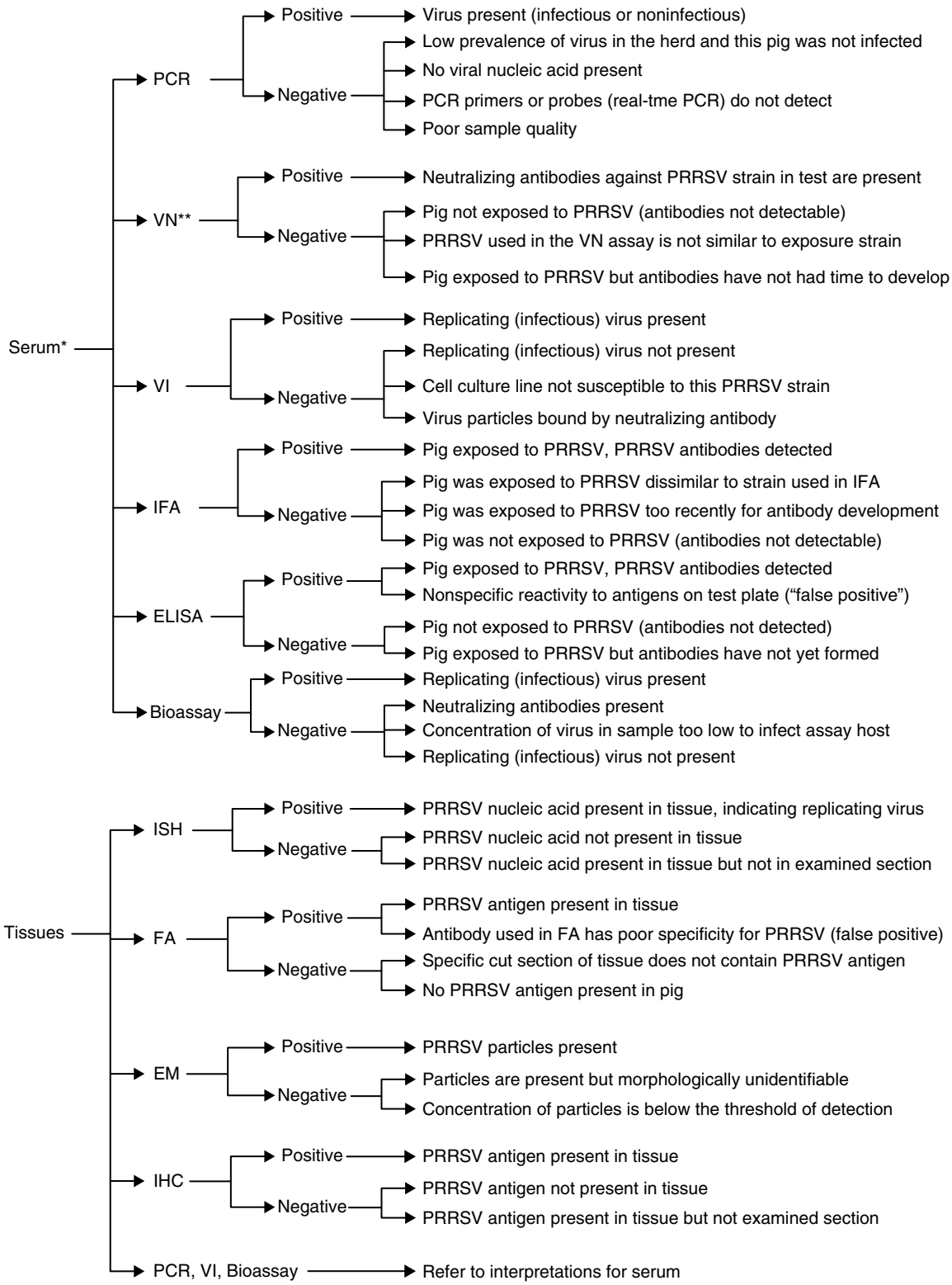


Figure 6.1 Possible interpretations of diagnostic test results using serum and tissues for PRRSV diagnosis. *Blood swabs and oral fluids can be tested by PCR. **VN, serum virus neutralization assay.

Table 6.1 Diagnostic tests for analyte types: infectious agent, antibody, antigen, or nucleic acid detection.

Antigen (or nucleic acid)-specific tests	Antibody-specific tests
	Agar-gel immunodiffusion (AGID)
Antigen ELISA	Antibody ELISA
Bacterial culture	
Bioassay	
	Buffered *Brucella* antigen test (BBAT)[a]
Clinical pathology[b]	
Complement fixation (CF)	Complement fixation (CF)
DNA sequencing	
Electron microscopy (EM)	
Fluorescent antibody (direct FA or indirect FA)	
Fluorescent microsphere immunoassay (FMIA)	Fluorescent microsphere immunoassay (FMIA)
	Fluorescent polarization assay (FPA)
	Hemagglutination inhibition (HI)
Immunohistochemistry (IHC)	
	Immunoperoxidase monolayer assay (IPMA)
	Indirect fluorescent antibody assay (IFA)
In situ hybridization (ISH)	
	Microscopic agglutination test (MAT)[c]
Parasite identification	
Polymerase chain reaction (PCR)	
Virus isolation (VI)	
	Virus neutralization (VN) or serum VN (SVN)

[a] For detection of *Brucella* sp. antibodies only.
[b] Indirect method of determining whether an antigen is present.
[c] For detection of *Leptospira* sp. antibodies only.

of nucleic acid does not discern between viable or nonviable organisms. Subsequently, bacterial growth is used for bacterial identification and subtyping (if applicable) to establish its significance. Not all bacterial isolates are associated with specific disease conditions, since some bacteria may be present in samples either as contaminants or commensals. Once the significance of bacterial isolates is established, their antimicrobial susceptibility pattern is determined to guide antimicrobial treatment decisions. Furthermore, isolates can also be saved for any future research or for vaccine production, making them a valuable source for retrospective studies and for disease prevention.

Before submitting samples for culture, it is important for clinicians to know how diagnostic laboratories process samples. There are a variety of artificial media, temperatures, and growth conditions that can be used to obtain bacterial growth from clinical samples (Markey et al. 2013). The conditions used primarily depend on sample type, animal age, and clinical history. Therefore, it is very important that the referring veterinarian provides this information at the time of sample submission to help guide sample setup and interpretation of results (Table 6.2). It is also beneficial to describe on the submission form any lesions observed and to specify if a particular bacterial disease is suspected. The next step is to select the appropriate bacterial test(s) if this option is available. Some laboratories offer a variety of bacterial cultures (e.g. aerobic, anaerobic, *Clostridium difficile*, *Mycoplasma*, *Brachyspira*, etc.) to help veterinarians make the selection at the time of sample submission. In general, a request for aerobic bacterial culture will ensure isolation of most porcine bacterial pathogens. They usually grow well aerobically on medium containing blood, and their growth is

Table 6.2 Guidelines for interpretation and troubleshooting of positive and negative bacterial culture results.

Pathogen	Analyte	Test	Outcome	Interpretation	Additional testing
Bacteria	Tissue Body fluids[a]	Culture in liquid or solid media	Positive	Bacterial agent is isolated from the sample	Identify agent to the species/subspecies/ pathotype level
	Blood Urine Feces		Negative	Bacterial agent is not isolated from the sample: • Antimicrobial treatment prior to sample collection • Inappropriate sample collection, submission, and processing • Commensal flora overgrowth • Other fast-growing pathogen overgrowth • Nonbacterial etiology	Submit samples for PCR or request IHC/PCR on histological sections/scrolls

[a] Cerebrospinal, thoracic, peritoneal, synovial.

improved in the presence of 5–10% CO_2. More fastidious aerobic pathogens (e.g. *H. parasuis* and *Actinobacillus pleuropneumoniae* biotype I) require additional nutritional supplements including nicotinamide adenine dinucleotide (NAD), whereas anaerobic bacteria (e.g. *Actinobaculum suis* and *Clostridium* spp.) must be grown in oxygen-free conditions, and therefore anaerobic culture must be requested. In some cases, enrichment methods are recommended for isolation of *Salmonella* and *C. difficile*. There are also a few swine bacterial pathogens that are very difficult to almost impossible to grow routinely. For example, *Mycoplasma hyopneumoniae* requires a specific medium, is extremely fastidious to grow, and can take up to 4 weeks to reach measurable levels (Thacker 2004). Similarly, *Lawsonia intracellularis* can only be grown in tissue cell culture that is not routinely available in veterinary diagnostic laboratories (Vannucci et al. 2012). Alternative methods such as polymerase chain reaction (PCR) or ELISA are available for detection of slow-growing or fastidious pathogens. More information about growth requirements and methods for culturing various porcine bacterial pathogens can be found in their specific chapters in this book.

When individual bacterial colonies (i.e. pure bacterial growth) are obtained, then bacterial identification and AST can be performed. Previously, the most common method of bacterial identification was based on specific growth characteristics, colony morphology, Gram staining, and a variety of biochemical tests using automated or manual identification systems. This relatively lengthy procedure took approximately 24–48 hours before bacterial identification was achieved. Although this approach is still in use in most diagnostic laboratories, it is being rapidly replaced by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). In contrast to biochemical procedures, this is a very efficient and sensitive method where a small amount of bacterial growth is directly transferred to a stainless steel target plate and mixed with matrix solution to co-crystallize the bacterial proteins. The plate can accommodate between 24 and 384 samples, and it is then transferred into the MALDI-TOF instrument where individual samples are exposed to short laser pulses, resulting in protein ionization. Ionized proteins travel through a linear flight tube and are separated based on their mass-to-charge ratio plotted against signal intensity, which is then used to create a specific bacterial protein fingerprint. This fingerprint, in most cases, is unique for a given bacterial species. It has been well documented that for species-level identification, an accurate protein fingerprint is typically in the range of 2–20 kDa. The predominant proteins in this range are ribosomal proteins, which ionize well, are abundant in cells, and vary minimally under different growth conditions. Once the bacterial protein spectra are collected, they are compared with reference spectra in the MALDI-TOF MS database to generate the bacterial identification in real time. In addition to generating bacterial identification, the software also generates a numerical score to indicate how well-generated spectra match the spectra in the database. Typically, a high score indicates that a specific genus and species identification is reached (e.g. *Actinobacillus suis*), whereas a lower score may indicate only a genus level of identification (e.g. *Actinobacillus* sp.). However, the score levels can be influenced by many factors such as the thickness of bacterial smear applied on the target plate and the method used for smear processing. Smears that are too thick will yield lower scores or occasionally result in no spectra generated. In contrast, use of an "extraction method" where bacterial proteins are purified before they are applied on the target plate will result in the highest scores. Because the "extraction method" is time consuming, it is not used routinely for MALDI-TOF MS identifications. Instead, laboratories opt to use either a "direct transfer method" or an "extended direct transfer

method." With the "extended direct transfer method," 70% formic acid (FA) is applied on smears deposited on the target to partially extract proteins before the matrix is added. In some instances, this can improve score levels over the "direct transfer method" where only matrix is used, but it also adds to time and cost of running MALDI-TOF MS. If no results are obtained, in most cases the species reference spectra are not present in the database. Since 2011, the numbers of entries into the database almost doubled, and with each manufacturer's annual database update, the number of reference spectra keeps growing. Although reference spectra of the majority of swine pathogens are in the database, there are still some gaps that need to be filled. For example, no spectra for *Actinobacillus porcitonsillarum*, a bacterial species very closely related to *A. pleuropneumoniae* biotype I, are present in the manufacturer's database. Because MALDI-TOF MS is an open platform system, this issue can easily be rectified by creation of a customized database entry within the laboratory. All customized entries, however, have to pass rigorous multiple quality checks, which start with proper bacterial identification. Isolates used for spectra creation must be well characterized, and their identity, at minimum, confirmed by either 16S rRNA or other housekeeping gene sequencing. Next, an "extraction method" must be used for protein purification. Once reference spectra are created, they should also undergo extensive manufacturer's recommended internal quality control checks before being added to the customized database. In addition, it is highly recommended to add reference spectra from a minimum of five isolates of the same bacterial species to account for their natural variability. Aside from biochemical and MALDI-TOF MS, molecular methods can also be used for bacterial identification of individual colonies. The most frequent method used is 16S rRNA gene sequencing (Janda and Abbott 2007). 16S rRNA genes are present in all bacteria and are well conserved, making them a reliable marker for bacterial identification. However, when dealing with closely related bacterial species and subspecies, occasionally other genes (i.e. *rpoB*, *sodA*, etc.) may need to be sequenced because the 16S rRNA gene sequence may not provide enough information for speciation (Angeletti et al. 2015; Shin et al. 2015). For the most accurate bacterial identification, whole genome sequencing can be used, but its cost makes it still prohibitive for routine use.

To date, numerous studies have shown that MALDI-TOF MS bacterial identification is almost as accurate (if reference spectra are in the database) as molecular identification (Bizzini et al. 2010; Carpaij et al. 2011; Randall et al. 2015). When compared with biochemical identification, MALDI-TOF MS is superior, particularly for gram-positive bacteria for which routine biochemical identification was always challenging (Cherkaoui et al. 2011; Dupont et al. 2010). The MALDI-TOF MS bacterial identification is currently limited in its ability to differentiate between very closely related species and subspecies (Randall et al. 2015). This issue is gradually diminishing with each database update, as more reference spectra are added for each problematic species and subspecies. In addition, the software is being upgraded to distinguish slight differences between species and subspecies. In the meantime, MALDI-TOF MS is replacing biochemical methods for bacterial identification. In 2016, almost half of American Association of Veterinary Laboratory Diagnosticians (AAVLD)-accredited laboratories were using MALDI-TOF MS. It is expected that this number will increase in the future since the MALDI-TOF MS provides highly reliable bacterial identification within the same day, requires minimum handling time and technical expertise, can be automated (FA and matrix deposition), and is cost effective. However, successful bacterial identification using MALDI-TOF MS still depends on growing individual bacterial colonies from clinical specimens, and AST needs to be done separately. In the future, diagnostic uses of MALDI-TOF MS may include direct detection of bacteria in clinical samples and AST (Ferreira et al. 2011; Mailhac et al. 2017; Oviano et al. 2017).

The mere isolation of bacteria from clinical samples does not automatically imply their significance. It is important to critically evaluate the isolation of specific bacterial species in association with clinical signs and lesions to assess their relevance in morbidity and mortality. This is particularly true when additional characterization is required to differentiate between virulent and non-virulent bacterial pathotypes. For example, isolation of *Escherichia coli* from the gut does not indicate its association with enteritis. Further serotyping (i.e. for presence of F4 [K88]) and/or genotyping (e.g. detection of genes for fimbriae and toxins) must be done to establish this association (Osek 2001). Furthermore, the significance of isolation can sometimes be confirmed only by the detection of toxins as in cases of *C. difficile* (Moono et al. 2016).

Despite typical clinical signs and proper sample collection and submission, bacterial pathogens may not necessarily be isolated, which may happen more frequently than anticipated (Table 6.2). Antibiotic treatment and lack of refrigeration following sample collection are two of the most frequent causes of negative culture (Oliveira 2007). Lack of bacterial isolation may also result from overgrowth by commensal flora or contaminants, particularly in respiratory and intestinal samples (Fittipaldi et al. 2003). Additionally, if field veterinarians do not specify clinical signs, lesions, and a tentative diagnosis during submission, no isolation of bacterial species with specific growth requirements will likely be achieved. If in any doubt, it is highly recommended to call the laboratory

for help with any questions related to sample collection, storage, and submission.

In summary, bacterial culture is still a widely used diagnostic method for bacterial isolation, identification, and susceptibility testing. In contrast to bacterial isolation, which has not changed in decades, bacterial identification underwent significant improvements in the last few years with the introduction of the MALDI-TOF MS. The introduction of this technology allows diagnostic laboratories to achieve bacterial identification the same day when bacterial growth is detected. This can help veterinarians with empirical treatment decisions if pathogens are isolated or help reduce antibiotic use when no bacterial pathogens are present.

After the clinical significance of bacterial species is established, then AST is recommended to help guide treatment decisions. Some clinically significant bacterial species have predictable susceptibility patterns, which do not warrant AST. For example, currently, *Trueperella pyogenes* is predictably sensitive to penicillin, and this drug can be reliably used for its treatment without AST. In general, AST should be routinely performed for bacterial species with unpredicted susceptibility patterns (e.g. *E. coli, Salmonella* spp.) and for bacteria where resistance is reported or expected (e.g. *Pseudomonas aeruginosa*). In North America, AST is performed in veterinary diagnostic laboratories following guidelines published by the Clinical and Laboratory Standards Institute (CLSI). CLSI guidelines provide recommendations for testing, including preparation of bacterial suspensions, media use, incubation conditions, and a list of antimicrobial agents per animal species that may be considered for testing to standardize test performance. They also include result interpretation guidelines, which are specific for bacterial species–antimicrobial agent combinations (CLSI 2015). Therefore, it is important to know the identity of bacterial species when interpreting AST results. However, guidelines are not available for all clinically relevant bacteria that do not have predictable susceptibility patterns. For example, guidelines are not available for slow-growing bacterial species with fastidious growth requirements such as *H. parasuis*. There are two AST methods frequently used in veterinary medicine: agar disk diffusion and broth dilution (CLSI 2015). Agar disk diffusion, also known as the Kirby–Bauer method, is a qualitative method where a suspension of pure bacterial culture is streaked onto the surface of nutrient agar and then paper disks impregnated with antimicrobials are applied on top of it. The plates are incubated overnight, and a diameter of zone of inhibition (i.e. lack of bacterial growth around disks) is measured the next day. The agar disk diffusion method is highly flexible as any drug can be easily included and/or omitted from the testing. It is cost effective, but is not suitable for testing of all pathogens because some will grow poorly or will not grow at all on the media used. It is primarily used for testing of fast-growing aerobic bacteria (e.g. *E. coli*, *Salmonella*, *P. aeruginosa*, etc.) and some fastidious aerobic bacteria (e.g. *A. pleuropneumoniae*).

In contrast to agar disk diffusion, broth dilution is a quantitative test, and the results are expressed as the minimal inhibitory concentration (MIC). Broth dilution is performed in microbroth format where an equal amount of bacterial suspension is applied to each well of a 96-well MIC plate and incubated overnight. Veterinary specific formats of MIC plates are commercially available, and they contain antimicrobials approved for use in a particular animal species in a twofold dilution range. For example, the bovine/porcine MIC plate contains penicillin in range of 0.12–8 μg/mL and tiamulin in range of 1–3 μg/mL. Therefore each well on the MIC plate contains different antimicrobial concentrations. The presence of bacterial growth indicates that a bacterium is resistant to that particular drug concentration, whereas the absence of bacterial growth indicates susceptibility. The lowest concentration of drug that will inhibit bacterial growth is called the MIC, and it is expressed in μg/mL. It is necessary to achieve that concentration at the infection site during the course of treatment to inhibit bacterial growth. As concentration of drug can vary in different body systems and fluids, it is important to have pharmacokinetic studies done in order to determine if therapeutic concentrations can be achieved at target site. Unlike the agar disk diffusion method, the MIC method is more laborious, more expensive, and less flexible because it may take a few months before any antibiotic changes to the commercial plate format can be made.

Interpretation of AST results is performed following CLSI guidelines with values expressed as susceptible (S), intermediate (I), resistant (R), or nonsusceptible (NI). MIC values (μg/mL) can also be reported if the broth dilution method is used. S implies that a particular drug used in a dosage recommended by a manufacturer will likely successfully treat infection. R, on the other hand, means that infection likely will not be treated under the same conditions and usually indicates the presence of a resistance trait. I is defined as a "buffer zone," meaning that under certain conditions, the drug can be used for a favorable clinical outcome. For example, a drug can be used for treatment of infection in a site where it can be physiologically concentrated (i.e. urine) or when a high dosage of drug can be used. The NI designation is more complex, and it is reported for bacteria where values are defined for susceptible interpretative criteria only because of lack or rare occurrence of reported resistance. When AST results do not conform within defined values, the isolates are reported as NI to the specific drugs. That does not mean automatically that they have a resistance mechanism. It is possible that within the wild bacterial population, some isolates may have susceptibility values

above the ones defined as susceptible by the current CLSI guidelines (CLSI 2015).

There are numerous antimicrobial drugs approved for use in swine, but there is no need to test all of them individually. The CLSI recommends using one representative for a specific class of related drugs. For example, tetracycline is used as a representative of the tetracycline group (tetracycline, doxycycline, minocycline). If bacteria are found susceptible to tetracycline, they are considered susceptible to other drugs within this class. However, if they are resistant, then doxycycline and minocycline need to be tested individually as they have a broader antimicrobial coverage. For the macrolide class, clindamycin is used for susceptibility testing for both clindamycin and lincomycin. As the CLSI does not endorse any commercially available drugs, only nonproprietary names are used for reporting purposes.

Overall, AST results can be used to predict the effectiveness of treatment. However, AST is an *in vitro* test performed under optimal conditions for the bacterium–antimicrobial agent interactions. Therefore, it should be used only as a general guide for drug selection, and other aspects of appropriate treatment choice(s) should be evaluated, such as pharmacodynamics and pharmacokinetics of the drug, intracellular versus extracellular location of the bacteria, and site of infection. These and other aspects of antimicrobial drug selection are beyond the scope of this chapter, and for more information, the reader is referred to the "Drug Pharmacology, Therapy, and Prophylaxis" chapter of this book.

Bioassay (swine bioassay)

A bioassay is a test performed using a live animal to determine the infectivity or potency of a particular pathogen or substance. Pigs have been used to measure the infectivity of various viruses (e.g. porcine reproductive and respiratory syndrome virus [PRRSV], porcine epidemic diarrhea virus [PEDV], porcine circovirus type 2 [PCV2], classical swine fever virus [CSFV], hepatitis E), and the conclusions are then compared to PCR results (Christopher-Hennings et al. 1995; Dee et al. 2014). The swine bioassay determines whether the detection of nucleic acid corresponds with the presence of live virus. Naïve pigs are inoculated with the infectious agent and monitored at regular intervals for the presence of viremia and/or seroconversion, which would indicate the presence of live virus in the inoculated material. Since live animals are used and need to be housed for several weeks before results can be obtained, the disadvantages of this test are cost, additional labor, and prolonged evaluation time. However, bioassays are one of the most sensitive and conclusive methods to determine whether a particular sample is infectious and could be transmitted to other swine. Swine bioassays have been utilized to determine the relative bioavailability of lead in soil (although this practice is being replaced by nonanimal assays) (Casteel et al. 1997), and mouse bioassays have been used to detect the presence of infectious *Toxoplasma gondii* in swine sausages (De Oliveira Mendonça et al. 2004).

Buffered *brucella* antigen test

There are several *Brucella* sp. tests available including the buffered acidified plate antigen (BAPA), buffered Brucella antigen test (BBAT) or card test, rapid automated presumptive (RAP), rivanol plate agglutination test (RIV), standard plate test (SPT), standard tube test (STT), complement fixation test (CFT), and the fluorescence polarization assay (FPA). Most *Brucella* sp. serology assays use the cattle antigen, *Brucella abortus*, to test swine sera for *Brucella* sp. antibodies. A positive reaction is visually discerned by agglutination of the sera with the antigen. None of these tests are specific for *Brucella suis* since there is extensive cross-reaction between the *Brucella* species, which can cause false-positive reactions (Nielsen et al. 1999). The card test is the most commonly used screening test for pigs. Serum reacting with the card test can also be tested using other assays to corroborate or refute a positive result. The FPA is commonly used as the follow-up test to sera reacting with the card test. Although the FPA is reportedly more sensitive and more specific than the current World Organization for Animal Health (OIE)-recommended BBAT, the fact that some pigs do not generate antibodies following *B. suis* infection restricts the use of these techniques for individual testing (Nielsen et al. 1999). State Board of Animal Health testing requirements vary from state to state as to what is an acceptable screening test and what follow-up test can be used for reacting sera. Some states will accept follow-up testing by state diagnostic labs, while some states insist that all follow-up testing be conducted by the National Veterinary Services Laboratory (NVSL). The NVSL result is accepted as the official final result. The best policy for *Brucella* serology testing is to know what screening test and follow-up test are required for a state or a foreign destination. State diagnostic labs will most commonly know the testing requirements for different states and countries.

Clinical pathology

Performing complete blood counts (CBCs) may indicate anemia, which could indirectly implicate an infectious agent such as *Eperythrozoon suis* (*Mycoplasma suis*). However, for direct identification, PCR tests might be used, since there are many other noninfectious causes of anemia. CBCs and clinical chemistries could also be useful in determining the presence, severity, and/or location

of inflammation, organ dysfunction, an infectious agent, or toxicant. Values of CBC or clinical chemistry parameters should be compared against normal ranges identified specifically for swine and may be dependent on age, sex, and breed (Evans 2006). Normal pig values within a specific farm are useful for comparisons.

Complement fixation

This is an immunological method used to detect antigens in infected tissues and fluids, measure antibody responses in naturally or experimentally infected pigs, and assay antigenic relationships among different strains or types of the same pathogen species (Rice 1960). For example, this test was commonly used to detect antibodies against *A. pleuropneumoniae* (Enøe et al. 2001) until a commercial ELISA test became available. Complement fixation (CF) testing is based on the ability of antigen–antibody complexes to bind to complement (plasma proteins that combine with antibody to destroy pathogens) and cause hemolysis of sheep red blood cells (sRBCs). A known concentration of sRBC and anti-sRBC antibodies is added to the assay and allowed to react with complement. In samples containing specific antibodies against the antigen of interest, antigen–antibody complexes will form and will consume the available complement prior to the addition of sRBC. Hence, a sample positive for the antibodies of interest will show minimum hemolysis. Serum samples lacking specific antibodies to the target antigen will show maximum hemolysis of sRBC.

The CF assay detects antibodies against any antigen and has been used as a regulatory test for interstate or international movement of animals. However, it is rarely used in diagnostic laboratories since it takes 2 days to complete, has extensive requirements regarding standardization of the necessary reagents, and, in most laboratories, has been replaced by ELISA testing.

Electron microscopy

Electron microscopy (EM) can be used to visualize pathogens, particularly new and emerging viruses, when diagnostic reagents are not available (Chen et al. 2014). EM can also be useful in determining the cellular pathogenesis of an agent as an aid in developing intervention strategies. The strength of EM is its ability to identify a virus family in both antemortem and postmortem sample. The weaknesses of EM include its lack of sensitivity, inability to differentiate viruses within a family, dependence on a stable and detectable virus structure in samples, and the cost of maintaining the scope and hiring trained personnel. EM has a lower limit of detection with negative stain methods of approximately 10^6 virus particles per milliliter of sample and therefore is useful for detecting viruses in enteric cases since crude fecal suspensions routinely have greater than 10^6 particles of pathogenic viruses per gram of feces. Screening for known agents by EM has largely been replaced by PCR. For example, EM cannot adequately detect and differentiate group A, B, and C rotaviruses in comparison with PCR, but it can be used as a method to corroborate the identification of virus by other techniques. Next-generation sequencing (NGS) has the potential to further reduce the value of EM in pathogen discovery.

Enzyme-linked immunosorbent assay (ELISA) (antibody ELISA and antigen ELISA)

A variety of ELISA-based tests are routinely used in herd health monitoring and disease diagnosis. ELISA technologies are particularly useful for rapid, high-volume sample analysis, and many ELISA kits are commercially available for agents associated with major disease syndromes in swine. Variations of ELISA technology can be used for the detection of antibodies against a given pathogen (antibody ELISA) or for the detection of the actual pathogen (antigen ELISA). The diagnostic sensitivity and specificity of ELISA tests are highly dependent on the selection and quality of reagents used in the assay and the intended purpose of the assay. A highly sensitive assay may be more desirable when monitoring for a reportable disease of low prevalence, and a highly specific assay may be more desirable as a confirmatory test. When used for the detection of antibodies against a particular pig pathogen, the most common ELISAs are the indirect and competitive or blocking assay. Indirect assays typically utilize a purified antigen that is coated on test wells, and unreacted well areas are subsequently coated by a protein solution to minimize nonspecific antibody attachment. Typically, a single dilution of test sera is then placed in the test wells and incubated. If antibody is present, it will bind to the test antigen. Next, an enzyme-labeled indirect or secondary antibody directed against swine antibodies is added, and when the substrate of the enzyme label is added, a color change results. The intensity of the color is measured as an optical density (OD), which is evaluated in the context of the OD of a positive and negative control. A formula is then used to obtain a sample-to-positive (S/P) ratio (the sample OD on the test well divided by the positive control OD). A "cutoff" level is designated for positive and negative results. The S/P is not generally considered a "titer" since it does not use a serial dilution of the serum to obtain a result that is immunologically meaningful, whereas a

serum titer is primarily defined as the reciprocal of the greatest dilution in a dilution sequence that produces an immunological response. For example, the titer of a serum neutralization assay (serum virus neutralization [SVN]) or hemagglutination inhibition (HI) antibody assay measures an amount of antibody in serum that neutralizes the virus or that prevents hemagglutination, respectively. In some cases, the S/P may be loosely correlated with a titer if a linear relationship can be established.

Sera may be screened for antibodies with an indirect ELISA. When unexpected positive findings are obtained, a blocking ELISA might be used to determine specificity of the findings for confirmatory purposes (Erlandson et al. 2005). A competitive or blocking ELISA is performed by coating test plates with an antigen lysate followed by blocking as for the indirect ELISA. Then, the diluted test serum is added to allow it to react with the test antigen. At the same time or after washing, a specific enzyme-labeled antibody directed against the test antigen is added, resulting in competition with the test serum antibodies. Negative serum samples result in maximum color development (lack of competition/blocking), whereas samples with specific antibodies show less color development (competition/blocking) with increasing antibody levels. This type of assay has been used for differentiation between pseudorabies virus G1- or gE-deleted vaccinated and infected pigs as well as with IAV, which uses a nucleoprotein antigen.

The greatest strengths of ELISA for antibody testing are high throughput volume, speed of testing, and sensitivity and specificity of the test. Antibody ELISAs are useful as herd screening assays; however, if ELISAs are used to determine an individual pig status, false-positive reactions have been observed in some cases and can be difficult to interpret. Repeating the test, obtaining a second serum sample for testing, or using another serological test for confirmation may be useful (O'Connor et al. 2002). However, other assays for antibody detection such as the indirect fluorescent antibody (IFA), immunoperoxidase monolayer assay (IPMA), HI, virus neutralization (VN or SVN), and CF tests may be more complex and typically require more time for antibody confirmation.

In addition to numerous applications in antibody detection, ELISA technologies can also be used for the detection of antigens. Antigen detection ELISAs may utilize various assay formats including traditional ELISA plate formats or lateral flow devices, often called immunochromatographic strips. Antigen detection ELISAs use test wells or plates coated with specific antibodies rather than antigens as would be used in an antibody detection ELISA. Lateral flow tests typically use a solid-phase membrane with test and control lines coupled with absorbent pads. The strips may be placed into a test sample, or the sample may be added to a designated area of the strip. These test formats may be used with serum or whole blood samples, tissue homogenates, or fecal samples, depending on localization of the targeted pathogen, available processing methods, and quantity of target antigen present in a given specimen. A variety of immunoenzymatic assays for swine diagnostics are available for pathogens such as IAV, classical swine fever (CSF), group A rotaviruses, and PCV2. The primary strengths of antigen detection immunoassays are that they are generally rapid, are simple to perform, and require minimal laboratory infrastructure relative to VI, PCR, or EM. Some lateral flow devices have been adapted for on-site application in field or farm settings. However, assay sensitivity may present challenges for the detection of some pathogens, and timing of sample collection may be critical. The antigen of interest must be present in adequate quantity to allow direct detection by these methods, and appropriate high-quality antisera or monoclonal antibody (mAb)-based reagents must be available for assay development.

Fluorescent antibody or indirect fluorescent antibody for antigen detection

Detection of virus-infected cells from frozen tissues of diseased animals is a classical diagnostic technique that is very rapid and specific. It is used as a presumptive test to quickly identify if a given pathogen is present, since a diagnosis is often completed in less than 6 hours of sample receipt. Another important use of fluorescent antibody (FA) is identifying viruses that may not cause a cytopathic effect (CPE) in cell culture. When monospecific antisera are used, immunological confirmation of the infectious agent and precise identification is rapidly confirmed. There are two basic FA procedures: direct and indirect. The direct FA utilizes a fluorescent-labeled primary antibody, while the IFA uses an unlabeled primary antibody followed by a labeled antispecies antibody that binds to the primary antibody. Both assay formats should use antibodies that are monospecific and do not react with other pathogens. Due to the stoichiometry/geometry of the assay systems, the indirect assay tends to be more sensitive than the direct assay. However, a properly prepared direct FA conjugate will provide brilliant fluorescence that is easily read with a fluorescent microscope. The direct staining procedure is usually shorter (about 45 minutes), whereas the indirect staining procedure takes longer (1–2 hours). Frozen section testing for specific pathogens is accomplished by mounting target tissue from a diseased animal onto a cryostat specimen holder (chuck), freezing the tissue in the cryostat, and skillfully cutting serial sections for

FA staining (usually for multiple pathogens). Once the sections have been collected on glass slides, they are fixed in acetone to keep the tissue on the slide during the staining procedure and permeabilize cells so primary antibodies can react with viral antigens in infected cells. Staining of the sections is accomplished by rehydrating the tissue, reacting with the primary antibody (with or without a fluorescent label) against the pathogen of interest, washing excess reagent from the slide, and, in the case of direct FA, mounting and adding a coverslip immediately prior to viewing with the FA microscope. If the indirect staining procedure is used, the washed section is reacted with the secondary antibody with a fluorescent label, washed, mounted, and coverslip for microscopic exam. For ease of viewing frozen section samples, counterstains like Evans blue are sometimes added to the conjugate. FA staining for pathogen detection in cell cultures is similar to the processes described above except aqueous acetone is typically used to fix the assay plates when the cells are grown on plastic. Staining of virus isolation (VI) cultures is usually done at the first appearance of CPE or at a fixed time, usually 3–5 days post inoculation to detect non-cytopathic viruses or cultures with minimal infection. FA staining for viruses is used almost daily in some diagnostic virology labs since it provides a quick, inexpensive, presumptive diagnosis. The quality of the primary antibody is critical to obtaining accurate results, so these reagents should be fully characterized for their specificity and sensitivity. False-negative results can occur if the cut tissue section does not have any infected cells. A shortage of highly trained technicians, along with newer techniques like PCR and ELISA, has decreased this testing in some diagnostic laboratories. In addition, immunohistochemistry (IHC) and *in situ* straining techniques are used in place of FA to show a correlation with histopathology and specific cell types.

Fluorescent microsphere immunoassay

Simultaneous detection of multiple targets within one sample has been developed using the fluorescent microsphere immunoassay (FMIA). These assays use multiple beads, each having a distinct dye ratio to distinguish them from each other in a flow cytometric instrument (Luminex xMAP™, Luminex Corporation, Austin, TX). Individual beads are then coated with different antibodies or recombinant proteins to capture antigen (e.g. bead is coated with a specific antibody to the target antigen) or antibodies (e.g. bead is coated with the specific antigen to the antibody) within a sample, and the instrument measures the fluorescence of a secondary fluorescently marked antibody if the target molecule binds to the bead. Beads may also be coated with nucleic acid probes, which can then bind to a complementary DNA (cDNA) target. This method may or may not use PCR as an initial step prior to detection (Mahony et al. 2007). Currently, the assay has been developed to detect swine pathogens and immune proteins (Deregt et al. 2006; Lawson et al. 2010; Okda et al. 2015). This assay may be highly relevant in the future for herd profiling and management decisions, since it simultaneously detects antibodies against multiple pathogens (Khan et al. 2006).

Hemagglutination inhibition (HI)

An inherent structural capability of some viruses to bind or agglutinate red blood cells (RBCs) is referred to as hemagglutination. This capability can be used to detect the presence of antibodies that bind to hemagglutination-associated structures or epitopes on the virus inhibiting the virus' ability to hemagglutinate RBCs (e.g. adhere to RBCs). HI activity tends to correlate well with protection – the higher the antibody titer, the greater the level of protection. HI testing is currently used most extensively for IAV serological testing, evaluation of IAV strains for autogenous vaccine formulations, and detection of antibodies to porcine parvovirus (PPV) and hemagglutinating encephalomyelitis virus. In the HI test, if specific antibodies in the test serum bind the hemagglutinating portion of the virion, hemagglutination of RBCs (which are added to the test) is blocked, resulting in a "button" of RBCs at the bottom of a microtest plate well (e.g. positive result, indicating antibodies to this antigen are present). If the RBCs do agglutinate with the virus after serum is added, this would indicate that the sera do not have antibodies, and a uniform mat of RBCs is observed at the bottom of the test well (e.g. negative result, indicating antibodies to this antigen are not present). Swine serum samples must be pretreated to remove nonspecific hemagglutinins and/or hemagglutination inhibitors. Generally, an initial serum dilution of 1 : 10 is used for the assays, and serial dilutions are then made to determine a titer, which is the highest dilution at which there is no longer sufficient antibody present to inhibit agglutination.

For influenza viruses of swine, the test is subtype specific, which means that the H1 or H3 type of the viral test antigen must be cross-reactive with the type of IAV present in swine herds. HI tests can also be developed with a farm-specific strain. Monitoring infection with a specific strain can be more informative. For North America, in 2010, a total of no less than six HI test antigens should be available for test purposes: alpha H1N1, beta H1N1, gamma H1N1, delta H1N1, novel (pandemic) H1N1, and H3N2. Fortunately, the 2001 beta H1N1 test antigen provided by the US Department of Agriculture to veterinary diagnostic laboratories cross-reacts well with sera from

swine that have been infected with either beta or gamma H1N1 viruses. Sera from pigs naturally infected by novel H1N1 can be reliably tested with gamma H1N1 or homologous test antigens. For non-vaccinated swine, HI antibody titers of 1 : 40 or higher are considered to be indicative of previous infection with H1N1 viral strains. A higher titer cutoff is used for H3N2, 1 : 80, and suspect titers of 1 : 40 in combination with other pigs having titers of 1 : 80 or higher are considered to be indicative of natural infection. However, results on acute and convalescent sera are more meaningful than a single HI result, and the timing of serum collection will affect the magnitude of the HI titer. A universal flu ELISA test detecting, but not differentiating, subtypes would be preferable for obtaining plus/minus results.

For PPV, HI titers of 1 : 256 or greater are usually considered to be indicative of natural exposure. Gilts vaccinated with a killed virus vaccine will commonly develop HI titers up to 1 : 128. It is very common to obtain titers of 1 : 2048 or 1 : 4096 for naturally infected swine. As with most diagnostic tests, developing a specific plan for whom to test and what to test will result in superior results.

Immunohistochemistry

IHC involves the detection of pathogen-associated antigens in formalin-fixed, paraffin-embedded tissues using specific antibody and an enzyme or fluorochrome label. It can be a highly sensitive and specific technique and is widely used in research and diagnostic laboratories. IHC is also of great value in retrospective studies using formalin-fixed, paraffin-embedded tissues. Excellent detailed reviews of IHC methodologies and applications in the diagnosis of swine infectious diseases are available (Ramos-Vara et al. 1999). The basic steps of most IHC procedures include tissue preparation with formalin fixation, paraffin embedding, and sectioning. Since formalin cross-links proteins, which can limit binding of antibodies to specific antigenic sites, various antigen retrieval methods are then used to unmask or uncover antigens for better recognition by antibody reagents. Common methods include enzymatic digestion or heat-induced antigen retrieval. Blocking agents may be required to reduce background staining due to endogenous enzyme activity. Next, the staining steps may involve direct or indirect procedures. Indirect staining protocols are the most common due to their greater sensitivity. A specific primary antibody is typically followed with a labeled secondary antibody. An avidin–biotin complex (ABC) method is commonly used whereby an unlabeled primary antibody is followed by a biotinylated secondary antibody, and then an avidin–biotin peroxidase reagent reacts with a substrate to produce a colored product.

A major strength of IHC is that it allows clear association of antigen detection with specific histological lesions. This is particularly useful in identifying whether a specific pathogen (e.g. PCV2) is the etiology for a given disease (e.g. post weaning multisystemic wasting syndrome [PWMS]), since some pathogens are detected more frequently than the syndrome. It may also allow for some level of antigen quantitation; however, the antigen may not be evenly distributed throughout a given tissue, and selection of appropriate specimens can be critical. IHC requires the availability of high-quality antibody reagents and highly optimized fixation and staining methods with the use of appropriate controls.

Indirect immunofluorescence (indirect immunofluorescence or indirect fluorescent antibody [IFA]) and immunoperoxidase monolayer assay (IPMA) for antibody detection

IFA and IPMA are used to detect the presence of antibodies against some infectious agents. IFA assays utilize a fluorescent-labeled secondary antibody and require a fluorescent microscope. IPMA utilizes a peroxidase-labeled secondary antibody and appropriate chromogen and can be read using a standard light microscope. The colored reaction of the IPMA is more stable than fluorescence. Three of the most common IFA assays routinely used in swine diagnostics are for PRRSV, PEDV, and PCV2 (Madson et al. 2014; Magar et al. 2000; Yoon et al. 1992). The basic procedure involves preparation of infected monolayers of permissive host cells on glass slides or 96-well cell culture plates, typically with paired wells of noninfected host cells as controls. Cell monolayers are fixed with aqueous acetone or acetone/methanol to permeabilize cell membranes and allow antibody access to internal viral proteins. Dilutions of test swine sera and control sera are incubated with the cells; then, a secondary labeled antiswine immunoglobulin G (IgG) or immunoglobulin M (IgM) antibody is added. After washing, cell monolayers are viewed under a fluorescent microscope, and antibody titers are reported as the highest serum dilution showing virus-specific fluorescence. Similar assays have been developed for porcine enteroviruses (Auerbach et al. 1994), *L. intracellularis* (Knittel et al. 1998), and other pathogens. Most IPMA procedures utilize a similar protocol except the secondary antibody is labeled with peroxidase and followed by a chromogen to provide color development (Guedes et al. 2002). Both the IFA and IPMA measure the binding of specific antibody to antigens in infected host cells, but the IPMA results can be detected without a fluorescent microscope. The IPMA has been used for detection of

PRRSV, particularly European strains, since these strains grow well in porcine alveolar macrophages (PAMs) rather than a continuous cell line, and the IPMA staining is easily observed in PAMs. One advantage of IFA and IPMA methods is that they can provide relative antibody titers by using serial dilution of samples. However, interpretation can be somewhat subjective and dependent on the experience of the technician. These assays also require replication of the indicator virus or intracellular bacterium in cell culture. When dealing with antigenically variable viruses, such as PRRSV, assay sensitivity can be affected by antigenic differences between the virus strain used in the assay and the strain infecting a given group of animals. Table 6.3 demonstrates the effect of strain variation on PRRSV IFA titer results.

Microscopic agglutination test

The microscopic agglutination test (MAT) is the reference test for serological diagnosis of leptospirosis in swine. This test is based on the reaction of specific antibodies and live *Leptospira* sp. bacterial cells. A mixture of test serum with live *Leptospira* sp. cells results in agglutination, which can be visualized using dark-field microscopy. Results are reported in titers, indicating the highest dilution that resulted in agglutination of 50% of the live *Leptospira* cells compared with the control (Chappel et al. 1992).

Laboratories that are able to maintain live *Leptospira* cultures for clinically relevant serovars affecting swine easily perform the MAT. It is a fairly sensitive test, inexpensive, and quick to perform, and the agreement between high MAT titers (>1 : 1024) and isolation of *Leptospira* from infected pigs is significant (Chappel et al. 1992). A limitation of the MAT is the subjective definition of a positive result, which varies among technicians and laboratories. Additionally, one single reading is of low diagnostic value. Different laboratories utilize different cutoff titers to define a positive sample, so two consecutive tests within a 2-week interval are recommended to detect convalescent titers indicative of infection. Considering that MAT detects both IgM and IgG, cross-reactions among serovars are commonly observed in acutely infected pigs, whereas the second test provides more specific results regarding the serovar affecting the herd (Ahmad et al. 2005).

In situ hybridization

In situ hybridization (ISH) uses either a radioisotope, fluorescent, or enzyme-linked nucleic acid (DNA or RNA) to hybridize to a specific cDNA or RNA sequence of a specific pathogen in a tissue section. This technique is distinct from IHC, which identifies protein antigens (rather than nucleic acid) in tissue sections. ISH can be used in infectious disease diagnosis since DNA or RNA probes specific

Table 6.3 Effect of strain variation on PRRSV IFA results.

	Days postchallenge					
	Day 0	**Day 4**	**Day 7**	**Day 11**	**Day 14**	**Day 28**
Pig # 1						
SD-23983	<20[a]	<20	40	640	1280	1280
Ingelvac PRRS MLV	<20	<20	40	1280	2560	2560
Ingelvac PRRS ATP	<20	<20	<20	160	320	640
Lelystad Isolate	<20	<20	<20	<20	<20	<20
Pig # 2						
SD-23983	<20	<20	20	640	1280	2560
Ingelvac PRRS MLV	<20	<20	20	640	1280	2560
Ingelvac PRRS ATP	<20	<20	<20	160	640	640
Lelystad Isolate	<20	<20	<20	<20	40	40
Pig # 3						
SD-23983	<20	<20	80	640	1280	2560
Ingelvac PRRS MLV	<20	<20	40	640	1280	2560
Ingelvac PRRS ATP	<20	<20	<20	160	320	1280
Lelystad Isolate	<20	<20	<20	<20	<20	40

[a] Endpoint titers expressed as the reciprocal of the greatest serum dilution showing detectable PRRSV-specific fluorescence.

for pathogen sequences allow direct visualization of the site of pathogen replication in a tissue. It is possible to use radioactive and nonradioactive probes to simultaneously detect multiple transcripts. The most common technique used in the veterinary diagnostic laboratory utilizes digoxigenin-labeled probes. A positive hybridization signal is visualized in tissue sections after using IHC staining methods. The general method for ISH involves permeabilization of the cells with proteinase K, binding of the labeled DNA or RNA probes, antibody–phosphatase binding to the probe, and staining of the antibody with alkaline phosphatase. ISH is particularly useful for pathogenesis investigations and the precise identification of target tissues where the pathogen is replicating. The ISH procedure is very useful when the nucleic acid sequence of the pathogen is known, but no antibody-based reagents are available. The technique is extremely sensitive and has been used to study a number of pathogens including PRRSV, PCV2, and torque teno virus. Typically, ISH is more sensitive than IHC, which requires higher numbers of target molecules to produce a positive reaction. In addition, for pathogenesis studies, ISH signals may be present longer postinfection (since the RNA or DNA of the organism is still present) when antigen production is below the levels of detection. ISH is not performed in every diagnostic laboratory and has been used primarily as a research tool rather than a standard diagnostic test.

Parasite (internal) identification

Fecal flotation is used to identify specific parasitic egg morphology since adult worms are often not readily speciated. Feces are mixed with a solution (e.g. sugar solution) that has a specific gravity higher than the parasite egg. With centrifugation or passage of time, the eggs float to the surface of the solution, a microscopic coverslip can be applied, and the egg morphology is evaluated via a light microscope (Corwin 1997). This is a quick, low-cost test. To distinguish eggs from other debris, it is also important to evaluate egg size. Very small parasitic eggs such as cryptosporidia may not be identifiable via morphology, and FA or fecal ELISAs might be used. For zoonotic parasitic agents such as *Trichinella* and *Toxoplasma* sp., serological antibody ELISAs have been utilized (Gebreyes et al. 2008).

Polymerase chain reaction (PCR)

DNA and RNA extractions for detection of pathogens by polymerase chain reaction

Prior to PCR detection, the nucleic acid (RNA or DNA) of the pathogen is extracted from the specimen. Extraction is the chemical, physical, or mechanical process needed to recover, concentrate, and purify the RNA or DNA from a mixture of proteins, lipids, carbohydrates, or other materials that might be found in the clinical specimen, and it allows the PCR to proceed without interference and inhibition from these substances. There are rare circumstances where extraction may not be needed prior to PCR, but a comparison with and without extraction would need to be performed, verifying that the PCR gives the same specificity and sensitivity. There is several commercially available extraction protocols designed for specific specimens (e.g. serum, tissues, cells, whole blood) and for the specific nucleic acid that is being extracted (e.g. total RNA, viral RNA, messenger RNA, DNA, total nucleic acid) that can be performed either manually or with automation. These extractions may differ in the mechanical processes used for extraction (e.g. boiling, vortexing, sonicating, physical disruption using glass beads or enzymes) and separation processes, whereby the nucleic acids are separated from other substances with organic solvents (e.g. phenol–chloroform) or by binding to silica or magnetic beads. Since various sample types are being used for swine diagnostics such as oral fluids, semen, or blood swabs (whole blood in saline) where commercial kits may not be specifically designed for these specimens, a comparative study between these protocols needs to be performed to ensure the best sensitivity and specificity. In addition, extraction protocols are frequently improved and further refined for various specimens, so the most current, well-validated extractions need to be used. In evaluating various extraction protocols, the quality, quantity, and how well the extracted nucleic acid works in PCR need to be tested using a wide range of amounts of extracted nucleic acid in the PCR assay.

Polymerase chain reaction process

PCR is a technique that utilizes the necessary reagents and conditions to exponentially amplify DNA or RNA *in vitro*. In a diagnostic laboratory setting, PCR is mainly associated with the amplification of species-specific nucleic acid sequences from clinically relevant viruses and bacteria present in clinical samples. Amplification of nucleic acid from selected pathogens can be followed by sequencing of target segments to improve pathogen identification or define strain groups.

The basic concept of nucleic acid amplification starts with RNA or DNA extraction, followed by exponential amplification of the DNA through thermal cycling at various temperatures. The temperature variations provide for enzymatic reactions that cause conversion of the RNA to DNA (a reverse transcriptase reaction, if RNA is the starting material) followed by denaturation of the DNA, primer binding, and elongation of the copy of

DNA with the Taq polymerase enzyme. The temperature cycles are then repeated approximately 30–40 times so that theoretically, doubling of the DNA occurs (100% efficiency) during each temperature cycle, and billions of DNA copies can be obtained from one copy. Traditional detection of amplicons using gel-based methods is still used by many laboratories worldwide; however, highly sensitive and specific automated detection systems such as real-time PCR are rapidly substituting gel-based methods.

Independent of the detection system, PCR is today the gold standard for sensitive and specific detection of viral and bacterial pathogens in clinical samples. It has major advantages compared with culture for detection of bacterial pathogens previously treated with antibiotics (i.e. nonviable). In addition, it improves turnaround time from many days to a day for detection of certain fastidious viral and bacterial pathogens.

Gel-based polymerase chain reaction

Gel-based PCR uses agarose gels for detection of amplicons produced during PCR. The PCR that precedes gel-based detection utilizes a pair of species-specific primers that will anneal to the target nucleic acid and initiate the replication of target sequences by the polymerase enzyme. Once amplicons are produced, PCR products are loaded onto precasted wells in an agarose gel, and an electric current is applied to the system (electrophoresis). PCR products will migrate through the gel and will be separated by size with the smaller fragments migrating faster through the gel and identified with a lower base pair (bp) size. A known positive control is used in every PCR to assure that the amplicon obtained from clinical samples has the expected bp size for the pathogen of interest. A specific band should have the same size as the band observed for the positive control. The absence of a band is interpreted as a negative result. Amplicons are visualized on agarose gels by utilizing an intercalating fluorescent dye that binds to double-stranded nucleic acid and fluoresces under ultraviolet (UV) light. Gel-based PCR methods can be adapted to detect multiple targets (multiplex PCR). The sensitivity of this method can be considerably improved by performing a two-step amplification method known as nested PCR. In nested PCR tests, an external set of specific primers is used for the initial detection and amplification of the target sequence in the clinical sample followed by a second amplification utilizing a nested (internal) set of primers. Gel-based PCR can also be used to genotype bacterial (Oliveira and Pijoan 2004) and viral isolates (Wesley et al. 1998).

Gel-based methods are easily developed and standardized, do not require expensive equipment, and have a lower cost compared with real-time PCR. The main limitations of gel-based PCR tests are the lower sensitivity (if nested PCR is not used), the subjective interpretation due to visual inspection of bp sizes on the gel, and the time required to obtain final results, since it requires four steps: extraction of the RNA or DNA from the clinical sample, a PCR, gel electrophoresis, and visualization of the gel under UV light for detection. Another main limitation of gel-based methods is the need to open the PCR tubes after amplification for electrophoresis. The millions of amplicons that are produced during PCR can aerosolize and contaminate the laboratory, especially when nested PCR tests are used where tubes are opened more often. Nested PCR tests may cause contamination, resulting in false-positive reactions unless the laboratory has stringent requirements for prevention. These would include the use of aerosol-resistant pipette tips, dedicated rooms, instrumentation and equipment for setup rooms, and rooms where the PCR is performed, adding positive controls after samples are set up and adjusting positive control samples to be at approximately the same quantities as to what might be in clinical samples. The limited number of samples that can be performed on an agarose gel is another drawback of gel-based methods (e.g. approximately 14–28 samples can be evaluated on a single gel) compared with real-time protocols (e.g. approximately 96–384 samples can be evaluated on a single instrument).

Real-time polymerase chain reaction

Real-time PCR uses an automated system that allows for detection and quantification of PCR products as they are amplified ("real-time" detection), without the need for gel-based detection ("endpoint" detection). Production of double-stranded nucleic acid amplicons is reported as it occurs by fluorescence, which is captured, analyzed, and reported by a computer attached to the real-time thermal cycler. Most diagnostic laboratories to identify swine pathogens in clinical samples utilize two main signaling systems: double-stranded DNA intercalating dyes and labeled hydrolysis probes (Hoffmann et al. 2009).

Intercalating dyes such as SYBR Green® (Life Technologies, Carlsbad, CA) bind specifically to double-stranded nucleic acids (amplicons in positive samples), resulting in fluorescence, which is captured and reported in real time by the computer-based detection system. A melting curve analysis, which compares the temperature needed to separate the double-stranded amplicons produced in positive samples and that of the positive control, is performed at the end of the reaction to confirm the specific detection of the target sequence.

TaqMan® probes (Life Technologies, Carlsbad, CA) (a specific type of hydrolysis probe) can also be used to

report the presence of pathogen nucleic acid in clinical samples using real-time PCR. Probes are short oligonucleotides labeled with a fluorescent dye at one end and a quencher at the other end. The quencher is responsible for inhibiting light emission by the fluorescent dye in intact probes. The probe, forward, and reverse primers are specific and complementary to the nucleotide sequence of the pathogen of interest. Once the probe binds to the target DNA (if the target is present), it will be cleaved by the DNA polymerase during the amplification process, the quencher will separate from the fluorescent dye, and the fluorescence will be captured and reported by the real-time equipment, confirming the presence of the target pathogen in the sample.

Real-time PCR has several advantages compared with gel-based methods. It is usually more sensitive, since detection of positive samples is based on computerized recognition of light emission instead of visual inspection, and highly specific, considering that positives are confirmed based on melting curve analysis or by species-specific probes. Real-time assays can be quantitative, allowing the characterization of pathogen load in the sample.

Quantitative polymerase chain reaction

Quantitative PCR in swine diagnostics is typically performed through a real-time PCR assay, whereby a standard curve is derived using a known amount of serially diluted RNA or DNA. The amount of nucleic acid in the clinical samples is then extrapolated from this standard curve. Since the nucleic acid is being amplified in a PCR assay, the number of DNA copies would be a standard method of reporting. When the DNA from a sample is amplified during real-time PCR, the fluorescent intensity that occurs will cross a specific threshold at a given cycle number during the PCR thermal cycling process. This cycle threshold (Ct) will be obtained, and the Ct is inversely proportional to the amount of DNA present in the sample (e.g. a sample that has a Ct of 25 typically has a higher amount of DNA present than a sample that has a Ct of 35). Quantitative PCR results are often used as a measure of the amount of infectious pathogen present within the individual or swine population. It has also been useful in research studies to determine the efficacy of vaccines (Zuckermann et al. 2007) and virulence of various strains of PRRSV (Johnson et al. 2004). However, PCR is only measuring the amount of nucleic acid present (RNA or DNA), and there may not be any infectious (replicating) pathogen within the sample, even though the nucleic acid is detected. As one scientist stated, "we can detect and measure the amount of DNA present in King Tut, but that doesn't mean he is alive and well and running around." For example, PRRSV may be detected in serum by PCR but may not grow in cell culture in all samples or be infective in pigs (Figure 6.2).

This needs to be considered when PCR results are obtained and used to evaluate the "infectivity" of clinical samples such as environmental samples. However, in cases where there is a fresh, well-maintained sample submitted, there will most likely be some relationship between the amount of nucleic acid detected and the amount of infectious pathogen detected. When VI and PCR detected serial dilutions of PRRSV, there was approximately a 3 log higher concentration by PCR (copy/mL) than by VI (tissue culture infective dose 50 [$TCID_{50}$]/mL) (Figure 6.3).

However, the difference between infectious dose and DNA copies obtained through PCR can be variable depending on cell culture and PCR conditions used by different laboratories. The higher levels in copies/mL versus $TCID_{50}$/mL have also been observed with PCV2 (Gilpin et al. 2003). A higher copy number can be observed since the sample may have some noninfectious or replication defective virus present; there may be a greater amount of subgenomic viral nucleic acid measured since purified virus is not typically obtained from clinical samples; cell culture does measure the presence of infectivity, but it is still an "artificial" system since the virus is grown on a cell monolayer that may not be porcine derived and on a plate or flask. Therefore, VI "may not count all particles present in a preparation, even many that are in fact infectious" (Condit 2007). Factors that could affect the infectious titer in cell culture include pH, the cell culture media used in the isolation, incubation time, cell type used, viral strain, sample submission and handling, and *in vivo* antibodies, which may neutralize virus. Therefore, some caution is indicated in extrapolating results from PCR and equating them with the amount of infectious virus ($TCID_{50}$).

Multiplex polymerase chain reaction

Multiplex PCR refers to the simultaneous detection of multiple targets by PCR within a single sample. Multiple primer sets (or primer/probe sets for real-time PCR) are used to detect the multiple targets. A significant amount of optimization is needed to obtain similar sensitivities and specificities as detecting each target individually, thus somewhat limiting the number of targets that can be detected simultaneously. Multiplex PCR assays have been used in swine diagnostics to determine *Clostridium perfringens* toxin genotypes (Meer and Songer 1997), *E. coli* toxin and fimbriae types (Zhang et al. 2007), and multiple viruses or viral genotypes (e.g. PCV1 and PCV2; type 1 and type 2 PRRSV; multiple IAV subtypes) within a single sample.

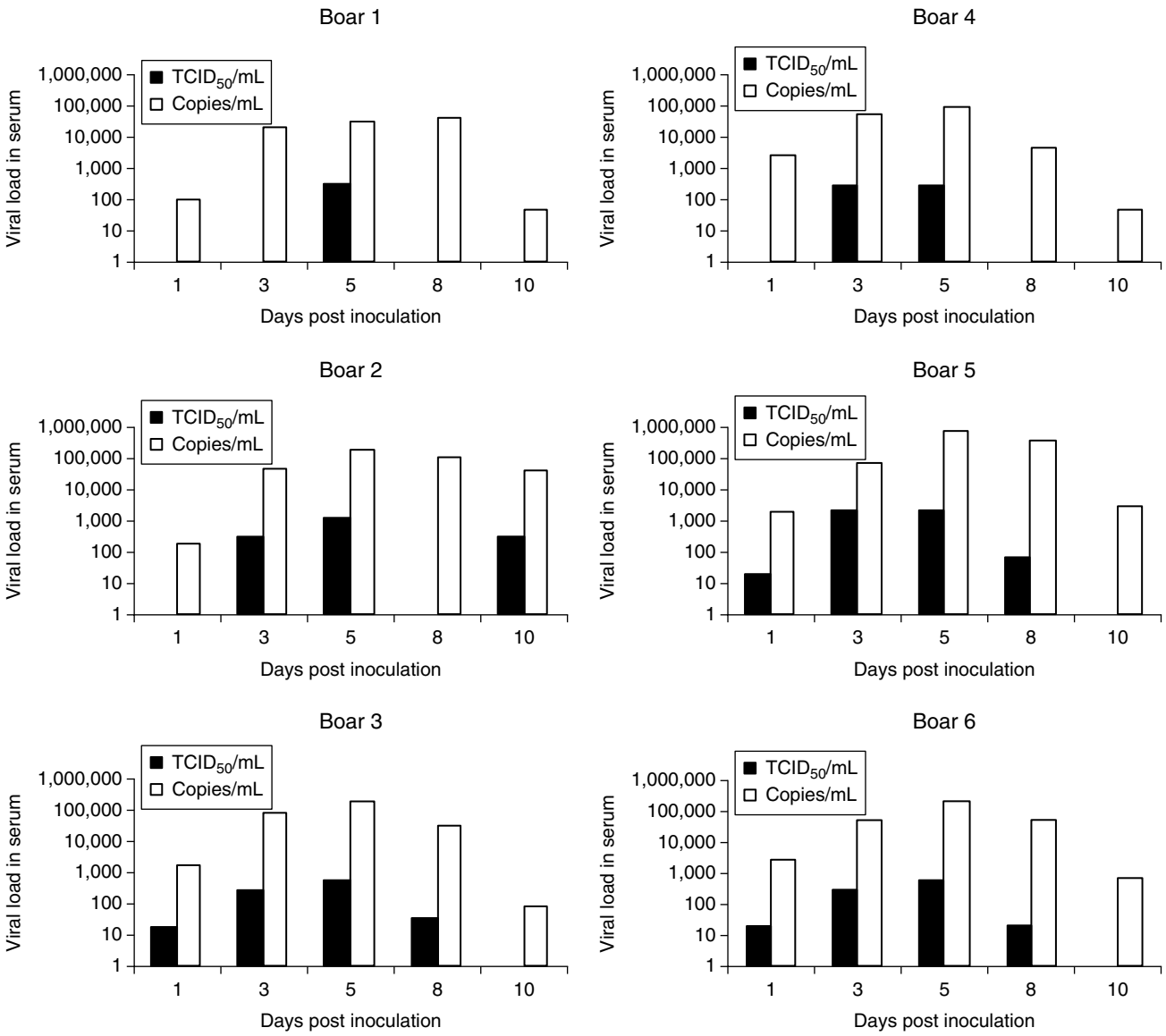


Figure 6.2 Viral load in serum, in $TCID_{50}$/mL, as determined by virus titration and in number of copies/mL as determined by quantitative real-time PCR. Values are for the first 10 days post inoculation for each PRRSV-inoculated boar. *Source:* Wasilk et al. (2004). Reproduced with permission of American Society for Microbiology.

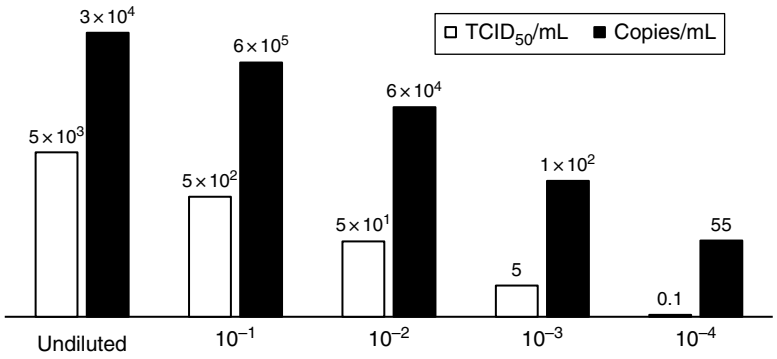


Figure 6.3 Serial 10-fold dilution of PRRSV showing the relationship between VI in TCID50/mL and real-time PCR results in copies/mL.

Sequencing (nucleic acid)

Nucleic acid sequencing is a powerful tool that adds specificity and discriminatory power to veterinary molecular diagnostics. It is a rapidly evolving technology, which has undergone three generations of development. Of these, dideoxy chain termination sequencing developed by Frederick Sanger is the first-generation sequencing technology that was widely used. Sanger sequencing complements detection by PCR by further confirming the detection of the pathogen of interest and also characterizing it to the strain level, for example, fastidious bacterial pathogens (e.g. *Mycoplasma hyorhinis*, *Mycoplasma hyosynoviae*, *Brachyspira* sp.). It is also used to type bacterial and viral isolates such as *M. hyopneumoniae*, PRRSV, PCV2, and IAV DNA or cDNA (produced by PCR from RNA).

The current Sanger sequencing method adopted by most diagnostic laboratories utilizes PCR amplicons obtained from clinical samples, which are purified and sequenced. Usually, the same gene utilized for detection of a specific pathogen is also sequenced for strain typing. Amplicons produced by PCR are purified and sequenced using an automated dye-terminator method. Each of the four dideoxynucleotide chain terminators (adenine, guanine, cytosine, and thymine) is labeled with a different fluorescent dye with distinct wavelengths. The sequencing reaction functions as a regular PCR, with the incorporation of labeled dideoxynucleotides being reported to a fluorescence reader. Each fluorescence peak is recorded in a chromatogram, which reports the sequence for the amplicon of interest. The chromatogram is useful for visual inspection of the quality and purity of the sequence. It can be edited and trimmed prior to reporting of the final sequence as a text file.

DNA sequencing is mostly used as a tool to investigate and characterize the molecular epidemiology of the pathogen of interest. It provides information on strain variability and allows swine veterinarians to troubleshoot biosecurity breaches, to determine whether vaccination or treatment was successful, and to identify the emergence of new strains. Although sequencing one single gene is feasible for diagnostic laboratories and affordable for swine veterinarians, it does provide limited information, and the significance of the differences between strains should be carefully evaluated. For example, at this time, there is little information in the literature regarding the biological importance of sequence data regarding virulence and cross-protection. Interpretation of sequencing information is unique for each pathogen, and inference of virulence and protection will depend on the gene that is being sequenced. Another limitation of sequencing is that it usually requires a higher pathogen load in the sample to generate accurate data compared with detection by PCR. It is not uncommon to have samples positive for a pathogen by PCR, which can generate unreliable or no sequence data. Specific genomic regions of several swine viruses have been sequenced including those for swine influenza virus, PRRSV, PCV2, CSFV, African swine fever virus (ASFV), PPV, transmissible gastroenteritis virus (TGEV), Teschen virus, swinepox virus, vesicular stomatitis virus, vesicular exanthema virus, and FMDV. The purposes of sequencing whole genomes or portions of the viral genome are to determine genetic relatedness between various viral strains, to provide information on changes in a virus over time, to identify mutations and recombination events, to develop molecular diagnostic assays to identify conserved areas of the genome, to confirm diagnostic testing results, to determine whether a vaccine or wild-type virus is present, and to identify novel viruses.

Prior to Sanger Sequencing and second- and third-generation sequencing, typing of bacterial strains has been traditionally performed using gel-based methods (Versalovic et al. 1991). Pulsed-field gel electrophoresis (PFGE) is a genomic fingerprinting method that displays a series of bands on a gel characteristic of a specific strain. Generally, PFGE has higher resolution than single-gene Sanger sequencing. It is generally used to type new variants of a pathogen or to trace pathogen transmission patterns. For example, PFGE was used to determine the transmission dynamics of a *Salmonella typhimurium* monophasic variant in a pig production cycle (Fernandes et al. 2016).

Multilocus sequence typing (MLST) is bacterial sequence-based genotyping method that has typing resolution that is comparable with PFGE. It consists of amplifying and sequencing seven housekeeping genes that generate a species-specific sequence type (ST). MLST schemes have recently been published for several clinically relevant swine pathogens, including *H. parasuis* (Olvera et al. 2006) and *M. hyopneumoniae* (Mayor et al. 2008). Although MLST is highly discriminatory, sequencing of seven genes for each strain is laborious and expensive for routine testing. Similar to viruses, routine bacterial strain typing by sequencing should ideally be based on the direct amplification and sequencing of a single discriminatory gene from submitted clinical samples. This is the case, for example, for *M. hyopneumoniae* strain typing based on P146 gene sequencing. P146 is an adhesion-like protein that is expressed on the surface of *M. hyopneumoniae* and is highly variable among different strains. *M. hyopneumoniae* P146 sequencing is almost as discriminatory as MLST (Mayor et al. 2008). This method was used to prove aerosol transmission of *M. hyopneumoniae* and can also be used to monitor introduction of new strains into the herd (Otake et al. 2010).

Second-generation sequencing is known by different names such as massively parallel sequencing or next-generation sequencing. It is called second-generation sequencing because this was the second successful nucleic acid sequencing technology developed after the Sanger method. Unlike the Sanger method, which can typically sequence about 1000–1500 bp of an amplicon, NGS can sequence entire viral and bacterial genomes. During NGS, sample DNA is sheared at random to generate small fragments. These fragments are then clonally amplified simultaneously to generate a sequencing library. The sequence of each fragment in the library is then determined by the addition of nucleotides complementary to those in the fragments rather than through chain termination chemistry as in the Sanger method. Consequently, the entire sample is sequenced in parallel reactions, resulting in several gigabytes of data from an NGS run. Although there are several commercially available platforms used for NGS, such as Life Technologies Ion Torrent and Roche 454, sequencers developed by Illumina are the industry's leading platform. Since this method can produce gigabytes of data, several bacterial or viral samples can be multiplexed and sequenced together in one NGS run. This is achieved by adding unique fragments of DNA known as bar codes to each sample at the time of sample library preparation and sorting out the reads using bioinformatics software. To ensure the accuracy and probability of pathogen detection, enrichment of samples is often required for NGS. Depending upon the pathogen to be detected and the sample type, this can be done by PCR enrichment or enzymatic methods. For example, primers designed to amplify various segments of the pathogen could be used to selectively amplify the pathogen genome. These amplicons are then used as the input for NGS. This approach has been used to produce full genomes of PEDV from clinical samples (Fan et al. 2017). Depleting the host and environmental DNA from the samples is a common approach used for enriching samples before metagenome sequencing. This is can be achieved by the use of nucleases that selectively degrade host DNA (Feehery et al. 2013). Since NGS produces a massive amount of sequence data, which covers the entire length of sample genome, it has the highest accuracy and power of resolution when compared with any other molecular testing method available today. Because of the unprecedented resolution of NGS, it is being used in several different applications in diagnostic testing. NGS is extremely useful for identifying new variants of rapidly evolving pathogens such as PRRSV (Lu et al. 2014). The application of NGS in PRRSV genome sequencing has allowed the study of PRRSV in unprecedented detail through reconstruction of the full PRRSV genome. This approach is broadly applicable to any of the swine viruses such as influenza (Clavijo et al. 2013). Another major utility of NGS is the traceback or determination of transmission routes of a pathogen. It is particularly useful in investigating zoonotic bacterial pathogens such as *C. difficile*. Recently, NGS-based sequencing of *C. difficile* isolated from humans and swine revealed that the isolates were identical, suggesting transmission between animals and people (Knetsch et al. 2014). Since NGS is highly accurate, it can also be used to rule out pigs as a source of human infections. One such example is an NGS-based finding that a US swine herd was not the source of a human *B. suis* infection. This NGS process and analysis averted a field investigation (Quance et al. 2016). Another major evolving application of NGS is the simultaneous detection, traceback, serotyping, and antibiotic resistance profile of bacteria such as *Salmonella enterica* and *Streptococcus suis* (Athey et al. 2016). During NGS, all the genes including antibiotic resistance genes and serotype-associated genes are sequenced. When NGS was used for analyzing *S. enterica*, a high concordance (99.74%) between phenotypic and predicted antimicrobial susceptibility was detected (Zankari et al. 2013).

Unlike the Sanger method, which requires prior knowledge about the target gene and a validated set of primers available for amplifying the target sequence, NGS does not require such knowledge and can sequence the entire genome. This makes it suitable for detecting unknown bacterial or viral pathogens. For example, when no known viral agent is detected by other testing methods, sample RNA could be converted into cDNA, and the entire cDNA could be sequenced using NGS. Resulting sequences are then used to search against all known viral sequences in public genomic databases to identify related viruses. This approach is called metagenome sequencing since it sequences all known and unknown genomes in the given sample. Metagenome sequencing is advantageous since it involves sequencing nucleic acids from the sample directly without the need for culturing. One example of the success of this approach is the recent discovery of a pestivirus that causes Parkinson's-like tremors and high mortality in pigs (Hause et al. 2015). Metagenomics is useful also for identifying the possibilities of the combined pathobiology behind disease complexes such as PMWS and for detecting the presence of various infectious agents in such complicated disease situations. Although PCV2 is known as the main etiologic agent behind PMWS, NGS-based metagenomics was used to establish that a novel bocalike parvovirus was also associated with PMWS (Blomstrom et al. 2010).

Although NGS is extremely accurate and powerful, it has several disadvantages. The first is the higher cost of the reagents and the sequencing infrastructure. Consequently, the cost per sample for NGS-based testing is much higher than Sanger sequencing-based methods. To be cost effective, several samples need to be

multiplexed in an NGS-based test. If a laboratory does not obtain a sufficient number of samples to multiplex, the test may need to wait for more samples to arrive, or it will need to be run at a very high cost per sample. Secondly, NGS-based tests typically require high capacity computing hardware and a dedicated bioinformatics staff to analyze the data. Because of this, adoption of NGS in veterinary diagnostic laboratories has been slow. However, the infrastructure costs are rapidly declining, making this method more user friendly.

Recently, a third-generation sequencing technology called nanopore sequencing has been commercialized by a company called Oxford Nanopore Technologies (ONT). This is called the third-generation sequencing method because it does not depend on chemistry-based reactions. An ONT sequencer is a handheld device that detects the base sequence of DNA when it passes through nanometer-sized pores in a membrane embedded in the device. Since it detects charge differences in the bases, it can produce extremely long sequences. Often it can identify the entire viral genome without the need for fragmenting samples as in the case of NGS. It is extremely portable and works by connecting to the USB port of a computer. This makes it suitable for field applications such as sequencing virus samples in the field during an outbreak (Quick et al. 2016). A major advantage of this method over NGS is that it is economical to sequence a single sample without the need for multiplexing. ONT nanopore sequencing also has very short detection times (e.g. approximately 1 hour), which is more rapid than PCR and Sanger sequencing. It has been employed to detect antibiotic resistance gene profiles in *Staphylococcus aureus* and *Mycobacterium tuberculosis* (Bradley et al. 2015). It is not yet widely available in the clinical diagnostic laboratories as this method is yet to be benchmarked against the current Sanger sequencing method. Secondly, the error rate in the sequence base calling in ONT sequencing is higher than the previous generation sequencing methods. However, this method is expected to become a common technique in clinical diagnostics. A comparison of sequencing methods is provided in Table 6.4.

Advances in nucleic acid sequencing such as second- and third-generation sequencing are making bioinformatics an integral part of clinical diagnostics. New sequencing methods generate massive amount of data. To be useful, the data need to undergo several steps of analysis. Typical steps involved are removal of error-prone reads, assembly of the sequencing reads into larger fragments called contigs, and then their comparison against a reference viral or bacterial genome. When there are no reference genomes available, reads are assembled using *de novo* assembly algorithms. Genes in the assembled genomes are then determined using a process called genome annotation. These computational steps have been an impediment in the adoption of new sequencing

Table 6.4 Comparison of nucleic acid sequencing technologies that are used (or have the potential to be used) in swine diagnostics.

Sequencing technology	Common uses	Advantages	Disadvantages
1. Amplicon sequencing by the Sanger dideoxy method	• Sequence single genes or PCR fragments amplified from viruses and bacteria (e.g. sequencing ORF5 of PRRSV to determine antigenic variability)	• Low cost • Extremely fast and well established • Data is simple to manipulate and analyze	• Requires prior knowledge about the target and standardized primers • Limited sequence information
2. Second-generation sequencing (NGS)	• Determining the whole genome sequence of viruses and bacteria • Detect emergence of new variants of bacteria and viruses • Traceback studies to identify the source of infection • Identification of unknown causes of infection	• Very high resolution • Can sequence several samples simultaneously • Can be applied to samples without a known sequence or primers available	• High cost per sample • Long turnaround time • Need for bioinformatics expertise
3. Third-generation nanopore sequencing	• Same as NGS	• Same as NGS with turnaround times faster than PCR • Very low infrastructure cost • Extremely portable	• Still evolving method. Data analysis requires a specialist lab with bioinformatics expertise • Needs to be benchmarked against standard methods • Higher error rate in sequence data compared with other methods

technologies in clinical diagnostics. However, easy-to-use bioinformatics analysis applications are being developed. Many of them are cloud-based applications that require uploading of raw sequence data and downloading the results in tabular graphical formats. Some examples for this include an application for determining the serotype of *Salmonella* (Zhang et al. 2015). Combined with declining costs of newer sequencing methods and cloud-based bioinformatics applications, NGS and nanopore sequencing methods are likely to be adopted increasingly in complex clinical cases that are not resolved by other diagnostic methods.

Toxicology/chemistry testing

Toxicities and vitamin or mineral deficiencies can be major contributors to disease syndromes, and diagnostic chemistry testing along with history, clinical signs, and postmortem findings is important in considering a diagnosis. Toxicities are more thoroughly covered in Chapter 70, and excellent references are available (Osweiler et al. 1976; Sachana and Hargreaves 2007).

Virus isolation (VI)

Since viruses are intracellular parasites, VI is used to grow and identify viruses from clinical samples within cells maintained in the laboratory. The variety of cell lines maintained by most laboratories depends on the permissiveness of various cell lines to viruses expected to be encountered. Primary and secondary cell cultures are often prepared from trypsinization of fresh tissues, including primary PAM cultures such as used for PRRSV diagnostics. Alternatively, established continuous cell lines can be used, since they are stable, easier to manage in laboratory settings, and very sensitive for many viruses. Embryonated eggs were widely used for viral diagnosis in the past but are now generally replaced by continuous cell lines. However, they are still used in some laboratories to grow certain strains of influenza A.

Received specimens are processed and inoculated onto susceptible cell cultures and monitored for CPE, which are characteristic morphological changes in a given cell type, such as lysis, syncytium formation, and inclusion body formation. CPE may be characteristic of certain virus infections but often are not definitive, so immunofluorescent staining or other methods are often used for verification. VI is a very sensitive procedure to detect certain viruses that readily replicate in cell culture. It can be highly definitive and may provide a viral isolate for further analysis such as for use in sequencing or production of autogenous vaccines. It can also be used as a test to detect new viruses where PCR is not available due to limited or no sequence information being available for PCR primer design.

Many viruses are very fastidious or may not replicate in the available cell cultures. VI requires freshly submitted samples maintained under refrigeration, and it may require an extended period of time (2 or more weeks) to obtain results. VI requires specialized equipment and skills and high-quality reagents to confirm isolation of a given virus. Additional challenges occur when dealing with specimens that have high levels of bacterial contamination.

Virus neutralization (or serum virus neutralization)

SVN assays can be used to measure the presence of neutralizing antibody to a given virus, indicating previous exposure to the virus. Classical SVN involves the binding of antibodies to virions, preventing infection of susceptible host cells. A variety of SVN assays can be applied to swine diagnostic serology. The assay can provide quantitative results, and testing of paired serum samples (acute and convalescent) can provide an indication of recent exposure. SVN can also be used as a tool in identification of an isolated virus or to determine the serotype or strain of an isolated virus, provided that appropriate monospecific antisera or mAb is available. The identification of group A rotavirus G and P types (which have importance in vaccine selection or development) is one example of this application.

SVN assays are generally highly sensitive and specific due to the very specific nature of antigen–antibody interactions. However, the assays may be too specific for useful general diagnostic applications when dealing with extremely diverse viruses, such as PRRSV, where limited cross-reactivity may be seen among different strains of the virus. Some viruses, such as PRRSV, may not induce robust levels of detectable neutralizing antibodies, or the neutralizing antibody response may be delayed until several weeks or more after initial infection, thus limiting the practical utility of VN assays in routine diagnostics.

When used for the evaluation of virus-specific neutralizing antibodies in serum samples, most SVN assays involve adding a constant amount of virus to dilutions of the serum samples to be tested, along with appropriate control sera. These mixtures are then incubated to allow any neutralizing antibodies present to bind to the virus. Susceptible host cells are then added to the serum–virus mixture, or the mixture is added to existing monolayers of host cells and incubated for 3–5 days until replicating virus induces a visible CPE in the host cells. If no neutralizing antibody is present, CPE will be apparent. If virus-specific neutralizing antibody is present in a given

serum sample, no CPE will be apparent at lower serum dilutions and an antibody titer or endpoint can be determined at a point in the dilution series where CPE becomes apparent.

Variations of the traditional CPE-based VN assays include the plaque reduction neutralization (PRN) assay. This assay is similar to the traditional approach except cell monolayers are overlaid with a soft agar prior to the 3- to 5-day incubation. Any non-neutralized infectious virions present at a given test serum dilution will infect host cells, and a "plaque" of lysed cells will develop. These plaques can then be visualized by staining cell monolayers with crystal violet, neutral red, or similar stains and by counting the clear plaques at selected dilutions of test and control sera. Endpoint titers are typically determined as the highest serum dilution, resulting in a 50–90% reduction in plaque-forming units.

Another variation is the fluorescent focus neutralization (FFN) assay that is of particular value when dealing with viruses that can infect a host cell but do not induce obvious CPE. The test is performed in a manner similar to the traditional CPE-based VN except cell monolayers are fixed with aqueous acetone or similar fixative at a selected time point after inoculation, generally 24–48 hours depending on the replication rate of the selected virus. Cells are then stained using standard FA staining techniques, and foci of infected cells in test serum and control wells are counted. Endpoint titers are determined as the highest serum dilution, resulting in a 50–90% reduction in fluorescent focus-forming units (FFUs).

Summary of diagnostics and interpretations

Diagnostic testing is used to answer several important questions: (1) Which pigs have what pathogen, and when and how did they acquire it? (2) Which pigs have maintained a negative pathogen status? (3) Which pigs have seroconverted to a planned exposure or vaccination? Each of these three categories has inherent challenges for test selection and interpretation of the test results. However, sample selection may be much more important than test selection. It is necessary to know what question(s) needs to be answered for collection of the appropriate sample(s) and to understand the basic pathogenesis of each disease and the effect of preexisting immunity so that the timing of sample collection is optimal.

Many of the frustrations and challenges with diagnostic testing interpretation occur due to living with endemic diseases. It can be difficult to assess the clinical relevance of a detected pathogen, when the ebb and flow of disease agents creates a moving target. Therefore, it is imperative to know what pathogen the pigs have, the timeline of when they became infected, the pathogen "load," and what effect it has on them to understand the results of a carefully planned sample and test selection. It is important to know what "normal" is to then determine what is "abnormal." Many times answering the question is straightforward. For example, if a client finds 30 dead, 5-week-old pigs one morning and the diagnostic lab reports a heavy pure growth of F4 (K88) or F18 *E. coli* from multiple intestines, it is known that the sudden death of 30 pigs is unexpected and a heavy pure population of a F4 (K88) or F18 *E. coli* in the intestine is not normal. Therefore, a cause and effect can be deduced. Knowing with certainty what caused the problem allows for a specific intervention and a probable return to the expected norm. Alternatively, finding 30 dead pigs over the course of 2 weeks in a group of 80 lb pigs experiencing respiratory disease may be an expected, albeit unwanted, event. The plan to understand what is causing the deaths may involve multiple submissions from younger pigs and samples from the farm or farms of origin. In this case, determining who has what, when did they get it, and how did they get it is a much more complex problem to unravel, treat, and prevent. An intrinsic bias to avoid in understanding endemic disease is diagnosis by "ego." With the myriad of agents and known or unknown interactions that result in clinical disease, determining which agents to specifically test for may lead to mistaken or inappropriate interventions, continuing morbidity and mortality, and client dissatisfaction. Negative test results can be as valuable as positive test results. Endemic disease is ever changing and should never be approached with the thought of conclusively knowing what is going on.

Determining if a group of pigs has maintained a negative disease status is usually a straightforward process and commonly involves testing for seroconversion to a disease agent along with looking for the infectious agent in pigs that have not yet seroconverted. Again, knowing when pigs will typically seroconvert from an infection, what samples to collect, and when to collect them is imperative to maintaining a negative herd status. Problems that arise with testing negative groups may involve both test sensitivity and specificity, but test specificity is the more common aggravation. Simply put, diagnostic testing is biology and not accounting. Diagnostic testing is subject to the inherent nonspecific reactions that can occur when people are involved with collecting and measuring samples produced by pigs. When judging test results that appear to be spurious, considerations for resolving the problem include determining if the pigs are in an early stage of infection or were actually vaccinated; if the lab, client, or veterinarian mixed up samples; if the correct pigs were tested; if

a new test kit or reagent at the lab was used; if there is a nonspecific reaction; and if that nonspecific reaction is reproducible with the same sample and with follow-up samples from the same animal (e.g. if you do not identify the animals tested on the submission form, you cannot answer this last question). Actions taken to resolve spurious results include asking the lab to make sure the right case was tested, retesting the original sample with the same test, testing the same sample with another test that has a different target for the agent, testing for the agent and for antibodies to the agent or vice versa, re-bleeding and recollecting samples from the group, re-bleeding and recollecting samples from the reacting animal(s), euthanizing the pig to look at tissues for evidence of an agent if you think the antemortem samples available are not adequate for determining the true status of infection, and inquiring at least three times to make sure the animals were not inadvertently vaccinated. These actions are important to investigate because people make mistakes, test wells are not always uniformly coated with antigen or antibody, pigs can produce nonspecific antibodies that react with one antigen but not another antigen to the same agent, pigs can temporarily produce nonspecific antibodies that react with multiple antigens of the same agent, pigs can permanently produce nonspecific antibodies that react with multiple antigens of the same agent, the PCR target for an agent may be inappropriate due to agent mutation, people are afraid to admit mistakes, pigs are mistakenly vaccinated, and bad test kits and reagents are produced and distributed to laboratories. The only way to avoid these problems is to never submit anything for testing. The more logical approach is to understand how problems can occur and how to resolve them. Leave a little time between testing and movement to allow for resolution, have a plan for resolving the problem, and stay calm. No one likes false positives or false negatives.

Confirming seroconversion to vaccination or live pathogen exposure is another testing event that is usually straightforward. For vaccinations, which tests to use and time-to-seroconversion are typically known and predetermined. Live pathogen exposure is not always a uniform or consistent event, but ensuring infection and an immune response is very important in preventing the introduction of pathogens into naïve populations. The inadvertent introduction of naïve animals into an infected group can greatly alter the disease dynamics affecting not only the naïve animals but also the existing population.

References

Ahmad SN, Shah S, Ahmad FM. 2005. *J Postgrad Med* 51:195–200.

Angeletti S, Dicuonzo G, Avola A, et al. 2015. *PLoS One* 10(3): e0120502.

Athey TB, Teatero S, Takamatsu D, et al. 2016. *PLoS One* 11(3): e0150908.

Auerbach J, Prager D, Neuhaus S, et al. 1994. *Zentralbl Veterinarmed B* 41:277–282.

Bizzini A, Durusell C, Bille J, et al. 2010. *J Clin Microbiol* 48:1549–1554.

Blomstrom AL, Belak S, Fossum C, et al. 2010. *Virus Res* 152(1–2):59–64.

Bradley P, Gordon NC, Walker TM, et al. 2015. *Nat Commun* 6:10063.

Carpaij N, Willems RJL, Bonten MJM, et al. 2011. *Eur J Clin Microbiol Infect Dis* 30:1169–1172.

Casteel SW, Cowart R, Weis CP, et al. 1997. *Fundam Appl Toxicol* 36:177–187.

Chappel RJ, Prime RW, Millar BD, et al. 1992. *Vet Microbiol* 30: 151–163.

Chen Q, Li G, Stasko J, et al. 2014. *J Clin Microbiol* 52:234–243.

Cherkaoui A, Emonet S, Fernandez J, et al. 2011. *J Clin Microbiol* 49:3004–3005.

Christopher-Hennings J, Nelson EA, Nelson JK, et al. 1995. *J Clin Microbiol* 33:1730–1734.

Clavijo A, Nikooieneiad A, Esfahani MS, et al. 2013. *Zoonoses Public Health* 60(5):327–335.

CLSI. 2015. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*, 3rd ed. CLSI supplement VET01S. Wayne, PA: Clinical and Laboratory Standards Institute.

Condit RC. 2007. Principles of virology, quantitative assay of viruses. In *Fields Virology*, 5th ed. Philadelphia: Lippincott Williams & Wilkins, p. 37.

Corwin RM. 1997. *J Swine Health Prod* 5:67–70.

De Oliveira Mendonça A, Domingues PF, Da Silva A, et al. 2004. *Parasitol Latinoam* 59:42–45.

Dee S, Clement T, Schelkopf A, et al. 2014. *BMC Vet Res* 10:176.

Del Río ML, Gutiérrez CB, Rodríguez Ferri EF. 2003. *J Clin Microbiol* 41:880–882.

Deregt D, Gilbert SA, Dudas S, et al. 2006. *J Virol Methods* 136:17–23.

Dupont C, Sivadon-Tardy V, Bille E, et al. 2010. *Clin Microbiol Infect* 16:998–1004.

Enøe C, Andersen S, Sørensen V, et al. 2001. *Prev Vet Med* 51:227–243.

Erlandson R, Evans R, Thacker B, et al. 2005. *J Swine Health Prod* 13:198–203.
Evans EW. 2006. Interpretation of porcine leukocyte responses. In *Schalm's Veterinary Hematology*. Ames, IA: Blackwell Publishing, p. 411.
Fan B, Jiao D, Zhao X, et al. 2017. Characterization of Chinese porcine epidemic diarrhea virus with novel insertions and deletions in genome. *Sci Rep* 7:44209.
Feehery GR, Yigit E, Oyola SO, et al. 2013. A method for selectively enriching microbial DNA from contaminating vertebrate host DNA. *PLoS One* 8(10):e76096.
Fernandes L, Centeno MM, Couto N, et al. 2016. *Vet Microbiol* 192:231–237.
Ferreira L, Sanchez-Juanes F, Munoz-Bellido JL, et al. 2011. *Clin Microbiol Infect* 17:1007–1012.
Fittipaldi N, Broes A, Harel J, et al. 2003. *J Clin Microbiol* 41:5085–5093.
Gebreyes WA, Bahnson PB, Funk JA, et al. 2008. *Foodborne Pathog Dis* 5:199–203.
Gilpin DF, McCullough K, Meehan BM, et al. 2003. *Vet Immunol Immunopathol* 94:149–161.
Guedes RMC, Gebhart CJ, Deen J, et al. 2002. *J Vet Diagn Invest* 14:528–530.
Hause BM, Collin EA, Peddireddi L, et al. 2015. *J Gen Virol* 96(10):2994–2998.
Hoffmann B, Beer M, Reid SM, et al. 2009. *Vet Microbiol* 139:1–23.
Janda JM, Abbott SL. 2007. *J Clin Microbiol* 45:2761–2764.
Johnson W, Roof M, Vaughn E, et al. 2004. *Vet Immunol Immunopathol* 102:233–247.
Khan I, Mendoza S, Yee J, et al. 2006. *Clin Vaccine Immunol* 13:45–52.
Knetsch CW, Connor TR, Mutreja A, et al. 2014. *Euro Surveill* 19(45):20954.
Knittel JP, Jordan DM, Schwartz KJ. 1998. *Am J Vet Res* 59:722–726.
Lawson S, Lunney J, Zuckermann F, et al. 2010. *Vaccine* 28:5356–5364.
Lu ZH, Archibald AL, Ait-Ali T. 2014. *Virus Res* 194:167–174.
Madson DM, Magstadt DR, Arruda PH, et al. 2014. *Vet Microbiol* 174(1–2):60–68.
Magar R, Müller P, Larochelle R. 2000. *Can J Vet Res* 64:184–186.
Mahony J, Chong S, Merante F, et al. 2007. *J Clin Microbiol* 45:2965–2970.
Mailhac A, Durand H, Boisset S, et al. 2017. *J Proteomics* 152:15–152.
Markey B, Leonard F, Archambault M, et al. 2013. Bacterial pathogens: Microscopy, culture and identification. In *Clinical Veterinary Microbiology*. Elsevier, pp. 9–47.
Mayor D, Jores J, Korczak BM, et al. 2008. *Vet Microbiol* 127:63–72.
Meer R, Songer J. 1997. *Am J Vet Res* 58:702–705.
Moono P, Foster NF, Hampson DJ, et al. 2016. *Foodborne Pathog Dis* 13:647–655.
Nielsen K, Gall D, Smith P, et al. 1999. *Vet Microbiol* 68:245–253.
O'Connor M, Fallon M, O'Reilly PJ. 2002. *Irish Vet J* 555:73–75.
Okda F, Liu X, Singrey A, et al. 2015. *BMC Vet Res* 11:180.
Oliveira S. 2007. *J Swine Health Prod* 15:99–103.
Oliveira S, Pijoan C. 2004. *Vet Microbiol* 99:1–12.
Olvera A, Cerdà-Cuéllar M, Aragon V. 2006. *Microbiology* 152:3683–3690.
Osek J. 2001. *J Vet Diagn Invest* 13:308–311.
Osweiler GD, Carson TL, Buck WB, et al. 1976. Diagnostic toxicology. In *Clinical and Diagnostic Veterinary Toxicology*, 3rd ed. Dubuque, IA: Kendall/Hunt Publishing Company, pp. 44–51.
Otake S, Dee S, Corzo C, et al. 2010. *Vet Microbiol* 145:198–208.
Oviano M, de la Luna Ramirez C, Barbeyto LP, et al. 2017. *J Antimicrob Chemother* doi:https://doi.org/10.1093/jac/dkw579.
Quance C, Robbe-Austerman S, Studber T, et al. 2016. *Emerg Infect Dis* 22(1):79–82.
Quick J, Loman NJ, Duraffour S, et al. 2016. *Nature* 530(7589):228–232.
Ramos-Vara JA, Segalés J, Duran CO, et al. 1999. *J Swine Health Prod* 7:85–91.
Randall LP, Lemma F, Koylass M, et al. 2015. *Res Vet Sci* 101:42–49.
Rice CE. 1960. *Can J Comp Med Vet Sci* 24:126–130.
Sachana M, Hargreaves AJ. 2007. Toxicological testing: in vivo and in vitro models. In *Veterinary Toxicology, Basic and Clinical Principles*. New York, NY: Elsevier, Academic Press, pp. 51–66.
Shin HB, Yoon J, Lee Y, et al. 2015. *Yonsei Med J* 56:550–555.
Thacker EL. 2004. *J Swine Prod* 12:252–254.
Vannucci FA, Wattanaphansak S, Gebhart CJ. 2012. *J Clin Microbiol* 50:1070–1072.
Versalovic J, Koeuth T, Lupski JR. 1991. *Nucleic Acids Res* 19:6823–6831.
Wesley RD, Mengeling WL, Lager KM, et al. 1998. *J Vet Diagn Invest* 10:140–144.
Yoon KJ, Joo HS, Christianson WT, et al. 1992. *J Vet Diagn Invest* 4:144–147.
Zankari E, Hasman H, Kaas RS, et al. 2013. *J Antimicrob Chemother* 68(4):771–777.
Zhang W, Zhao M, Ruesch L, et al. 2007. *Vet Microbiol* 123:145–152.
Zhang S, Yin Y, Jones MB, et al. 2015. *J Clin Microbiol* 53(5):1685–1692.
Zuckermann F, Alvarez Garcia E, Luque I, et al. 2007. *Vet Microbiol* 123:69–85.

7

Optimizing Sample Selection, Collection, and Submission to Optimize Diagnostic Value

Paulo H.E. Arruda and Phil Gauger

Diagnostic question and identification of the problem

The diagnostic process requires a systematic and unbiased collection and eventual integration of history, signalment, and clinical signs that should eventually be correlated with gross pathology, histologic lesions, and molecular, bacteriology, and/or serology test results that will reliably and accurately address the veterinarian's diagnostic question. Using an evidence-based medicine approach, a final diagnosis should be supported by objective evidence generated throughout the diagnostic process. Producing diagnostic evidence is truly a partnership between producer, farm employee, veterinarian, and the diagnostic laboratory. Direct communication between the diagnostician and veterinarian is highly recommended to develop a diagnostic plan that includes appropriate animal selection, thorough and proper sample collection, and selection of relevant diagnostic assays. Selection of the most appropriate diagnostic assay(s) can be optimized by the collaborative effort between the veterinarian and the diagnostician. The level of communication is often directly correlated with the complexity of the diagnostic case to be investigated. The diagnostic information generated through this process will be the foundation for the development of strategies to prevent and treat diseases as well as to guide the improvement of management practices.

Optimizing the value of the diagnostic process fundamentally begins with an objective and unbiased collection of detailed clinical information based on thorough observations of the environment and affected animals (Chapter 1; four-cycle approach) followed by the formulation of a well-defined diagnostic question. The environmental and clinical evidence collected as well as the diagnostic question should be included on the laboratory submission form to maximize information that will allow the diagnostician to thoroughly investigate the case. A straightforward and unambiguous diagnostic question is necessary to avoid potential misunderstandings and confusion that can affect diagnostic outcomes. The diagnostic question should dictate the diagnostic plan such as selecting the most appropriate sample type, number of sample(s) necessary to answer the question, and the most suitable diagnostic test(s) (Chapter 6). Common questions include but are not limited to diagnosis of clinical disease(s) in an individual animal, diagnosis of disease(s) in a population, detection of a pathogen(s) in an individual animal, detection of current pathogen(s) in a population, detection of prior exposure in an individual animal, detection of prior exposure in a population, estimation of prevalence (within a specific subgroup or other well-defined denominator) and estimation of incidence (number of cases over time divided by the population at risk), monitoring the presence of pathogens over time, estimation of efficacy of a particular intervention, and continuous improvement tools.

Evidence collected and shared by the veterinarian should paint a "clinical picture" and guide the diagnostician through the investigation process. Inclusion of information such as geographic location of site, animal age, detailed and accurate description of clinical signs, medication and vaccination history, feed and water source, and morbidity and mortality data is highly recommended. From a chronological perspective, the collection of information at the farm level should precede the formulation of the diagnostic question(s) and hypothesis regarding the presumptive causative agent (the final diagnosis may or may not be infectious). A failure to respect this order might lead to known cognitive biases such as confirmatory or selective bias. To illustrate this, the proper collection and quality of information, number and nature of samples, and diagnostic assays selected can be significantly influenced if the hypothesis has been subjectively constructed prior to gathering evidence. This process often occurs unconsciously and tends to confirm our own purported

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

hypothesis that may potentially lead to incorrect or partially correct diagnosis.

If necropsy is performed at the farm, veterinarians should provide an accurate description of macroscopic lesions preferably using standard pathology terminology. This should include organ(s) affected, chronicity of lesions, lesion distribution, the presence and type of exudate, and overall severity. For instance, animals suffering from influenza infection might demonstrate the following morphologic diagnosis: acute, cranial ventral, multifocal and lobular, pneumonia (Khatri et al. 2010). Macroscopic lesions are often not pathognomonic; however, gross pathology will help refine the differential diagnostic list. Furthermore, complete and appropriate description of clinical signs using proper medical terminology is highly desired as this could directly impact the course of the investigation chosen by the diagnostician. For instance, "central nervous system (CNS) signs" are commonly reported by veterinarians as a descriptor for clinical signs that include CNS, lameness, paralysis, or metabolic disease. However, these descriptions are vague and nonspecific. Investigation of hind limb paralysis in swine suffering from teschovirus-associated disease will require a completely different diagnostic process when compared with swine experiencing convulsions and paddling as observed in cases of bacterial meningitis or water deprivation/sodium toxicity. Both of these scenarios are often considered CNS clinical signs despite the marked differences in clinical presentation (see Table 19.5 for CNS terminology).

Unfortunately, the importance of these initial diagnostic processes are often overlooked and underestimated despite their essential nature. Thoroughly collecting all relevant clinical information will decrease the turnaround time of results, decrease unnecessary testing, and lower the economic impact of diagnostics. Furthermore, diagnostic results should always be interpreted in the clinical context that is provided by the veterinarian on the submission form.

Identification of diagnostically representative animals (individual vs. population medicine) and proper collection of samples

Once the clinical information has been collected and the well-defined diagnostic question has been formulated, sample selection and collection should proceed. The first major aspect to consider is the fundamental differences between individual and population medicine and how this could impact the interpretation of results. From a commercial swine perspective, the majority of diagnostics are conducted using a population medicine strategy. By definition, population medicine uses the diagnostic results generated from a group of individuals to make recommendations to the remainder of the herd with the ultimate goal of improving the health of the population. While this concept has been widely utilized and provides numerous advantages, proper selection of the individuals within the population to be sampled becomes crucial. Similarly, the diagnostic question should dictate which animals and sample types to be collected. If the goal is to diagnose clinical disease, the selection of acutely affected animals is recommended. For instance, if the objective of the diagnostic investigation is to determine why pigs are actively coughing, submission of samples from acutely affected and non-medicated animals is recommended. The stage of the disease process based on an individual or a population basis must be considered to optimize sample selection. The likelihood of pathogen detection or isolation is directly influenced by the stage of the disease process (acute, subacute, or chronic) and the pathophysiology of the pathogen to be investigated. For example, pigs with subacute or chronic cough due to influenza infection might lead to negative influenza PCR results despite the presence of histologic lesions (necrotizing bronchiolitis) suggesting a previous infection with an epitheliotropic virus. In this case, resubmission of samples including oral fluids from a population can dramatically increase the chances of detecting influenza virus given that this is the objective of the diagnostic investigation. Oral fluid samples represent a population that includes different animals at various stages of the disease process. In contrast, if the objective is to determine the seroprevalence or identify evidence of previous exposure through the detection of antibodies against a specific pathogen, samples must be collected after sufficient time has allowed the induction of detectable antibodies, or often after the virus or bacteria are no longer present in the affected animals. This dynamic is often agent dependent; additionally, different types of serologic assays for the same agent can also alter the dynamic of detection.

From an individual animal standpoint, the onset and duration of clinical signs as well as likelihood of pathogen detection can be influenced by the stage of the disease process as well as animal age, host immune status, route of infection, dose, pathogen strain (virulence factors), the presence of coinfections, and management practices. Clear understanding of clinical signs, lesion distribution, and pathogen transmission rates provides an opportunity to identify individual pigs that may be at different stages of the disease process. This allows a variety of sample types to be collected from within the population. In general, fever can be used as a reasonable predictor of success for detecting infectious agents in live pigs; if the pig has a fever, the odds of detecting and isolating the agent increase. Thus, if the objective is to

detect the agent, antigen, or genetic material, finding acutely affected, febrile pigs to sample is the first step. Selection of pigs prior to antibiotic treatment is essential if bacterial isolation is desired. In contrast, if the objective is to demonstrate antibodies, selecting pigs that are beyond the febrile stage is more likely to be successful. While selection of chronically affected animals might lead to a large spectrum of macroscopic and histologic lesions, diagnostic results often do not represent the herd problem and therefore should be avoided in the context of population medicine.

The biology of the etiologic agent must also be considered in the diagnostic planning. For example, *Clostridium difficile* is found exclusively in the large bowel; therefore sections of colon (large intestine) and colon contents are essential for an accurate diagnosis of *C. difficile* clinical disease. Similarly, influenza virus does not have the capability to spread systemically in swine; therefore submission of whole blood or serum for influenza RNA detection in a clinically affected animal will not be appropriate. Furthermore, when investigating an abortion outbreak where influenza is suspected, submission of sow blood or fetuses will not help with its diagnosis; nasal swabs or oral fluids from clinically affected sows are the recommended samples for influenza virus detection.

The endemic nature of an infectious agent adds another level of complexity when investigating clinical cases. The majority of agents causing disease in swine are ubiquitous and considered part of the normal microbiota at some point in the pig life. Therefore, mere identification (detection) of the agent does not necessarily indicate clinical disease and only suggests a possible diagnosis. Knowledge regarding the pathogen biology, ecology, and distribution within the environment and host must be considered when assigning clinical relevance. Characterization of macroscopic and histologic lesions, differentiation between strains of bacteria through the presence of virulence factors, and anatomic location from which the pathogen was detected should be taken into consideration when determining causation. For example, the pig intestinal tract contains approximately 800 different bacterial species (Looft et al. 2012) including some potential pathogens such as *Clostridium* sp., *Escherichia coli*, and *Salmonella* sp.; however, these agents will only cause clinical disease under certain circumstances. If the intent is to find virulent strains of *E. coli*, samples should be taken from clinically affected pigs rather than random fecal samples. Isolates can be further characterized by genotyping to detect the presence of toxin and attachment genes to help support potential clinical significance (Moon et al. 1999). Similarly, the upper respiratory tract is colonized by a complex and diverse microbiota. For instance, simply detecting or isolating *Haemophilus parasuis* or *Streptococcus suis* by PCR or routine culture, respectively, from the nasal cavity and nasopharynx does not confirm clinical relevance or suggest that they are involved in a disease process. Thus, infection does not guarantee clinical disease. If the purpose of an investigation is to find an *H. parasuis* isolate responsible for causing polyserositis or meningitis, cultures should be attempted from fibrinous exudates, affected joints, or the brain from clinical animals rather than lung parenchyma or nasal swabs (Oliveira 2004).

Diagnostic sample selection and collection

Antemortem

Antemortem diagnostic testing has increased in popularity in the swine industry as production systems consolidated and incorporate disease monitoring and surveillance programs. These programs track changes in agent detection over time, survey a population at a single point in time, or evaluate potential exposure. Depending on the sample type and test availability, antemortem diagnostic testing can be used to detect the presence of viruses, bacteria, or an immune response indicating previous exposure. The veterinarian should use caution when interpreting antemortem test results that may indicate the presence of a pathogen or antibody that suggests only a presumptive diagnosis. Further diagnostic testing with histopathology and ancillary tests are necessary to assign potential causation (see Chapter 8).

Common antemortem diagnostic samples used for swine include serum, oral fluid, nasal swabs, and fecal or rectal swabs. Serum serves the dual purpose for detection of systemic pathogens during acute infection or presence of antibodies after sufficient time has been allowed for seroconversion. Approximately 2–5 mL of serum is desired for most diagnostic tests, and subsequent retesting if needed. Depending on the laboratory, serum can be pooled in groups of five for some PCR assays without decreasing sensitivity (Gerber et al. 2013).

The use of oral fluids for diagnostic testing has dramatically increased over the previous decade due to their ease of collection, minimal welfare implications, and broad applicability for pathogen detection. Oral fluids are most often collected as a pen-based sample and represent a population unless swine were trained for individual collection (Ramirez et al. 2012). Oral fluids are validated for PRRSV antibody tests but have more broad application for pathogen detection assays such as PCR. It is important to check availability of current tests with each diagnostic laboratory. Again, caution is urged when interpreting antemortem test results from oral fluids. Using ancillary tests that are analytically sensitive, such as PCR, will detect pathogens considered common flora

in the respiratory tract as well as residual genetic material that has contaminated the environment from a previous infection.

Oral fluids are easy to collect using cotton rope suspended in the pen at a length accessible to the size of pig (shoulder length). Use 1.3 cm rope for nursery pigs and 1.6 cm rope for grow/finish/adult swine. Allow 20–30 minutes in active pens for pigs to chew on the rope and collect the oral fluid. Extract the fluid by squeezing the rope into a plastic bag so that the sample accumulates into one corner. Transfer the sample to a plastic falcon tube (approximately 4–5 mL) or use a conical tube with a secure cap. It is best to chill or freeze the sample as quickly as possible and transport to the laboratory using ice packs. Use 1 rope for each 150 animals and space the ropes evenly throughout the barn. Avoid extraneous contamination of the rope with feed, feces, or soil as this may affect PCR tests through inhibition of the assay.

Nasal swabs are most commonly used to detect viral agents that replicate in the upper respiratory tract such as influenza A virus (IAV) with less application for other respiratory agents such as PRRSV, *Mycoplasma hyopneumoniae*, and other bacteria. Snout wipes have also been used to detect IAV but may be less sensitive for most diagnostic tests and have increased chance of cross-contamination occurring between animals during collection. Nasal swabs should have a synthetic tip with a plastic shaft; if the goal is PCR analysis, avoid gel-based transport media that are only applicable for bacterial culture. Transport media should prevent the swab from desiccation and preserve the viability of the pathogen. After proper restraint and clearing the nasal planum of debris, insert the swab deep into the nasal cavity (at least into the middle third of the nose), rotate the swab gently, and withdraw. Return the swab to the transport media provided, chill, and prepare for shipment to the laboratory.

Collection of feces or rectal/fecal swabs is often appropriate to detect most enteric viral pathogens and can be used for routine bacterial culture. Swabs should be collected on an individual basis in sufficient numbers to account for the expected prevalence of the pathogen in the population. The same type of swab can be used for either nasal or fecal collection. After collection, swabs should be placed in a transport medium, chilled, and submitted to the laboratory.

Postmortem

Swine practitioners are among the few medical professionals who routinely perform postmortem examinations (necropsies) of their patients (King and Meehan 1973; Pinto Carvalho et al. 2008). The postmortem examination is a valuable opportunity for veterinarians to understand not only the disease processes involving the individual pig under examination but also to initiate the process of identifying emerging health threats. The postmortem examination is the first step in an inquiry that goes beyond "dissection and microscopic examination of tissues" to include a vast array of assays and techniques to find disease agents or evidence of their presence (Dada and Ansari 1996). It is important to develop a routine that is consistent, thorough, logical, and systematic. The routine will vary among individuals but should be consistent for an individual.

One means of developing a systematic approach is to have necropsy kits prepared in advance, including labeled specimen containers for complete sample selection and correct grouping of samples. Specimen containers can be pre-labeled using permanent markers. Another approach is to carry packets of preprinted labels that can be placed on the containers at the time of collection. The primary purpose of using labels is to provide the checklist function to ensure complete sample collection is performed.

Collection and submission of fresh samples from affected animals is essential for pathogen detection, isolation, and identification. Sample collection should focus on salient features or macroscopic lesions and should include a section of both affected and non-affected tissue. If gross lesions are not observed, select multiple but random samples from tissues that are related to the diagnostic question. Samples should be refrigerated immediately after collection and should not exceed 5 cm × 5 cm in dimeter. If needed, fresh samples can also be frozen. If possible, samples should be placed in individual bags that are tightly sealed to avoid cross-contamination. At the very least, organs such as the brain, spinal cord, lung, heart, liver, spleen, and kidney should be bagged separately from intestines or gastrointestinal content.

When submitting samples to the diagnostic laboratory, make sure the box is appropriately labeled and in accordance with the specific requirements of that country. In general, fresh samples should be accompanied by ice packs or immersed in dry ice. Amounts of ice packs or dry ice will vary depending of the mail system to be utilized, time of year (season), shipping distance, and time to arrival.

Formalin-fixed specimens are invaluable aids for assessing pathological significance of suspected disease processes or infectious insults. Histopathology is relatively inexpensive such that multiple tissues and lesions can be examined to confirm a role of suspected agents or disease as well as suggest the presence of alternative insults that have not been considered. For some disease processes, immunohistochemistry is a very useful diagnostic test for demonstrating a causative agent within compatible lesions.

Samples for histopathology should rarely exceed 1 cm in thickness to allow proper fixation. One hundred percent formalin is the aqueous solution of dissolved formaldehyde gas available as a 37% saturated solution in water. Tissue fixation is optimized in 10% formalin. Buffered formalin concentrates are commercially available that can be diluted with water to achieve a 10% neutral buffered formalin solution. Ten percent neutral buffered formalin can be prepared by combining ingredients in the following proportions: 900 mL distilled water, 100 mL 37% formaldehyde solution, 8.0 g NaCl, 4.0 g potassium phosphate monobasic, and 6.5 g potassium phosphate dibasic. Alternatively, non-buffered 10% formalin can be substituted using a 1 : 9 ratio of 37% formaldehyde solution (100% formalin) to water.

Sample selection for histopathology should focus on salient features or lesions that include a section of affected and non-affected tissue. Five sections of lung that includes the anterior and cardiac cranioventral lung, intermediate lung, and two sections from the caudodorsal lobe of lung are recommended. Lesions are often segmentally distributed in the small intestine and colon. Select 4–6 sections of the ileum and jejunum and 2–3 sections of the spiral colon. In general, one section of the liver, kidney, spleen, and heart should be sufficient for proper evaluation. Samples to be submitted for histopathology examination should not be frozen as this process will lead to the formation of artifacts that can potentially impact histopathology examination. Frozen samples can be used for culture and PCR testing.

Pig necropsy: external examination

It is of the utmost importance to select pigs that represent the herd issue to achieve an accurate final diagnosis. If the pigs are alive and to be euthanized, it is recommended to collect serum and whole blood prior to euthanizing if these samples are desired. Approved euthanasia methods must be chosen and administered carefully to ensure the safety of the people and the welfare of the animal.

An external examination of the skin, extremities, umbilicus, joints, oral cavity, upper respiratory tract, and the perineum are appropriate prior to conducting the necropsy. Assess and record the general body condition of the pig. Physical abnormalities or gross pathology should be described and ultimately guide the sample collection process that will complement the diagnostic investigation. The carcass and extremities such as the ears and legs can be evaluated for discoloration (pale, purple, red), necrosis, edema, or the presence of vesicles; when noted, these features should be considered when formulating the differential list as well as the selection of diagnostic assays to be performed.

Evaluation of the skin should include the anatomical location, size, shape, and descriptions of the type and amount of exudate. Common descriptors include but are not limited to confluent or raised, pustular or plaques, rhomboid, ulcerated, hyperkeratotic, vesicular, necrotic, or exudative. It is also appropriate to note the presence of abnormalities in the oral cavity, ocular or nasal discharge, palpebral edema, fecal staining, swollen joints, trauma/fractures, and hernias. Diagnostic specimens should be collected prior to the examination of internal organs to avoid cross-contamination. Sections of affected skin, ears, and whole legs with affected joints should be collected to preserve the integrity of the sample. Fresh and formalin-fixed skin samples are appropriate for bacterial culture, molecular assays, and histopathology examination. Whole legs or specific joints should be submitted chilled using ice packs with no further processing to prevent contamination and preserve tissue architecture.

Pig necropsy: internal examination

Entire texts have been published regarding the proper procedure to necropsy swine and help suggest a presumptive diagnosis of swine diseases (Guillamon and Jalon 2002). The following is a general guide to follow in concert with specific recommendations from the veterinary diagnostic laboratory. Individual laboratories and diagnosticians within laboratories may have preferences that differ from the recommendations provided here. Healthy dialogue with the diagnostic pathologist is always recommended.

The necropsy can be performed with larger pigs and adult swine lying on their left side or on their back. For smaller pigs, necropsy is usually conducted with the pig lying on its back. Make the initial incision by holding the foot of the right forelimb and abducting the limb while making a cut from the angle of the right jaw through the skin and axillary musculature and along the right ribs to reflect the right forelimb. Making the cuts through the skin from the subcutaneous tissues outward reduces the dulling of the knife. A second incision similarly reflects the right hind limb. Raise the foot to abduct the hind limb and cut starting at the right flank at the juncture of the leg with the abdomen through the interior thigh musculature and hip joint capsule (severing the ligament) posteriorly through the proximal ham to just lateral to the tail. Reflect the skin past the midline ventrally and dorsally by cutting the subcutaneous connective tissue and muscles. Examine and collect fresh and fixed samples of the inguinal lymph nodes at this time.

Open the abdominal cavity, being careful not to puncture any of the digestive tract, by making an incision along the posterior margin of the ribs. Start at the sternum and continue dorsally to the vertebral column. Continue the cut to the posterior limit of the abdominal

cavity and then continue ventrally to the midline. Make sure not to touch any abdominal organs at this time as to minimize contamination.

Thoracic cavity

The rib cage is opened next by one of several methods, depending on the size of the pig and the tools available. In immature pigs, the costochondral junctions between the ribs and the sternum can usually be cut fairly easily using a knife by cutting through the cartilaginous bone at the costochondral junction. Alternatively, if a pruning shears is available, the pruners can be used to snip the ribs individually along the sternum up to the ventral limit of the thoracic inlet. If the pruner is available, the ribs can be snipped along the vertebral column beginning at the last rib and extending to the dorsal limit of the thoracic inlet and the ribs can be cut free by incising the diaphragm posteriorly and the muscles and vessels at the thoracic inlet anteriorly. Alternatively, the ribs can be reflected individually or in groups of two or three by incising the intercostal muscles lengthwise between every one, two or three ribs, and then each section of ribs can be manually reflected back over the vertebrae to expose the thoracic cavity.

The ribs can be examined at this time for evidence of bone weakness or prior fractures. Assess bone density by trimming a single rib out entirely and bending it back against the greater curve of the rib manually until it breaks. In growing pigs the break should produce a pronounced snapping noise and sensation when the rib cracks. Lack of this snap sound indicates bone weakness and may suggest a mineral or vitamin imbalance that warrants further investigation. In adults, the ribs should be very difficult to break and produce a sharp snap if it can be broken manually. While metabolic or nutritional bone disorders are rare in pigs, this assessment is simple and inexpensive and has proven sensitive with practice. The practice gained by routinely snapping ribs from normal pigs provides the basis for comparison when bone density is low.

Determine which bone is preferred by your diagnostic laboratory to evaluate bone density. Submitting an entire unfixed long bone such as the second rib for mineral analysis and a fixed section of the costochondral junction of another rib is indicated if bone density is suspected. Ribs have been reported as more sensitive than other bones to dietary changes and thus present a more diagnostically useful sample (Walker et al. 1993). Submission of serum is recommended if vitamin D_3 analysis is desired.

For smaller pigs, the opening of the carcass is conducted with the pig in dorsal recumbency. Both forelimbs are reflected laterally using the same cutting procedures as described for larger pigs. At least one of the hind limbs is reflected as described previously. Then the thoracic and peritoneal cavities are opened by first making cuts through the skin on the lateral aspects of the ventral jawline and then using this skin as the starting point for a full-length bilateral sagittal section to reflect the ventral body wall caudally, maintaining the plane of the cut above the organs being exposed.

At this point the thoracic and abdominal cavities are entirely exposed. This represents an opportunity to evaluate the presence of macroscopic lesions and/or abnormalities *in situ,* which should be noted on the submission form. Prioritization of specific anatomical areas should be based on the clinical signs noted as well as the presence of macroscopic lesions; a different order of tissue examination and collection will follow to optimize sample quality and integrity.

The next step depends on the primary clinical problem to be addressed. In most cases, the necropsy will proceed with examination and collection of tissues from the thoracic cavity. However, if neurologic disease or lameness is the primary concern, there is merit in collecting samples to investigate these problems prior to contaminating equipment and surfaces with thoracic or other tissues.

Examination and sampling (Table 7.1) of thoracic organs involve removal of the tongue, trachea, and lungs by first extending incisions bilaterally along the medial aspects of the jawbones, extending from the angle of the jaw rostral to the apex. Using blunt dissection, the tongue is lifted ventrally from the jaw and carefully dissected away, noting the tonsils of the soft palate. Excise the tonsils and place one-half in 10% formalin and retain one-half unfixed. The trachea and esophagus are further exposed by pulling the tongue ventrally along with a combination of blunt dissection and careful trimming through to the thoracic inlet. The trachea can be incised and examined grossly at this time. The lungs and heart are then lifted out of the thoracic cavity with continued careful trimming, with final cuts through the esophagus and blood vessels at the diaphragm.

The mediastinal lymph nodes are located near the thoracic inlet and proximal to the bifurcation into the lungs. These and the submandibular and tracheobronchial lymph nodes should also be examined and collected if enlarged or hemorrhagic. When lesions appear similar in different lung lobes, 1–2 samples are sufficient. However, if there are areas that differ in texture, color, or appearance, each unique lesion should be sampled (fresh and fixed sections). Fixed and fresh section/samples should always include at least one section of major airway especially when IAV or *M. hyopneumoniae* is suspected. Lung lesions in pigs are often most severe in the cranioventral lobes. Color can be a deceiving indicator of pneumonia, especially with hypostatic congestion or even modest autolysis. Lung texture is a more reliable indicator of lung disease, with firm, rubbery, congested, or non-collapsing areas of particular interest. However, if gross

Table 7.1 Porcine respiratory disorders – specimen collection.

Tissue/sample	Fresh (preferred chilled than frozen)	Fixed (10% buffered formalin)
Serum	4–5 mL	
Swabs	Caudal nasal passage, turbinate, bronchial swab	None
BAL	~10 mL	None
Nasal turbinate	2 × 2 × 2 cm	1 cm thickness
Lung	6 × 6 × 6 cm – 2–5 sections per pig with visible airways	Three pieces per pig from affected areas with different gross appearances (2 × 2 × 1 cm)
Lymph nodes	Mandibular, mediastinal, tracheobronchial, mesenteric, and superficial inguinal	Mandibular, mediastinal, tracheobronchial, mesenteric, and superficial inguinal
Tonsil	½	½
Heart	4 × 4 × 4 cm piece	2 × 2 × 1 cm including L and R ventricles and septum
Liver	4 × 4 × 4 cm piece	2 × 2 × 0.5 cm
Kidney	Half of a kidney	0.5 cm slice through center
Spleen	5 cm piece	1 cm thickness

Animal selection – three euthanized pigs with representative clinical signs, acutely affected, and untreated (if available) or three freshly dead pigs.
Sample submission – package and identify specimens from pigs individually.
BAL, Bronchoalveolar lavage.

lesions are not present, a random sample of five sections of lung is appropriate for a thorough histological evaluation of the lung.

Collecting samples for histologic examination was described previously, but in the specific case of the lung, there is an added caution to handle the tissues very gently during examination and sample collection. If too much pressure is applied to the lung prior to fixation, histologic examination is compromised due to the compression of airways and alveolar spaces.

Fresh lung samples should be of sufficient size to allow for bacterial culture in addition to other tests (PCR, virus isolation, etc.). Generally, unfixed pieces that are at least 5 cm cubes are adequate. Fixed and fresh lung samples should include pleura. If septicemia (Table 7.2) or pleuritis is present, affected areas of the lung should be sampled specifically.

The heart is examined by first cutting through the pericardium to expose the heart. Collect excess or abnormal pericardial fluid in a syringe, if present. Fibrinous exudates can be collected with swabs. Examine the surface of the heart for evidence of inflammation or hemorrhage. Incise both ventricles and the septum and examine the valves for inflammation and the muscle for hemorrhage or fibrosis. If indicated by clinical or pathological examination, collect sections of any lesions as well as septum, papillary muscle, and right and left ventricles for fixation and an unfixed piece of ventricle.

Abdominal cavity

The location of intestinal organs should be noted so as to make sure no volvulus or torsions are present. The spiral colon should be located in the pig's left side with the apex of the cecum on the pig's right side. If indicated, fibrinous exudates can be collected with swabs and urine with a syringe. Solid organs including liver, spleen, lymph nodes, and kidneys are sampled next, collecting fixed and fresh samples. In adult animals, the female reproductive tract and bladder can be examined for gross pathology as routine sample collection of these organs is not typically included. The ovaries are also evaluated grossly for abnormalities as well as matching the findings to the animal's recorded stage in the reproductive cycle. Intestinal lymph nodes (especially the mesenteric and gastrohepatic) should be examined next for size, texture, and the presence of hemorrhage.

When enteric disease is suspected (Table 7.3), longitudinal incisions of multiple sections of small and large intestine to examine mucosa should be routine. The ileum is the next section sampled. The ileum is easily located by finding the cecum and following the ligament to the ileum (Figure 7.1). Fixed samples (3–4 sections) are collected from the proximal and distal portions of the ileum, including a 15 cm section of fresh intestinal segments for bacterial culture. The jejunum and duodenum are sampled similarly, along with all areas with suspected lesions.

Fixed and fresh samples are also collected from the cecum (optional) and spiral colon (2–3 sections). When sampling the spiral colon, samples should be taken from the proximal, middle, and distal portions. A short cut into the lumen of the descending colon or rectum will help determine fecal consistency at the time of death.

Table 7.2 Porcine septicemia – specimen collection.

Tissue/sample	Fresh (preferred chilled than frozen)	Fixed (10% buffered formalin)
Serum	5 mL	None
Whole blood	3 mL in EDTA	None
Swabs	Brain, epicardium, joint (periarticular tissue including synovium), fibrin	
Cerebrum,[a] cerebellum, and brain stem	Cut brain in half longitudinally, slightly off the midline. Submit larger half fresh/chilled	Fix the smaller half in formalin
Lung	6 × 6 × 6 cm – two sections per pig with visible airways	Three pieces per pig from affected areas with different gross appearances (2 × 2 × 1 cm)
Heart	4 × 4 × 4 cm piece	2 × 2 × 1 cm including L and R ventricles and septum
Liver	4 × 4 × 4 cm piece	2 × 2 × 0.5 cm
Kidney	Half of a kidney	0.5 cm slice through center
Spleen	5 cm piece	
Lymph nodes	Mandibular, sternal, tracheobronchial, mesenteric, and superficial inguinal	Mandibular, sternal, tracheobronchial, mesenteric, and superficial inguinal
Ileum	10 cm segment	2 cm segment

Animal selection – three euthanized pigs with relevant clinical signs, acutely affected, and untreated (if available) or three freshly dead pigs.
Sample submission – package and identify specimens from pigs individually.
[a] Submission should include representative sections of the cerebrum (frontal, parietal, occipital, and temporal cortices), brainstem (midbrain, pons, and medulla), and cerebellum.

Table 7.3 Porcine diarrhea – specimen collection.

Tissue/sample	Fresh (preferred chilled than frozen)	Fixed (10% buffered formalin)
Serum	5 mL	None
Oral fluid	5 mL	None
Swab	Rectal swabs, environmental swabs	
Tonsil	½	½
Liver	4 × 4 × 4 cm piece	2 × 2 × 0.5 cm
Stomach	3 × 3 × 3 cm piece	1 cm thickness
Spleen	5 cm piece	1 cm piece
Jejunum	10 cm segment	Three sections, 2 cm long, unopened
Ileum	10 cm segment	Three sections, 2 cm long, unopened
Mesenteric lymph node	Entire lymph node	1 cm thickness
Spiral colon	Approximately ¼ to ½ of colon bagged separately from small intestine	Three sections, 2 cm long, unopened
Fluid contents from cecum or colon	In leakproof container	

Animal selection – three euthanized pigs with relevant clinical signs, acutely affected, and untreated (if available) or three freshly dead pigs.
Sample submission – package and identify specimens from pigs individually.
Note: Ideally, fix intestines within 15 minutes of death for best preservation.

Once all abdominal organs have been sampled, the stomach can be opened. This is done by cutting along the greater curvature. Presence or absence of feed is noted as well as presence or absence of ulcers or hemorrhage. In pigs, gastric ulcers are located in the non-glandular portion of the stomach, also known as the pars esophagea. The pars esophagea should be carefully examined to rule out gastric ulcers. The stomach is rarely sampled for routine diagnostic purposes.

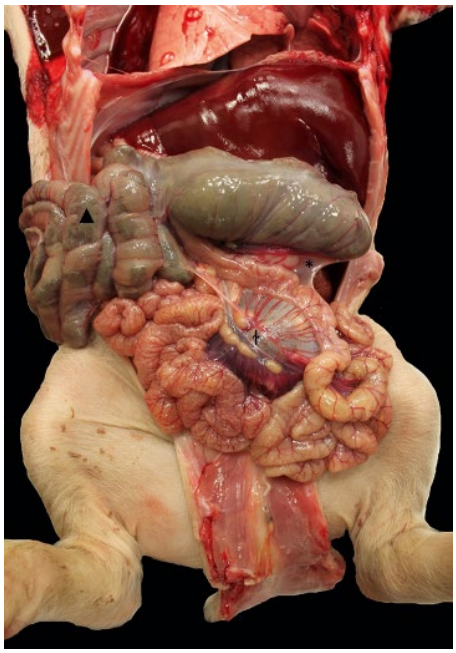


Figure 7.1 Pig necropsy. Pig lying on his back after the front and back legs have been reflected. Visualization of the gastrointestinal organs – ileum, ileocecal ligament, spiral colon, cecum, and mesenteric lymph nodes. ▲Mesocolon. *Ileocecal ligament – cecum is located in the cranial portion and ileum located ventrally. ‡Mesenteric lymph nodes.

Table 7.4 Porcine abortion – specimen collection.

A. *Optimum specimens*, chilled

1) At least three intact fetuses and placenta each from aborted litters or different parities – including the freshest fetus
Note: If there are mummified fetuses, submit nine mummies – three smallest, three medium, and three largest (freezing the fetuses is acceptable if they cannot be sent immediately to the laboratory)
2) Sow serum (5 mL) – If attempting to diagnose PRRSV, sera are best collected when sows are acutely affected (off-feed and febrile)
3) Nasal swabs or oral fluid from sows with aborted litters and/or showing respiratory signs

B. *Alternate specimens* – Note: Pooling tissues from multiple fetuses is acceptable

Tissue/sample	Fresh (preferred chilled than frozen)	Fixed (10% buffered formalin)
Heads	3	
Thoracic fluid	2 mL	
Stomach content	3 mL	
Lung	3	Three samples: 1 cm × 1 cm × 1 cm
Heart	3	Three samples: 1 cm × 1 cm × 1 cm
Liver	3	Three samples: 1 cm × 1 cm × 1 cm
Kidney	3	Three samples: 1 cm × 1 cm × 1 cm
Spleen	3	Three samples: 1 cm × 1 cm × 1 cm
Placenta	3	Three specimens: 3 cm × 3 cm
Superficial inguinal lymph nodes	3	Three samples
Mummies (intact; if available)	9 (see above)	

Collection of reproductive and abortion tissues

Porcine abortions can be primarily divided as follows: (1) sporadic abortions and (2) abortion storm outbreaks. Determination of the primary cause or pathogen leading to abortion is difficult particularly in the sporadic events, although diagnostic tests are routinely utilized to rule out an infectious etiology or an occasional noninfectious cause. The majority of abortions are considered idiopathic; this occurs when diagnostic tests fail to detect an etiologic agent and histopathology is unremarkable.

Diagnostic efforts often focus on the aborted fetuses particularly when dams are not showing clinical signs. It is recommended to submit 4–6 fetuses per litter from at least 3 affected litters (Table 7.4). When mummified fetuses are present, submission of three from each of the smallest, medium, and largest mummies is recommended. Necropsy of neonatal piglets, aborted fetuses, or mummified fetuses should be performed as described for nursery, grower, or finisher swine. Measuring the crown-rump length of the smallest to largest fetus in a litter will provide a rough estimation of gestation length. Gross pathology is often absent in aborted fetuses. However, it is important to recognize and note any lesion or abnormality that should be included in the history and signalment. Fresh and formalin-fixed lung, heart, liver, kidney, and spleen should be submitted for molecular assays, histopathology, and bacteriology to rule out the primary differentials of abortion that include PRRSV, PCV2, porcine parvovirus, leptospirosis, ascending bacterial infection, and bacterial septicemia. Placenta, when available, is an important diagnostic specimen to include in all abortion investigations. In addition, fetal thoracic fluid and stomach content are important diagnostic samples for ancillary tests and bacteriology, respectively. Although mummified fetuses are rarely useful for histopathological evaluation, fresh samples are necessary for parvovirus diagnostic assays. All samples should be chilled prior to shipment on ice packs.

It is important to focus diagnostic efforts on the dam when clinical signs are observed and systemic or localized illness is suspected. Anorexia, lethargy, fever, or respiratory signs such as coughing will help determine the appropriate sampling strategy for a diagnosis.

Collecting serum from acutely affected dams is appropriate to detect systemic agents such as PRRSV (PCR is the most appropriate diagnostic assay in this circumstance). Nasal swabs or oral fluids are appropriate when respiratory disease is observed. Submitting samples from both affected dams and fetuses is recommended when clinical signs are suggestive of maternal illness. Agents such as IAV are not systemic and will not be detected in fetal tissues; samples from affected dams are essential to achieve an accurate diagnosis. Serum and nasal swabs should be chilled as soon as possible after collection and submitted on ice packs during transit to the laboratory.

Collection of CNS tissue

In cases where neurologic signs are the primary complaint, submission of brain and spinal cord is essential (Table 7.5). Neurologic tissue is highly specialized and structurally and anatomically complex. In general terms, the brain is divided into four major regions: cerebrum, cerebellum, diencephalon, and brainstem. These anatomic locations have different functions and when affected will lead to different clinical signs. For instance, in cases of teschovirus-associated disease, animals often show loss of coordination and posterior paralysis but remain alert and responsive to environmental stimuli. As a result, the frontal cortex might have minimal diagnostic value; lesions are concentrated primarily in the spinal cord and brain stem. Proper sample selection is essential to reach an accurate diagnosis. To exemplify, when investigating cases of edema disease caused by Shiga toxin-producing *E. coli,* submission of approximately 6 sections of small intestines is crucial as the bacteria do not go systemic and are not evenly distributed in the intestine. Lesions and clinical signs are a consequence of the effects of the Shiga toxin toward endothelial cells lining blood vessels within the nervous tissue (Boyd et al. 1993). Additionally, the stage of disease process plays an important role in pathogen detection; submission of an acutely affected animal is highly advised.

Submission of brain swabs in addition to three to four formalin-fixed sections of the cerebrum and cerebellum is recommended to properly diagnose a bacterial meningitis such as *S. suis* and *H. parasuis* infection. The atlas–axis junction can be exposed in pigs lying on their back and tilting the head dorsally while incising just below the pharyngeal area. The spinal cord is exposed as the head is tilted more acutely, and a swab can be inserted directly into the foramen magnum and pushed back into the brain cavity until it stops (Figure 7.2a). The swab will stop at the junction of the cerebrum and cerebellum, a common site for recovering bacteria that cause meningitis in pigs. The first cervical vertebrae can then be cut off with the spinal cord in place for histologic examination. This can be cut using a knife through the intervertebral disk or by use of a pruner shears or a hacksaw. Submit the swab for bacteria culture and the vertebrae with spinal cord fixed in formalin.

The brain is removed by first skinning the skull in the area bounded by the eyes rostrally, the ears laterally, and the foramen magnum caudally. The calvaria is removed by making a circle of cuts through the skull from the occipital condyle to the base of the ear, from the base of the ear to the eye socket, across the skull to the other eye socket, back to the base of the other ear, and finally back to the occipital condyle. These cuts can be made using a saw or hatchet, depending on availability and preference. The calvaria is pried free, exposing the meninges and brain, which can be dissected free by cutting cranial nerve attachments. The brain can be split longitudinally, with half preserved in formalin and half submitted unfixed.

In younger pigs, the skull can be split using a necropsy knife that is thrust through the nasal cavity and extending the knife to the apex of the mandible followed by inserting the knife with blade facing distal and extending the atlas–axis junction or back of the head. Leverage is applied carefully to the knife handle to cut through the skull longitudinally, and the brain can be cut loose from the cranial nerves as previously described.

For cases in which some or all of the vertebral column is needed, remove the entire pluck and offal from the carcass and trim away the muscle from the region to be sampled. If thoracic vertebrae are needed, the ribs can be disarticulated from the spine by cutting with a knife or pruner. If only cervical or lumbar vertebrae are needed, these sections can be cut away from the thoracic vertebrae with a knife, saw, or pruner and submitted intact. Alternatively, the spinal cord can be excised *in situ* using a Barnes dehorner as a bone rongeur to scoop out the ventral portion of the vertebrae to expose the vertebral canal (Figure 7.2b–d). Spinal ganglia will be exposed using this process and should be collected. Severe lymphoplasmacytic ganglioneuritis can be observed in animals suffering from teschovirus-associated encephalomyelitis (Yamada et al. 2014). Ideally, sections of the cervical, thoracic, and lumber regions should be collected (half fresh and half formalin fixed). Alternatively, selection of spinal sections can be targeted based on clinical assessment. Spinal cord samples should be aseptically collected as pathogens such as teschovirus and sapelovirus can be endemic within feces (Arruda, et al. 2017; Buitrago et al. 2010; Cano-Gomez et al. 2013; Chen et al. 2012), making interpretation of results difficult.

Locomotor tissue: arthritis and lameness sampling

If arthritis or lameness is the primary concern, sampling joints prior to taking thoracic or abdominal tissue samples is preferred to avoid contamination of joint samples with endemic bacteria that may be present as normal microflora within other tissues. The important step for

Table 7.5 Porcine neurological disorders and septicemia – specimen collection.

Tissue/sample	Fresh (preferred chilled than frozen)	Fixed (10% buffered formalin)
Serum	8 mL	
Swabs of: 1) Meninges 2) Brain	1) Swab meninges immediately after exposed 2) Swab one cerebral hemisphere into a lateral ventricle	None
Cerebrum,[a] cerebellum, and brain stem	Cut brain in half longitudinally, slightly off the midline. Submit larger half fresh/chilled	Fix the smaller half in formalin
Spinal cord[b]	5 cm segments of the cervical, thorax, and lumbosacral regions	5 cm segments of the cervical, thorax, and lumbosacral regions
CSF	3 mL	None
Lymph nodes[c]	½ of the sternal, tracheobronchial, mandibular, superficial inguinal, and mesenteric enlarged lymph nodes	½ of the sternal, tracheobronchial, mandibular, superficial inguinal, and mesenteric enlarged lymph nodes
Lung	5 × 5 × 5 cm pieces. 3–5 pieces with macroscopic lesions	2 × 2 × 1 cm sections (2 or 3) – include gross lesion if present
Pleura or pericardium	Swabs, pericardium fluid, fibrin (if present)	1 × 1 × 1 cm
Heart	4 × 4 × 4 cm section	2 × 2 × 1 cm section
Liver	4 × 4 × 4 cm piece	2 × 2 × 0.5 cm
Kidney	Half of a kidney	0.5 cm slice, including cortex and medulla
Spleen	5 cm piece	1 cm piece
Jejunum	10 cm segment	Two sections, 2 cm in length
Ileum	10 cm segment	One section, 2 cm in length

Animal selection – three euthanized pigs with relevant clinical signs, acutely affected, and untreated (if available) or three freshly dead pigs.
Sample submission – package and identify specimens from pigs individually.
CSF, cerebrospinal fluid.
[a] Submission should include representative sections of the cerebrum (frontal, parietal, occipital, and temporal cortices), brainstem (midbrain, pons, and medulla), and cerebellum.
[b] Collection of enlarged lymph nodes should be preferred regardless of anatomical location.
[c] Aseptic collection of spinal cord is highly recommended.

obtaining optimal joint sample begins with an aseptic and systematic technique; thorough examination of all accessible joints, including the carpus, shoulder, elbow, stifle, and hock, is also highly recommended.

Obtaining clean samples from joints can be a challenge in field settings. As a result, some practitioners prefer to submit intact joints for careful and proper dissection at the diagnostic laboratory. This approach can be successful, but some bacteria that cause infectious arthritis are fastidious and less likely to be cultured a day or two after joint samples are collected. Therefore, careful dissection of joints and collection of joint fluid and tissue in the field can increase the likelihood of isolation and/or detection of the pathogen(s) involved.

Joint fluid can also be aspirated with a needle and syringe once the skin is reflected or percutaneously if the skin has been aseptically prepared (Figure 7.3a and b). The joint fluid can be submitted for culture, molecular testing, (PCR) and clinical pathology. Antemortem joint fluid collection under short-term injectable anesthesia is also a useful tool especially in populations where necropsy is not an option. More details regarding investigation of lameness in swine and collection of diagnostic material can be found in Canning and Karriker (2016). Recently, clinical pathology reference intervals have been created for commercial finisher hogs and will be an additional tool available for practitioners (Canning et al. 2016). Changes in clinical pathology parameters such as protein, pH, and total nucleated cell counts can provide practitioners with evidence of inflammatory changes within the joint. As infectious pathogens often have a temporary residence within the joint, clinical pathology will be helpful in providing context to PCR and culture results. It is important to examine multiple joints from pigs suspected of having lameness or arthritis problems. Specific joints of interest may depend on the age of the pig and clinical signs present.

Clean samples can also be obtained from joints by first reflecting the skin away from the joints to be sampled and then using a new #22 scalpel blade to trace the outline of the bones through the joint capsule. Making

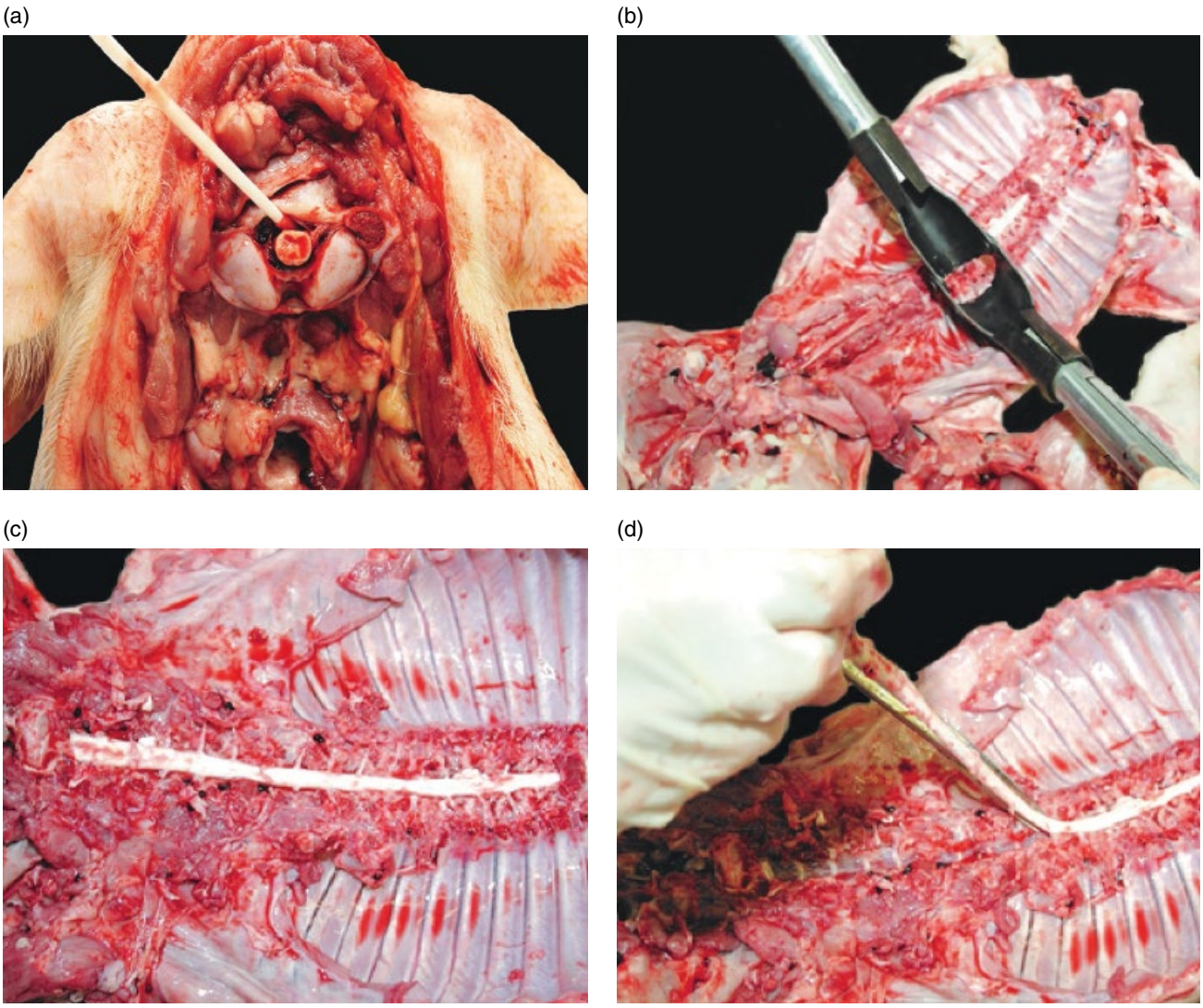


Figure 7.2 Illustration of brain swab and spinal cord collection. (a) Collection of a brain swab prior to extracting the brain tissue. Expose the atlas–axis junction and insert the swab directly into the foramen magnum. (b) Collection of spinal cord *in situ* using a Barnes dehorner. (c) Spinal ganglia can also be viewed and collected during this process. (d) Removal of the spinal cord for fresh and fixed sample collection.

this cut is simpler on the medial aspect of the joint where less muscle is present. The clean scalpel blade is less likely to contaminate the joint than the necropsy knife. Leverage applied to the distal portion of the limb exposes the joint so that fluid and joint sample materials can be collected (Figure 7.3c and d). With practice, each joint can be opened with minimal cutting and potential for contamination. Once exposed, examine the synovial tissue, synovial fluid, and cartilage for abnormalities. Joint swabs should be collected for bacteriology and/or molecular assays (Figure 7.3c). In addition, synovial tissue should be collected for histologic examination (Figure 7.3d). Histologic examination plays an essential role in the interpretation of PCR and culture results from joint fluid. Similar to the investigation of other tissues, test results from joints should always be interpreted in a clinical context. Additionally, identification of macroscopic and histologic lesions should add confidence to the causative role of potential agent detected. For instance, isolation of *S. suis* from joint swabs of pigs with no clinical history suggestive of a bacterial sepsis nor synovial histologic lesions should be interpreted with caution.

Necropsy safety

Safety is always a concern when performing postmortem examinations on pigs. Pigs harbor microorganisms that can potentially infect humans (Tucker 2006), floor

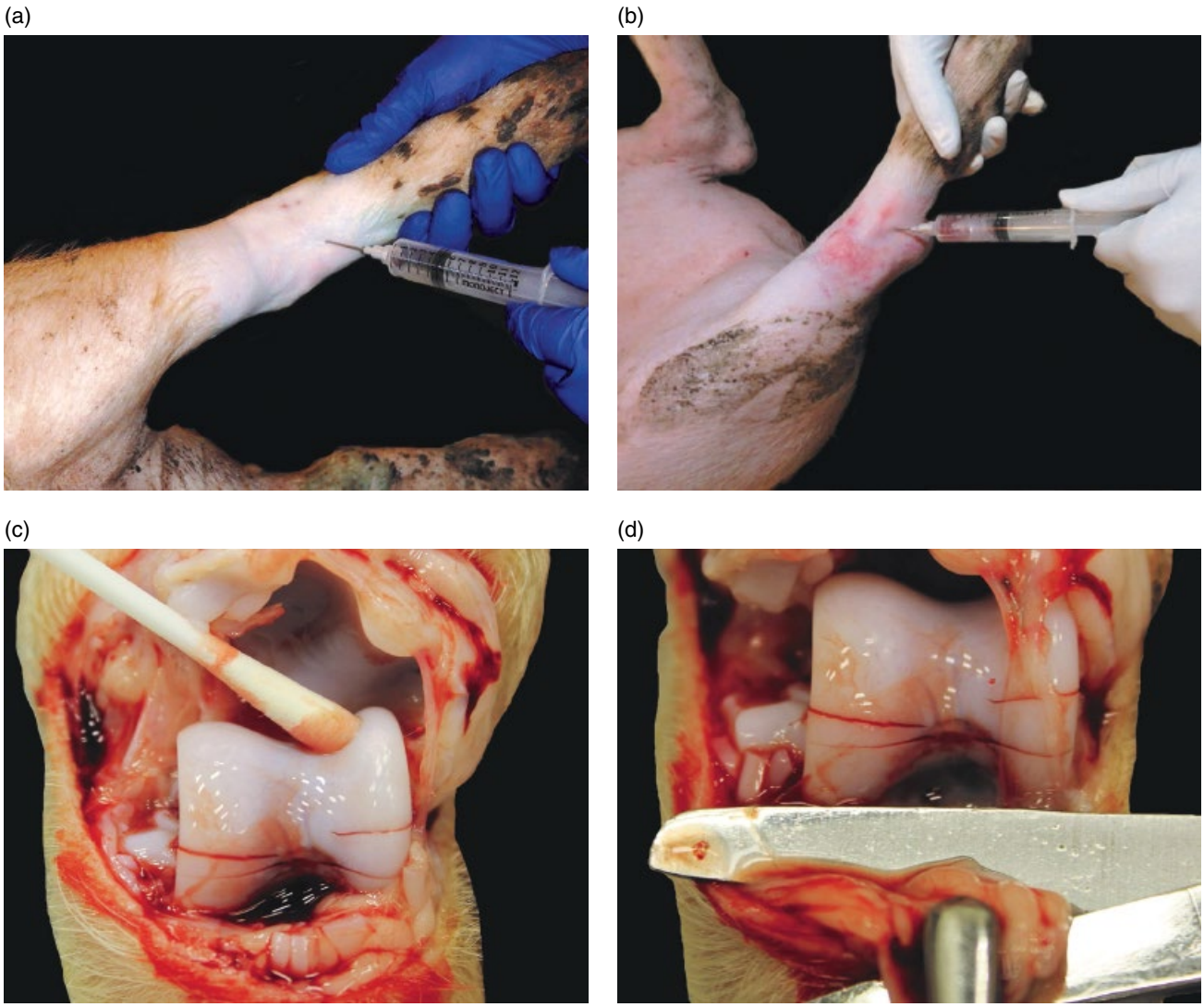


Figure 7.3 Collection of synovium fluid, joint swab, and synovium tissue. (a, b). Collection of synovial fluid prior to exposing the joint cavity. Collection can be performed from either the dorsal (a) or lateral side (b) of the joint. (c) Complete exposure of the joint cavity for macroscopic examination and swab collection. (d) Collection of synovial tissue for histopathologic examination.

surfaces can become slippery from body fluids, and the sharp tools used in the course of the examination can cut the prosecutor as well as the pig. The environment in which field necropsies are performed is often not ideal from a safety standpoint, so care should be taken to reduce the risk of injuries when planning postmortem examinations. Personal protective equipment should be worn that is appropriate for the risk posed by the examination. At the very least, waterproof gloves should always be worn to reduce the risk of skin contamination with body tissues and fluids.

Safe knife handling is obviously important during the necropsy. Most knife injuries can be avoided by adhering to three knife safety rules: (1) always cut away from yourself, (2) use your knife only as a knife, and (3) keep your knife sharp. These three points might seem obvious, but practitioners often find themselves violating one or more of these to save time or make do with the available resources, leading to avoidable injuries. Using a postmortem knife for purposes other than a pig necropsy can damage the knife and the operator. Using a sharp knife is safer because cutting time and force are reduced, which decreases fatigue and the probability that the blade will slip on the surface being cut. There is also less opportunity for the operator's hand to slip off the knife handle (McGorry et al. 2003).

Keeping track of knives and other sharp instruments is also important and can be a challenge during an extensive necropsy. Storing the knife by inserting the blade into large muscles in the carcass when not in use during the necropsy saves time and prevents injuries.

Conclusion and final comments

In summary, the diagnostic process should start with the accurate assessment of the primary complaint and formulation of a well-defined diagnostic question. Formulating a well-defined diagnostic question is imperative to additional steps in the diagnostic process that includes sample selection, proper collection, adequate preservation, and secure submission. The investigation should be based on the unbiased and objective collection of accurate history, signalment, and clinical observations, which should be included in the submission form to be sent to a diagnostic laboratory. Following an assessment of the clinical scenario, representative animals must be selected for necropsy that includes the identification and documentation of gross pathology that may or may not be associated with the primary problem. A systematic process including the selection of the most appropriate animals, sample types, and numbers of samples is essential to achieve a final accurate diagnosis that should align with the clinical assessment. Diagnostic data generated through this process should provide the empirical foundation necessary to the implementation of strategies needed to address the issues of concern.

References

Arruda PH, Arruda BL, Schwartz KJ, et al. 2017. *Transbound Emerg Dis* 64(2):311–315.

Boyd B, Tyrrell G, Maloney M, et al. 1993. *J Exp Med* 177:1745–1753.

Buitrago D, Cano-Gomez C, Aguero M, et al 2010. *J Vet Diagn Invest* 22: 763–766.

Canning P, Karriker L. 2016. Diagnostic considerations for infectious lameness. In Ontario Association of Swine Veterinarians Fall Conference Proceedings.

Canning P, Karriker L, Viall A, et al. 2016. Diagnostic considerations for Mycoplasma Hyosynoviae and lameness in growing pigs. In Allen D. Leman Swine Conference.

Cano-Gomez C, Garcia-Casado MA, Soriguer R, et al. 2013. *Vet Microbiol* 165:115–122.

Chen J, Chen F, Zhou Q, et al. 2012. *J Virol* 86:10898.

Dada M, Ansari N. 1996. *J Clin Pathol* 49:965–966.

Gerber PF, O'Neill K, Owolodun O, et al. 2013. *J Clin Microbiol* 51(2):547–556.

Guillamon M, Jalon J. 2002. *A Guide to Necropsy Diagnosis in Swine Pathology*. Greenfield, IN: ELANCO Animal Health.

Khatri M, Dwivedi V, Krakowka S, et al. 2010. *J Virol* 84,11210–11218. doi: https://doi.org/10.1128/JVI.01211-10.

King L, Meehan M. 1973. *JAMA* 73:514–544.

Looft T, Johnson TA, Allen HK, et al. 2012. In-feed antibiotic effects on the swine intestinal microbiome. *Proc Natl Acad Sci U S A* 5:1691–1696.

McGorry R, Dowd P, Dempsey P. 2003. *Appl Ergon* 34:375–382.

Moon H, Hoffman L, Cornick N, et al. 1999. *J Vet Diagn Invest* 11:557–560.

Oliveira S. 2004. *J Swine Health Prod* 12:308–309.

Pinto Carvalho F, Cordeiro J, Cury P. 2008. *Pathol Int* 58:568–571.

Ramirez A, Wang C, Prickett JR, et al. 2012. *Prev Vet Med* 104:292–300.

Tucker A. 2006. *Pig J* 57:178–191.

Walker GL, Danielson DM, Peo ER Jr, et al. 1993. *J Anim Sci* 71: 3003–30010.

Yamada M, Miyazaki A, Yamamoto Y, et al. 2014. *J Comp Pathol* 150: 276–286.

8

Collecting Evidence and Establishing Causality

Eric R. Burrough, David H. Baum, and Kent J. Schwartz

Introduction

Assigning cause (or blame) for an event is a seemingly visceral compulsion of humans. Assigning cause by providing a diagnosis is a core competency of swine veterinarians, often aided by laboratory testing, but it bears noting that establishing causation is not as simple as obtaining a test result. A single test result may suggest cause for an epidemic of new disease related to introduction of a single agent and can be relatively straightforward. However, in modern swine production, diseases are often multifactorial involving multiple infectious agents and risk factors. The expression of endemic potential pathogens as disease is often associated with noninfectious risk factors or compromised herd immunity. Disruption of herd immunity, incomplete elimination protocols, or early weaning stressors can lead to disease epidemics from previously subclinical infections such as Glasser's disease or mycoplasmosis. Management and environmental risk factors also predispose to disease expression. For example, changes in piglet processing can result in expression of inguinal abscesses and umbilical hernias, or changes in pig flow and commingling can exacerbate systemic viral or bacterial disease expression. In those cases, the identification of a specific infectious agent as cause of a specific lesion or disease can appropriately be considered as "proximate cause." Veterinarians are well trained and highly adept at identifying proximate cause(s). A broader view would be to seek and identify those risk factors that underlie or contribute to disease expression, most appropriately viewed as "ultimate cause(s)," which typically are population or herd-level diseases or risk factors. Ultimate causes are often the focus of intervention for disease control, prevention, or elimination through appropriately applied intervention or management practices. Therefore, causality in swine production is a synthesis of both proximate cause (the infectious agent or specific insult type associated with lesion or disease state) and ultimate cause (risk factors leading to disease expression). Both are important for deriving appropriate interventions for sustainable production.

Accuracy in assigning causation is a matter of confidence in the quality and interpretation of all available evidence within the context of the presenting clinical scenario. An awareness of laboratory, experiential, institutional, and personal sources of bias is prudent if accuracy and scientific confidence is desired in assigning cause. Accuracy is enhanced when a diagnosis is deduced with due consideration of all available evidence, a process that uses evidence to limit possibilities. In contrast, diagnosis by inductive reasoning is extrapolation of a diagnosis from limited evidence, such as assigning cause from a single diagnostic test or report without the context of relevant history and/or clinical signs and data. The nature of biology is that there is will always be some level of uncertainty in the data used to assign a final diagnosis. The presence of an insult type in an individual or population can be confirmed with near certainty, but mere presence is not always sufficient to assign causation. Multiple insult types and risk factors can affect the extent of disease expression. Diligence is required to not interpret association as causation. Diagnosis by exclusion or having a negative test for an insult type in an individual or population will always have a level of uncertainty as it is not possible to prove a negative.

These concepts are essential to integrate purposefully into collection of evidence and establishing causality using objective clinical skills and current scientific knowledge as a foundation for diagnostic investigation. In simple terms, and above all else, a proper diagnostic investigation must begin with one or more well-defined questions or specific objectives. All subsequent aspects of the investigation, including animal selection and sampling, the number of samples required, and the selection of particular diagnostic tests with estimates of diagnostic sensitivity and specificity, are wholly dependent upon the clinical context and specific questions raised.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Determining if the goal of diagnostic testing is for confirming diagnosis of a clinical disease or is for pathogen monitoring or surveillance is a critical decision in the process and might best be viewed dichotomously. It is like a fork in the road where the sampling approach commits to one or the other with specific efforts to increase confidence that the diagnostic question can and will be answered (Figure 8.1).

There are times when a practitioner might want to determine both causality (a disease diagnosis) and get an estimate of potential related coinfections (pathogen monitoring/surveillance); however, these situations will likely require two different sets of purposefully collected samples to accurately answer these two separate objectives. While pathogen monitoring can take many forms, pathogen surveillance requires some specific quantity of a sample type to estimate presence and prevalence of a pathogen in a population at a calculated level of confidence. Disease diagnosis generally relies on a complete and broad set of samples from typically affected animals to determine relative contribution of a pathogen or pathogens to disease expression with some level of confidence. Often,

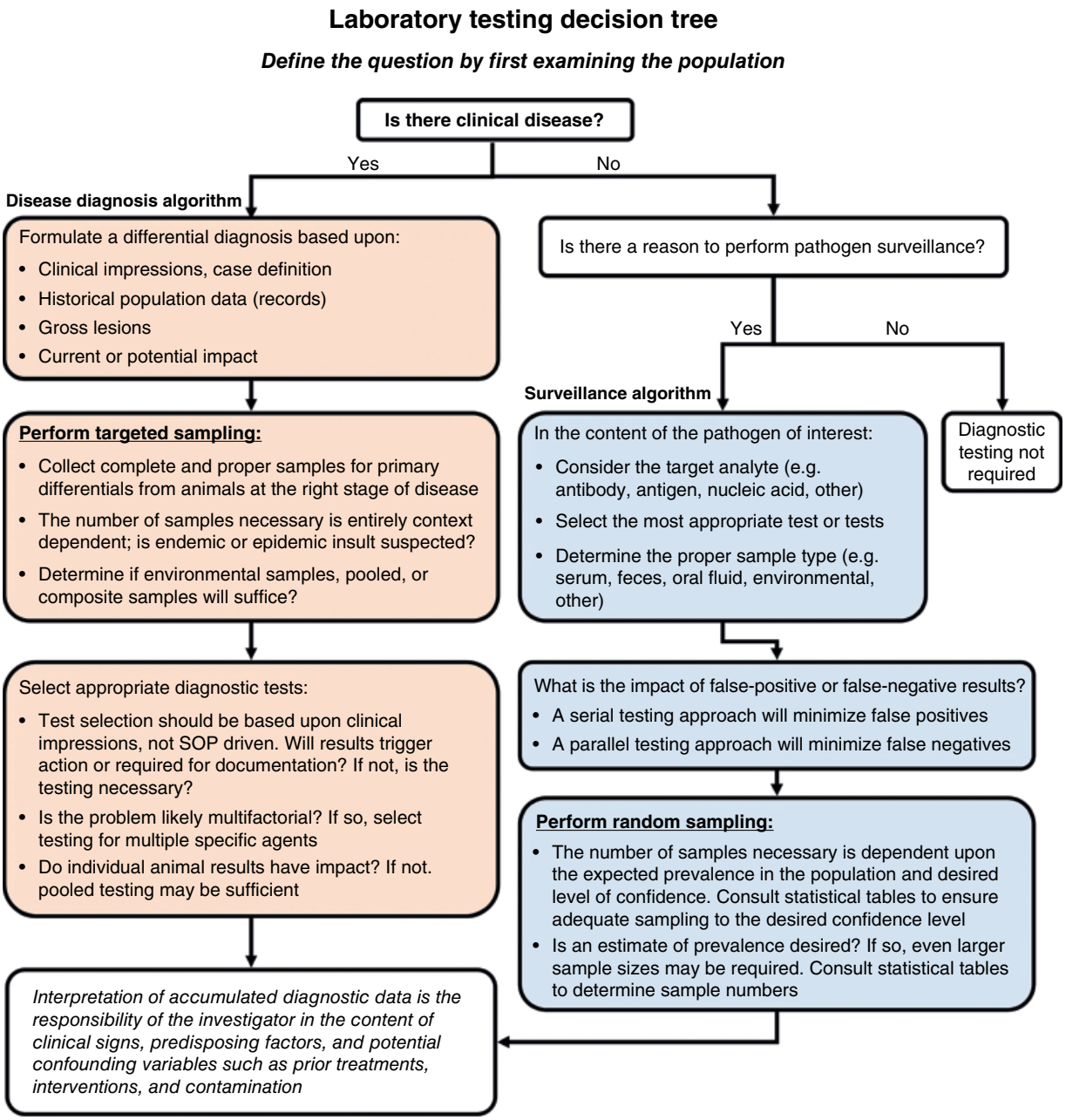


Figure 8.1 Decision tree for laboratory testing in diagnostic investigations. Proper clinical context is essential prior to diagnostic sampling and generation of data.

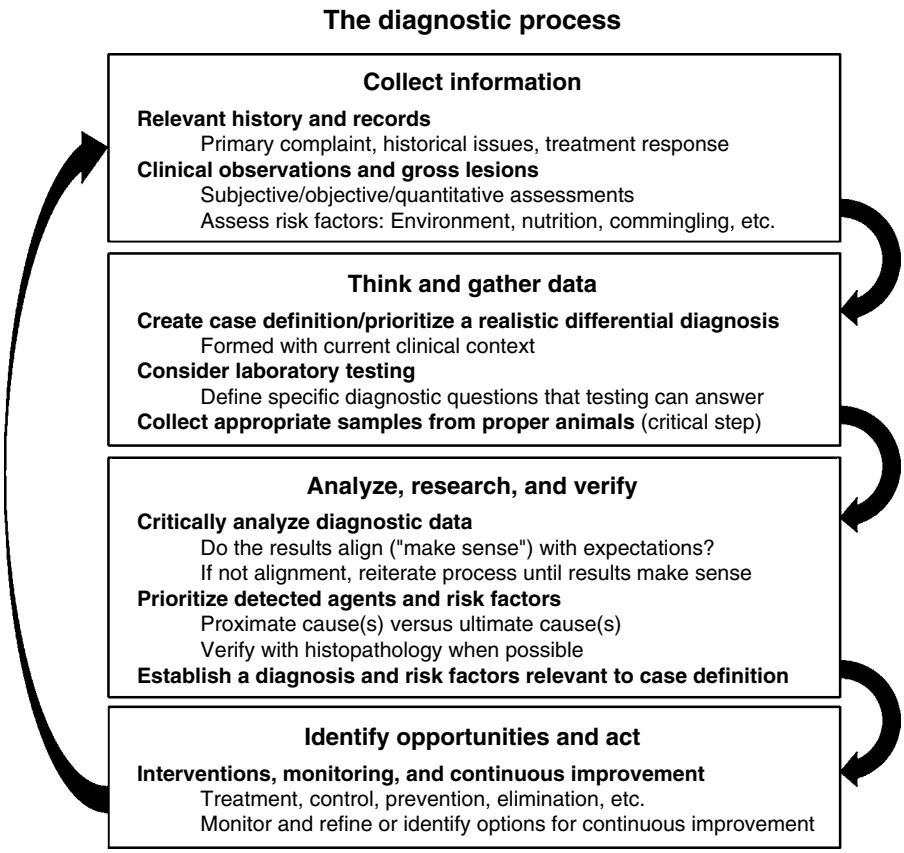


Figure 8.2 The diagnostic process as a continuum. When properly executed, the diagnostic process involves a consistent, systematic approach with continual evaluation of the validity of data generated and alignment of diagnostic results.

laboratory submissions for proper disease diagnosis are of inadequate quantity (not enough animals sampled) for confident pathogen surveillance or estimates of prevalence. Similarly, submissions for pathogen surveillance are often of insufficient depth (too few tissues per animal sampled) for confidence in etiologic or disease diagnosis. Accordingly, this chapter has two primary subsections, each outlining best practices and methods to increase confidence in the interpretation of data collected. It is assumed the reader has a solid understanding of the technical aspects of individual diagnostic assays (Chapter 6) and methods for optimal diagnostic sampling (Chapter 7).

Collecting evidence for disease diagnosis

Theory

Diagnostic process

There are three main reasons to pursue a diagnosis: (1) to accurately label an observed condition, (2) to satisfy scientific curiosity, and (3) to determine appropriate therapeutic action (Crombie 1963). The latter of these is often of greatest concern to swine practitioners and underlines the importance of accuracy in the diagnostic process to reduce errors and unnecessary treatment costs. Accordingly, a proper diagnostic investigation should be a systematic repeatable process within a scientifically validated framework to deduce the presence, etiology, epidemiology, and/or risk factors associated with a suspected disease or infection from all available evidence. The diagnostic process outlined in Figure 8.2 assures a systematic approach to collect data and to evaluate the validity of that data. The narrative and final diagnosis generated from this process should align all relevant observations, data, information, and current scientific knowledge to ultimately "make sense." If these data (observations or laboratory data) do not completely align with conclusions, there is likely some error in the data or deductive process, and this incongruency should trigger the practitioner to begin the process again.

While the swine practitioner often has a short-term goal of establishing or confirming a diagnosis in a cohort of pigs, it is important to remember that the diagnostic process is a continuum within the population, often with many intermediate diagnoses along the way. Examples of diagnosis types may include a clinical diagnosis,

differential diagnosis, morphologic diagnosis, etiologic diagnosis, and laboratory diagnosis. These intermediate diagnoses all have implications with individual constraints and limitations that must be incorporated and aligned within the context of the case as the practitioner attempts to assign a final or herd diagnosis and determine the proper course of action.

When an animal or population of animals has clinical signs of disease, a swine practitioner often uses knowledge gained from textbooks and scientific journals to formulate a prioritized differential diagnosis, typically accompanied by a strong belief as to which differentials are most likely present. While this belief may be true, it cannot be considered knowledge without proper evidence or justification. If confirmed through empirical evidence and experience, this belief becomes knowledge and serves as a framework to guide future clinical investigations. This is an example of deductive reasoning where the application of existing knowledge is used to generate new knowledge. Accordingly, it is imperative that such new knowledge be acquired and validated through a purposeful, repeatable diagnostic process. If new knowledge is acquired by luck, chance, or random events, as is often the case with inductive reasoning, it is not a justified true belief. In other words, the process of justification or acquiring evidence is equally important as the justification or conclusion itself.

For example, the knowledge gained from a positive PCR test for *Mycoplasma hyopneumoniae* derived from a lung sample selected from an animal with clinical signs and gross and microscopic lesions of enzootic pneumonia would have far greater knowledge value than would a positive test derived by chance from a random sample of lung. The former has more evidence to support association with disease than the latter. Diagnostic accuracy is enhanced because the process that selected the lesioned tissue is more likely to produce future disease confirmations than the random method and therefore creates more complete knowledge from which to base future investigations.

Sensitivity and specificity

Another key to understanding disease diagnosis by laboratory testing is that analytical sensitivity and specificity of a laboratory test are not the same as diagnostic sensitivity and specificity, though many will inadvertently confuse the two. Diagnostic specificity is reflected in the positive predictive value of an assay regardless of the analytical sensitivity and specificity (Saah and Hoover 1997). In other words, diagnostic specificity describes the likelihood that an animal with a positive test actually has disease. Similarly, diagnostic sensitivity is a reflection of the negative predictive value of an assay. The fact that an assay has high analytical sensitivity (often also referred to as limit of detection) and can detect minute quantities of an analyte in the laboratory, as is the case for nucleic acid in many PCR assays, does not automatically make it the best diagnostic assay.

Diagnostic sensitivity is, by definition, the probability that a test correctly identifies animals with the condition of interest: true positives/(true positives + false negatives). For example, a test with 80% diagnostic sensitivity for detecting infection with an agent would correctly identify an average of 80% of infected pigs in a population as test positive and would incorrectly identify 20% as noninfected because they tested negative (false negatives). Herein the original diagnostic question put forth by the practitioner again becomes important as the diagnostic sensitivity of the same assay may be different for detecting infection versus detecting disease. Consider that if a diseased animal is more likely to shed the organism of interest than subclinically infected animals, then the same assay described as 80% sensitive for detecting infection may be 98% sensitive for detecting disease. The diagnostic question matters.

Diagnostic specificity is the probability that a test correctly identifies animals without the condition of interest: true negatives/(false positives + true negatives). A test with 90% diagnostic specificity would correctly classify 90% of non-affected pigs on average as negative and would falsely classify 10% of pigs as affected (false positives). Here again the diagnostic question is important: is the practitioner interested in detection (presence or absence of a pathogen) or disease (impact on the animal or population)? Many new diagnostic assays have high analytical sensitivity and analytical specificity, which makes them highly efficient at detecting infectious agents; however, for many pathogens, particularly those that can become endemic in a population, the mere presence of the agent is not highly correlated with a diseased state.

In most field situations, high diagnostic sensitivity and specificity are both desirable although nearly impossible to obtain from a single test. In a general diagnostic context, sensitivity and specificity, once established, are inversely related (as one increases, the other decreases). Lowering the minimum detection limit of a test, such as increasing the cutoff value for a positive PCR result, may improve diagnostic sensitivity but will undoubtedly lower the diagnostic specificity of the test. Buyers of pigs and regulatory officials in importing states and countries typically want tests approaching 100% diagnostic sensitivity for detecting specific pathogens to minimize the risk of introducing new pathogens to an area. A similar line of reasoning can also be applied for tests to detect agents of public health concern (i.e. *Salmonella* spp., *Trichinella* spp., etc.) and antibiotic residues. On the other hand, owners of breeding herds prefer tests of high diagnostic specificity to maximize their chance of selling replacement animals and eliminate issues related to false-positive results. High diagnostic specificity is also

desirable for commercial producers participating in eradication programs where economic losses from false-positive results can be substantial.

Application and examples

Many common diseases can be diagnosed by clinical observations and history; however, confidence in accuracy is increased when the framework of a diagnostic investigation is customized around the context of the case. A diagnostic investigation, then, is executed in much the same fashion as a research protocol with a clear clinical (case) definition, identification of the specific population involved, clearly defined diagnostic objectives, and strategically selected tests, samples, and methods to acquire the evidence from which to identify cause, propose solutions, or monitor interventions.

Preparing diagnostic submissions

Properly executed, each step of the diagnostic process (Figure 8.2) provides at least a portion of the information necessary for confidence in accuracy and utility of identifying and implementing solutions to clinical concerns and/or monitoring outcomes for continuous improvement. The concepts embedded in the diagnostic process and testing can be applied to individual animals, to a group of animals, to successive groups in a particular flow or herd of animals, or to an entire production system. The population should always be defined with estimates of accuracy and confidence in place as part of the diagnostic process. Remember the goal of the diagnostic investigation process is to generate the evidence in a systematic fashion that will allow deduction of diagnosis of one or more insults with the greatest confidence that the conclusions are accurate.

Case definition relies on clinical skills and suppression of bias through objective observation and proper data collection. This initial case definition and sample selection forms the basis for all further diagnostic testing, interpretation, and analysis, and it cannot be stressed enough that outsourcing this critical task to field managers and livestock producers is a significant risk that may derail the entire diagnostic process from the outset. A thorough clinical examination of individual animals and tabulation of clinical signs should allow one to write one or more case definitions for the affliction of individual animals as well as multiple insults encountered in populations of pigs. Mortality is not a clinical sign or diagnosis; it is an outcome and should not be viewed as a case definition or clinical sign. Avoid the temptation to force all clinical signs into one box or assign causation to one etiology. In fact, it is preferable to not think of a specific etiology when creating the case definition(s). A case definition provides the criteria that will be used to classify, categorize, and quantify the afflictions present in the group. The use of specific clinical descriptors will allow more accurate communication of the clinical issue at hand and can be augmented by visual images or video. For example, diarrhea is a nondescript term often used to describe enteric disease and provides little information on potential proximate causes. On the other hand, descriptors such as watery, pasty, bloody, mucoid, voluminous, and projectile not only invoke a vivid mental picture but also help to narrow a differential diagnosis and direct diagnostic testing. Principles of epidemiologic investigation coupled with clinical examination should allow for localization of where lesions may be expected and direct diagnostic sampling.

Necropsy is one of the most valuable tools a practitioner has for presumptive diagnosis. Necropsy of affected or dead animals that meet the case definition can reveal lesions compatible with a particular insult type or rule out some of the differentials developed from the case definition. This is also the opportunity to collect and preserve appropriate samples for laboratory investigation (see Chapter 7). Often the greatest source of error in defining true status by testing is in the process around sample selection and preservation. No amount of effort or test sensitivity can overcome the limitations of improper sampling and preservation. As noted above, careful attention to collection of data from history, clinical signs, case definition, and gross lesions will allow prioritization of a differential diagnosis. Clinical and pathological data objectively collected can usually localize a likely source of insult into one or more body systems. Carefully consider the potential causes; do not assume a diagnosis. Differential diagnosis – which is essentially pausing to ask the question "what could this be?" – is the primary driver for selection of the most appropriate samples and tests for continuing the diagnostic investigation through laboratory testing. Samples should then be collected accordingly and completely. When there is any doubt, seize the opportunity to collect samples in a systematic, organized, thorough, and complete manner. There is no better time, and perhaps no other opportunity, to collect appropriate samples than at the initial site visit. Samples can include serum or whole blood (affected and non-affected cohorts), all relevant tissues from representative animals (both fresh and formalin fixed), oral fluids, water, feed, and/or environmental samples (see Chapter 7).

In general, tissue samples from two to three acutely affected animals that truly represent the case definition are sufficient for diagnosis of disease. However, in some situations, a cross-sectional or sequential approach to sample collection is warranted. For example, arthritis or chronic polyserositis in grower stages often has origins in the nursery phase. For an accurate herd diagnosis, it is prudent to design a cross-sectional or sequential

diagnostic investigation to better understand which agents are contributing and the sequence in which they occur. Samples from animals later in course of disease and/or mortalities can be useful to implicate sequential insults or involvement of multiple agents or insult types. As multiple diseases can be simultaneously present and have cumulative and interacting effects within a population, it is often difficult to obtain a rapid and accurate diagnosis from a single or limited number of sample submissions for diagnostic testing.

Similarly, nonrandom antemortem sampling can be useful if the clinical status of uniquely identified individual animals is known. For example, serologic titers for a particular agent such as *Leptospira*, Aujeszky's disease, or PRRSV would be expected to be positive and relatively high 2 weeks after abortion. Such nontrivial change in antibody titer between two time points (seroconversion) is useful supporting evidence for disease diagnosis. This type of targeted sampling is not random; hence fewer samples are required to establish the presence of herd infection than in random sampling approaches described for surveillance.

Selecting the best diagnostic test for a given situation requires a thorough understanding of the stage of disease in the population, treatment history of the animals sampled (including past vaccinations), and the potential pathogen(s) at hand. The array of tests, technologies, and protocols available can make selection of laboratory tests confusing; however, the selection and interpretation of laboratory tests can be another significant source of error within the diagnostic process. In general, a diagnostic test should not be performed unless the practitioner can anticipate all of the likely results of the test and can interpret the results in the context of the animal or population sampled and at least one of the potential results of the test will trigger purposeful action. Testing solely for the sake of generating data is a perilous approach that is often uninterpretable, wastes valuable resources, and muddies the diagnostic picture.

Good communication with knowledgeable diagnosticians is a necessary component of the diagnostic process to avoid pitfalls and misdiagnosis. Figure 8.3 provides examples of situations where microbial culture, serology, and molecular diagnostics may be useful in the diagnostic process for pathogen detection as well as situations where each may be of little or no value. Such assays help determine if a host is infected (e.g. a microbe is present and proliferating within the host) but may not be sufficient to differentiate between subcategories of infection, which include colonization, subclinical infection, and diseased. Thus, clinical context is essential in the selection and interpretation of diagnostic assays. The practitioner should strive to communicate specifically and effectively with the diagnostic laboratory using the telephone, digital images, video, written descriptions on submission forms, historical data, charts, records, and whatever other methods are available to improve mutual understanding of issues at hand in the population sampled and to document and archive for ongoing reference and herd history.

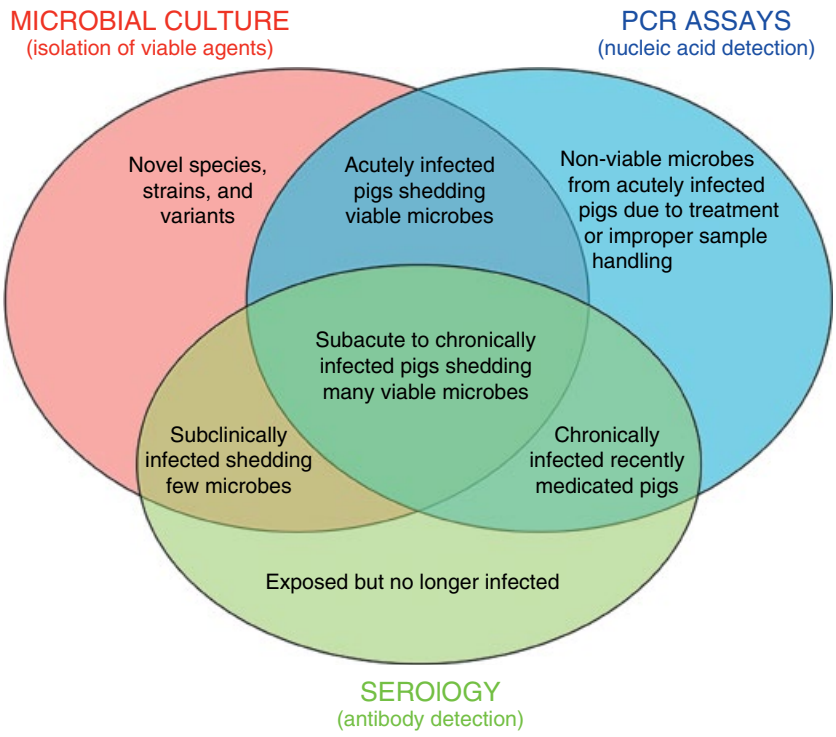


Figure 8.3 Venn diagram of different clinical scenarios related to infectious disease and the diagnostic assays expected to be effective in detecting an etiologic agent from individual pigs. Note that there is no universally "best" test and a parallel testing approach may be warranted in many situations.

The value of data accumulated over time and application of appropriate data management tools offer the possibility that disease occurrences, interventions, and outcomes can be continually analyzed with real strides toward continuous improvement and identifying best practices. However, the "big data" approach to swine disease control will only be valid if the original diagnosis and assumptions as to etiology (cause) are valid and accurate within a known and accurate context. Databases must have accurate inputs, gathered with an understanding of where the numbers come from, how they are derived, accurate denominators, specific clinical context, and the likely impact of any detected pathogen/analyte. Furthermore, databases must be continuously monitored and improved through appropriate controls and feedback loops (Nielsen 2011).

Combining multiple diagnostic tests

Methods to improve accuracy of disease diagnosis often include the application of serial or parallel testing, depending upon the diagnostic objective, as described in the following paragraphs.

Serial testing with a stepwise combination of multiple tests increases diagnostic specificity at the expense of diagnostic sensitivity (more likely to miss a few cases but with added confidence that those identified are diseased) and is useful in answering questions regarding pathogen impact and the presence of disease. One of the most common serial approaches in routine diagnostics is to first use a test with high analytical sensitivity, such as PCR, and to follow up on positive results with histopathology to confirm compatible lesions. If further confirmation is desired, immunohistochemistry or *in situ* hybridization can be applied to the affected tissue sections to demonstrate the presence of pathogen antigen or nucleic acid within lesions, respectively – the proverbial smoking gun. Requiring all tests in serial to be positive in order to classify the animal or population as diseased will greatly improve the specificity of the diagnosis.

Parallel testing, on the other hand, requiring only one test in a battery to be positive in order to classify the animal or population as affected greatly increases diagnostic sensitivity (a wider net) at the expense of diagnostic specificity (more likely to have some false positives) and is most useful in addressing pathogen surveillance questions. This loss of specificity comes as a consequence of using multiple imperfect tests, which therefore increases the chance of abnormal results in otherwise healthy and/or non-exposed pigs. The probability of at least one abnormal test result increases as the number of independent tests is increased. For example, suppose that a sow was screened by two different unrelated assays for a specific bacterial infection. If the sow was never really exposed to the agent (unknown to the veterinarian) and each test had a specificity of 95%, the probability that both tests are negative would be approximately 90% ($0.95 \times 0.95 = 0.9025$). Hence, the probability that at least one test would yield a (false) positive result is now 10%.

As diagnostic cases become more complex, and submitting practitioners more sophisticated, diagnostic laboratories can aid in refining test strategies and expectations. Diagnosticians generally appreciate the opportunity to be involved in protocol development for difficult cases, case series, field studies, or field research. Repeated submissions to gain insight on a complex problem, or cases series to better define compromises in a system or flow over time, are becoming increasingly common requests. A diagnostician's insight can be quite helpful in formulation of an action plan once the submitter's concerns, issues, objectives, and resources are known. The quality of investigation in these circumstances is often greatly enhanced with a consistent specific protocol and standardized submission. A single diagnostician providing oversight in the laboratory can also assure continuity and greater insight into the question at hand. The same is true for those requesting testing as part of a field trial or actual research trial. As stated throughout this section, communication is essential in the diagnostic process. When a laboratory result does not make sense, investigate until it does. While it may be laboratory error, the adept practitioner should be open to the possibility of something totally unexpected or even something new.

Sampling for surveillance and prevalence estimation

Theory

In veterinary diagnostic medicine, it is useful to remember the following truisms: (1) there is no perfect diagnostic test, (2) there is no perfect diagnostician, (3) it is not possible to prove a negative, and (4) time and observation are allies. Accordingly, the swine practitioner does well to use their knowledge of diagnostic medicine with due respect for that which is known, but also objective perspective of that which is unknown. "We've never heard or seen that before! How can it be true?" is the practitioner's confession of reticence to new paradigms. In this mindset, one tends to ignore or procrastinate the opportunity to hone their diagnostic framework through new observations, data, and time, which become allies to new knowledge. In the 1880s, Robert Koch proposed four straightforward postulates for proving causality related to infectious agents and disease; however, with time it was shown that not all infectious agents fit this model (viruses, co-pathogens, prions, etc.) and refinements and amendments to these postulates were required (Evans 1976). Such is the background to understanding principles of causality and in particular to

approaching methods for surveillance and monitoring in animal populations as new diagnostic tests, sample types, and advanced methodologies become available. As science and technology continue to evolve and expand, further discoveries will likely continue to test accepted models of causation and will require practitioners to be open to new interpretations. When such new developments arise, the practitioner is implored to approach and adopt new knowledge with common sense and sound reasoning.

Monitoring versus surveillance

There are significant differences between pathogen/disease monitoring and surveillance in an epidemiologic context (Christensen 2001). Both involve continuous collection of data; however, the intent of monitoring is to assess the disease status of a given population over time, while surveillance describes situations where purposeful actions will occur when the measurement reaches a certain level (Christensen 2001). In a general sense, monitoring is performed for diseases that are known or suspected to be present in a population, whereas surveillance is for those diseases or agents that are not expected to be present. Thus, the differentiating factor is if the intent is to take immediate action or not.

When submitting samples for pathogen monitoring or surveillance in a population, it is important to remember that absence of evidence should not necessarily be interpreted as evidence of absence. Put another way, it is prudent to exercise caution when interpreting a zero numerator (all samples test negative) and to always do so in the context of the denominator (number of samples from a defined population). To this end, the number of samples necessary (the denominator) to detect a pathogen or disease in at least one animal in a population at a given point in time is inversely related to the expected prevalence within the population sampled. Further, the number of samples increases dramatically as the desired confidence level for detection increases or approaches 100% and even more so if the expected prevalence is low. Accordingly, truly random sampling from a population for detection of a disease or infection of low expected prevalence requires large numbers of samples to be confident that the population is truly negative when all test results are negative. For example, even with a perfectly sensitive test at least 60 animals should be randomly sampled for a disease of 5% expected prevalence if the desired confidence level is 95%. If the assumed prevalence approaches 50% or more, then the number of samples required for detection drops to 5 or fewer with 95% confidence. Tables describing specific sample numbers necessary to detect a pathogen or disease within a population at various confidence levels and with varying disease prevalence are available elsewhere (DiGiacomo and Koepsell 1986).

Proving a population negative for a particular attribute with absolute certainty is impossible. Hence, cases where all of the individual results of a particular test are negative require thoughtful interpretation, particularly when there is little context regarding the samples themselves. A useful rule for interpreting negative results from random samples is the "rule of three," which stipulates it can be safely concluded with 95% confidence that the upper limit of prevalence of an outcome (in this case a positive test result) is at most $3/n$ where n is the number of samples with negative results (Hanley and Lippman-Hand 1983). For example, if 20 samples are collected and all samples test negative for a pathogen, it would be tempting to conclude that the population is negative for this pathogen; however, the "rule of three" provides a more conservative and appropriate interpretation such that one can confidently conclude the prevalence of the pathogen within the tested population is less than or equal to 3/20 or 15%. This conclusion still includes the possibility of a truly negative population but suggests additional testing may be required in the context of the diagnostic question. As the total number of negative samples increases, the upper limit of the prevalence estimate decreases. A derivative of this rule can also be used to quickly estimate the number of samples necessary to detect a pathogen with 95% confidence and is calculated as $3/P$ where P is the expected prevalence in decimal form. For example, if a pathogen is assumed to have a prevalence of 25% in a population, then the number of samples needed to confidently screen for the pathogen is 3/0.25 or 12. This estimate aligns well with published table values when disease prevalence is less than 50% and is a slight over estimate when disease prevalence is high.

Estimating prevalence

Understanding disease prevalence and infection rates in a large population may be desired in efforts to determine the effectiveness of prevention and control programs; however, this requires a proactive monitoring or surveillance program. Due to feasibility and financial constraints, it is often desired to estimate prevalence in a herd from observed prevalence in a sampling of the population. The precision of the estimate is dependent upon the true prevalence in the population and the sample size. Accordingly, larger sample sizes are necessary to accurately estimate prevalence (5% maximum error), and estimates derived from less than 10 samples are only applicable for conditions of very high (>90%) or very low (<10%) prevalence with an expected error rate of 15–20%. Thus, attempts to estimate population prevalence require a specific proactive random sampling approach designed with regard to the expected prevalence and population size, and attempts to estimate prevalence from cumulative data derived from routine diagnostic submissions would be tenuous at best as common sampling sizes (5–10 samples) are too small

for precise comparisons. Tables describing specific sample numbers necessary to estimate the prevalence of a pathogen or disease within a population and at various error limits are available elsewhere (DiGiacomo and Koepsell 1986).

Application and examples

The swine practitioner, having determined the desire to perform pathogen monitoring or surveillance, must ultimately decide the nature of the data they wish to generate: qualitative or quantitative.

Qualitative data and cutoff values

Qualitative results (positive, suspect, or negative) are laboratory interpretations based upon statistics generated during the assay validation process. Qualitative results are certainly helpful and can be definitive; however, the assignment of the proper test interpretation category (positive or negative) is dependent upon a predetermined, somewhat arbitrary cutoff value. Depending on the context of the diagnostic question, this can affect final test interpretation when samples have test results near the cutoff value. This is most relevant when considering results of serologic and PCR-based tests, for which cutoff values may differ between laboratories, versus results of microbial culture that are not dependent upon a specific cutoff.

Cutoff values are determined with one of three objectives: (1) to maximize the diagnostic sensitivity and diagnostic specificity of the assay, (2) to maximize only the diagnostic sensitivity, or (3) to maximize only the diagnostic specificity. The chosen cutoff value therefore determines the interpretation of each result as positive or negative, regardless of the assay platform. Ideally, the data for determining assay cutoff values come from controlled infection studies of known naïve animals. Samples collected at consistent intervals from the known-infected and negative control animals afford investigators the opportunity to understand antibody, antigen, and nucleic acid detection over time. For example, samples containing nucleic acid at the threshold of detection for a given PCR assay will be sometimes positive and sometimes negative based on the cutoff threshold (CT) of a particular test, the efficiency of extraction in the laboratory, and other sources of laboratory error. Rarely is this an issue with acute disease diagnosis (see previous section) as dogma suggests that there should be abundant pathogen nucleic acid in appropriately collected samples from animals with acute disease. However, generalizing these concepts to assign "positive or negative status" to environmental samples for surveillance is often an inappropriate interpretation. Depending upon the expected impact, test–retest strategies, confirmation testing, or resampling may be more appropriate options.

The same is true of most modern antibody assays. The cutoff used to assign "positive" or "negative" is the interpretation of continuous data generated from a spectrophotometer. Results of repeated testing of the same sample, with continuous data assays, vary around an average of their values. That is, the same sample will produce test results (which are continuous data) with different interpretations if the result is near the value defined as the cutoff for the assay.

Quantitative data and time

Quantitative data, by definition, provide a numerical value for each sample and are useful to display the variation within a sample set and among sample sets over time. This variation and associated sample set averages build an objective understanding of overall system output. The results are also independent of any subjectively defined cutoff value. This can be particularly useful with ELISA sample-to-positive (S/P) ratios, clinical pathology results, and other continuous data.

Time is your ally when considering the conceptual framework and connectivity of infection, disease, clinical recovery, and serologic response as well as relationships between time of onset and duration of infection as depicted in Figure 8.4. Exposure can lead to infection in which the offending agent replicates and is shed by the host. Clinical disease may or may not occur. The agent or its antigens or genes may be detectable by some method for some duration. Infection typically results in an immune response often measured by an antibody assay. In most cases, the agent is eventually cleared from the host and no longer directly detectable, while antibody remains for a longer period and provides options for indirect detection. Each offending agent and each individual animal will have its own timeline, usually hovering around averages but commonly with significant outliers. Variation is expected in clinical outcomes and in

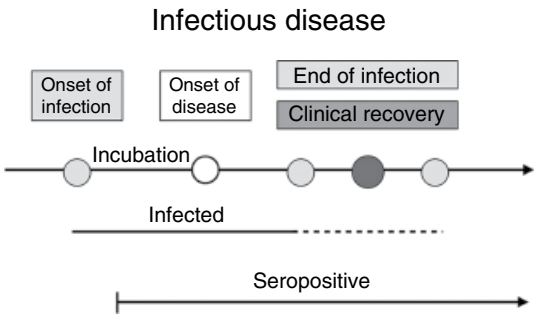


Figure 8.4 General relationships between infection, disease, recovery, and serologic response over time. Note that clinical recovery does not necessarily mark the end of infection or impact serologic status. Knowledge of these relationships helps determine appropriate diagnostic testing (direct vs. indirect methods) for specific pathogens. *Source:* Courtesy of Dr. Greg Stevenson.

magnitude and duration of various assays along the timeline. This illustrates the infrequency of black-and-white outcomes and solutions for the multifactorial nature of infectious diseases. The absence of clinical signs does not mean absence of infection.

Using diagnostic data for process improvement

A model for process improvement was proposed by Walter Shewhart and later promoted by W. Edwards Deming as the Shewhart Cycle of Plan, Do, Study, Act (Deming 1986). This cycle provides a framework for learning about a process and then working to improve the process. The Shewhart Cycle of Plan, Do, Study, Act (Deming 1986) directly applies to swine health management and the use of diagnostic data. A plan for using diagnostic data for major pathogens, particularly those for which elimination has merit, likely involves herd health stabilization, pathogen elimination, and prevention of reintroduction. At all stages of the health management process, there is a need to collect proper diagnostic data to measure success and drive future action. Without applicable and appropriate measurement tools, there will be no accurate information to analyze and study.

If health management is the objective of sampling and testing, the swine practitioner has made the decision to be engaged in disease monitoring and/or surveillance. Prior to collecting this health management data, an

Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
0.048	0.190	0.390	0.917	0.347	0.386	0.031	0.302	0.106
0.204	0.071	0.130	1.010	0.403	0.456	0.252	0.020	0.132
0.301	0.299	0.148	0.545	0.063	0.131	0.018	0.031	0.349
0.258	0.041	0.107	0.844	0.126	0.122	0.062	0.033	–0.004
0.181	0.213	0.500	1.124	0.157	0.085	–0.005	0.014	0.020
0.155	0.041	0.557	0.427	0.622	0.600	0.003	0.096	0.056
0.053	0.395	1.114	3.035	0.558	0.542	0.021	0.007	0.005
0.132	0.406	0.137	1.618	0.829	0.083	0.044	0.208	0.084
0.047	0.420	1.082	1.229	0.707	0.709	0.000	0.051	0.390
0.161	0.325	0.374	0.076	0.207	0.194	0.000	0.009	0.118
0.141	0.217	0.907	2.197	0.443	0.170	0.472	0.136	0.285
0.378	0.235	0.447	0.936	0.953	0.118	0.413	0.060	0.135
0.439	0.173	0.601	0.726	0.061	0.246	0.270	0.183	0.024
0.365	0.453	1.100	0.299	0.044	0.337	0.259	0.259	0.210
0.227	0.701	0.470	1.414	0.402	2.124	0.347	0.392	0.493

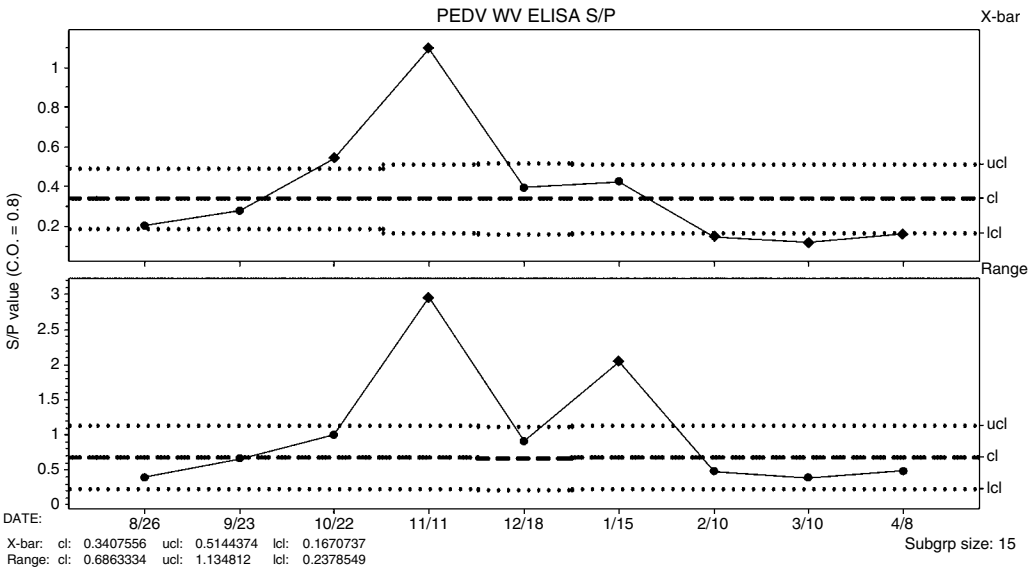


Figure 8.5 Statistical process control (SPC) chart for PEDV exposure using PEDV whole virus ELISA results (S/P) from monthly collection of 15 serum samples. Prior to collecting these samples, it was determined that data from the first three months would serve as baseline data for determining upper and lower control limits (UCL and LCL). The table contains the raw S/P data for each month, and the averages of each month's S/P values are reflected in the upper graph, while the lower graph shows the variation (range) for each month. There are signals of abnormal variation in the data from November and January, suggesting recent exposure to PEDV ahead of those sampling periods.

operational definition (adapted from Deming 1986) must be created and includes:

A specific test to be applied for a specific agent, disease process, or physiological parameter – for example, testing for antibody, antigen, or nucleic acid in animal or environmental samples to detect evidence of the agent.

A criterion or criteria for judgment (e.g. presence of evidence of an agent within an animal or population).

A decision (yes or no) if the sample did or did not meet the criterion (or criteria); thus an action is or is not required.

As laboratory data related to an operational definition are collected, a consistent and purposeful method to analyze the results should be implemented. A statistical process control (SPC) chart is one effective method for examining quantitative data over time, and an example is provided in Figure 8.5. In this example, a specific test (PEDV WV ELISA on serum collected monthly), with specific criteria for judgment (average S/P ratios with expected upper and lower limits as defined by the first 3 months of data), is used to monitor population status and screen for potential PEDV exposure events. In this case the assumption would be that averages outside the calculated upper limit are evidence of potential exposure to PEDV and would trigger additional action including diagnostics and confirmatory testing as described in the first half of this chapter. Additional information related to SPC charts and understanding variation is available elsewhere (Wheeler 2000; Wheeler and Chambers 1992). SPC charts are dependent upon not only how the data are generated but also the frequency of data collection. Assuming quality data are collected, SPC charts then require proper creation, interpretation, and use.

References

Christensen J. 2001. *Acta Vet Scand Suppl* 94:11–16.

Crombie DL. 1963. *J Coll Gen Pract* 6:579–589.

Deming WE. 1986. Out of the Crisis. Massachusetts Institute of Technology, Center for Advanced Engineering Study, Cambridge, MA.

DiGiacomo FR, Koepsell TD. 1986. *J Am Vet Med Assoc* 189:22–23.

Evans AS. 1976. *Yale J Biol Med* 49:175–195.

Hanley JA, Lippman-Hand A. 1983. *JAMA* 249:1743–1745.

Nielsen AC. 2011. *Acta Vet Scand* 53(Suppl 1):S3.

Saah AJ, Hoover DR. 1997. *Ann Intern Med* 126:91–94.

Wheeler DJ. 2000. *Understanding Variation: The Key to Managing Chaos*. Knoxville, TN: SPC Press.

Wheeler DJ, Chambers DS. 1992. *Understanding Statistical Process Control*. Knoxville, TN: SPC Press.

9

Disease Control, Prevention, and Elimination

Eric J. Neumann and William F. Hall

Introduction

Biosecurity, or preventing the transmission or introduction of diseases, is a cornerstone of modern veterinary service in the pig industry. Eradication of diseases from pig farms remains an important goal, though it is one rarely achieved. Fortunately, through the process of attempting to eliminate diseases, transmission of diseases and hence their frequency or occurrence (and often their severity) are reduced. This chapter describes a framework for understanding an infectious disease through knowledge of the pathogen's interaction with the host, the production setting, and its broader environmental landscape, a framework described as "disease ecology." Disease ecology is prefaced on the categorization of pathogens based on the mechanisms they rely on to perpetuate themselves between leaving one host and finding a new one. By considering pathogens in terms of their ecology, rational approaches to disease control and eradication can be developed even when complete information about a pathogen is lacking.

Disease transmission

Disease transmission includes any mechanism by which an infectious agent is spread from an infected host, animate or inanimate vector, or environmental reservoir to a susceptible host. Implicit in a transmission event is the requirement that a new host becomes infected. This situation is in contrast to an exposure event whereby an infectious agent is presented to a potential host with transmission as one possible outcome. The possibility of transmission is a function of the combined likelihood of several serially occurring events. Transmission requires that a pathogen successfully exits an infected host, escapes potential threats to its existence in the environment, breaches the defense systems of a new and susceptible host, and then reaches an anatomical site suitable for further replication or perpetuation in that host (Zimmerman 2003).

Some authors have elaborated on the need to draw distinction between modes of transmission and routes of infection (Smith 2006). Modes of transmission can be classified as horizontal (between contemporaries or animals of the same generation) or vertical (between infected animals of one generation and uninfected animals of the succeeding generation, *in utero* or through the colostrum). Within horizontal modes of transmission, further distinctions can be made to categorize transmission events that occur as a result of direct or indirect contact with an infected animal or through exposure to an airborne pathogen.

Route of infection specifies the means by which a pathogen enters the host including the alimentary, respiratory, or urogenital tracts, skin, or conjunctiva. To gain a thorough appreciation of the determinants of disease transmission, one must resist the urge to systematically classify pathogens based on their most likely, prevalent, or important pathway of travel between infected and uninfected animals. The advent of molecular-based diagnostic tests and advanced epidemiological analyses has enabled a more complete understanding of the complex interactions between host, pathogen, and the environment. An alternative approach to understanding disease transmission in the context of this new information is through a framework that attempts to explain where the pathogen hides or resides when it is not in the host animal.

An important objective for veterinarians, animal scientists, and farmers is the prevention, control, and, when possible, eradication of disease in commercial livestock. Fundamental to these objectives is knowledge of the habitat in which the pathogen can persist. "If we can combat the pathogen only when it has invaded our stock we shall never eradicate it; if it has any other habitat to hide in, we must find that habitat" (Halpin 1975).

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Ecology of disease: host–pathogen-environment

In broad terms, disease is either a physiological or psychological dysfunction and includes both cellular pathology and clinical signs; these may be a result of infection with a pathogenic agent or be associated with any number of noninfectious causes. For the sake of this chapter, disease will be taken to mean only those pathologies and clinical signs caused by infection with a pathogenic agent.

Disease may persist long after a pathogen has been cleared from the host. Disease may be classified based on temporal staging (peracute, acute, subacute, chronic, persistent/latent), severity (unapparent, mild, moderate, severe), or the organ systems that are affected. Under the right circumstances, organisms from all taxonomic kingdoms are represented among the list of potentially infectious pathogens of the pig.

An understanding of why disease occurs and how it can be controlled requires some knowledge of contributions made by the environment in which the host and pathogen reside. A more apt term that incorporates the complex interactions between host and pathogen, including environmental variables, is "disease ecology." After making its first appearance in the scientific literature nearly 60 years ago (Bejarano 1960), the term was used primarily in discussion of arthropod-borne and wildlife diseases until the 2000s. However, in recent years, use of the term has expanded into epidemiological studies of animal and human diseases as analytical techniques capable of simultaneously integrating spatial, climatological, temporal, and demographic data with traditional measures of disease occurrence have become more widely used.

Ecology is the study of the distribution and abundance of organisms and their interactions with the environment (Begon et al. 2006). Implicit in the term is a sense of balance, an imperative that an ecological system will evolve over time into a stable circumstance that reacts to external influences in such a way to maintain that stability. In the context of disease ecology, one could use as an example the relationship that evolves between *Mycoplasma hyopneumoniae* bacteria and young pigs in the environment of a conventional continuous-flow growing-finishing barn. Over time, a high prevalence of infection will be established in the pig population but with few overt clinical signs (endemicity). The prevalence of infection, its phenotypic and genotypic characteristics, systematic environmental variables (throughput, pig density, and pig husbandry and management), and the pig's own evolutionary progression (body functions, body size, social behavior, and body defense mechanisms) will remain stable. In the absence of external stimuli, there is no obvious imperative for the host, the pathogen, or the environment to change.

From a more philosophical perspective, one can even incorporate the influence of public policy (as an environmental contributor to pig disease) into the disease ecology paradigm. Law and Mol presented a compelling discussion on the "politics of boiling pigswill" in a 2008 paper that offered their reflections on the influence that the regulation of waste-food feeding to pigs had on the 2001 foot-and-mouth disease virus (FMDV) outbreak in the United Kingdom (Law and Mol 2008). They suggested that through regulation of the "mundane and material" practice of boiling pigswill, competing politico-environmental ethics were established whereby the rich were divided from the poor through separation of FMDV-free countries from those in which the disease was endemic. The authors stated, "To boil pigswill at Heddon on the Wall [the index farm] is to reproduce a distinction between a productive and a less productive agriculture. It is also a technique for making and maintaining a specific geographical distribution of that productivity." We are no longer raising pigs in the isolation of our own farms; we are subject to the effects of the political winds of the day, and this should be considered in the modern framework of disease ecology.

A specialized discipline within disease ecology has evolved to deal with the unique mode of disease transmission carried out through arthropod vectors, that being "landscape epidemiology." Like disease ecology, landscape epidemiology had its origins in the scientific literature of the 1960s but experienced resurgence in use during the 2000s. Landscape epidemiology has a spatially defined focus with the intent of measuring changes in the dynamics of host, vector, and pathogen interactions and their association with the risk of disease transmission to the host species (Reisen 2010). Geographical information systems in combination with analytical techniques accessible to a broader group of scientists have allowed those working with vector-borne diseases (specifically arthropod-borne pathogens) to describe nidalities of infection. Nidalities are pathogen specific and are fundamentally determined by the concurrent spatial overlap of landscapes that offer the requisite combination of vegetative cover, elevation, latitude, and microclimate capable of supporting populations of competent and infectious vectors, competent vertebrate reservoir species, and susceptible host species.

Japanese encephalitis virus (JEV) is a mosquito-borne Flavivirus and is the most frequent cause of arboviral-induced encephalitis of people around the world; domestic pigs are the most important reservoir for this virus. Recent work utilizing a landscape epidemiology approach has shown that the seasonal increase in the

number of human infections in Southeast Asia and Oceania is now occurring up to 2 months earlier in the year and persisting for up to two months longer than typically expected (Hsu et al. 2008). The explanation appears to be related to the local effects of climate change on ambient temperature and the increased frequency and intensity of rainfall, both of which have contributed to a change in the ecology of the mosquito vector. Similar findings have been reported for West Nile virus and Bluetongue virus (Weaver and Reisen 2010); evidence of both infections can be found sporadically in domestic pigs though their role in further transmission is unlikely (Sugiyama et al. 2009; Teehee et al. 2005). A landscape epidemiology approach enabled researchers to identify these important public health trends that have the potential for substantially impacting the pig industries in affected regions.

Measuring the occurrence of disease

There are several ways to measure disease occurrence depending on the question one is trying to answer. Important to these measures is defining the number of animals in the population that are at risk of becoming infected. Commonly used measures of disease occurrence are shown in Table 9.1. Two important distinctions should be recognized when interpreting prevalence and incidence: (1) prevalence measures the occurrence of infection at a single point in time, while incidence incorporates the frequency at which new infections occur over a defined period, and (2) prevalence assumes all individuals in the population are (or were) at risk of becoming infected some time before being diagnosed, while incidence considers only those animals susceptible to infection at the beginning of a defined time period.

Incidence measures tend to be of more use than prevalence in study of disease transmission as calculations of both incidence risk and incidence rate consider only those animals known to be free of infection at the beginning of a defined time period and relate this occurrence to the accumulation of new infections over time. Incidence measures inform one of the "force of infection" or the rate at which new cases occur over time, a particularly useful determinant in predicting the course of a disease epidemic. In calculating incidence rate, the cumulative time at risk for individuals in the population is recorded in order to precisely account for the time that has elapsed prior to an animal becoming infected.

For relatively stable populations in which the incidence rate of a disease is constant (rare for most infectious diseases in commercial pig production), the relationship between prevalence, incidence rate, and disease duration can be represented by the following formula (Dohoo 2003):

$$\text{Prevalence} = \frac{(\text{incidence rate} * \text{average duration of disease})}{(\text{incidence rate} * \text{average duration of disease}) + 1}$$

A cascade of successful events must occur in order for disease transmission to take place starting with exit of the pathogen from an infected host and ending with the pathogen finding a suitable habitat or cell to occupy in a susceptible host. Each step in this cascade has an associated probability of success, sometimes conditional or dependent on events that occurred in a previous step. The combination of these probabilities results in a determination of the likelihood that the new host becomes infected and can be expressed qualitatively (low, medium,

Table 9.1 Commonly used measures of disease occurrence.

100 pigs are monitored two times, over a 30-day period for infection with pseudorabies virus, based on seroconversion to the virus.

Measure	Formula	Day 0 Positive = 10 Negative = 90	Day 30 Positive = 25 Negative = 75	Interpretation at day 30
Prevalence risk	$\frac{\text{No. positive}}{\text{No. in population}}$	$\frac{10}{100} = 0.10$	$\frac{25}{100} = 0.25$	25% of pigs have evidence of prior infection with PRV
Incidence risk	$\frac{\text{No. becoming positive during period}}{\text{No. at risk at start of period}}$	NA (unknown time at risk)	$\frac{(25-10)}{90} = 0.17$	There is a 17% chance of a pig becoming infected with PRV during a 30-day period
Incidence rate	$\frac{\text{No. becoming positive during period}}{\text{Accumulated "time at risk"}}$	NA (unknown time at risk)	$\frac{(25-10)}{((90*15)+(75*15))} = 0.006$	6 pigs become infected with PRV per 1000 pig-days at risk[a]

NA, not applicable.

[a] Assumes all pigs became infected halfway through the period (i.e. 15 days after monitoring began).

or high likelihood) or quantitatively (10, 50, or 90% likelihood). In common terms, a pathogen might simply be described using descriptors such as "highly contagious" or "very infectious," for example, when it is perceived that transmission between pigs or farms is inevitable. Given that current laboratory techniques allow precise enumeration of the number of pathogens shed by an infected host and determination of the minimum dose required for transmission to occur, some clarification of terms related to the likelihood of successful transmission is useful. "Infectious(ness)" describes those diseases (as distinct from pathogens) that are capable of being transmitted between individuals or can be alternatively described as the inverse of those diseases having a noninfectious cause. For example, neoplasia (perhaps excepting those with suspected viral origins) is considered noninfectious, while most conventional diseases of pigs have an infectious cause (bacteria, viruses, internal and external parasites, and fungi/yeasts). "Contagious(ness)," by contrast, is a qualitative descriptor synonymous with the combined likelihood of success of all the events necessary for transmission to occur. The combination of host, pathogen, and environmental variables that would make transmission from an infected host to a susceptible host very likely to occur would also promote rapid spread between individuals; these types of diseases would be considered very contagious. By these definitions, a pig in the chronic stages of a resolving pneumonia that was initiated by infection with influenza virus two weeks previously would be considered to have an infectious disease (the pathology was initiated by influenza virus, and the lung continues to harbor transmissible secondary bacterial pathogens) but is currently in an ecological state whereby the disease is not very contagious (influenza virus is no longer present, and the secondary bacteria are unlikely to be transmitted to an otherwise healthy pig).

The number of pathogens that must be delivered to the host in order to successfully establish infection is referred to as the infectious dose. This does not represent the number of pathogens that must be delivered to the site of replication in the host, as this number would theoretically be the same for a given pathogen, regardless of the route of exposure. As all infectious pathogens (and the pig host) are subject to normal biological variation (related to virulence attributes of the pathogens and active or innate immune effectiveness for the pig), one should anticipate that there will be variation in the infectious dose associated with a given pathogen. To standardize calculation and reporting of this information, infectivity of a pathogen is generally stated as an infectious dose 50, or "ID_{50}." The ID_{50} of a pathogen represents the dose level that will result in successful infection of 50% of those hosts that were exposed (to that dose level). Using porcine reproductive and respiratory syndrome virus (PRRSV) as an example, the ID_{50} for oral, intranasal, and parenteral routes of exposure were reported to be $10^{5.3}$, $10^{4.0}$, and $10^{2.2}$ tissue culture infectious dose 50 ($TCID_{50}$, interpreted similarly to ID_{50}), respectively (Hermann et al. 2005). In this case, oral exposure required a 1263-fold higher dose as compared with parenteral exposure, reflecting the additional steps involved in the transmission pathway for PRRSV to reach its site of replication through oral exposure; presumably the same number of virus particles reached the site of replication in both instances.

Patterns of disease occurrence

Understanding the pattern of disease occurrence at a population level is an important consideration when hypothesizing the cause of a disease and how it might best be prevented, controlled, or eradicated. Critical to determining the pattern of disease occurrence is establishment of a case definition. This may be as simple as accepting a positive diagnostic test result but more often includes multiple criteria such as the presence of one or more clinical signs, the presence of a characteristic histological lesion, or perhaps even the lack of a confirmed diagnosis of a known pathogen. Once a case definition is established (recognizing the definition may need to be refined over time), one can proceed in establishing the disease's orientation in space and time. Examination of the course of disease events over time is important in establishing associations between cause and effect. This is particularly important considering the difficulty in establishing the incidence rate of the disease when pigs are raised in "open populations" characterized by the continuous movement of pigs into and out of the population over time (e.g. continuous-flow growing-finishing facilities).

From a temporal perspective, disease occurrence is often described as sporadic, endemic, or epidemic. Sporadic disease occurrence refers to episodes of disease that occur randomly over time; cases are clustered in time but with variable intervals of time between the clusters. This may occur when opportunities for contact between a pathogen and a susceptible host are infrequent or when the presence of multiple factors (or agents) necessary for disease to occur is absent. Endemic disease generally refers to a situation whereby disease occurs at a stable prevalence with a predictable and invariable incidence rate; cases occur at an expected rate. While endemic is often thought to indicate a disease of low prevalence, *M. hyopneumoniae*-induced pneumonia of growing pigs presents an obvious example of an endemic disease that can establish at a high prevalence. Seasonal fluctuations do not preclude the use of the term endemic as seasonal effects tend to be reasonably predictable. Endemic disease occurrence often suggests

long-term adaptation by the host and pathogen, usually accompanied by little or no mortality. Epidemic disease occurs when incidence rate increases to the extent that prevalence rises above an expected level. The interval between epidemics can be variable or invariable based on the specific ecology of the disease condition. Epidemics are often a result of an imbalance between host and agent due to a change in virulence, a change in the disease ecology, or introduction of a novel agent.

Epidemic curves are a useful graphical means of determining the pattern of disease occurrence; examples of sporadic, endemic, and epidemic occurrence are shown in Figure 9.1. Examination of an epidemic curve can provide insight as to the type of pathogen causing the disease and the stage of the epidemic (early, peak, or declining). More detailed discussion of epidemic curves and their interpretation are available from other sources including the previous edition of this text (Neumann 2012).

In describing epidemics, it is useful to classify animals in the population according to a simple system: susceptible, infectious, or recovered. Of course, pathogenesis varies among different infectious organisms such that other disease states may also need to be represented: incubating (infected but not yet infectious to others), latent (infected but not continuously infectious to others), recovered (but not resistant to reinfection), and others. To put these terms in perspective, Kennedy published the following description using a grass fire as an analogy for the evolution of an epidemic disease outbreak (Kennedy and Roe 1987):

> An infectious epidemic behaves very much like a grass fire. Whether an epidemic (grass fire) develops or not will depend on current conditions, the 'pressure of infection' (amount of sparks/flames), a threshold proportion of susceptibles (dry grass) in the population, and the size and density of the susceptible population. The more resistant individuals (green grass) in a population, the more the epidemic is impeded. However, if the pressure of infection (fire) is high enough, even the resistant individuals may succumb (burn). Human intervention may also affect the course of the epidemic. Susceptibles may be immunized (back-burning) and cases may be treated (sprayed). Also, the infective and susceptibles may be isolated and quarantined (fire-breaks). Eventually, an epidemic may burn itself out as the number of susceptibles falls below the threshold, or may smolder on at endemic level until conditions are again conducive to another epidemic.

There exist several motivations for the development of tools that can describe or predict the nature of disease transmission events within animal populations. One obvious reason is to facilitate exotic disease response planning, but other less obvious reasons include animal welfare (minimizing the number of animals affected by disease), economic evaluation of disease management strategies, and support for public policy around international trade of animals and animal products. Software tools utilizing either mathematically based modeling techniques or outbreak simulations have been developed to provide information on the likelihood, extent, and time course of disease epidemics.

The mathematically based tools generally utilize a state transition approach whereby all individuals are assumed to exist in one of the three states: susceptible (S), infectious (I), or recovered (R). Assumptions are made about the contagiousness of the disease and the duration for which an individual remains contagious to others, and then a series of differential equations are solved to transition individuals from one disease state to another at the predicted rates (Anderson 1982). While SIR models may seem overly simplistic, they are particularly useful in evaluating the effect of potential interventions, when attempting to identify the start of an outbreak (predicting backward in time to identify an index case or time), or predicting when the peak of an epidemic is expected to occur. For complex diseases, SIR models can be expanded to incorporate the effect of open populations (introduction of new susceptible individuals), latency, or the potential for reinfection of recovered individuals.

The pattern of disease occurrence is related to spatial factors as well as time. An important assumption underlying SIR modeling is that all individuals in the susceptible population have an equal probability of becoming infected. In recent years, spatially relevant modeling procedures have become available as an alternative to, or enhancement of, traditional transmission modeling methods (Pfeiffer 2008). By combining spatial and temporal information about animal populations, the extent and frequency of contact between individuals, and the relevant ecological factors in the environment, accurate assessments of the likelihood of disease transmission in a real-life setting are possible.

Mechanisms of disease persistence

The essence of veterinary medicine lies in control of animal diseases. Stopping transmission of pathogens between infected and susceptible hosts is a prerequisite for disease control as the economic, moral, and animal welfare implications of disease control programs that rely entirely on treatment postinfection are substantial. There exist numerous modes of transmission including aerial spread, direct contact with an infected host,

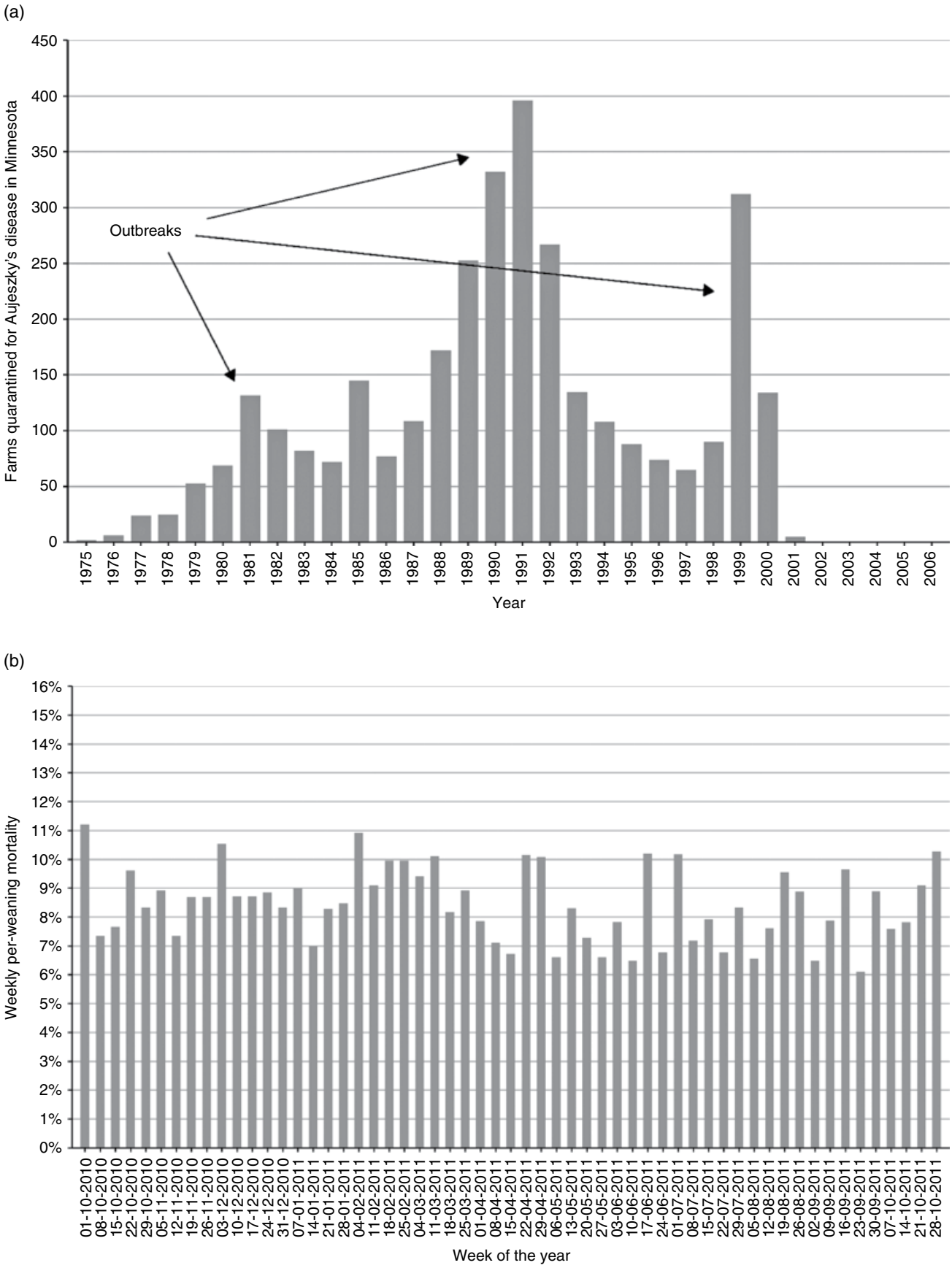


Figure 9.1 Epidemic curves representing sporadic (a; ADV outbreaks from 1975 to 2006 in Minnesota [*Source:* Anderson et al. 2008]), endemic (b; weekly preweaning mortality rate on a typical US farm [*Source:* Gillespie 2009. Reproduced with permission of Dr. Gillespie, American Association of Swine Veterinarians]), and epidemic (c; number of farms infected with classical swine fever in the Netherlands during 1997–1998 outbreak [*Source:* Elbers et al. 1999]) disease occurrences.

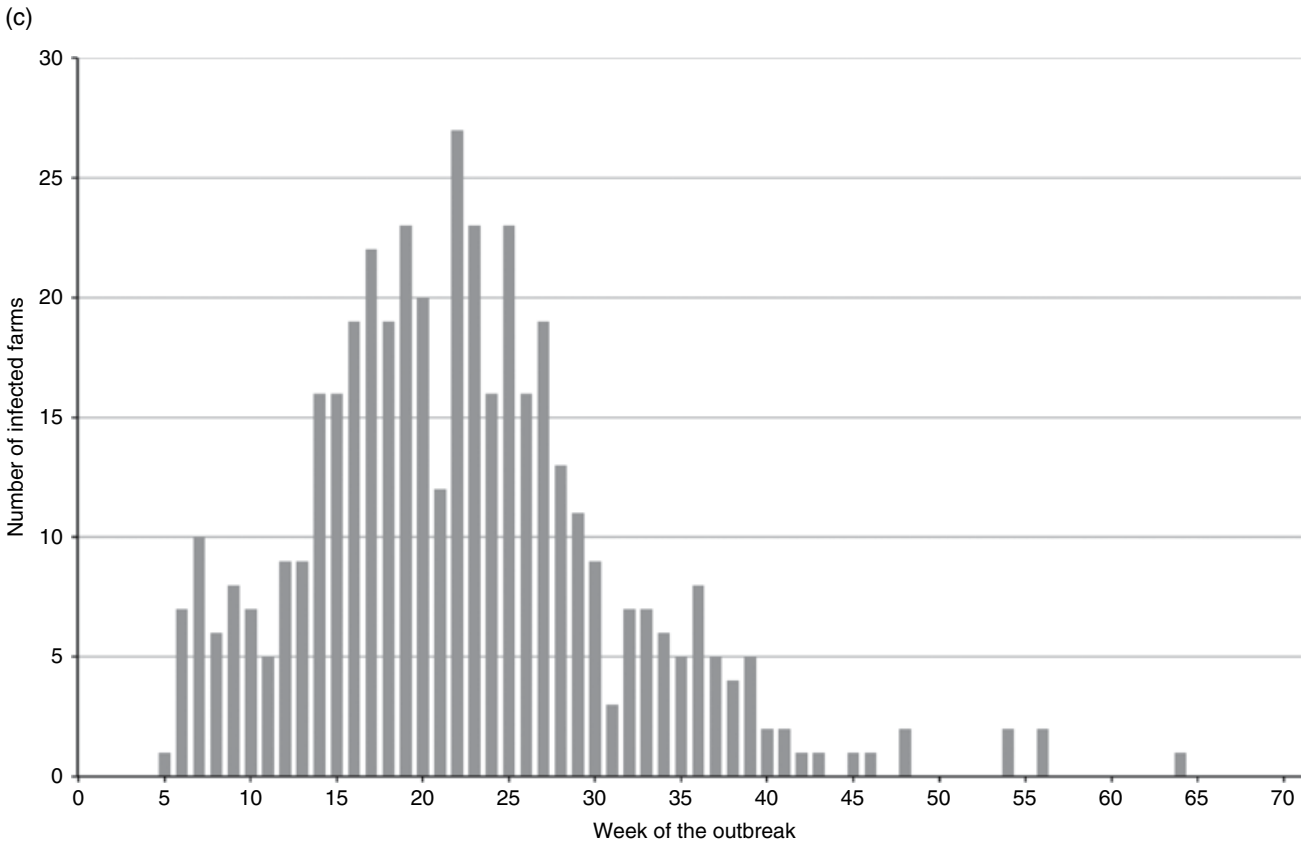


Figure 9.1 **(Cont'd)**

or indirect contact with an infected host through an animate or inanimate vector. Many pathogens take advantage of more than one of these modes in order to gain entry into the susceptible host through the alimentary tract, reproductive organs, or respiratory system, across the integument, or by inoculation (iatrogenic or wounds). Despite our best efforts, management of these modes of transmission or routes of entry does not reliably result in eradication of disease unless one also considers the mechanism by which a pathogen is able to persist in the absence of a suitable host. For a pathogen to perpetuate, a mechanism must be available for it to survive in nature. Thorough study of the mechanisms by which pathogens are able to perpetuate themselves will also have the advantage of giving insight into potential reservoirs of emerging agents. With this information, we may be better equipped to anticipate the impact of the changing ecology of traditionally important pig pathogens, as production settings are altered to meet the challenges of global food distributors, exacting consumers, and environmental mandates.

Classification of pathogens based on their mechanism for perpetuation leads to sensible guidance on the complexity of control and eradication programs that are required for their management. Through modification of early published insight on the topic of pathogen perpetuation (Matumoto 1969), five categories are proposed and described in order of the least to the most complexity required for pathogen eradication: vector-borne pathogens, short-cycle pathogens, long-cycle pathogens, resistant pathogens, and commensal pathogens.

Vector-borne pathogens

For the current discussion, pathogens that absolutely require a vector for successful transmission between infected and susceptible hosts will be considered vector-borne pathogens. Two features of this criterion should be clarified, namely, that the vector is animate and that the vector is required for transmission to occur. Inanimate vectors (e.g. boots contaminated with pig feces containing *Salmonella*) are essentially an extension of the infected pig itself and suggesting that there is any particular role the infected boot is playing in the transmission pathway is nonsensical. Also for the sake of clarification, pathogens such as African swine fever virus (ASFV) or PRRSV that are known to utilize insect vectors in their transmission but are not limited only to arthropod-borne spread will not be considered here. As an example, ASFV has three distinct transmission pathways, namely, a sylvatic cycle involving *Ornithodoros* ticks and wild suids, an *Ornithodoros* tick and domestic pig cycle, and a direct pig-to-pig transmission cycle,

suggesting that simply controlling the tick vector will not ensure eradication of the disease from a population of infected commercial pigs (Arzt et al. 2010). The essence of a vector-borne disease in the context of disease eradication is that control of the vector will ensure eradication of the disease.

At least two important infectious agents of pigs should be considered as exclusively vector-borne pathogens: JEV and vesicular stomatitis virus (VSV). Both agents are relevant to this discussion but for distinctly different reasons. Though the clinical disease due to JEV can be mild in pigs, pigs serve as an important reservoir of infection for maintaining a population of infected mosquitoes in a geographical region. Japanese encephalitis is a globally important pathogen of humans for which pigs play an important role in the disease's landscape epidemiology. Similar to JEV, VSV is also transmitted between pigs by an arthropod vector (the virus has been recovered from several different genera of mosquitoes, flies, and midges). However, the disease occurs relatively infrequently though the New Jersey serotype of VSV is particularly well adapted to pigs (Martinez et al. 2003). The significance of the disease, at least among FMDV-free countries, is that VSV produces vesicular lesions in pigs that are grossly indistinguishable from other notable vesicular diseases including those produced by FMDV, vesicular exanthema virus (VEV), and swine vesicular disease virus (SVDV). Clinical outbreaks of VSV in pigs will produce predictably significant short-term effects on international trade until other exotic pathogens are ruled out.

For these examples, introduction of the pathogen into a population of pigs, maintenance of the pathogen in the pig population, and transmission of the pathogen between pigs in the population can be absolutely controlled by preventing contact with their respective arthropod vectors. This makes their control and eradication a straightforward, though practically, very difficult process.

Short-cycle pathogens

Short-cycle pathogens include those agents whose greatest imperative is to quickly be transmitted to the next susceptible host as they generally have a very limited ability to survive outside the host. At first, one might assume this category would include all viruses as viruses are absolutely unable to replicate outside their host. However, there are at least a few notorious viruses and many bacteria that are perfectly content to bide their time (waiting, though not increasing in number) in a suitable environment until the right combination of ecological factors occurs that facilitates their entry into a new host where replication can begin again. Characteristics of short-cycle pathogens include rapid inactivation outside the host, short incubation periods, commencement of shedding shortly after infection becomes established (but without persisting for an extended time), rapid development of protective immunity, and a requirement for access to large populations of susceptible hosts in order for continuous transmission to occur.

Both bacterial and viral agents are represented in the short-cycle pathogen category with influenza A virus (IAV) in swine being an obvious member. IAV enters the respiratory tract as part of an oronasal or aerosol transmission event. Clinical signs of the disease become apparent in a matter of 3–5 days concurrent with establishment of high viral loads in lung tissue. High levels of virus are expelled in sputum or droplet nuclei over a relatively short period (less than 1 week) at which point clinical signs rapidly abate and solid immunity is conferred. Dense populations of susceptible individuals are necessary for the virus to propagate into an epidemic form. IAV epidemics are seasonally apparent in people due in some respect to the increased congregation of people indoors due to colder winter conditions. However, this seasonality is less apparent in modern pig production as large production cohorts are established in pig-dense regions on a continual rather than seasonal basis, thus meeting the requirement for access to large populations of susceptible hosts. The virus has evolved in a way that ensures an adequate size population of susceptible pigs (or a population of cohorts of pigs) remains available; IAV has the propensity to genetically drift and recombine at a rate faster than immunity from a prior infection is able to persist. Though having a different pulmonary cell tropism, porcine respiratory coronavirus follows a pattern similar to that of IAV and thus is also classified as a short-cycle pathogen.

Several enteric agents can also be considered short-cycle pathogens. Transmissible gastroenteritis virus (TGEV) and a related coronavirus, porcine epidemic diarrhea virus (PEDV), share similar features of oral exposure, rapid incubation (hours), high levels of virus shedding over a short period of time, and generation of a robust immune response (although short in duration).

Given their need for large populations of susceptible hosts, their infected host's short duration of contagiousness, and their limited ability to remain viable outside the host, control and even eradication are achievable goals for short-cycle pathogens. The fundamental element in eradication plans for these pathogens is the creation of closed populations so that a steady supply of new susceptible host is unavailable (by definition, transmission is primarily limited to direct contact); facilitating exposure of the pathogen to remaining susceptible hosts (when appropriate) in the face of an outbreak can also be utilized in order to shorten the duration of an epidemic and subsequently the risk of exposure to other susceptible populations.

Long-cycle pathogens

Long-cycle pathogens are those characterized by the ability to establish an infection in such a manner that the host remains contagious for an extended period of time. While these pathogens may share some attributes of short-cycle pathogens such as a rapid incubation phase, their hallmark feature is the ability to find an ecological niche in the host that will allow them to resist clearance by the normal immune mechanisms of the pig. Congenital and other forms of vertical transmission are also common features among long-cycle pathogens. Because of their propensity to establish a long-term contagious state in the host, these pathogens are not dependent on having access to large or dense populations of susceptible hosts; their ability to persist is largely independent of population size.

Several noteworthy viruses fall into the category of long-cycle pathogens as do a number of rather unique bacterial species. *Actinobacillus pleuropneumoniae*, *M. hyopneumoniae*, and *Mycobacterium* species (especially *Mycobacterium avium*) are respiratory bacterial pathogens characterized by their ability to induce chronic, smoldering infections in the lung. While sporadic outbreaks do occur, particularly with *A. pleuropneumoniae*, all three establish long-term residency in their own unique biological niche (pulmonary abscesses for *A. pleuropneumoniae*, the extracellular surface of the bronchial epithelium for *M. hyopneumoniae*, and lymph node or pulmonary abscesses for *M. avium*) that keeps them well isolated from the direct effects of the pig's immune system and promotes an extended period of contagiousness. Similarly, long-cycle viral pathogens exist that have a pulmonary tropism including Aujeszky's disease virus (ADV) and PRRSV. Despite both demonstrating the short-cycle attribute of rapid incubation period (and creating explosive epidemics as a result), their most significant epidemiological feature is the ability to establish a persistent infection. As a member of Herpesviridae, ADV creates a classic latent infection through invasion and residency in the trigeminal nerve where it remains ready to be reactivated to systemic sites (in preparation for an impending transmission event) when biological stress is experienced by the pig. Neurons are a site preferentially sequestered from most of the usual pathogen clearance mechanisms of the pig as most of these mechanisms are lethal to the host cell that is harboring the pathogen; with little redundancy in the nervous system, any immune response that results in the destruction of neurons is likely to have a profoundly negative effect on the host. While much of the pathogenesis related to PRRSV infection has been reported, the exact means by which it escapes clearance by the immune system remains unclear. The virus has a strong preference for long-term residency in various immune cells of monocytic lineage, and evasion mechanisms including production of quasispecies, presentation of decoy epitopes, and induced dysregulation of various cytokines and other immune messengers have been hypothesized though not fully explained (Mateu and Diaz 2007).

Long-cycle pathogens also occur in other body systems beyond the respiratory system. Various serovars of *Leptospira interrogans* occur in pigs and establish long-term infections in the kidney and urinary tract, *Mycoplasma suis* resides in the blood and hematopoietic system for extended periods, and *Brucella suis* establishes persistent infection of male reproductive tissues. The enteric system is home to several unique long-cycle pathogens. *Lawsonia intracellularis* presents in several different clinical forms but all share the unique histological feature of chronic inflammation of the small intestine caused by persistent intracellular infection of enterocytes and lamina propria cells by the bacterium. Spirochetal diseases of the colon (*Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*) can create clinical signs reminiscent of the diarrhea associated with *L. intracellularis* but are generally more severe. Intracellular residency in the epithelial cells of the colon again provides refuge from the immune system as it attempts to resolve the ongoing infection. *Salmonella* species, particularly *S. choleraesuis* var *kunzendorf*, also take advantage of the intracellular niche to avoid detection by the immune system. Erysipelas is another disease caused by a bacterial pathogen that finds a location preferentially sequestered from the immune system. Often as sequelae to the acute stages of the systemic illness (producing the unique erythematous dermatitis lesions that are the hallmark of erysipelas), the bacterium will often lodge in joint fluid, synovial membrane, and surrounding tissues to cause chronic arthritis. The acute bacteremia apparently violates the integrity of the joint–blood barrier, allowing *Erysipelothrix rhusiopathiae* to enter the joint space; after resolving the acute infection, the integrity of the barrier is restored, effectively separating the bacterium from the full range of immune clearance mechanisms.

The significance of long-cycle pathogens is the difficulty for which successful eradication programs can be established (excepting, of course, through complete depopulation of herds). Similar to short-cycle pathogens, most long-cycle pathogens are not particularly well equipped to thrive outside the living host for extended periods. What they are well equipped for is thriving inside the host for extended periods. Few reliable protocols for eradication of these pathogens exist at the herd level, and even fewer at an industry level. Quality diagnostic tests become the critical arbiter for most eradication programs designed for these pathogens. ADV stands out as a uniquely eradicable long-cycle pathogen given the combination of a highly efficacious vaccine, the availability of diagnostic tests that can differentiate infected

from vaccinated animals (DIVA), and accessibility to a wide array of highly accurate, sensitive, and specific diagnostic tests for both ruling in and ruling out infection with ADV. For the foreseeable future, efforts in managing most of the long-cycle diseases will be through control plans rather than eradication efforts. However, over time and with good compliance, effective control plans can lead to pathogen elimination but with whole herd test and removal procedures often required to identify the last of the persistently infected pigs.

Resistant pathogens

Some pathogens adopt a perpetuation strategy that involves creation of a stable form resistant to environmental degradation or inactivation. Whether this strategy is a result of evolutionary selective pressure or simply in response to the failed ability to transmit between hosts at short intervals is unknown, but regardless of the reason, it has developed into a highly successful strategy by a few bacteria and viruses and also by most of the pig nematodes. The characteristic feature of resistant pathogens is simply their resistance to inactivation in the environment (many remaining infective over months to years); resistant pathogens have no reliance on access to large or dense populations of susceptible animals. An additional common, though not requisite, feature of many long-cycle pathogens is their ability to indiscriminately affect different host species.

Two important bacteria will serve as fitting examples of resistant pathogens. The first bacterium is *Bacillus anthracis*. *B. anthracis* can produce various lesions and clinical signs in pigs ranging from acute sudden death to less dramatic syndromes that induce multifocal lymphadenitis. It is a historically important disease (though rarely seen any more in modern pig industries) but remains an important zoonotic risk for abattoir workers, particularly in those plants processing grass-fed ruminants. *B. anthracis* is a large gram-positive bacillus that when shed into the environment (often as a result of the death of an acutely affected animal) is signaled through exposure to oxygen to form a capsular spore that makes the organism highly resistant to inactivation by heat, desiccation, ultraviolet light, and disinfectant chemicals. Contaminated environments (notably, burial pits established as part of cleanup efforts after large outbreaks of anthrax) can remain a hazard for susceptible hosts for years. *B. anthracis* is able to infect nearly any mammalian species. The second bacterium that will be used as an example of a long-cycle pathogen actually represents most of an entire genus of bacteria: *Clostridium* spp. *Clostridium perfringens*, given its high prevalence in many commercial pig populations, will serve as a convenient example of the genus though a similar case could also be made for *Clostridium novyi, Clostridium botulinum, Clostridium chauvoei*, or other clostridial species. *C. perfringens*, like *B. anthracis*, is a relatively non-species-specific, gram-positive bacillus, proficient at spore formation under appropriate environmental conditions. Infection of young pigs by this particular species of *Clostridium* generally results in neonatal diarrhea with high within-litter prevalence. Farrowing sows that are housed outdoors often have a higher prevalence of clostridial diarrhea in their nursing piglets compared with their peers that farrow indoors, likely due to the simple fact that routine and periodic cleaning/disinfection of indoor farrowing facilities reduces (though does not eliminate) the infectious dose of bacteria presented to the neonatal pig. Genotyping of *C. perfringens* occurs as part of diagnostic investigation on some farms in order to support appropriate choice of pre-farrowing vaccinations, but once diagnosed on a farm, the disease tends to be managed rather than any attempt being made to eliminate the organism. *C. perfringens* is commonly found in commercial bovine, sheep, and goats with no or minor genetic changes relative to those isolates recovered from pigs.

Internal parasites comprise most of the membership in the group of resistant pathogens. For most of the pig nematodes of which the reader will already be familiar, the adult form of the parasite is relatively fragile in an environment outside the host. It is the parasite's ova that present the long-cycle perpetuation opportunity for the pathogen. Life cycles vary extensively between *Ascaris, Ostertagia, Trichuris*, and other significant pig nematodes, but the parasites do share some common features: they have long and complex prepatent periods ("incubation periods" relative to bacteria and viruses), require sexual reproduction, and produce ova capable of lasting years or decades in typical pig-raising environments. These ova can successfully resist desiccation, chemical inactivation, and in some cases (*Ascaris suum*) even physical removal from the environmental surface itself. Notable microscopic parasites that share some of these same features include *Trichinella spiralis* and *Toxoplasma gondii*; while previously mentioned nematodes such as *A. suum* do periodically infect man, *T. spiralis* and *T. gondii* have the well-deserved reputation of being substantial risks to public health in some countries.

In the context of disease transmission, control, and eradication, pathogens that perpetuate through use of environmentally resistant life stages present both opportunities and challenges. The bacteria used as examples demonstrate the difficulty that would be faced in attempting to establish eradication programs as the life span of the resistant organism likely exceeds the life span of the pig and the farmer! If one chooses to raise pigs in an environment that was used by a pig in a former time, there can be reasonable surety that the resistant pathogens have remained behind. Often, only low infectious doses are required to establish infection with resistant

pathogens, and once the first successful transmission event occurs, the infected individual can effectively recontaminate the environment with a sufficient number of the agent to repeat the cycle again in a week, a month, a year, or a decade later. Similarly, the internal parasites described above can be difficult to eradicate depending on the environment in which one chooses to raise the pigs. Over time, contaminated indoor facilities can be rid of parasite ova with fastidious hygiene, persistent use of anthelmintics, and screening of new stock to avoid reintroduction of the pathogen. In an outdoor environment that has previously housed pigs, eradication is likely an unachievable goal. Conceptually, for control of resistant pathogens, all one has to do is avoid contact between the susceptible host and the agent. Practically, that is also the biggest problem. "Where once a pig has lived, the roundworm will remain."

Commensal pathogens

Commensal, derived from the Latin word c*ommensalis* (*com-* meaning together and *-mensalis* indicating a feature *of the table*), was originally cast to describe those infectious organisms habituating the alimentary tract but causing no apparent harm to their host. The term has come to more broadly indicate the co-association of any two organisms that are not either dependent or parasitic on each other. Using this definition, one is provided with an apt descriptor for a class of pathogens that seem to be found in association with otherwise healthy pigs but have the notorious reputation for periodically transforming into a virulent state with expression of fulminate clinical disease. Mechanisms involved in this periodic occurrence are poorly understood for most of the pathogens in this group, though concurrent or recent infectious conditions, the presence of an external stressor causing a disruption in homeostatic mechanisms, and diminished barrier function have been implicated as contributing factors in humans (Tlaskalova-Hogenova et al. 2004).

Multifactorial causation is another term for the requirement that specific cofactors must be present in order for a commensal pathogen to cause clinical disease. Perhaps the prototypical pig diseases in this respect are the porcine circovirus-associated diseases (PCVADs); the causative agent porcine circovirus type 2 (PCV2) only recently emerged in pigs (Firth et al. 2009). Its associated clinical expressions remain a significant challenge to reproduce experimentally despite its widespread occurrence across pig farms around the world (Madec et al. 2008). Its classical presentation as a cause of nonresponsive wasting in juvenile pigs cannot be reliably reproduced in an experimental setting even when using low-passage field virus from affected farms. Characteristic tissue lesions can be generated in this setting but only rarely to the degree of severity and prevalence common in field outbreaks. Infection in the laboratory or in a field setting results in high levels of viral shedding with subsequently high levels of transmission occurring. However, the overt clinical signs described for PCVAD only seem to occur when the virus is present in combination with other infectious and noninfectious cofactors. Vaccination of pigs infected or expected to become infected with PCV2 reliably prevents the occurrence of clinical signs of PCVAD in affected farms (Kixmoller et al. 2008; Neumann et al. 2009), and even more interestingly, anecdotal evidence is accumulating that suggests vaccination against PCV2 can improve the performance of pigs on farms with known presence of the virus but without any clinical signs of PCVAD (Agten et al. 2010; Brons et al. 2010; Luppi et al. 2010; Sidler et al. 2010).

Commensal pathogens that appear to require the presence of an external stressor in order to be induced into causing clinical disease include *Haemophilus parasuis* and *Streptococcus suis*. Vertical and horizontal transmission pathways have been described for both bacteria; indeed it is very likely that no pig farm in the world is free of either of the pathogens. While genetically distinct strains of each organism are known to occur and these strains possess different combinations of identified virulence factors, it is rare that the presence of one of these virulence factors in a given strain is enough to simply explain the occurrence of clinical disease. The anecdotal literature presents examples of farms that operate with little or no clinical disease related to either pathogen for extended periods of time until without clear explanation, a spontaneous outbreak occurs (MacInnes and Desrosiers 1999; Tokach 1993). External stimuli proposed to induce outbreaks of clinical disease for *H. parasuis* or *S. suis* include temperature changes, feed outages, and coinfection with other agents (Drum and Hoffman 1998; Oliveira and Pijoan 2004; Villani 2003). Another streptococcal bacterium, *Staphylococcus hyicus*, is a common inhabitant of the pig and its environment and, in most instances, appears to simply exist as part of the external skin flora. However, the agent is implicated as the causative agent of exudative epidermitis or "greasy pig disease." Both the anecdotal and peer-reviewed literatures report that the disease appears both sporadically and epidemically on farms often without explanation; hypothesized cofactors for expression of the disease include low herd-level parity, hygiene, and overall poor quality of farm management and pig husbandry (Clark 2002; Murray and Rademacher 2008; Schwartz 2002; Zoric et al. 2009).

Colibacillosis, presenting both as neonatal diarrhea (primarily *Escherichia coli* possessing the fimbrial antigens F4, F5, F6, and F41) and as post weaning diarrhea (*E. coli* F41 or F18), presents an interesting situation with regard to diseases that are caused by commensal

pathogens. Numerous members of *Enterobacteriaceae* are considered commensal organisms, generally categorized as such by the absence of one of these recognized fimbrial types. However, reports describing typical clinical cases of neonatal colibacillosis or post weaning diarrhea but associated with non-typical or non-typeable *E. coli* are becoming more common (Harel et al. 1991). The extent to which an *E. coli* is either "pathogenic" or "commensal," or whether a given pathovar can move between these states, has not been fully resolved. Genetic mechanisms (horizontal gene transfer [HGT]) for exchange of virulence factors between pathovars of *E. coli* that allow for rapid shifts in the severity of the infection have been described in human infections (Croxen and Finlay 2010). In pigs, virulence factors similar or identical to those described for human isolates of the bacterium have been described, and it is likely that the same genetic mechanisms for their exchange occur (Wu et al. 2007; Zhang et al. 2007). Data from field studies of pig farms have shown that at least on a population basis, numerous fimbrial types of *E. coli* are present in pigs from shortly after birth (including those presumed to be pathogenic) and that the relative contribution of each fimbrial type changes with pig age (Katouli et al. 1995). The F4 fimbrial type has been studied extensively in this regard, and a particular pathovar expressing the O149 somatic antigen has been isolated from both healthy and diarrheic pigs (Amezcua et al. 2002, 2008; Melin et al. 2004). An explanation for its apparent presence both as a commensal and as a pathogen on a single farm remains unexplained but may involve contributions from the host, the environment, and additional virulence factors that are expressed or acquired in response to these contributing factors. The extent to which these genetically based changes are random or occur as a result of selective pressure is not entirely known.

The significance of recognizing the existence of a commensal pathogen type is that one has to recognize the fact that some pathogens are extraordinarily unlikely to be eradicable. Management of commensal pathogens will rely on establishing control measures (vaccination, treatment, and husbandry) that temporarily shift the balance of power into the hands of farmers and veterinarians and away from the pathogens.

Causal inference

Formal postulates for determining disease causation had their beginnings during the mid-nineteenth century and were most famously incorporated into an essay written by Jakob Henle (1838). His postulates were subsequently revised by one of his former pupils, Robert Koch, and presented in their more commonly accepted form in 1890 (Koch 1890): the agent must be present in every case of the disease under appropriate circumstances, the agent should occur in no other disease as a fortuitous and nonpathogenic agent, and the agent must be isolated from the body in pure culture, repeatedly passed, and induce disease when reintroduced to the host. The inadequacy of these postulates in covering all the potential relationships between pathogen and host became apparent within 10 years of their publication but nonetheless remain today as a useful framework for establishing infectious disease causality. Because of limitations introduced by strict interpretation of the Henle–Koch postulates, authors have attempted to generate other lists of causal criteria that incorporate an epidemiologically sensitive perspective of the agent–host–environment relationship embodied in disease ecology. A set of criteria for determining disease causation was published by Hill (1965) that seems to have captured the essence of an epidemiological diagnosis (Table 9.2).

Reading these criteria points out the need to understand the distinction between necessary and sufficient causes of disease. Multifactorial diseases are those cre-

Table 9.2 Epidemiological criteria for assessing disease causation.

Causal criteria	Definition
Strength of association	There is a strong association between the putative factor and disease (as measured by relative risk, odds ratio, and others)
Consistency	The association should be repeatedly observed by different persons, across different places, circumstances, and times
Specificity	The occurrence of one (or a group of) causal factor(s) should lead to only one disease, and the disease should result only from that cause
Temporality	Cause must precede effect; the putative factor must precede the occurrence of the disease
Biological gradient	When exposure to the factor falls along a gradient, higher exposure should result in more severe (or frequent) disease; a dose–response effect should exist
Plausibility	The association should be plausible with respect to biological knowledge, recognizing that our knowledge is limited to facts known at the time
Coherence	The cause and effect interpretation of the association should not seriously conflict with the generally known facts about the natural history and biology of the disease
Experiment	It may be possible to generate prospective experimental evidence whereby removal of the putative factor results in less severe (or frequent) disease
Analogy	Similar known examples of cause and effect can support the existence of a causal association

Source: Hill 1965.

ated by exposure to a combination of several independent factors. A necessary cause is considered to be an exposure (to an agent or risk factor) without which the disease cannot occur. Implicit in this definition is that this exposure, when occurring in isolation, may not cause disease in all individuals. Other risk factors (or agents) may need to be present simultaneously in order for the disease to occur. When, in combination, exposure to a defined group of factors results in disease in all the individuals, this group of factors is considered to be a sufficient cause. Necessary and sufficient cause provides a flexible framework for undertaking disease outbreak investigations in which the true cause of the outbreak is unknown. A cautionary note is, however, required. When sorting through the myriad potential causal factors associated with the occurrence of a disease, it is inevitable (particularly in the early phases of an outbreak investigation) that factors will be identified that have the appearance of being causally related to the disease but in truth are not causal. These factors are termed confounding variables or "confounders." By definition, a variable is a confounder if it is associated both with the true causal exposure variable and with the occurrence of the disease; however, the confounding variable must not be caused by the disease. A simple example illustrates the concept: as facts, we know that (1) smoking is strongly associated with the occurrence of lung cancer, (2) people that smoke tend to have yellow staining on the skin of their forefingers, and (3) only people that smoke have yellow staining on their fingers. If an early researcher was investigating the cause of lung cancer and did not consider the impact of smoking on the occurrence of lung cancer, he/she might erroneously report yellow fingers as the cause of lung cancer (i.e. strong evidence of a causal relationship as described above by Hill's criteria)! Unfortunately, most real-life situations are not this clear as we may have very little prior knowledge about risk factors and their relationship to a disease outcome. In order to organize one's thought processes during disease investigation activities, path diagrams or "webs of causation" can be created to evaluate the potential role of confounding variables in the outbreak. A causal web illustrating the association of various risk factors with respiratory disease in pigs is shown in Figure 9.2 as an example. Analytical methods

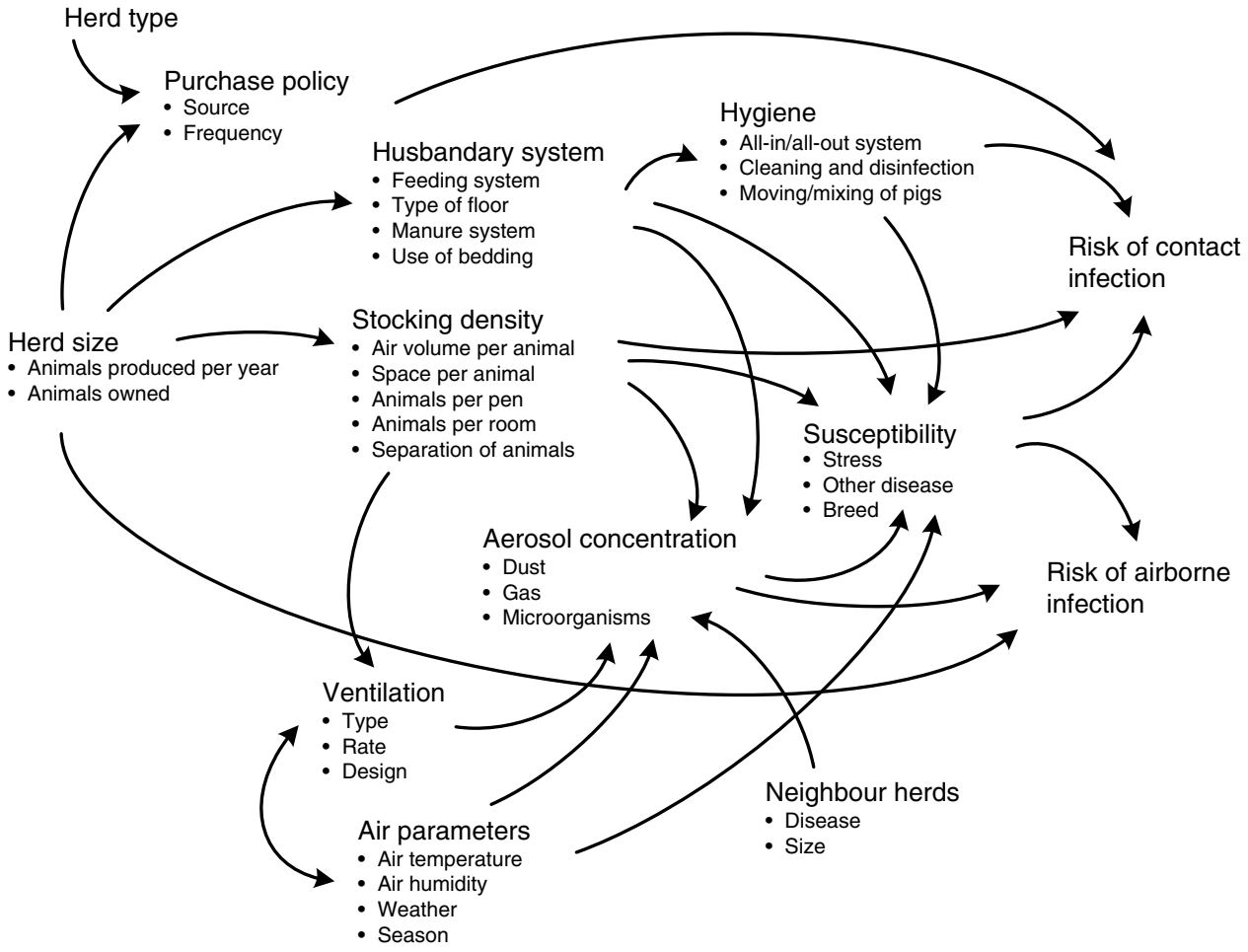


Figure 9.2 Causal pathway of risk factors associated with respiratory disease of pigs. *Source:* Stark (2000).

are available for assessing the certainty, strength, and interaction of risk factors and disease outcome when presented as causal webs (Stage et al. 2004).

Biosecurity

Biosecurity plans at their most rudimentary level attempt to manage the risk of introducing new pathogens into farms, livestock industries, or countries and to minimize the transmission of endemic diseases between farms within these levels. These goals are accomplished through segregation of uninfected from infected animals (or the pathogen itself) and thorough cleaning and disinfectant of livestock premises and facilities (Madec et al. 2010). However, when considering biosecurity, there is a greater imperative at stake than just the health of livestock species. Veterinarians and farmers have an opportunity and perhaps even an obligation to develop a more holistic approach to biosecurity that appreciates agriculture's contribution to zoonotic disease transmission, farmed animals as a source of human foodborne pathogens, and the broader impact of livestock farming on the environment.

Biosecurity is fundamentally about managing two sources of special cause variation on farms: pathogens and people. Sources of variation in the biological or financial performance of a farm can be attributed to either normal (i.e. random) variation or special cause variation. Normal variation – be it related to a pig's genetic potential for growth, the lysine content of a soybean, or the average ambient temperature in Ames, Iowa, during December – is by definition predictable given some current and historical knowledge of the biology, chemistry, or physical properties of that particular variable. By contrast, special cause variation describes those fluctuations in the value of a variable that exceed the boundaries of what we consider to be "normal"; this exceptional fluctuation may be defined as one that falls beyond plus or minus three standard deviations from the mean value, one that contributes more to the risk of a particular outcome than what would normally be expected, or any other measure one may determine is appropriate for the purpose. Systematic fluctuations due to normal variation in livestock production systems cannot be eliminated, though one strives to reduce the range of normal variation. The occurrence (and magnitude or frequency) of special cause variation in production systems, however, can absolutely be managed with the ultimate goal being to eliminate it entirely.

Biosecurity as a discipline began making an appearance in the livestock-related literature during the 1990s with several authors presenting work aimed at creating consensus around definition of the term and the scope of activities that it might encompass (England 2002; Pyburn 2001). A review of relevant literature on the subject around the same time (Amass and Clark 1999) confirmed a paucity of facts that could scientifically support the recommendations being made by veterinarians to their clients about biosecurity measures. The same author then embarked on a series of innovative studies to debunk biosecurity myths (Amass et al. 2000), establish the efficacy around commonly used disinfects and their appropriate usage (Amass 2004), and understand the role of people and inanimate objects in disease transmission (Amass et al. 2003a,b,c).

With the advent of real and perceived global worry over terrorism and the occurrence of large-scale outbreaks of animal disease, including the emergence of novel zoonotic pathogens (Table 9.3), a plethora of organizations began publication of biosecurity guidelines for use by livestock producers. One author surveyed the literature and the Internet for farmer-targeted publications on biosecurity in the United States and found 111 publicly available sources representing all the major livestock species (Moore et al. 2008). Despite a similarity in recommendations made across many of the publications, the author speculated that the sheer mass of information available likely contributed to poor biosecurity compliance due to confusion over "which guidelines do I use?" Indeed, several authors have reported poor farmworker compliance with biosecurity recommendations (Nespeca et al. 1997; Vaillancourt 2005) with at least one study suggesting that farmers could not perceive which of the biosecurity practices (that they were asked to perform) were most useful or important (Casal et al. 2007).

Biosecurity planning

Within farm (herd level)

Biological risk management (BRM) has been suggested as a term for use of management tools that help to identify infectious hazards to a farm (or veterinary practice, other livestock premises, etc.), to assess the risk presented by each hazard, and then to develop plans for managing each of the hazards (Bickett-Weddle 2005; Ramirez and Zaabel 2012). Based on traditional thinking, a farm's biosecurity plan would be considered a failure at the moment a disease was introduced to that farm. However, a more modern approach to biosecurity planning such as that embodied in BRM planning recognizes that disease risk cannot be completely eliminated, but only managed. Also, because few farms are constructed or operated in an identical manner, it would be illogical to think one biosecurity plan could meet the needs of all farms. Several risk-based methodologies have been established for development of biosecurity plans including a public–private partnership in the United States called the Secure Pork Supply (http://www.

Table 9.3 Twenty-five years of pig disease: emergence or reemergence of pig pathogens (1990–2016).

Year	Disease	Location/emergence	Species	References
1991	PRRSV	United States, Europe	Pigs	Terpstra et al. (1991)
1992	*Brucella* spp.	United States	Humans	Centers for Disease Control and Prevention (1994)
1993	*Salmonella enterica* spp. *enterica* serovar *infantis*	Denmark	Humans, pigs	Wegener and Baggesen (1996)
1994	Porcine circovirus type 2	Europe, Canada	Pigs	Edwards and Sands (1994)
1995	Japanese encephalitis virus	Australia	Humans, pigs	Hanna et al. (1996)
1996	*Brachyspira (Serpulina) pilosicoli*	United Kingdom	Humans	Trott et al. (1996)
1997	Porcine hepatitis E virus	United States	Pigs	Meng et al. (1997)
1998	Nipah virus	Malaysia	Humans, pigs	Centers for Disease Control and Prevention (1999)
1999	West Nile virus	United States	Humans	ProMED-mail (1999)
2000	Classical swine fever virus	United Kingdom	Pigs	ProMED-mail (2000)
2001	Foot-and-mouth disease virus	United Kingdom	Multiple	ProMED-mail (2001)
2002	Aujeszky's disease virus	United States		ProMED-mail (2002)
2003	Porcine myocarditis (Bungowannah) virus	Australia	Pigs	Kirkland et al. (2007)
2004	Methicillin-resistant *Staphylococcus aureus* (MRSA)	United States		Voss et al. (2005)
2005	*Streptococcus suis*	China	Humans, pigs	ProMED-mail (2005)
2006	Porcine high fever disease	China	Pigs	ProMED-mail (2006)
2007	African swine fever virus	Georgia	Pigs	ProMED-mail (2007)
2008	Ebola-Reston virus	Philippines	Primates, pigs	ProMED-mail (2008)
2009	Pandemic H1N1 influenza virus	Worldwide	Humans, pigs	Centers for Disease Control Prevention (2009)
2010	Foot-and-mouth disease virus	Japan	Multiple	ProMED-mail (2010)
2011	Novel influenza virus	United States	Pigs, cattle	Hause et al. (2013)
2012	Porcine deltacoronavirus	Hong Kong	Pigs	Woo et al. (2012)
2013	Porcine epidemic diarrhea and porcine deltacoronavirus	United States		Stevenson et al. (2013)
2014	Atypical porcine pestivirus	United States	Pigs	Hause et al. (2015)
2015	Senecavirus A	United States, Brazil	Pigs	Hause et al. (2016)
2016	Porcine circovirus type 3	United States	Pigs	Palinski et al. (2017)

securepork.org/index.php), an audit-based approach developed specifically for use on pig farms called the Production Animal Disease Risk Assessment Program (http://vdpambi.vdl.iastate.edu/padrap/default.aspx), and an Australian swine-specific biosecurity assurance program called the Australian Pork Industry Quality Assurance Program (http://www.apiq.com.au/) that has its basis in hazard analysis and critical control point (HACCP) methodology.

HACCP methodology is a useful tool in constructing BRM plans. It had its origin in the US National Aeronautics and Space Administration (NASA) in the 1960s. Logistical planners involved in design of systems that would support astronauts while they were in space recognized a need for systematic methods of quality assurance (QA) around food products that would accompany the astronauts. Recognizing the inadequacies of a quality system that relied on end-product testing of food (accuracy and reliability of testing, determination of which hazards should be tested, high cost, and time factors related to testing), NASA began working with process engineers at Pillsbury to develop a method that would ensure the creation of safe food products through quality monitoring of the production process rather than

the production output. The full history and development of HACCP is reported elsewhere, and the reader is encouraged to read further for more detail (Dunkelberger 1995; Sperder and Stier 2009). Process monitoring rather than end-product testing is a useful paradigm for establishing BRM plans.

HACCP methods are based on seven principles: (1) identification of potential hazards; (2) determination of critical control points (CCPs) at which a process can be established to mitigate any hazard that is introduced; (3) establishment of critical limits around each CCP; (4) creation of a process to monitor each CCP; (5) establishment of corrective actions to be taken in the event a critical limit is exceeded; (6) institution of recordkeeping procedures that can document CCP monitoring activity; and (7) verification of HACCP system performance through periodic quality testing of the end product. As described above, pathogens and people present the two most important sources of special cause variation. The fact that both are animate variables (as opposed to a kernel of corn, a concrete floor, or a mechanical fan) gives them the unique ability to respond to feedback from the farm operator, and they are thus likely to generate special cause variation. Pathogens respond to this feedback through evolutionary mechanisms such as acquiring resistance to antibacterials (Aarestrup et al. 2008), modifying their epitopic presentation to a pig's immune system (Ostrowski et al. 2002), acquiring virulence mechanisms (Villa and Carattoli 2005), or simply emerging from nature as a novel pathogen (Kirkland et al. 2007). People respond to feedback through all of the predictable behaviors that typify the human condition: avoidance of disliked tasks, poor compliance with procedures having little direct or short-term benefits to themselves, reluctance to seek clarification when requested assignments are poorly understood, and the "creative will" or tendency to modify established processes for their own best fit. Using HACCP methods as a basis for development of BRM plans, especially when they are developed in conjunction with the farmworkers expected to comply with the BRM plan, provides a platform whereby biosecurity compliance can become the norm rather than the exception. HACCP helps us to separate special cause variation from normal variation, identify the cause of the special variation, and then remediate the process.

A simple example using HAACP to manage the hazard of disease introduction (e.g. leptospirosis, toxoplasmosis, swine dysentery, others) through exposure of pigs to rodents will illustrate the process. For this example, step one or hazard identification is straightforward: lack of an effective rodent control program will increase the likelihood of contact between pigs and the disease vector. Step two is identification of appropriate CCPs; this step may be assisted through construction of process flow diagrams or causal webs as has been described above. Logical CCPs in this rodent control process might include placement and management of baiting stations and environmental management around the periphery of buildings (avoiding accumulation of materials that may be used by rodents as nesting/hiding spaces, controlling vegetation, and avoiding feed spills). Establishment and monitoring of critical limits around these CCPs are straightforward and will fulfill the third and fourth steps in HACCP. For example, the expected rate of bait usage can be estimated with assistance from a pest management company, and monitoring the usage rate of the farm can be as simple as tracking the history of bait purchase and ensuring the appropriate amount is being used each month. Establishment and monitoring critical limits around the environmental management CCP requires a bit of creative thinking but little hard work: grass and weeds should be kept mowed to a height less than 10 cm, a walking space the width of a person's outreached arms should be maintained clear of debris around the exterior of all buildings, and all feed spills should be cleaned up on a daily basis (effectively, zero tolerance). Step five or the establishment of corrective actions is again straightforward in this example but has to be considered in two phases. Phase one is giving immediate attention to the CCP that is out of control, e.g. "go mow the grass" or "go clean up the feed spill." Phase two of the corrective action is as important as phase one but is often forgotten – that being the need to remediate any product (sick pigs) that may have been negatively affected during the period, the CCP was out of compliance. In our example, it may be the case that the farm was participating in a *Leptospira*-free production scheme and that the potential exposure to rodents may require a self-imposed quarantine period, a "no-ship" period, temporary use of vaccines or medication, or undertaking targeted surveillance in order to reenter the scheme. Step six in HACCP is creation of a system of written documentation that provides evidence of all management activities related to each CCP. This can seem an onerous task to the farmer but actually provides a value-added opportunity for the willing veterinarian, as well as providing information useful when working backward in time to determine the cause of a disease outbreak or production loss. Periodically, HACCP-monitored processes require validation to ensure they are actually doing the job for which they were designed. Validation is the essence of the seventh and final step in HACCP. Returning to the rodent control example, validation steps might include an inspection of the building spaces for evidence of rodents (feces, holes in walls, bedding material), a nighttime visit to maximize the chance of visualizing rodents, rodent trapping, and serological disease testing of the pigs.

Establishing a thorough understanding of a risk-based process for developing a BRM plan is critical for both the farmer and the veterinarian. It offers an intuitive process for engaging the farmer and the staff, it provides a logical framework for organizing one's ideas about the specific biosecurity needs of a given farm, it forces one to methodically analyze effectiveness of a BRM plan, and it creates a clear understanding about the consequences of noncompliance with the plan.

The specific elements to be included in a BRM plan can vary extensively between farms and geographic regions. Resource documents are widely available from producer and veterinary organizations (Moore et al. 2008), state and national animal health departments, and international organizations such as the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO). The reader is encouraged to familiarize himself/herself with the resources available, much of which can be freely accessed via the Internet. A recent and comprehensive guide that defined a number of good practices for pig farm biosecurity was produced by FAO in 2010 (Madec et al. 2010). Given the global audience of FAO, the guide necessarily discusses the unique issues presented by several types of pig industries including village-based low-intensity pig scavenging production, small-scale but confined pig production, and large-scale intensively managed production. The guide avoids an explicit discussion of risk-based BRM plan development but instead provides a succinct discussion of the major causal pathways for disease introduction related to a failure in biosecurity; this is a fitting addition to the early planning stages of risk-based BRM plan development in modern intensive production systems.

Between farms (national level)

Biosecurity planning does not stop at the farm gate and, in many respects, is not confined to one's own sector of the agricultural industry. National-level biosecurity planning has achieved a much higher profile than even 10 years ago for reasons including real and perceived anxiety over national security, terrorism, negative responses to large-scale euthanasia campaigns associated with animal plagues, and emergence of novel human, animal, and zoonotic pathogens. These factors when combined with increasing public worry over our care of the environment, concerns about global climate change, growing sensitivity to animal use and well-being, and the recent occurrence of several unusual foodborne disease outbreaks have created a paradigm shift among national policymakers. This paradigm shift is all about harmonization.

In a key guidance document published by FAO in 2007, the following definition of biosecurity was proposed: "A strategic and integrated approach to analyzing and managing relevant risks to human, animal and plant life and health, and associated risks to the environment" (Anonymous 2007). Through the use of this definition, the authors developed arguments that supported development of harmonized national biosecurity policies that could take advantage of synergies that may exist across different economic sectors and between industries within a single economic sector. Intuitively, there would seem to be an advantage that could be gained from establishing a single authority that could manage all the biosecurity risks across primary production (animal feed and livestock), food processing (slaughter and processing), and the environment (water and air quality) as the activities involved in all these economic sectors are interdependent. This national biosecurity model was proposed by authors of the FAO report *a priori*, meaning there existed little prior evidence at the time to establish that the harmonized approach would, as a matter of fact, be an improvement over the traditional approach to biosecurity whereby primary production, food processing, and the environment are managed under separate authorities. At the same time, we do have compelling evidence that inadequacies do exist in traditional national biosecurity models, so the current momentum to shift toward a more modern approach will likely continue with some advantages and disadvantages yet to be realized.

Risk-based biosecurity planning remains important at the national level just as it did at the local farm level described above. While the seven steps in HACCP allow one to work at a relatively high level of detail for planning that is customized to the specific needs of a farm, a modification of the approach is required for use at the national level. The HACCP model places particular emphasis on hazard identification, recognition of special cause variation, and then remediation of the process or product after an out-of-control event has been detected. The modifications to HACCP that have been made in order for it to function at a national level shifts the emphasis to gaining a better understanding of the nature of a particular identified hazard: what is the probability that a recognized hazard will occur, what are the consequences if the hazard does occur, and what is the cost-to-benefit ratio of mitigation strategies that might be employed to prevent the hazard from occurring?

The OIE pioneered some of the early efforts to establish a harmonized framework for assessing risk around national biosecurity and animal health, and the framework is widely used as the basis for many types of risk analysis today (Anonymous 2010). The OIE risk analysis process includes four steps: hazard identification, risk assessment, risk management, and risk communication (Figure 9.3).

The OIE hazard identification step is identical to step one in HACCP and attempts to answer the question

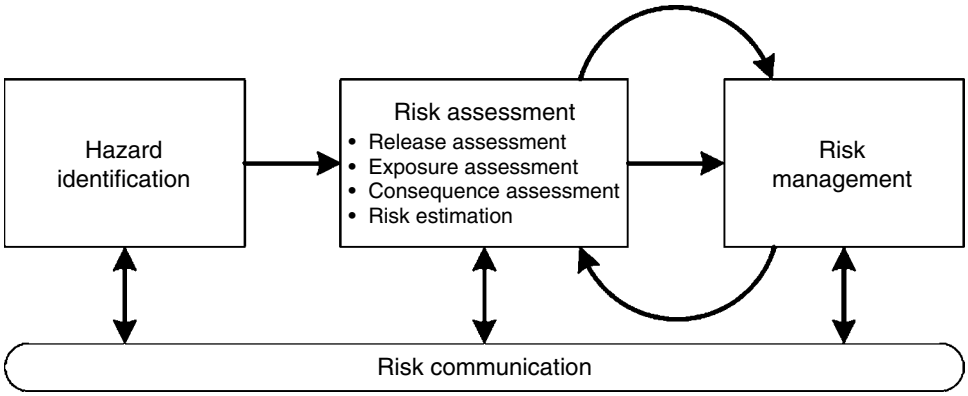


Figure 9.3 OIE risk analysis framework for development of import health standards.

"What can go wrong?" and, depending on the reason for conducting the risk analysis, may be answered simply through creation of a list of unwanted livestock pathogens or diseases, or may be more complex including identification of hazards that cross species or economic sectors. Step two of the OIE risk analysis is the actual risk assessment itself. The distinction between "risk analysis" and "risk assessment" is important. Risk analysis reflects the entire process from hazard identification through to the communication plan around the process and its outcomes. Risk assessment, however, refers specifically to the characterization of the risk associated with each identified hazard and includes an assessment of the likelihood of occurrence and the likely severity of an occurrence. In the context of a risk analysis conducted to inform policy around proposed changes to importation of a risk item (animals, animal products, semen, etc.), OIE specifies four tasks that should be completed as part of a complete risk assessment: First, a release assessment describing the biological pathways necessary for an importation event that would result in introduction of the pathogenic agent into a country should be prepared; this should include an estimate of the probability of those events occurring. Second, an exposure assessment should be conducted to described the pathways and associated probability of a domestic animal (or human, or farm) becoming exposed to an imported pathogen that was able to successfully negotiate the barriers designed to prevent its release. The third task that should be carried out is a consequence assessment that explores the consequences (production losses, welfare implications, financial impact) of a successful exposure event. This necessarily includes determination of the farm, national, and cross-sector consequences of a successful exposure. At the conclusion of these three tasks, a fourth task called the risk estimation is undertaken. The risk estimation combines the probabilities of all the events occurring, from hazard identification through the consequence assessment, to arrive at an overall estimate of the risk associated with the proposed activity; the risk estimation may be quantitative, qualitative, or descriptive depending on the quality of data inputs and the needs of the relevant stakeholders. After completion of hazard identification and risk assessment, the risk management step is initiated. Risk management, as the name implies, explores the possible mitigation strategies that will be considered to manage any residual risk that was identified during the risk estimation step. In a thorough risk analysis, the risk management proposals will be analyzed using a process similar to the process used for risk assessment and will yield three important risk estimates for comparison: the baseline likelihood of entry (the *status quo* risk that existed prior to the proposed activity being undertaken), the unrestricted likelihood of entry (the expected *post facto* risk if the proposed activity is undertaken, but no risk mitigations are implemented), and the restricted likelihood of entry (the expected *post facto* risk that remains if the proposed activity is undertaken and the recommended risk mitigations are implemented). These three estimates of risk are compared in light of what is considered an acceptable level of risk (ALOR) and may include a macroeconomic cost-to-benefit analysis as well. The fourth and final step in the OIE risk analysis framework is referred to as risk communication. Ideally, conducting risk analyses should be a participatory process (consultation with stakeholders at each step in the analysis), iterative, transparent, and public (inform stakeholders of the conclusions that were reached).

Across borders (international level)

Methodology for management of transboundary animal diseases at an international level is similar to that described for national policy on biosecurity. The additional influence presented by the current global political situation, the global economy, and international treaties and agreements to which a country is bound bears mentioning. Regulation of international biosecurity is only complicated if a country wishes to conduct trade with another country. In the absence of trade, there is no

mandate to follow any other country's recommendations or requirements. However, most countries do not have the ability (or desire) to remain independent from international trade of goods, and nearly always, some give-and-take is required in order for two countries to agree on the terms of trade.

International standard setting organizations such as the Codex Alimentarius Commission (CAC), the OIE, and the World Trade Organization (WTO) develop standards that can serve as a unifying framework, if not obligatory standard, by which countries can reach consensus around their differing trade requirements. The CAC is a body that was created in 1963 by FAO and the World Health Organization (WHO) for the purpose of establishing standards relating to foods, food production, and food safety. The OIE was established through international agreement in 1924 (in response to an outbreak of Rinderpest in Belgium in 1920); the organization is responsible for improving animal health around the world and currently has 176 member countries and territories. The WHO is an agency within the United Nations that has a role similar to that of OIE but focused primarily on public health rather than animal health; WHO was established in 1948. The WTO is responsible for the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement). The SPS Agreement is an international treaty managed by the WTO since 1995. Under the SPS Agreement, guidelines relating to the safe importation of food, animal, and plant products are negotiated and maintained.

Rushton in 2006 presented four principal threats that could contribute to the occurrence of a transboundary biological emergency (Rushton and Upton 2006): (1) emergence or occurrence of transmissible disease from a local wildlife population, (2) disease relocation as a result of movement of migratory wildlife or birds, (3) disease introduction through legal or illegal exportation of wildlife and livestock products, and (4) emergence of a disease or difficulty related to changes in the way we raise livestock.

Other factors of unknown significance might also be added to the list of global contributors to animal, human, and zoonotic diseases. It seems the extensive interconnectivity of modern society has created numerous complex systems, defined by nonlinear flow processes that by their very nature tend to be unstable, unpredictable, and interdependent and that when they fail produce sequelae that cannot be anticipated. Who could have anticipated the chain of events that would be required to link purposeful melamine contamination of wheat gluten by a Chinese supplier to contaminated pet food in California and to melamine exposure in pigs in various regions of the United States (and the subsequent worry over human exposure through consumption of tainted pork) (Baynes et al. 2008; Burns 2007)?

Plant pests, and biosecurity plans to manage them, have their own historical narrative, but a theme that runs through the commercial agronomy sector (but with application to the commercial livestock sector) is the risk presented by monoculture of particularly desirable crop species. Given the introduction of a suitable pest, monoculture plantings create a bounty of susceptible hosts of which a pest can take advantage; the introduction of soybean rust (*Phakopsora pachyrhizi*) into the Midwestern United States (Li et al. 2008) and the epidemic infestation of pine bark beetles (*Dendroctonus* spp.) in forests and timber plantations on North America's Pacific Coast (Flint et al. 2009) are classic examples of the vast biological niches available to an appropriately positioned pest in today's modern agriculture. Do commercial pigs essentially represent a mammalian monoculture? It is a challenging task to qualify what differentiates one pig breed from another in modern commercial pig genetics, but at least at a phenotypic level, there exists a huge similarity between pigs across the world, a population ready to be exploited by a fortuitous or cunning pathogen.

The significance of the two most important sources of special cause variation, pathogens and pigs, cannot be underestimated. The role of pathogens has been described in the preceding paragraphs. However, the role of people deserves another special mention – that being the risk of intentional introduction of infectious diseases to livestock populations. Agricultural bioterrorism has been an increasingly worrisome topic for national biosecurity managers and policymakers alike. Though one author has identified the occurrence of only two intentional biological attacks on North American agriculture (purposeful iatrogenic contamination of veterinary supplies with anthrax and glanders around the time of the First World War and contamination of dead stock that were destined for processing into animal feed, with pesticides in 1997) (Ackerman and Giroux 2006), the world investment in modification of infectious agents as weapons (for people or animals) is extensive (Szinicz 2005). Indeed our ability to create life from the pool of available molecules and laboratory accessories appears to become more proficient each day (Gibson et al. 2010).

Distinguishing special cause from normal variation (or signal vs. noise) will be an important requisite skill for risk analysts, biosecurity managers, and veterinarians in the future. It presents the evidence for distinguishing risk from reality in the spectrum of livestock disease.

Diagnosing failures of biosecurity

People working at all levels in animal agriculture benefit from good biosecurity practices and therefore have a role in behaving responsibly with regard to actions that may increase the risk of diseases occurring. Whether one is part of a commercial farming enterprise, raises a few

animals or birds as hobby, or is only affiliated as a service provider such as a veterinarian, feed supplier, or livestock hauler, everyone involved in livestock or poultry production has a responsibility to develop good biosecurity habits. Animal agriculture is highly interconnected, meaning that one person's bad behavior will not only negatively affect that person's animals but is also very likely to have a negative consequence for someone else such as a neighbor or business relation.

When disease occurs, some investigation of the cause of the disease is usually undertaken. In the case of endemic pathogens or for relatively low-consequence diseases, the farmer may initiate an investigation himself/herself armed with historical knowledge of his/her own farm and its disease history. However, in those situations where a disease outbreak is caused by a new, unusual, or high-consequence pathogen, a more formal investigation to determine the cause of the outbreak is warranted and may involve a number of different people.

In an earlier edition of this book, Dial et al. (1992) presented a series of hierarchical trees that described the interrelationships between various factors that contributed to reproductive failure in swine breeding herds. These flow diagrams challenged the reader to look beyond the symptom of a reproductive failure (such as "low numbers of pigs weaned per sow per year") when investigating a problem and instead focus on identifying and resolving the points of failure that actually created the observed symptom. By analogy in a clinical setting, a veterinarian tends not to focus on examination and treatment of the erythematous dermatitis lesions on pigs that are produced as a result of infection with *Erysipelas rhusiopathiae*. Instead, the clinician should focus on risk factors for the disease itself including vaccination status, vaccine efficacy, sources of exposure to the organism, and others to ensure that in addition to providing supportive care to the infected individuals, adequate resources are put toward reducing the likelihood of occurrences of the disease in the future.

Using a similar approach, a framework for logically exploring the potential areas of biosecurity failure that might have contributed to a disease incursion is described in the sections below. Risk factors contributing to biosecurity failure have been aggregated into four themes: live animals, transportation, feed and feed ingredients, and people. While additional themes could be developed, these four provide sufficient guidance to ensure that the individuals involved in conducting most disease outbreak investigations probe deeply enough into the "symptoms" of biosecurity failure to discover their underlying root causes.

Live animals

Contact between diseased live pigs (including all the body fluids they excrete) and non-diseased pigs provides the best opportunity for successful transmission of pathogens. In modern commercial pork production, the notion of "population medicine" drives investigation, treatment, and prevention of disease. One of the tenets of production in this respect is the maintenance of closed herds, which do not permit the routine introduction of live animals not reared by that farm, versus open herds, which permit introduction of live pigs not reared by that farm. Production systems in the United States frequently operate as a series of "multisite" farms, which complicates the definition of a closed versus open farm. However, as a basis for exploring risk factors related to live animals, this provides a convenient first criterion (Figure 9.4).

Closed herd (farmer raised)

As the name implies, in a closed herd, all gilt replacements are selected from animals raised on the farm, and no additional pigs are brought onto the site for finishing. Closed herds must be further separated in order to deal with the significant differences presented by closed herds that operate in multisite production systems (which are often large, complex, and involving commingling of pigs from different sites) and more traditionally defined closed herds that operate on a single site. In the case of multisite closed herds, one can most simply manage their biosecurity risk by considering them to be a special case of an open herd; risk factors related to live animals on these herds will be discussed in the section below on open herds.

Disease management The cornerstone of managing live animal biosecurity planning is implementation of an overall disease management strategy for the farm. This will likely include standard protocols for managing the overall health of the herd such as identification and treatment of sick animals, vaccination programs, and biofeedback procedures but will also describe specific actions related to ongoing monitoring of herd health.

Whole herd disease monitoring Key to ensuring healthy internal replacement gilts on a closed herd is maintaining excellent health across the entire herd. While needs vary between farms, many veterinarians recommend the use of routine slaughter checks and review of data collected at the processing plant, review of performance records, and ongoing diagnostic testing such as routine serology and submission of tissues to a diagnostic laboratory.

Replacement gilt disease monitoring Though in many respects one can consider all pigs on a single-site farm to have the same health status, acclimatization and monitoring internally selected replacements prior to their introduction into the breeding herd are warranted. Most

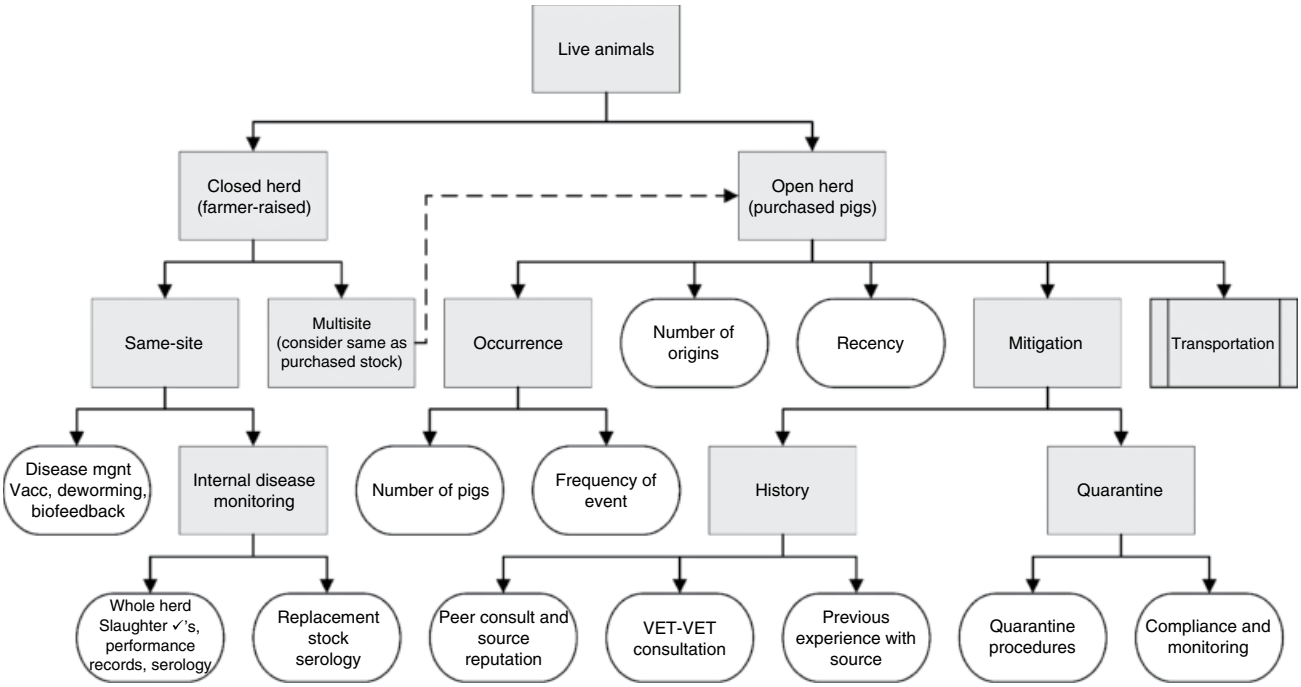


Figure 9.4 Root causes of biosecurity failure related to introduction of live animals.

typically, the focus for these animals is acclimatization to ensure they have been safely exposed to (and recovered from) reproductive pathogens that are circulating in the breeding herd. Serological monitoring can be a useful to tool to determine if these animals have had adequate controlled exposure (through biofeedback or vaccination) to agents such as porcine parvovirus and PRRSV (if present) before breeding occurs. As with all diagnostic testing procedures, attention should be given to collecting samples from a statistically valid, representative sample of animals.

Open herd (purchased pigs)

Open herds may be composed of breeding animals, growing animals, or both. In all cases, it is ideal that pigs managed as open herds are derived from a single source. In addition, for grower herds, it is ideal that these pigs be managed as a single all-in/all-out (AIAO) cohort with cleaning and disinfection (C&D) between cohort groups. When the open herd includes breeding females and the stock being introduced are replacement gilts, additional steps should be included to ensure their biosecurity risk to the existing breeding herd (which is not typically operated in an AIAO manner) is managed.

Number of origins Reducing the number of different origins from which purchased pigs are sourced is an effective means of managing the risk of disease incursion. This is true specifically in the case of significant endemic agents (such as PRRSV in the United States) that are known to exist as divergent variant strains for which immune cross-protection is unlikely or unpredictable. By limiting the number of pig sources, the likelihood of introducing new strains will decrease. The same holds true for exotic pathogens but to a lesser extent.

Recency Considering the time period between observation of a disease event and the last introduction of purchased animals can be very helpful in assessing the likelihood of the purchased animals being responsible for the disease incursion. In most cases, as the interval increases, the likelihood of the new pigs being the source of a new disease decreases.

Related to the number of origins and recency, two additional risk factors associated with the occurrence of new pig introductions should also be considered by those managing the risk or responding to a disease outbreak: number of pigs being introduced and the frequency with which new pigs are introduced.

Number and frequency of pig introductions Though their significance can be either hidden or overinterpreted when assessed using "one-size-fits-all" outbreak questionnaires, information related to both the number of pigs introduced per event and the number of introduction events per time should be examined carefully. Personal experience and review of the scientific literature are often relied in assessing this information as there is no single rule of thumb that separates a "risky" from

a "safe" introduction of pigs. Knowledge of pig management factors, housing, environment, pathogen factors (route of transmission, ID50), and host factors (immunity, age) are required to interpret the significance of number and frequency risk factors.

There are few activities that contribute risk to disease introduction that cannot be mitigated to some extent. When developing biosecurity plans related to the introduction of live animals, mitigation measures can be applied both ahead of the event (evaluation of historical information) and after arrival of the pigs (quarantine activities).

Peer consultation and source reputation, "vet-to-vet" consultation, and previous experience with source People or businesses that make a habit of knowingly supplying diseased pigs or whom fail to act responsibly when it occurs by chance are often well known by their own reputation. Veterinarians and farmers should take action prior to the purchase of pigs to establish a consensus opinion on the health status of the pigs to be purchased from a potential supplier. This can be done by each contacting individuals in their own peer groups, talking to other customers, speaking to the veterinarian that works for or is employed by the supplier, and considering one's own previous experience with the supplier.

It is important to establish whether the source herd has had any recent disease outbreaks, determine what diseases have or have not been detected or tested for in the source herd, and become familiar with the vaccines and antimicrobials used on the source farm. Determine if the source herd routinely conducts serological surveys of the herd, and if so, request recent results of the surveys.

In addition to collecting as much historical information as possible about the source herd, isolation and quarantine procedures should be formally developed for the recipient farm. Once developed, a monitoring program should be established to ensure the established isolation and quarantine program is being followed by farm staff.

Quarantine procedures Because the incubation period for different disease agents is highly variable and the incoming stock may not exhibit any disease symptoms for some time, it is essential that these animals remain in quarantine until test results and clinical observations provide reasonable assurance they are healthy. The quarantine period also allows time for any new diseases that have incurred on the source farm to be recognized and to inform customers. Quarantine also provides insurance in the rare event that the incoming pigs became infected with a pathogen while in transit. The duration of isolation will depend on the disease agents that are of the most concern but typically lasts between 30 and 60 days.

Isolation allows pigs to recover from the stress of transport to the farm and adapt to a new environment and provides a window of time for any incubating infections to become evident. When possible, isolation should be accompanied by an acclimatization program that ensures the incoming pigs have time to be effectively vaccinated against (or exposed to through controlled biofeedback) for those diseases known to be endemic on the recipient farm. Pigs should be moved through the quarantine facility as an AIAO group. When possible, a separate person and equipment should be dedicated to work in the isolation facility, or if not possible, work at the facility should be left as the last work task of the day.

Activities related to the quarantine and acclimatization program such as vaccinations should be recorded on a daily log along with any health observations including the presence of coughing, excessive sneezing, diarrhea, blood or mucus in the feces, loss of appetite, skin lesions, or lameness. It is typical that some type of serological monitoring program be undertaken as part of the program. This may involve blood sampling at entry and again prior to release from quarantine to look for the presence of disease antibodies or to confirm acclimatization procedures have provoked an immune response in the pigs. Importantly, enough time needs to be allowed for the return of laboratory results and the potential need to retest animals if necessary.

Compliance and monitoring In HACCP terms, quarantine and acclimatization can be considered a CCP, which is monitored through review of the daily activity log and serological monitoring of pigs at arrival and departure. However, given the importance of quarantine and acclimatization, farms should take the extra step to have a "third person" (someone without daily responsibility for care of pigs in the isolation facility) periodically assess the level of compliance with the procedures that have been established for the facility. This may involve reviewing the daily log of activities from several AIAO cohorts of animals that transited the facility, review of any serology results that are available, interviews with staff members charged with its daily operation, and possibly retesting of pigs that were introduced to the farm 6–12 months prior.

Transportation

Transportation, as a risk factor for biosecurity failure, is related primarily to movement of animals (live and dead), feed and ingredients, and other general supplies on or off the farm. The following sections will deal with transportation of animals and other supplies; feed will be covered in its own section.

This section on transportation will focus on processes related to the act of transportation, as distinct from the risk associated with the goods that are being transported.

In this respect, the risks associated with transportation of live or dead animals can be combined and discussed as one. These risks can be categorized into those that are associated with the driver, those associated with the truck, and those that occur as a result of transportation management or methods (Figure 9.5).

Live and dead pigs

Hygiene and behaviors of the driver Drivers contribute a very tangible risk to the transportation process because in many instances, the driver is either not involved with the rearing of pigs being hauled (and hence disconnected from any of the negative consequences of his behaviors) or is acting as an external contractor to the farm (and therefore may not be directly responsible to the farm but instead is responsible to his employer). In any event, one must accept that drivers themselves are a distinct risk factor for which training and minimum standards of performance should be developed.

While describing the exact standards for managing this risk is beyond the scope of this text, a few basic tenets are offered. Drivers should be provided with and expected to wear clean clothes or coveralls and boots during loading and unloading procedures. Related to this is a requirement to train the driver in donning and removing this gear in a manner that avoids him cross-contaminating his clothing or the inside of the driver compartment. The driver is expected to comply with all biosecurity measures that are in place on the farm, which again requires that the farmer invest in providing some basic level of training to the driver. Drivers should not enter farm buildings under any circumstance, and therefore a clear virtual or physical line of separation needs to be established at all loading and unloading areas.

Vehicles and their drivers that present a risk of transmitting diseases into a pig production unit include straight trucks, semi-tractors, semi-trailers, pickups and trailers, cars, all-terrain vehicles, motorcycles, farm tractors, livestock carts, farm equipment, etc. The degree of risk depends on how recently the vehicle has been exposed to other pigs or livestock farms and if the pigs on the farm have direct or indirect contact with the vehicle. Specific aspects of risk factors related to the vehicle are described below.

Cleaning and disinfection Proper C&D of vehicles used to transport live pigs is one of the key methods to prevent transmission of disease to a swine operation. In addition to commercial vehicles, farm vehicles hauling market pigs and cull sows need to be properly cleaned and disinfected prior to returning to the farm perimeter. When commercial truck wash facilities are not available, a dedicated cleaning area for pig transport vehicles that have been used off the farm should be established. There is extensive literature on development of C&D processes for a given farm including required equipment, use of detergents, high-pressure steam, disinfectants, and drying.

In some regions, recycled wash water is commonly used in the early stages of trailer cleanout protocols, and when this is the case, strict policies should be in place to manage the potential for cross-contamination of "cleaned" trailers. It is important to thoroughly clean and disinfect the tires, wheel arches, and underside of the vehicle as well as the transport compartment itself. Special procedures need to be developed to ensure the driver compartment is cleaned at the same time as the trailer and that the driver is trained in how to minimize contamination of the area during deliveries.

Drying and downtime Drying of all surfaces within the truck prior to moving fresh batches of animals is a critical step in the C&D process. In and of itself, drying, especially if done under heating conditions, can serve as a very effective disinfectant process for surfaces that have already been cleaned. When C&D has been properly applied, the additional risk-reducing contributions of drying or downtime are difficult to quantify. However, in reality, C&D procedures are rarely perfect, and given the enormous negative consequences of a disease incursion related to transportation, many farms willingly invest in the extra transportation capacity required to allow for drying or downtime of trailers. To improve the economics, some farms opt to build these procedures into high-consequence transport events such as the movement of replacement females but forego the risk in lower-consequence movements such as the final movement of finishing pigs to a processing facility.

As described above, in addition to driver- and vehicle-related biosecurity risks for transportation, there are also aspects of how transportation is managed that can contribute to or mitigate the risk.

Multispecies hauling In some parts of the world, the use of trucks to sequentially or simultaneously move several species of livestock is common practice. While for explicitly species-specific pathogens this practice would seem to be a reasonable way to improve the economics of haulage due to less downtime for cleaning and perhaps better vehicle usage efficiency, the problem is that one never knows what pathogens are on a truck, and therefore the potential for "the next load" of animals (of a different species) to become infected is also unknown. The practice also tends to propagate a sense that hauling livestock in contaminated vehicles is acceptable, which, when combined with the likelihood that the driver is disconnected from the rearing of these same livestock, does little to contribute to good overall biosecurity habits.

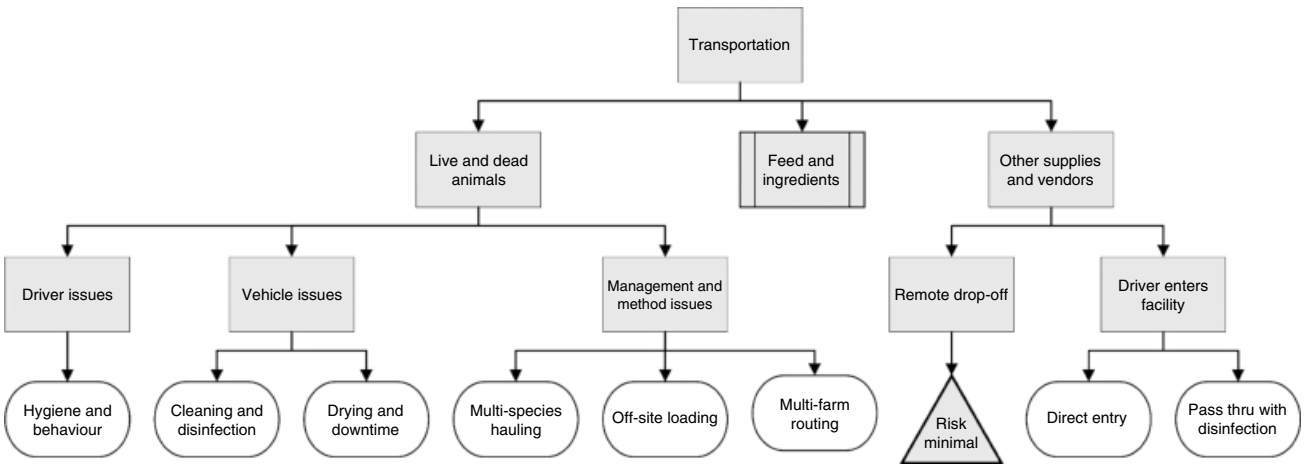


Figure 9.5 Root causes of biosecurity failure related to transportation of animals and other materials.

It goes without saying that adoption of multispecies haulage practices will lead to larger-than-expected outbreaks of high-consequence multispecies pathogens such as FMDV, when they occur.

Off-site loading Loading and unloading pigs is a common means of bringing disease onto farm. To manage this risk, the loading ramp should be sited at the perimeter of the farm whenever possible and connected to the rearing facilities by covered alleyways. However, this is an uncommon setup on most commercial farms that more often resort to attaching the loading ramp directly to the facility. When this is the case, clear procedures need to be established to avoid drivers entering the facilities, farm staff entering the transport vehicle, and pigs inadvertently returning to the facility after having entered the trailer.

In many cases, particularly when the transport event is infrequent, "off-site loading" can be implemented. While there are many variations of this practice, the basic principle is to use a dedicated farm vehicle to remove pigs from a facility, which is then driven a short distance outside the farm perimeter where a commercial transport is waiting. The two vehicles can then reverse to each other and allow pigs to be transferred directly from the farm vehicle to the commercial hauler. The hauler can then depart for its destination having never entered the farm premises, and the farm vehicle can then be cleaned and disinfected at the dedicated location on the farm premises. Obviously, an identical procedure but in reverse order can be used to move animals into the farm facility.

While on-farm composting is becoming increasingly common, many regions still rely on pickup of dead stock by commercial animal rendering companies. Under no circumstances should rendering pickup vehicles enter the farm premises. Instead, a small mortality depot station should be constructed that spans the farm perimeter. The depot should have one door on the farm side for staff to deliver dead stock and a second door on the opposite of the depot that can be accessed by the rendering hauler. In this way, cross-contamination between the parties can be easily avoided.

Multi-farm routing

In an ideal world, all pig deliveries would arrive as a single point-to-point delivery. Often however, particularly in the case of breeding stock deliveries, this is not practical. In this case, the best option for the farm is to be the designated first delivery point. Of course, not every farm can be the first delivery point, and therefore adoption of an off-site loading procedure may be the most appropriate way to manage the risk. In a multi-farm routing situation, adherence to good biosecurity practices outlined above related to the driver and vehicle is singularly important.

Other supplies and vendors

Pigs are only one of the biosecurity risks that arrive (or depart) from farms. Feed and feed ingredients represent another important potential biosecurity risk that will be covered in a later section. However, other supplies (or vendors) routinely arrive at farms and present a biosecurity risk. These supplies and vendors may carry goods that have an inherent risk (such as semen), but the risk being described here again is related to the carrier, not the goods themselves. Delivery companies bringing goods and supplies to farms often organize their routing such that dedicated drivers and vehicles are assigned to rural routes. In other words, it is highly likely that a delivery person will be making multiple stops and different farms, all within a few minutes or hours of each other. Depending on how the delivery is managed at each farm, the delivery person, or their vehicle, or both can have a high risk of carrying infectious agents from farm to farm. Fortunately, options exist to manage this risk.

Remote drop-offs

Remote drop-off points provide an excellent way to prevent vehicles and drivers from entering the farm. A drop-off point might be as simple as a locked shed at the perimeter of the farm premises, a cooperating local rural business, or a staff member's garage. While it is possible that the delivery person contaminates the area around the drop-off point, the farm staff has the advantage of knowing that contamination potentially occurred and takes measures to mitigate the risk; the farmer takes responsibility for managing the risk rather than entrusting his herd's health to the postal service! This method is only suitable for small items and may not be appropriate for perishable items such as vaccines or semen, so other options are needed, which require the delivery person to enter the farm premises. In the special case of service personnel (electricians, plumbers, engineers, etc.), these people usually must enter the farm facility itself.

Direct entry

If a driver must enter the farm premises to make a delivery, suitable procedures or physical barriers should be established to prevent the driver from entering the facility itself. While more elaborate systems are possible, the simplest rely on fencing the farm perimeter, allowing for only a single entry point through a locked gate. On the gate should be a sign that provides two phone numbers: mobile number for the farm manager and a land line for the farm office.

Service personnel should be notified well ahead of time that they will be required to follow all the biosecurity procedures required of farm staff before being allowed inside the farm facilities. Often, only certain vendors cater to the specific needs of pig farms, and they will be familiar with the need to attend to biosecurity during their visit.

Pass-through with disinfection Some farms make use of a pass-through box that allows a delivery person to place items into a small chamber accessed outside the farm (office usually) and the farm staff to retrieve the item from inside the office. Some pass-through boxes have inbuilt disinfection equipment, but more often, the pass-through box is located in a known "high-risk" location where the outer packing material can be disinfected (or destroyed), thereby managing the potential for the packing material itself to act as a physical vector for pathogens.

Feed and ingredients

Feed and ingredients present a special biosecurity risk to farms for several reasons. First, feed or ingredients typically require delivery to the farm and therefore present a transportation risk (covered above). Second, because of their high usage rate, transportation events are frequent, and even rare feed-associated risks are highly relevant because of the high quantity of the products that are used. Third, pig feed in many countries contains ingredients that are derived from pigs or other animal (or fish) species, thereby opening the possibility of direct pathogen introduction into the pig farm. This section describes the risk of biosecurity failure related to feed and feed ingredients, categorized by risk associated with delivery and risks associated with manufacturing (Figure 9.6).

Delivery issues

Multi-farm routing and other transportation issues While this issue was covered in some detail in the transportation risk section above, it is worth noting a few unique transportation issues related to feed. Feed delivery trucks are commonly on multiple farms each day (different loads) and are often scheduled on multi-farm delivery routes; this occurs much more commonly than with livestock transport vehicles. In principle, managing the transport risks for feed and ingredients is much the same as for livestock trucks, but one must be diligent in their management because the frequency of delivery is much higher than for livestock. It is rare for more than 1 week to pass without a feed delivery truck entering a farm. Ideally, trucks delivering feed should be able to unload the feed without entering the pig site. This procedure requires that all bulk bins and facilities storing bagged feed be on the inside edge of the pig site perimeter. Having the feed truck fill all bulk bins without entering the pig site is not practical on many pig farms because numerous bulk bins are located at different sites within the pig unit, some bins hold a small volume of feed, and several different diets are simultaneously used (Levis and Baker 2011). Therefore, other procedures to minimize risk of disease transmission by the feed truck and driver must be used.

Cross-contamination of compartments and loads While feed trucks come in many different configurations, nearly are constructed in such a way that multiple diets or ingredients can be kept separate during transport. With this convenience comes the potential for cross-contamination. Specific procedures for loading, unloading, and cleaning out feed trailers are well established but are beyond the scope of this text. Cross-contamination between compartments within a truck can result, for example, in feed specified and correctly manufactured as "animal protein-free" being delivered to a farm with low levels of animal protein contamination as a result of inappropriate truck cleanout procedures. A similar risk exists for contamination with noninfectious yet unspecified ingredients such as antimicrobials. Further complicating the issue of cross-contamination in trucks is that much of the manipulation of the unloading controls is done blindly by the driver as he is not able to easily visualize what is happening inside the truck until after unloading has been completed.

Manufacturing issues

Manufacturing issues that contribute to biosecurity failures may be associated with the manufacturing process, the ingredients themselves, or inadequate QA processes aimed at detecting and remediating manufacturing faults. Each of these issues will be described in turn in the following sections.

Biosecurity risks related to the feed manufacturing process may occur as a result of cross-contamination events inside the manufacturing plant or as a result of defective further processing steps that occur after feed ingredient mixing.

Cross-contamination in the facility may occur between ingredients, between finished feeds (or between finished feeds and ingredients), or by finished feed or ingredients coming into contact with non-feed contaminants.

Between ingredients All commercial feed manufacturing facilities are under managed under strict state and federal regulations aimed at controlling the use and distribution of antimicrobials, ingredients of animal origin, and potentially toxic ingredients, ingredients that are a potential risk to humans, and others. To the extent possible, manufacturers use dedicated ingredient storage bins (or disposable bags) to manage the potential for cross-contamination of these ingredients. However, for high-volume ingredients or high-throughput ingredients, bulk storage is common. This creates the possibility that a bulk storage bin is not completely emptied prior to a subsequent load (or perhaps even a different ingredient) being added. Commercial feed mills can be relied upon to have processes in place to manage these issues, but

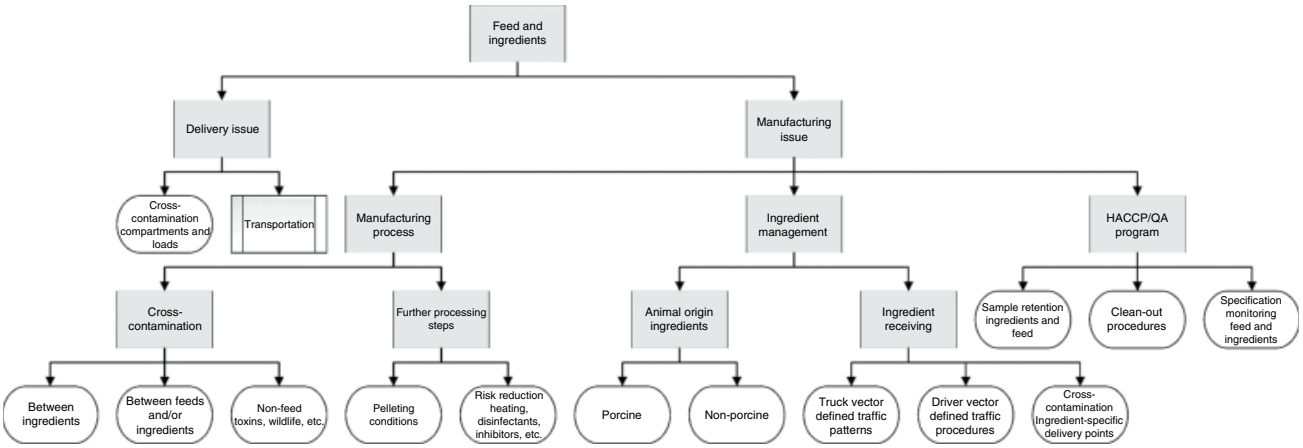


Figure 9.6 Root causes of biosecurity failure related to feed ingredients and manufacture.

on-farm feed manufacturing is exempted from many of the regulations enforced at commercial facilities. In any event, when unexpected or unexplained disease events occur on farms, one is expected to thoroughly investigate all possible points of failure including feed ingredient cross-contamination.

Between feeds and other ingredients The potential exists for cross-contamination to occur during latter stages of the feed manufacturing process, both at commercial facilities and on-farm mills. Managing the issue and investigating cause of disease outbreaks that may be related to a feed contamination event are identical to those described above for ingredients.

Non-feed toxins, wildlife, etc. Feed and ingredients are presented with an opportunity for contact with numerous non-feed contaminants. Among others, these can include fungal spores, industrial fluids from manufacturing or delivery equipment, pesticides, herbicides, rodenticides, and feces or urine from wildlife, birds, or pets. Some of these contaminants may produce no harm to animals or the public, but others can have quite significant effects. Those charged with investigation of animal disease outbreaks need to keep an open mind and seek to identify all risk factors that might have contributed to what has been expressed as an infectious disease outbreak.

For various reasons including minimizing risk associated with feed-borne animal pathogens, further processing steps are often employed as part of feed manufacturing. When animal diseases occur despite the implementation of these further processing steps, investigation of the processes should be undertaken.

Pelleting conditions Pelleting is a very common process involving application of steam, heat, and pressure that serves to improve feed digestibility, improve feed handling characteristics, and lower the concentration of infectious agents in which they exist. Importantly, the time and temperature required to sterilize feed through the pelleting process could have the simultaneous effect of substantially decreasing the nutritional value of the feed. Pelleting (when done correctly) does not sterilize feed but can substantially reduce pathogen numbers if they are present; this effect varies according to the pathogen, and therefore a uniform reduction of risk should not be anticipated for all pathogens.

Remember that poor manufacturing, delivery, or storage practices post pelleting can result in the feed becoming recontaminated, again reinforcing the need to evaluate all steps in the feed manufacturing and delivery processes to determine their contribution to a disease outbreak.

Risk reduction, heating, disinfectants, inhibitors, etc. Besides pelleting, other further processing steps may be implemented, or other ingredients may be added in an effort to minimize the risk of infectious agents being delivered to pigs through their feed. These will not be covered in detail here, but similar to pelleting, when the role of feed is being investigated as a contributing factor in a disease outbreak, efforts should be made to identify and assess all further processing steps that are being used in the manufacturing facility.

As described earlier in this section, some feed ingredients themselves present a biosecurity risk to pigs. Specifically, ingredients derived from animals are frequently included in diets as a high-quality source of amino acids or energy (as fat). Managing the risk associated with these ingredients requires special attention and is a particular area of focus for authorities that regulate manufacture of animal feeds. More generally, commercial and on-farm feed manufacturers receive frequent deliveries of ingredients (animal origin and otherwise), and if good procedures are not in place to ensure their careful receipt and storage, the potential for cross-contamination abounds.

Porcine origin In some countries, the general restriction on feeding same-species ingredients to livestock is exempted for pigs as there is no clear evidence supporting the existence of an endogenous porcine prion. For this reason, feeding of porcine blood products and some tissue extracts continues in these countries. Under perfect conditions, the likelihood of most pathogenic bacteria and viruses relevant to pigs surviving the manufacturing process for these ingredients is very low, and any that do appear to be present in extremely low concentrations are unlikely to provide an infectious dose to a pig. However, manufacturing processes are imperfect, and so occasional batches of product can be assumed to have higher than anticipated levels of contamination or have somehow become cross-contaminated with untreated raw ingredient during manufacture, storage, or transport. When these ingredients are being included in pig diets or when feed being used on a farm is being sourced from a manufacturer that uses these ingredients, they should be included in the investigation of a disease outbreak.

Uncommon in most commercial pig farming industries but common among noncommercial and backyard farmers is feeding of human food waste (e.g. swill) to their pigs. Virtually all countries with a functional national agriculture ministry or department have regulations that stipulate heat treatment of food waste before it can be fed to pigs; many countries have banned the practice altogether. Nonetheless, the practice of feeding untreated food waste (often derived from large institutions such hospitals, restaurants, schools, or prisons) appears to be relatively

common in some countries with modern commercial pig industries. When an outbreak of an unexpected disease occurs in a commercial herd and noncommercial pigs are known to be kept in the vicinity of the farm, the possibility of the noncommercial farm becoming infected through feeding of untreated waste food (and subsequently infecting the commercial farm through aerosol, indirect, or local spread) should be investigated.

Non-porcine animal origin Ingredients derived from ruminant, poultry, and fish are common in many feed manufacturing facilities, even if not being purposefully included in pig diets. Animal pathogens are promiscuous, and given an opportunity, many have the potential to cross-species and infect pigs. As all of these animal-origin ingredients have the potential to be contaminated with pathogens, they need to be included in any investigation of feed as a contributing factor to a pig disease outbreak.

Regardless of their origin, all feed ingredients have the potential to become contaminated during the receiving process. The contamination can occur as a result of truck or driver mismanagement or through cross-contamination in the receiving equipment (dumping pits, augers, etc.).

Truck vector (defined traffic patterns) It is impossible for feed transport vehicles to avoid dirty roads and possible contamination. This can be a particular problem during the winter when liquids and debris can freeze on the exterior surfaces of the truck, only to fall off once arriving at the feed mill premises. Traffic patterns should be developed for the mill premises that will minimize ingredient delivery trucks (i.e. not typically traveling to farms) coming into contact with feed delivery trucks (i.e. traveling to and from farms on a frequent basis). In addition, hygiene procedures should be developed that minimize the opportunity for debris and liquids that dislodge from the truck to fall into the receiving equipment.

Driver vector (defined driver procedures) Similar to procedures developed for truck drivers (feed or animals) making deliveries to farms, procedures should be implemented to ensure ingredient delivery truck drivers do not contaminate the ingredient they are hauling, as well as contribute to keeping their own personal cleanliness (including the driver compartment) to a high standard. Just as the trucks themselves should follow designated traffic patterns at the feed manufacturing facility, the drivers should also have dedicated footpaths that keep them separate from drivers making deliveries to livestock farms.

Cross-contamination (ingredient specific delivery points) Some ingredients due to their volume, frequency of delivery, or risk profile require dedicated receiving areas or equipment. As is the case with all other activities related to feed or ingredient manufacture, storage, or distribution, any failure in process management will lead inevitably to a cross-contamination event.

The final element to consider in assessing the contribution of feed to an animal disease outbreak is related to implementation of a QA program at the manufacturing facility. These QA programs are typically designed using HACCP principles and importantly should cover all activities that occur at the manufacturing facility as well as prescribe quality standards or specifications for their ingredient suppliers. There are at least three functional areas that should be included in a QA program: sample retention, cleanout procedures, and specification monitoring of ingredients and finished feed.

Sample retention (ingredients and feed) Regardless of whether feed is being manufactured at a commercial facility or on farm, a policy for sample retention (ingredients and finished feed) should be implemented. The specifics of a sample retention policy are beyond the scope of this text but are available from other sources. Sample retention is not a simple procedure as it requires an investment in time, space, and recordkeeping. However, the rapid rate of feed disappearance on most farms means that any feed considered to have the potential for involvement in an animal disease outbreak has typically been consumed prior to investigation of the disease. In these cases, sample retention programs are the only way to go back in time and determine the true contribution to the outbreak.

Cleanout procedures There are numerous points at which cross-contamination of feed or ingredients can occur during manufacturing, storage, or delivery. Aside from having good procedures and training in place designed to avoid the occurrence of cross-contamination, having clearly defined cleanout procedures at every stage in the process where contamination might occur is the best way to mitigate the risk.

Specification monitoring, feed, and ingredients Fundamental to HACCP-based QA programs is the need for quality specifications at all CCPs, a program to monitor the specifications through collection and analysis of data, and finally period verification processes to ensure the specifications are adequate to achieve the desired level of quality in the final product.

All feed mills should demand quality of themselves through production of safe feed manufactured to the specifications demanded by the customer, as well as demanding quality from their suppliers. Because of the regulatory environment around commercially manufactured feeds and ingredients, quality specifications are generally available. However, it is up to the customer to know what they are getting. Specifications do not necessarily equate to high quality; specifications simply state the extent to which a

product meets some standard (within a given level of tolerance) when in fact the standard may be inadequate for the desired purpose! Determine the appropriate standard for your purpose, inform your supplier, and then periodically test the supply at an independent laboratory to verify compliance with the specification.

People

People comprise the fourth and final risk factor that can contribute to a failure in biosecurity and result in an outbreak of disease. The discussion of people-related risk factors that follows first explores the risk contributed by people who have recently traveled internationally (and hence potentially been exposed to pathogens exotic to their country of residence) as compared with people that have only traveled nationally in their recent history. In this respect, we are chiefly concerned with the risk associated only with those people that may have direct or indirect contact with the pig farm of interest: risk contributed by the remainder of the general population can be ignored with a few notable exceptions, which will be described later (Figure 9.7).

People can act as either a biological vector (the person is truly infected with an agent that can also infect pigs) or a physical vector, meaning they are acting as a fomite carrying the agent on their person, clothes, gear, or perhaps their oronasal surfaces.

The risk of people acting as a vector should be considered in light of their anticipated extent of interaction with pigs on the farm. For farm visitors (including people such as vendors or delivery personnel), direct contact with pigs is not expected, and therefore they present only a minimal risk of introducing a pathogen onto the farm. This of course assumes that these individuals follow the prescribed protocols developed as part of the transportation section of a farm biosecurity plan described above.

Regardless of the above, it should be standard practice to have a designated visitor parking area away from any of the pig facilities, that visitors are wearing visibly clean clothes and footwear, and that they sign a visitor log that must include, among other items, their most recent contact with pigs.

Farm staff or other people that can be expected to have either direct or indirect contact with pigs on the farm require much more scrutiny. A number of conditions might reasonably be required of these people including such things as not being permitted to own or work with other pigs, not being allowed to live at another pig farm, not being allowed to come to work if they have influenza-like symptoms, and not being permitted to bring raw or untreated meat products onto the farm as part of their personal lunch. In addition, standard processes should be established and trained around implementation of a number of key risk-reducing practices.

Shower-in/shower-out

Many farms, particularly in the United States, require all visitors and staff to shower in to the farm at the beginning of the work day and then shower out at the end of the day. While providing an opportunity to remove and pig pathogens being carried on the person, the real benefits of shower-in/shower-out systems are that there is a clear demarcation of where "dirty" (i.e. outside the farm facility) ends and "clean" (i.e. inside the farm facility begins) begins and the process forces staff to leave their personal clothing and footwear outside the farm and only use gear that is

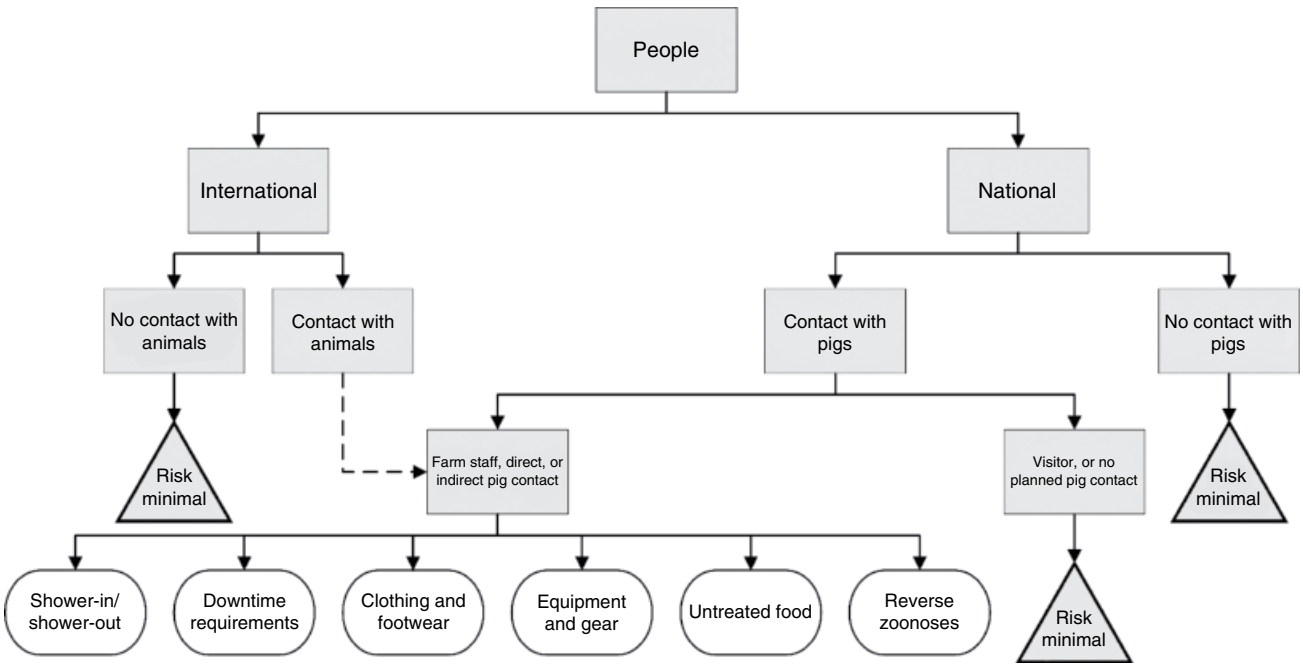


Figure 9.7 Root causes of biosecurity failure related to animal contact with people.

known to be clean while coming into contact with the pigs. The process has the side benefit of allowing staff to leave the farm in a clean state, minimizing contamination of their personal vehicle and home. Farms that implement shower-in/shower-out typically provide all the necessary clothing and footwear for all staff and provide facilities to launder everything inside the farm facility, avoiding the need to carry any farm clothing outside the facility.

Downtime requirements

The duration of time a visitor needs to be away from all pigs before allowed entry into a pig farm is quite variable, almost certainly related to the paucity of actual data on the subject. However, intuitively it makes sense to avoid contact with pigs prior to entering a farm. Some farms vary the requirement based on the consequences that might result if a disease incursion was to occur. For example, a boar stud might require 5 days of downtime, while a commercial finisher might only require an overnight downtime.

Clothing and footwear

Given the low cost of coveralls and boots, farms should not allow staff or visitors to bring their own gear onto the farm. Instead, they should keep a small inventory of gear in a range of sizes that can be made available to the occasional visitor as required. While coveralls can be easily laundered, boots typically are difficult to get completely clean. In both cases, it is simplest for the farm to provide gear rather than permit visitors to bring their own.

Equipment and gear

All equipment and consumable supplies brought into the pig operation should arrive clean and undamaged. Many farms bring supplies through a fumigation room or spray them with disinfectant at the entry point. The greatest risk that accompanies consumables is that they may have been delivered to another farm, returned to the supplier, and then redistributed by the supply company.

Specialist equipment and supplies often need to be brought onto a pig farm by service people such as electricians or plumbers. It is not unusual that these specialists have brought their gear onto other pig farms. As their services are often needed on an emergency basis, farmers typically have little choice but to allow the equipment into the farm. However, it is perfectly reasonable to inspect all equipment that is to be brought onto the farm, to clean and disinfect it as best as possible, and importantly to try to position the equipment once in the facility in locations that minimize the opportunity to come into direct contact with pigs.

Farm machinery and equipment can also be a risk for transmitting disease to a pig operation, especially if the machinery and equipment were used at or nearby other pig operations. Whenever possible, avoid borrowing equipment from other farms and when it is necessary, following procedures described above for gear used by service contractors.

Untreated food

If left untreated, all products of animal origin have the potential to harbor animal pathogens, and this includes raw meat products or any food items that come into contact with raw meat. Some farms or staff members are in the habit of preparing meals at the farm rather than preparing food at their home and then bringing to the farm for direct consumption or reheating. Given the level of international trade in meat products, it is likely that at least some raw meat products brought onto a farm were sourced internationally and therefore may be contaminated with high-consequence exotic pathogens. Rather than trying to mitigate the risk through some other means, the best policy is to avoid bringing untreated meat products into the farm facility.

Zooanthroponosis

Some pathogens are able to move between animals and humans (zoonosis). Those zoonotic agents (or diseases) that have a propensity to travel from humans to pigs are termed zooanthroponosis. Influenza, specifically the 2009 pandemic strain, is notable for this behavior. For this reason, people with influenza-like symptoms should avoid contact with pigs. More broadly, people and pigs can impact each other's health by acting as reservoirs of the infection. Encephalitis B virus is an important cause of viral encephalitis in people across much of South Asia. The virus moves between people, pigs, and mosquitoes, reminding one that livestock farming cannot be done in isolation from the rest of the general public. Farmers have a responsibility to stay engaged and aware of larger public health issues and not ignore the role of pigs for some human diseases.

Principles of evidence-based biosecurity

All the features of size, production flow, commercial imperative, geographical location, local industry density, connectedness to other farms, and physical building characteristics that describe a farm are the same features that make it impossible to develop a one-size-fits-all BRM plan suitable for use across the pig industry. As an alternative, the following ten principles are proposed as fundamental criteria that must be fulfilled when developing biosecurity plans for pig farms. Scientific evidence is available to support application of each principle that is appropriate for most farm and disease settings that will be encountered. Readers are encouraged to develop their own studies that can further our knowledge of these topics for use by an even broader cross section of the international industry. The principles are not complicated, though they can be difficult to institute effectively and with adequate levels of compliance (Figure 9.8).

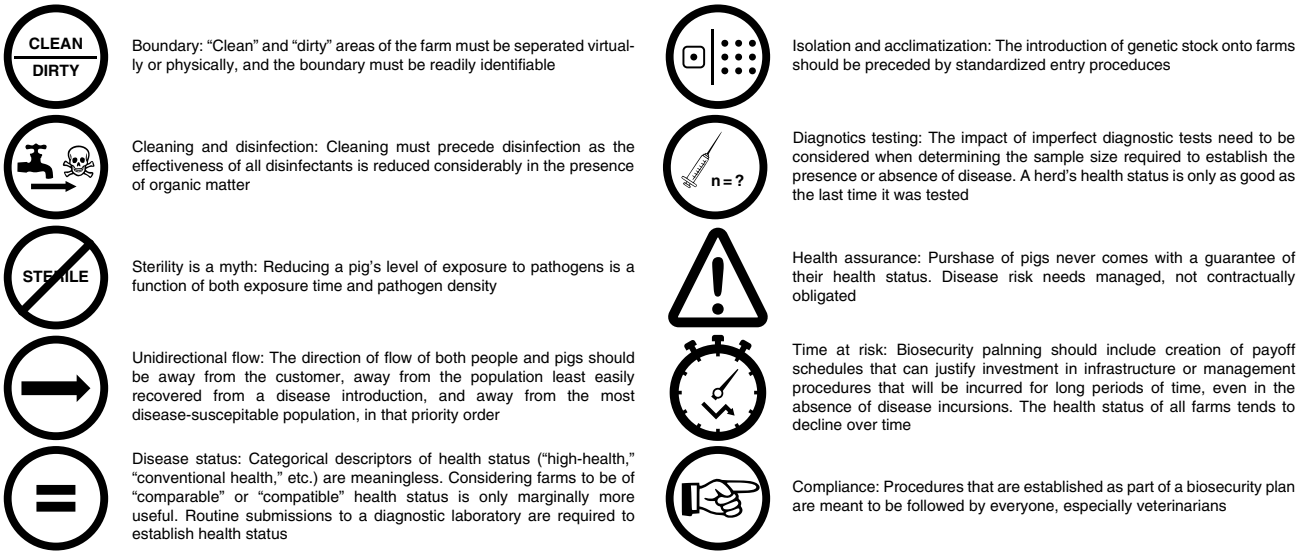


Figure 9.8 Ten principles of evidence-based biosecurity. Adapted from Neumann (2012).

References

Aarestrup FM, Wegener HC, Collignon P. 2008. *Expert Rev Anti Infect Ther* 6(5):733–750.

Ackerman GA, Giroux J. 2006. A history of biological disasters of animal origin in North America. *Rev Sci Tech* 25(1):83–92.

Agten S, Van Gorp V, Ballis BM. 2010. Effect of Porcilis PCV one shot vaccination on slaughter weight and mortality in a subclinically infected pig farm. Paper presented at the 21st International Pig Veterinary Society Congress, Vancouver, Canada, p. 115.

Amass SF. 2004. *J Swine Health Prod* 12(2):82–83.

Amass SF, Clark LK. 1999. *J Swine Health Prod* 7(5):217–228.

Amass SF, Vyverberg BD, Ragland D, et al. 2000. *J Swine Health Prod* 8(4):169–173.

Amass SF, Halbur PG, Byrne BA, et al. 2003a. *J Swine Health Prod* 11(2):61–68.

Amass SF, Pacheco JM, Mason PW, et al. 2003b. *Vet Rec* 153(5):137–140.

Amass SF, Schneider JL, Ragland D, et al. 2003c. *J Swine Health Prod* 11(6):277–283.

Amezcua R, Friendship RM, Dewey CE, et al. 2002. *Can J Vet Res* 66(2):73–78.

Amezcua R, Friendship RM, Dewey CE. 2008. *Can Vet J* 49(1):39–45.

Anderson RM. 1982. *The Population Dynamics of Infectious Diseases: Theory and Applications*. London; New York: Chapman and Hall.

Anderson LA, Black N, Hagerty TJ, et al. 2008. Pseudorabies (Aujeszky's Disease) and Its Eradication: A Review of the U.S. Experience (Technical Bulletin Number 1923). Washington, DC, USA. https://www.aasv.org/documents/pseudorabiesreport.pdf.

Anonymous. 2007. *FAO Biosecurity Toolkit*. Rome, Italy: Food and Agricultural Organization of the United Nations.

Anonymous. 2010. Terrestrial Animal Health Code 2010. Section 2. Risk analysis. http://www.oie.int/eng/normes/mcode/en_titre_1.2.htm. Accessed June 16, 2017.

Arzt J, White WR, Thomsen BV, et al. 2010. *Vet Pathol* 47(1):15–27.

Baynes RE, Smith G, Mason SE, et al. 2008. *Food Chem Toxicol* 46(3):1196–1200.

Begon M, Townsend CR, Harper JL. 2006. *Ecology: From Individuals to Ecosystems*, 4th ed. Malden, MA: Blackwell Publishing.

Bejarano JF. 1960. *Rev San Militar Argen* 59:366–368.

Bickett-Weddle D. 2005. An Overview of Biological Risk Management. Ames, Iowa, USA. http://www.cfsph.iastate.edu/Infection_Control/Overview/OverviewofBRMMar2005.pdf.

Brons N, Hughes AM, Adam MJ. 2010. Improved production parameters and profit as a result of PCV2 piglet vaccination in the United Kingdom. Paper presented at the 21st International Pig Veterinary Society Congress, Vancouver, Canada, p. 134.

Burns K. 2007. *JAVMA* 230(11):1600–1620.

Casal J, De Manuel A, Mateu E, et al. 2007. *Prev Vet Med* 82(1–2):138–150.

Centers for Disease Control and Prevention. 1994. Brucellosis outbreak at a pork processing plant – North Carolina, 1992. *MMWR Morb Mortal Wkly Rep* 43(7):113–116.

Centers for Disease Control and Prevention. 1999. Update: Outbreak of Nipah virus – Malaysia and Singapore, 1999. *MMWR Morb Mortal Wkly Rep* 48(16):335.

Centers for Disease Control Prevention. 2009. Swine influenza A (H1N1) infection in two children – Southern California, March–April 2009. *MMWR Morb Mortal Wkly Rep* 58(15):400.

Clark T. 2002. *Staphyloccocus dermatitis* and PDNS. Paper presented at the 33rd Annual Meeting of the American Association of Swine Veterinarians, Kansas City, Missouri, USA.

Croxen MA, Finlay BB. 2010. *Nat Rev Microbiol* 8(1):26–38.

Dial GD, Marsh WE, Polson DD, et al. 1992. Reproductive failure: Differential diagnosis. In Straw BE, D'Allaire S, Mengeling WL, et al., eds. *Diseases of Swine*, 7th ed. Ames, IA: Iowa State University Press, pp. 88–137).

Dohoo IR. 2003. *Veterinary Epidemiologic Research*. Charlottetown, PEI: AVC Inc.

Drum SD, Hoffman LJ. 1998. *J Swine Health Prod* 6(5):217–218.

Dunkelberger E. 1995. *Food Drug Law J* 50(3):357–383.

Edwards S, Sands J. 1994. *Vet Rec* 134(26):680–681.

Elbers AR, Stegeman A, Moser H, et al. 1999. *Prev Vet Med* 42(3–4):157–184.

England JJ. 2002. *Vet Clin North Am-Food Anim Pract* 18(3):373–384.

Firth C, Charleston MA, Duffy S, et al. 2009. *J Virol* 83(24):12813–12821.

Flint C, McFarlane B, Müller M. 2009. *Environ Manag* 43(6):1174–1186.

Gibson DG, Glass JI, Lartigue C, et al. 2010. *Science* 329(5987):52–56.

Gillespie TG. 2009. Managing 7% preweaning mortality: Is this possible with total born levels increasing? Paper presented at the 40th Annual Meeting of the American Association of Swine Veterinarians, Dallas, Texas, USA.

Halpin B. 1975. *Patterns of Animal Disease*. London: Baillière Tindall.

Hanna JN, Ritchie SA, Phillips DA, et al. 1996. *Med J Aust* 165(5):256–261.

Harel J, Lapointe H, Fallara A, et al. 1991. *J Clin Microbiol* 29(4):745–752.

Hause BM, Ducatez M, Collin EA, et al. 2013. *PLoS Pathog* 9(2):e1003176.
Hause BM, Collin EA, Peddireddi L, et al. 2015. *J Gen Virol* 96(10):2994–2998.
Hause BM, Myers O, Duff J, et al. 2016. *Emerg Infect Dis* 22(7):1323–1325.
Henle J. 1838. *On Miasmata and Contagie* (translated from German by G Rosen). Baltimore, MD: Johns Hopkins Press.
Hermann JR, Munoz-Zanzi CA, Roof MB, et al. 2005. *Vet Microbiol* 110(1–2):7–16.
Hill AB. 1965. The environment and disease: association or causation? *Proc R Soc Med* 58:295–300.
Hsu SM, Yen AM, Chen TH. 2008. *Epidemiol Infect* 136(7):980–987.
Katouli M, Lund A, Wallgren P, et al. 1995. *Appl Environ Microbiol* 61(2):778–783.
Kennedy DJ, Roe RT. 1987. *Basic Epidemiology for Field Veterinarians*. Sydney, New South Wales, Australia: New South Wales Department of Agriculture.
Kirkland PD, Frost MJ, Finlaison DS, et al. 2007. *Virus Res* 129(1):26–34.
Kixmoller M, Ritzmann M, Eddicks M, et al. 2008. *Vaccine* 26(27–28):3443–3451.
Koch R. 1890. Ueber bakteriologische Forschung. Paper presented at the International Medical Congress, Berlin, Germany.
Law J, Mol A. 2008. *Geoforum* 39(1):133–143.
Levis DG, Baker RB. 2011. Biosecurity of pigs and farm security. Lincoln, Nebraska, USA. http://extensionpublications.unl.edu/assets/pdf/ec289.pdf.
Li X, Engelbrecht CJ, Mueller DS, et al. 2008. *Plant Dis* 92(6):975–975.
Luppi A, Bonilauri P, Mazzoni C, et al. 2010. Swine herd with a prevalent PCV2 subclinical infection: diagnostic investigation results before and after PCV2 vaccination. Paper presented at the 21st International Pig Veterinary Society Congress, Vancouver, Canada, p. 126.
MacInnes JI, Desrosiers R. 1999. *Can J Vet Res* 63(2):83–89.
Madec F, Rose N, Grasland B, et al. 2008. *Transbound Emerg Dis* 55(7):273–283.
Madec F, Hurnik D, Porphyre V, Cardinale E. 2010. Good practices for biosecurity in the pig sector – issues and options in developing and transition countries, Rome, Italy. www.fao.org/docrep/012/i1435e/i1435e00.pdf. Accessed June 16, 2017.
Martinez I, Rodriguez LL, Jimenez C, et al. 2003. *J Virol* 77(14):8039–8047.
Mateu E, Diaz I. 2007. *Vet J* 177(3):345–351.
Matumoto M. 1969. *Bacteriol Rev* 33(3):404–418.
Melin L, Mattsson S, Katouli M, et al. 2004. *J Vet Med Series B* 51(1):12–22.
Meng X–J, Purcell RH, Halbur PG, et al. 1997. A novel virus in swine is closely related to the human hepatitis E virus. *Proc Natl Acad Sci* 94(18):9860–9865.
Moore DA, Merryman ML, Hartman ML, et al. 2008. *JAVMA* 233(2):249–256.
Murray D, Rademacher C. 2008. System wide sick pig management. Paper presented at the 39th Annual Meeting of the American Association of Swine Veterinarians, San Diego, California, USA.
Nespeca R, Vaillancourt JP, Morgan Morrow WE. 1997. *Prev Vet Med* 31(1–2):73–86.
Neumann, EJ. 2012. Disease transmission and biosecurity. In Zimmerman JJ, Karriker LA, Ramirez A, et al., eds., *Diseases of Swine*, 10th ed. Ames, IA: Wiley-Blackwell, pp. 141–164.
Neumann EJ, Simpson S, Wagner J, et al. 2009. *J Swine Health Prod* 17(4):204–209.
Oliveira S, Pijoan C. 2004. *Vet Microbiol* 99(1):1–12.
Ostrowski M, Galeota JA, Jar AM, et al. 2002. *J Virol* 76(9):4241–4250.
Palinski R, Piñeyro P, Shang P, et al. 2017. *J Virol* 91(1).
Pfeiffer D. 2008. *Spatial Analysis in Epidemiology*. Oxford; New York: Oxford University Press.
ProMED-mail. 1999. West Nile-like Virus – USA (New York City). Archive Number: 19990925.1708. http://www.promedmail.org/post/19990925.1708. Accessed June 16, 2017.
ProMED-mail. 2000. Swine Fever, Classical – UK. Archive Number: 20000809.1331. http://www.promedmail.org/post/20000809.1331. Accessed June 16, 2017.
ProMED-mail. 2001. Foot & Mouth Disease – UK. Archive Number: 20010220.0325. https://www.promedmail.org/post/2199413. Accessed June 16, 2017.
ProMED-mail. 2002. Pseudorabies, Porcine – USA (Pennsylvania). Archive Number: 20020825.5147. https://www.promedmail.org/post/20020825.5147. Accessed June 16, 2017.
ProMED-mail. 2005. *Streptococcus suis*, Porcine, Human – China (16): Background. Archive Number: 20050819.2436. http://www.promedmail.org/post/20050819.2436. Accessed June 16, 2017.
ProMED-mail. 2006. Undiagnosed Die-off, Porcine – China (Jiangsu): RFI. Archive Number: 20060412.1087. http://www.promedmail.org/post/20060412.1087. Accessed June 16, 2017.
ProMED-mail. 2007. African Swine Fever – Georgia: OIE. Archive Number: 20070607.1845. http://www.promedmail.org/post/20070607.1845. Accessed June 16, 2017.
ProMED-mail. 2008. Ebola-Reston, porcine – Philippines. Archive Number: 20081211.3896. http://www.promedmail.org/post/20081211.3896. Accessed June 16, 2017.
ProMED-mail. 2010. Foot & Mouth Disease, Bovine – Japan: (MZ) OIE. Archive Number: 20100420.1284. https://www.promedmail.org/post/20100420.1284. Accessed June 16, 2017.
Pyburn D. 2001. Biosecurity: How do you define it? Paper presented at the Iowa State University Swine Disease Conference for Swine Practitioners, Ames, Iowa, USA.

Ramirez A, Zaabel P. 2012. Swine Biological Risk Management, Ames, Iowa, United States. http://www.cfsph.iastate.edu/pdf/swine-biological-risk-management
Reisen WK. 2010. *Annu Rev Entomol* 55:461–483.
Rushton J, Upton M. 2006. *Rev Sci Tech* 25(1):375–388.
Schwartz K. 2002. Infectious causes of skin necrosis in finishing pigs. Paper presented at the 33rd Annual Meeting of the American Association of Swine Veterinarians, Kansas City, Missouri, USA.
Sidler X, Kurmann J, Buergi E, et al. 2010. Economic impact of CIRCOVAC in a PCV2 subclinically infected breeding farm in Switzerland. Paper presented at the 21st International Pig Veterinary Society Congress, Vancouver, Canada. p. 099.
Smith RD. 2006. *Veterinary Clinical Epidemiology*, 3rd ed. Boca Raton: CRC/Taylor & Francis.
Sperder WH, Stier RF. 2009. *Food Saf Mag* 15 (6) 42–46.
Stage FK, Carter HC, Nora A. 2004. *J Ed Res* 98(1):5–12.
Stark KD. 2000. *Vet J* 159(1):37–56.
Stevenson GW, Hoang H, Schwartz KJ, et al. 2013. *J Vet Diagn Invest* 25(5):649–654.
Sugiyama I, Shimizu E, Nogami S, et al. 2009. *J Vet Med Sci* 71(8):1059–1061.
Szinicz L. 2005. *Toxicology* 214(3):167–181.
Teehee ML, Bunning ML, Stevens S, et al. 2005. *Arch Virol* 150(6):1249–1256.
Terpstra C, Wensvoort G, Pol JM. 1991. *Vet Q* 13(3):131–136.
Tlaskalova-Hogenova H, Stepankova R, Hudcovic T, et al. 2004. *Immunol Lett* 93(2–3):97–108.
Tokach LM. 1993. *J Swine Health Prod* 1(1):29–30.
Trott DJ, Stanton TB, Jensen NS, et al. 1996. *Int J Syst Evol Microbiol* 46(1):206–215.
Vaillancourt, J. 2005. Effective biosecurity: The case for compliance and regional perspective. Paper presented at the 36th Annual Meeting of the American Association of Swine Veterinarians, Toronto, Ontario, Canada.
Villa L, Carattoli A. 2005. *Antimicrob Agents Chemother* 49(3):1194–1197.
Villani DJ. 2003. *J Swine Health Prod* 11(1):27–30.
Voss A, Loeffen F, Bakker J, et al. 2005. *Emerg Infect Dis* 11(12):1965–1966.
Weaver SC, Reisen WK. 2010. *Antiviral Res* 85(2):328–345.
Wegener HC, Baggesen DL. 1996. *Int J Food Microbiol* 32(1–2):125–131.
Woo PC, Lau SK, Lam CS, et al. 2012. *J Virol* 86(7):3995–4008.
Wu X–Y, Chapman T, Trott DJ, et al. 2007. *Appl Environ Microbiol* 73(1):83–91.
Zhang W, Zhao M, Ruesch L, et al. 2007. *Vet Microbiol* 123(1–3):145–152.
Zimmerman J. 2003. Epidemiology and ecology. In Zimmerman JJ, Yoon, KJ, eds., *2003 PRRS Compendium*, 2nd ed. Des Moines, IA, USA: National Pork Board, pp. 157–161.
Zoric M, Nilsson E, Lundeheim N, et al. 2009. *Acta Vet Scand* 51:23.

10

Drug Pharmacology, Therapy, and Prophylaxis

Locke A. Karriker, Johann F. Coetzee, Robert M. Friendship, and Michael D. Apley

This chapter is an overview of the major considerations specific to drug therapy in swine. Major drug classes and biological agents used in swine, with a focus on antimicrobial drugs and the basic principles on which effective drug use is based, are included. Readers seeking a general review of antimicrobial therapy principles are referred to excellent reviews elsewhere (Boothe 2006). Anesthetic drug properties, tranquilizers, and anesthetic protocols are discussed in Chapter 11. A useful additional reference for anesthetic information in swine is the comprehensive bibliography by Smith (2011). A key consideration in selecting drug therapy in swine is compliance with local, state, territorial, national, and international regulations that impose limitations on use or expectations for the safety of products derived from the animals treated. Due to the international audience of this book and the highly variable regulation of drug treatment, it is incumbent on the reader to understand applicable law for their case context and apply discretion when using information from this chapter. Specific regulatory impacts on drug treatment will not be discussed further in this chapter.

Major considerations for drug therapy

Managing the effective use of drugs or biological agents for prevention and treatment of disease is an important responsibility of swine veterinarians, which involves detailed knowledge of these agents, the risks involved to both humans and animals by their use, and the applicable local, national, and international regulations. In swine, priority is placed on the production of safe meat and protection of animal welfare. Additional considerations include cost, efficacy, and ease of application. Within each of these broad categories exist several swine-specific issues that must be managed by the animal caretaker and veterinarian (Table 10.1).

All decisions to initiate drug therapy must recognize that any chemical addition to the animal has the potential to negatively impact homeostasis. Energy, enzyme pathways, and metabolic substrates must be diverted from growth and homeostasis to eliminate exogenous chemical additions including therapeutic drugs. Therefore, clear evidence that the potential benefit to the animal is greater than the negative impact on homeostasis is a prerequisite to treatment. Demonstrating a clear benefit is predicated on correct identification of existing or potential clinical compromise, most commonly infectious disease. All drug use involves a calculation that benefits of use exceed the risks involved. The goal is to minimize use of drugs in pork production while ensuring the production of healthy animals in a humane, cost-effective, safe, and environmentally sustainable manner. Changes in swine production practices continue to reduce reliance on antimicrobial drugs and improve pork safety in modern production.

Selecting appropriate drug therapy consists of several specific steps: (1) determine the treatment objective using the sum of clinical and diagnostic evidence available, (2) identify the best treatment option while considering swine-specific physiology, (3) establish the treatment regimen in light of production logistics and regulation constraints, (4) facilitate implementation and compliance, and (5) assess the outcomes and modify protocols as indicated. The most complex treatment decisions in swine are generally those where an antimicrobial is used for treatment of clinical disease. A useful organization scheme for the swine veterinarian when making a comprehensive antimicrobial selection is the "S.P.A.C.E.D." mnemonic described in Table 10.2.

Determining treatment objective

The current standard for determining a treatment objective in the context of antimicrobial therapy is a diagnosis that includes bacterial culture and identification accompanied

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 10.1 Considerations for drug use in swine.

Major consideration	Further considerations
Human safety	Direct drug toxicity to user, toxicity to consumer through tissue residues, contamination of environment
Animal welfare	Prevention of reduction of disease, ease of administration for animal, need for restraint
Host animal safety	Direct toxicity to pig, tissue damage, adverse drug reactions, indirect adverse effects such as disruption of microflora
Regulations	Availability of products, national regulations on use, international regulations for export, use authority, compliance with established withdrawal periods, veterinarian–client relationship
Efficacy	Assessment of efficacy, setting client expectations for efficacy, cost/benefit of treatment, effects of concurrent disease
Drug dosage and administration	Route required, ease of administration, physiochemical properties, frequency of administration
Pharmacology/ pharmacokinetics	Evidence for dose, route, duration, frequency, estimations of drug clearance rates, distribution to target tissues, mechanism of action, expected routes of elimination, mechanism of drug metabolism
Recordkeeping	Requirements for dispensing such as prescriptions or feed directives, documenting treatment, tracking disposition of treated animals, data collection to assess efficacy
Drug storage/accessibility	Physiochemical conditions impacting drug concentration or viability during storage or use, drug-specific legal requirements for storage and possession
Resistance/tolerance	Antimicrobial resistance resulting in animal or human treatment failures, increasing tolerance of the animal to drug effects

Table 10.2 Antimicrobial selection considerations (S.P.A.C.E.D.).

Spectrum	Drug efficacy against pathogen; pathogen Gram staining; pathogen metabolism (aerobic or anaerobic)
Pharmacokinetics and pharmacodynamics	Drugs' ability to reach the pathogen at the site of infection in high enough concentrations to be effective; plasma antimicrobial concentration vs. time profile; concentration dependent or time above MIC dependent or AUC above MIC dependent; volume of distribution; protein binding
Adverse reactions	Safe to use in this disease state, breed, age group, or class of animal; potential contraindications/toxicities; potential risks to the person administering the drug
Compliance	Caretaker's ability to provide the full course of treatment; legality of use in this class of animal
Environment	Site of infection characteristics; potential for resistance development
Diagnostics	Pathogen identification; actual or estimated susceptibility profile

with antimicrobial sensitivity testing of that isolate. In swine cases, this isolate should be the consequence of a complete diagnostic investigation that confirms that lesions consistent with the pathogen are present in appropriate tissues. Further, the case needs to be representative of the primary problem in the target swine population. It is often necessary to confirm culture and sensitivity data for several representative cases in a specific population as pathogens are not necessarily a homogeneous species or even genus in a swine population. Several pathogenic bacteria can coexist with nonpathogenic strains of the same genus and species in the pigs such as the case with intestinal *Escherichia coli*.

When culture and sensitivity confirmation is not available or the urgency of intervention on behalf of swine welfare requires initiating therapy before results are available, several sources of information are available to supplement empirical treatment. Practitioners have the capability with some diagnostic laboratory cooperation to maintain antimicrobial sensitivity summaries for their clients or practice region compiled from a series of diagnostic cases. Several diagnostic laboratories summarize the percentage of isolates that are susceptible to selected antimicrobials on a routine basis. These summaries are generally available online and may represent a more current and broad view of microbial susceptibilities than even randomized controlled trials using single isolates.

Treatment options and specific impacts of swine physiology

Major classes of antimicrobial drugs

A brief overview of some key aspects of the major classes of antimicrobial drugs, their antimicrobial activities, pharmacokinetic properties, toxic or other adverse effects, and major clinical applications is given in Table 10.3. Further

Table 10.3 Overview of the major classes and identities of antimicrobial drugs used in swine, their antimicrobial activities, pharmacokinetic properties, toxic and other adverse effects, and major clinical applications.

Drug class	Specific agent or example of agents	Antibacterial activity, resistance	Pharmacokinetic properties	Toxic or adverse effects	Major applications
Sulfonamides	Sulfamethazine, intermediate acting; others used	Bacteriostatic; broad-spectrum, gram-positive and gram-negative aerobes; anaerobes; acquired resistance very widespread; active intracellular bacteria, protozoa	Rapidly absorbed from intestine, well distributed in tissues	Violative kidney residues from feed use through recycling; feed contamination if feed not withdrawn 15 days before slaughter	Minor value; largely growth promotional; possible disease prevention
Sulfonamide–diaminopyrimidine combinations	Sulfamethazine/trimethoprim	Bactericidal; gram-positive and gram-negative aerobes; anaerobes; *Mycoplasma* and *Leptospira* resistant	Rapidly absorbed from intestine, well distributed in tissues; crosses uninflamed blood–brain barrier	Wide safety margin	Largely water use for acute infections (pneumonia, streptococcal meningitis); in feed and water for atrophic rhinitis
Beta-lactam	Penicillin G, potassium penicillin	Bactericidal; highly active many gram-positive aerobes; some fastidious gram-negative aerobes, for example, *Haemophilus parasuis* and *Pasteurella multocida*; anaerobes; *Leptospira*, enteric bacteria, and *Mycoplasma* resistant	Poorly absorbed from intestine, relatively poorly distributed in tissues; crosses only inflamed blood–brain barrier; procaine penicillin G has more prolonged plasma concentrations due to slower release from the injection site	Safe drug; possible anaphylaxis or procaine-induced excitement	Excellent for IM use in *erysipelas*, *streptococcal* infections including meningitis, and *Clostridial* infections; some bacterial pneumonias. Potassium pen in water
Beta-lactam	Ampicillin, amoxicillin	As penicillin G, broader activity against gram-negative aerobes, but resistance widespread	As penicillin G, but better absorbed orally and distributed through tissues	Safe drug	Similar to penicillin G; addition of beta-lactamase inhibitors (e.g. clavulanic acid) has resurrected penicillin use in other species
Beta-lactam	Third-generation cephalosporins; ceftiofur	Bactericidal; gram-negative aerobes especially, including *Escherichia coli* and *Salmonella*; gram-positive aerobes; anaerobes; *Mycoplasma* resistant	Poorly absorbed from intestine, relatively poorly distributed in tissues; crosses only inflamed blood–brain barrier	May predispose to *Clostridium difficile* colitis if used in neonatal pigs; resistance emerging in *Salmonella* may represent human health hazard	Excellent for IM use in gram-negative aerobic infections, including colibacillosis and salmonellosis, and gram-negative bacterial pneumonias
Aminoglycoside	Gentamicin, neomycin	Bactericidal; gram-negative aerobes, including enterics	Poorly absorbed from intestine, relatively poorly distributed in tissues	Nephrotoxic with prolonged parenteral use; persistent kidney residues	Gentamicin IM for neonatal *E. coli* infections; neomycin orally for *E. coli* infection
Aminocyclitol	Spectinomycin	Bactericidal; gram-negative aerobes, including enterics	Poorly absorbed from intestine, relatively poorly distributed in tissues	Safe drug in swine	Orally for *E. coli* infection
Lincosamide	Lincomycin	Bacteriostatic; gram-positive aerobes; anaerobes including *Brachyspira hyodysenteriae*; *Mycoplasma*	Well absorbed from intestine and well distributed in tissues	Safe drug in swine	Oral use for control of *Brachyspira*; oral or IM use for control of *Mycoplasma*

Macrolide	Tylosin, tulathromycin, tylvalosin, tilmicosin	Bacteriostatic; gram-positive aerobes; anaerobes; some gram-negative aerobes; *Mycoplasma*	Well absorbed from intestine and well distributed in tissues	Safe drug in swine except injected tilmicosin is fatal; others are IM irritant; may cause rectal edema; pruritis; anal protrusion	Oral use for control of proliferative enteropathy, atrophic rhinitis, and possibly leptospirosis
Pleuromutilin	Tiamulin	Bacteriostatic; gram-positive aerobes, anaerobes, some gram-negative aerobes; *Mycoplasma*; more active than tylosin	Well absorbed from intestine and well distributed in tissues	Safe drug in swine	Oral use for control of *Brachyspira*, *Mycoplasma*, chronic pneumonias, proliferative enteropathy, and leptospirosis
Tetracyclines	Oxytetracycline, chlortetracycline	Bacteriostatic; classically broad spectrum, gram positive, gram negative but acquired resistance extremely widespread; *Erysipelothrix*, *Haemophilus*, *Leptospira*, and *Pasteurella* are exceptions	Well absorbed from intestine and well distributed in tissues	Safe drugs in swine	Oral use as "feed" drugs for growth promotion and nonspecific disease prophylaxis in countries where allowed; used in feed, occasionally IM, for treatment of infections caused by bacteria listed as being susceptible
Phenicols	Florfenicol	Binds 50s ribosomal subunit; bacteriostatic may be bactericidal at high concentrations	Well absorbed orally	Florfenicol relatively safe; chloramphenicol is toxic to humans and banned in US food animals	Labeled for *Actinobacillus pleuropneumoniae, P. multocida*, *Salmonella choleraesuis*, and *Streptococcus suis* in water. Use of injectable is extra label in swine
Quinoxaline derivatives	Carbadox	Bactericidal; mechanism is unknown; primarily active against gram-positive bacteria with little efficacy against some gram negatives	Orally absorbed	It is carcinogenic and genotoxic in rodents, and, consequently, the usage of carbadox is prohibited in Europe and Canada	Carbadox is most commonly used as a feed additive to promote growth in swine; controls swine dysentery (*Serpulina hyodysenteriae*); nasal infections (*Bordetella bronchiseptica*)
Fluoroquinolones	Enrofloxacin	Bactericidal; inhibits bacterial DNA gyrase or topoisomerase IV, preventing DNA supercoiling and cell replication	Well absorbed with a high volume of distribution. Enrofloxacin is de-ethylated to form ciprofloxacin, which comprises <10% of the amount of enrofloxacin present in the plasma	The long-term effects on articular joint cartilage have not been determined in pigs above market weight. US federal law prohibits the extra-label use of fluoroquinolones in food-producing animals	Treatment and control of swine respiratory disease (SRD) associated with *A. pleuropneumoniae*, *P. multocida*, *H. parasuis*, *S. suis*, *B. bronchiseptica* and *Mycoplasma hyopneumoniae*. Control of colibacillosis in groups or pens of weaned pigs where colibacillosis associated with *E. coli*. has been diagnosed

details are available through manufacturer's package inserts and through pharmacology-focused textbooks (Giguere et al. 2015).

Antimicrobials may be classified as bactericidal or bacteriostatic based on the outcome of specific *in vitro* drug–bacteria interactions. Typically, a drug is considered bactericidal when there is a 3-log reduction in bacterial cultures over a 24 hour period. It has been suggested that bactericidal drugs are preferred for treatment of serious life-threatening infections, when host defenses are impaired, and infections of vital tissues such as meninges, endocardium, and bones, where host defenses are also not fully functional. In other cases, bacteriostatic agents may be equally useful. It should be recognized that this classification is only one of many aspects that should be considered when making appropriate antimicrobial selections.

To some extent, drug dosage can be tailored to the susceptibility of the organism, the site of infection, and the pharmacokinetic and pharmacodynamic properties of the selected antimicrobial agent. However, *in vitro* susceptibility data are laboratory derived, and the standardized conditions under which the susceptibility data are generated do not exist at the site of infection. When interpreting antimicrobial susceptibility testing, it is important to know if the laboratory is conforming to Clinical and Laboratory Standards Institute (CLSI) standards as well as applying CLSI interpretive criteria. Where CLSI approved standards and interpretive criteria exist for a combination of the animal species, pathogen, disease, and drug regimen, then more confidence may be placed in the clinical application of these results. Where there are no CLSI interpretive criteria, such as for any enteric disease in swine, much more care must be taken in interpretation.

Factors involved in tailoring a dosing regimen include, among other things, the susceptibility of the pathogen in terms of minimum inhibitory concentration (MIC), the concentration of the antimicrobial agent at the site of infection in active form (pharmacokinetic properties of the drug), and the pharmacodynamic properties of the antimicrobial agent. Some antimicrobials (aminoglycosides, fluoroquinolones) are concentration dependent (optimum action of the drug depends on concentration of the drug above MIC), whereas others (beta-lactams, lincosamides, macrolides, trimethoprim/sulfamethazine) are time dependent (optimum activity depends on how long they remain at concentrations above MIC). The complex issues involved in optimal antimicrobial therapy are beyond the scope of this chapter although it can be concluded that some dosage recommendations for drugs licensed in the past have not taken modern understanding into account and are suboptimal or inappropriate based on more recent data. Although several factors determine optimal dosage, the factor that most frequently limits dosage is toxicity. The upper level of the recommended dosage (when provided as a range) should not be exceeded, because this is often determined by toxicity. In some instances, a drug's antibacterial effects may be limiting and may determine the upper level of dosage. For example, the killing rate of beta-lactam drugs has an optimal concentration with little benefit of the plasma concentration exceeding 2–4 times the MIC, whereas the killing rate of the aminoglycosides or fluoroquinolones is proportional to drug concentration. Penicillin G is virtually nontoxic in nonallergenic patients, but its dosage is limited by marginal return in the form of antibacterial action above a certain concentration. By contrast the dosage of aminoglycosides is limited not by antibacterial effects but by its toxicity.

In terms of duration of treatment, the variables affecting length of treatment have not been adequately defined in swine. Responses of different types of infections to antimicrobial drugs vary, and clinical experience with many infections is important in assessing response to treatment. For acute infections, it will usually be clear within 2 days whether therapy is clinically effective. If no response is seen by that time, both the diagnosis and treatment should be reconsidered.

Drug selection for specific diseases

It is beyond the scope of this text to discuss all product indications, cautions, and regulations, nor is it within the scope of this text to include all possible treatment options. Readers are referred to the relevant specific disease chapter for further suggested treatments.

A few swine-specific treatment examples are noted here to guide veterinary practice and illustrate general principles. For example, parenteral tilmicosin is fatal to swine even at very low concentrations. However, oral administration is approved for *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* (Backstrom et al. 1994).

Bimazubute et al. (2010) demonstrated that concentrations of oxytetracycline could be achieved in nasal secretions of swine that were greater than the MIC for *P. multocida* and *Bordetella bronchiseptica*, key pathogens in atrophic rhinitis. However, this could only be achieved with intramuscular (IM) injection at 40 mg/kg body weight. This dose level was four times the swine label recommendation. Although peak concentrations in plasma and nasal secretions both occurred 4 hours after administration, the maximum concentration of the drug in nasal secretions was 6.29 µg/mL compared with 19.4 µg/mL in plasma. Efficacious levels were not achieved with lower IM doses nor feed formulations containing oxytetracycline at 400 mg/kg of feed. In many species including pigs, enrofloxacin is metabolized to ciprofloxacin, which also has potent antimicrobial activity. Variations in the amount of enrofloxacin converted to ciprofloxacin occur with age in swine. None of the

original dose of the parent enrofloxacin was detected as ciprofloxacin in 10 kg pigs compared with 52% of the original enrofloxacin detected as ciprofloxacin in 76 kg pigs (Bimazubute et al. 2009). It is important to consider that the majority of swine in the world are marketed before they achieve maturity, and therefore most treatments are applied to juvenile animals. In most species, including pigs, age-specific variation in metabolism is recognized and must be considered. Unfortunately, swine-specific data for most drugs have not been developed.

Cornick (2010) demonstrated that fewer pigs fed tylosin or chlortetracycline shed *E. coli* O157:H7 for longer than 2 weeks compared with pigs fed no antimicrobials. Experimentally, antimicrobial-free pigs can be infected and shed for longer than 2 months. However, field studies rarely recover this organism from domestic pigs. Potentially, feed medication protocols used in commercial swine production have reduced the presence of this food safety risk. As antimicrobials are removed from swine populations worldwide, it will be necessary to monitor for unintended consequences to animal welfare or food safety.

Most pharmacokinetic parameters are studied in healthy, disease-free pigs. There are few studies evaluating how disease impacts the pharmacokinetics of drugs and the implications for modifying treatment regimens. In one study, the mean of maximum plasma tetracycline concentrations was lower and achieved significantly later postinjection in pneumonic pigs relative to healthy pigs (Pijpers et al. 1991). Pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV) had decreased plasma ceftiofur concentrations relative to healthy pigs (Tantituvanont et al. 2009). Vaccination of pigs with a modified live PRRSV vaccine prevented the changes in pharmacokinetics induced by wild-type virus infection (Sparks et al. 2017).

Establishing treatment regimens considering production logistics and regulatory constraints

Routes of drug treatment

In general, individual animal treatment through injection is preferred for serious, rapidly developing infections such as acute systemic infections (septicemia, acute pneumonia, or streptococcal meningitis), but medication of an entire group is preferred when ease of administration can be considered, identifying sick individuals in population is challenging, or it is necessary to avoid the animal welfare implications or human safety concerns of handling and restraining the animals. IM injection is preferred for serious infections since it usually results in more complete absorption of drug and higher tissue concentrations than oral administration. Parenteral therapy of individual animals by IM injection is administered just behind the ear on the lateral side of the neck. This site is chosen in case the drug preparation causes local tissue damage as well as to prevent the possible additional effect of sciatic nerve damage if the ham muscles were used. Needleless injection devices are available for vaccines but are so far infrequently used for treatment due to dose limitations imposed by the design of the devices.

Oral medication is easier to apply to groups of pigs and reduces injection-related food safety problems of broken needles, abscesses, and tissue damage. Oral medication for infections outside the gastrointestinal tract generally represents a less efficient use of the total antimicrobial applied to a population. For example, the range in reported oral bioavailabilities of amoxicillin is 11–47% (Hernandez et al. 2005). Water medication is a more rapid method of treating a group of sick pigs than feed medication, with the advantages of immediate implementation and that sick pigs will continue to drink when they will not eat. However, the disadvantages are that not all drugs are water soluble, that water may be spilled or wasted, and that some drug carriers may occlude nipple watering systems. Administration of drugs by water is through an in-line proportioner containing a concentrated drug solution or by a water tank containing the appropriately dosed drug. Pigs drink about 8–10% of their body weight daily, depending on environmental temperature and palatability of the drug.

As an example, amoxicillin has been demonstrated to achieve therapeutic levels in swine (Agerso et al. 1998) via water delivery. Mean plasma concentrations fluctuated between 0.5 and 1.3 μg/mL during the treatment period and were suspected to reflect pig drinking behavior. Consumption of amoxicillin-containing water also increased as pen temperature increased during the study. Several studies have demonstrated relatively large individual animal variation (Agerso et al. 1998) relative to group plasma means. This variation in effective dose, and corresponding variation in response to therapy, must be considered when setting treatment expectations for water medication protocols. In fact, when tetracycline plasma levels were evaluated in pigs administered water medicated at labeled concentrations, plasma levels were highly varied and generally lower than 0.3 μg/mL, which suggests that therapy would have questionable value for most target pathogens (Dorr et al. 2009). Another study of tetracycline water medication in swine revealed that the oral bioavailability is very low (Mason et al. 2009).

In-feed medication has historically been the most common route of administration of anthelmintic and antimicrobial drugs. The disadvantage for treatment of acute infections is not only that sick pigs may not eat but also that existing non-medicated feed needs to be either removed or eaten. For this reason, in-feed medication is

often reserved for long-term use in the prevention, control, or treatment of chronic infections.

Principles of prophylaxis

Antimicrobial drugs are administered to swine for the prevention of diseases. The timing of prophylaxis is especially critical and may not vary significantly from the optimum for treatment except that prophylaxis implies clinical signs are not yet occurring although the animal or population may already be infected. The generally accepted principles of antimicrobial prophylaxis are as follows:

- Medication should be directed against specific pathogens or diseases.
- Prophylaxis should be used only where efficacy is established. It should be of a duration that is as short as possible consistent with efficacy.
- Dosage should be the same as that used therapeutically.
- Adverse effects itemized earlier should be minimized.
- Prophylactic use of antimicrobials should be restricted for the treatment of animals that are most likely to respond to treatment.
- Alternatives to these antimicrobial use practices need to be found and employed when possible.

One prophylactic practice in swine is that of "pulse medication," whereby a therapeutic level of a specific drug is included in the feed at therapeutic concentrations periodically for a short duration to significantly reduce clinical disease while allowing the animal to develop natural immunity because of infection by the pathogen. This approach requires sufficient diagnostic history from the population to anticipate a predictable onset of the disease on a particular farm. When applied correctly, this approach can limit total antimicrobial use while preserving animal welfare. A comparison of pulse dosing to continuous dosing in feed demonstrated improved performance and survivability in the presence of naturally occurring disease for both treatments. However, pulsing permitted sufficient natural exposure to stimulate active humoral immunity to *Mycoplasma hyopneumoniae*, while continuous treatment did not (Walter et al. 2000). The implications of this approach for the development of antimicrobial resistance are not completely understood.

In a study of ceftiofur administered as a single injection of the crystalline-free acid, pigs injected 13 and 10 days prior to challenge did not have removal rates significantly different than untreated controls after challenge with *A. pleuropneumoniae* (Crane et al. 2006). However, treatment 7, 4, and 1 days prior to challenge resulted in fewer removals than the control group. Understanding the duration of antimicrobial effect for the selected treatment is an important component of treatment success.

Multidrug therapies must be considered for their potential to create toxicities that would not occur because of either treatment independently. In swine, the most commonly described example of this is toxicity due to concurrent exposure to an ionophore (narasin, for example) and tiamulin. Radke (2017) described three cases where the toxicity was manifested as neurologic/musculoskeletal signs, ataxia, tremoring, lameness and increased vocalization, especially when forced to move. In all three cases, an error in the manufacture of feed led to the accidental administration of an ionophore concurrently with the intentional administration of tiamulin.

Regulation

The use of antimicrobial drugs in food animals is regulated by law in many countries, and veterinarians need to know and abide by the regulations. The regulations involve an approval process of drugs produced by a manufacturer only if they meet human and animal safety standards as well as being shown to be efficacious at specified dosages for particular purposes. In the United States, general limits to how antimicrobials are used exist. Extra-label use is allowed when drugs are approved for use in humans or food animals and the specific requirements of the Animal Medicinal Drug Use Clarification Act are met. However, extra-label use via feed delivery is not allowed, and there are specific drugs that are banned in food animals in the United States. None of these circumstances are swine specific and will not be discussed further here, but the swine veterinarian is cautioned to be aware of the most current regulations for their practice location.

Drug withdrawal

Most drugs must not be used near slaughter to avoid any significant residues in meat products. While generally understood for antimicrobials, it is often overlooked that most vaccines have required withdrawal times as well. The use of vaccine near market in growing animals is unlikely, but timing the vaccination of sows that might be candidates for culling requires observation of the withdrawal time. The precise period varies with the drug and the dosage. For drugs used at the labeled dosage, this will be specified on the package insert. For extra-label drug use, withdrawal information may be obtained from the manufacturer or in some cases from national or international databases such as, in the United States, the Food Animal Residue Avoidance Databank (www.usfarad.org). Veterinarians should be aware that there is robust international trade of pork products, acceptable residue limits in pork vary among countries, and the withdrawals included on the label may only be valid for the country in which the drug label was approved. Consequently, some harvest plants have extended withdrawal periods for specific

drugs to prevent residues using the limits of the destination country. It is important to accommodate the requirements of the destination market with the lowest maximum residue limit (MRL). In most cases, the harvest plant will know the potential destinations for pork products they export and will have withdrawal suggestions. Dialogue with the harvest plant is imperative to create treatment withdrawals that protect a producer's ability to market at the desired plant.

Limiting development of resistance

Numerous reports have recommended that all stakeholders concerned with the use of antimicrobials in both food animals and humans must be involved in an overarching global strategy to contain resistance (e.g. World Health Organization 2000a) and have recommended steps to enhance the prudent use of antimicrobials in animals, including the removal of growth promotion use of antibiotics if they are important in human medicine (e.g. World Health Organization 2000b). It is critical to note that resistance mechanisms are not limited to antimicrobials but have also been reported for copper and zinc (Fard et al. 2011). Further, as mentioned previously, direct evidence that even low levels of antimicrobials reduce the shedding of human pathogens by swine (Cornick 2010) must be considered in the formulation of risk mitigation regulations at the national level. At the international level, the World Organization for Animal Health continues to formulate recommendations and options for risk management relating to antimicrobial use in animals (OIE 2003; WHO 2004). Outside the EU, other countries are in the process of assessing or starting to reassess the use of antimicrobial drugs in food animals based on the importance of the drug in human medicine and the likelihood of exposure of humans to resistant bacteria or resistance genes arising from animals (e.g. Health Canada 2002; Center for Veterinary Medicine, US FDA 2004).

There are opportunities to apply innovation to reduce the amount of antimicrobials used in production. While the inclusion of antimicrobials in semen extenders does not represent a significant portion of the total use in swine, a recent study demonstrated the potential for innovation to limit antimicrobial use. In this study, Morrell and Wallgren (2011) used single layer centrifugation to separate sperm from bacteria to create semen doses free of bacterial contamination.

In recent years many countries have started to monitor resistance in both important pathogens (e.g. *Campylobacter jejuni*, *Salmonella*) as well as "indicator" commensal bacteria isolated from animals, foodstuffs, and humans. For example, in the United States, the National Antimicrobial Resistance Monitoring System (NARMS) established in 1996 is designed to document emerging resistance problems, as well as to provide data on which public health policy decisions can be made for the use of antimicrobial drugs in food animals. In Canada, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) has taken a similar approach to NARMS. In 2010, CIPARS reported (Deckert et al. 2010) that in sentinel grower/finishers, antimicrobials considered very important to human health were not frequently used. Additionally, resistance as determined by culture and sensitivity of samples obtained on farms was characterized as low.

One emerging resistance problem that will likely receive greater attention in the future is extended-spectrum cephalosporin resistance in multidrug-resistant *E. coli* and *Salmonella* serovars (Winokur et al. 2001; Zhao et al. 2003), in which the *cmy-2* gene encoding expanded-spectrum cephalosporin resistance may be found on several different plasmids that can readily be transferred through bacterial conjugation (Carattoli et al. 2002). However, as deftly pointed out by Davies (2010), "Genes encoding for antimicrobial resistance are not foodborne pathogens."

Tracking genetic changes provides warning of adaptation and spread of the capability to transcribe cellular modifications that would make foodborne pathogens harder to combat. One example of an opportunity for swine practitioners to guide more judicious use involves the use of colistin (a cationic polypeptide antibiotic) in pig production. There is very little pharmacokinetic data to guide colistin use in swine, and it has reemerged in human medicine to treat infections caused by gram-negative bacteria that are more frequently resistant to aminoglycosides. Since the 1980s, it has been understood that colistin resistance was in part conferred by the chromosomal *mcr-1* gene mutation in bacteria. More recently, the *mcr-1* gene has been found on a plasmid in *E. coli* isolated from swine. Plasmid transfer can occur within bacterial species but also between different species, which allows it to be disseminated more readily. The reader is referred to the review by Rhouma et al. (2016) for further explanation of the relationship of colistin use and emergence of the plasmid-mediated resistance.

Prudent use guidelines

The widespread concern about antimicrobial resistance and the animal–human resistance link has led most major national veterinary organizations to improve antimicrobial drug use by development of prudent use guidelines. Such guidelines represent first steps in the more judicious use of antimicrobial drugs that may become considerably more complex over time if they address antimicrobial drug choice for diseases. An example of such guidelines, that of the American Association of Swine Veterinarians, is shown in Table 10.4.

Table 10.4 American Association of Swine Veterinarians (AASV) Judicious Therapeutic Use of Antimicrobials Principles for Swine Veterinarians.

1) Implement preventive strategies, such as appropriate husbandry and hygiene, routine health monitoring, and immunization to optimize pig health.
2) Recognize that in spite of best efforts toward preventive strategies, there are times when swine veterinarians need to prescribe treatment for individual and herd conditions. This is a part of the veterinarians' oath to "protect animal health and welfare." Use the following suggested steps to assess necessity for judicious therapeutic use of antimicrobials.
3) Consider other therapeutic options prior to, or in conjunction with, antimicrobial therapy.
4) Meet all requirements of a veterinarian–client–patient relationship (VCPR).
5) Prescribe extra-label antimicrobial therapy only in accordance with the Animal Medicinal Drug Use Clarification Act amendments to the Food, Drug, and Cosmetic Act and its regulations.
6) Work with those responsible for the care of pigs to use antimicrobials judiciously regardless of distribution system through which the antimicrobial was obtained.
7) Optimize regimens for antimicrobial therapy using current pharmacological information and principles.
8) Use antimicrobials following careful review.
9) Follow label directions or, when extra-label use is clinically necessary, follow AMDUCA regulations.
10) Use historical outcomes and clinical experience in the selection of antimicrobials.
11) Use adequate laboratory support for antimicrobial decision-making.
12) Devise a plan for treatment of ill or at-risk animals.
13) Minimize environmental contamination with antimicrobials.
14) Record all antimicrobial treatments.
15) Periodically reevaluate antimicrobial use.

Note: The AASV website elaborates on these basic guidelines (https://www.aasv.org/documents/2014AASVJUG.pdf).

Effects of antimicrobial treatment on immunity

Effects on the immune system of drugs intended to provide antimicrobial therapy must be considered as part of treatment plan development. For example, Pomorska-Mol et al. (2016) found that ceftiofur, doxycycline, and tiamulin decreased, while amoxicillin and tulathromycin increased, humoral immune response to *Erysipelothrix rhusiopathiae* vaccination. Treatment at the time of vaccination for influenza A virus (IAV) of swine and pseudorabies virus (PRV) affected the immune response of pigs (Pomorska-Mol et al. 2015). Ceftiofur hydrochloride administered at the time of vaccination delayed the humoral response and affected the cellular response to PRV. Ceftiofur hydrochloride also significantly suppressed production of antibodies against IAV. The effect of disease treatment on subsequent immunity is relevant in production scenarios where reexposure or recrudescence of disease is possible, and it may be necessary to balance treatment efficacy with development of immunity. For example, when pigs were infected with *A. pleuropneumoniae* serotype 2 for a second time, pigs treated with enrofloxacin during the first infection had the least immunity and the worst clinical disease. At the time of the first infection, treatment with enrofloxacin resulted in the best clinical improvement compared with tetracycline and penicillin. However, there was insufficient exposure of the immune system to produce protective immunity, and clinical performance at reinfection was worst among the antibiotic treatment groups. Tetracycline reduced clinical impact at the first infection and allowed pigs to develop immunity that protected them at reinfection (Sjölund et al. 2009). In another study, enrofloxacin treatment before low dose exposure did not interfere with infection, development of immunity, or subsequent protection against challenge. Enrofloxacin treatment after low dose exposure did impair development of immunity, and pigs were susceptible to subsequent challenge (Macedo et al. 2017).

Assessing outcomes or treatment failure

Treatment failure has many causes. The antimicrobial selected may be inappropriate because of misdiagnosis, inactivity at the site of infection, failure to culture infections, inaccurate or inapplicable laboratory results, resistance of pathogens, chronic nature of the infection (which may affect metabolic state of the pathogen), or errors in sampling. These factors are more likely to cause failure than inadequate dosage although this may also be important. It is important that producers comply with dosing instructions. When failure occurs, diagnosis must be reassessed, and samples collected for laboratory analysis.

Non-antimicrobial therapeutics and strategies

Manipulating passive immunity

Spray-dried animal plasma has been widely used in diets for newly weaned pigs and is associated with increased growth rates on the order of 27% (van Dijk et al. 2001). The mode of action of spray-dried animal plasma is not fully understood but is assumed to be at least partly due to the presence of immunoglobulins, which may provide a certain level of protection to the newly weaned piglet at a time when the supply of immunoglobulins from sow's milk has ceased. The ability of plasma proteins to neutralize the effect of specific organisms is dependent on the immunizations and disease history of the pigs from which the blood is collected.

Specific antibodies from chicken egg yolk have been examined as a source of passive immunity for newly weaned pigs as well. Laying hens are vaccinated against specific pig pathogens such as *E. coli*. Antibodies are secreted into the yolk of the egg (IgY) in large quantities (up to 200 mg/egg) (Marquardt and Li 2001), and dried yolk is incorporated in nursery pig rations. Trials using specific egg-yolk products to prevent post weaning *E. coli* diarrhea have produced inconsistent results (Chernysheva et al. 2004). Stability of the product during feed processing and passage through the pig's gastrointestinal system are major concerns.

Direct-fed microbials (probiotic)

Probiotics are defined as live microbials provided in the feed in an attempt to encourage proliferation in the intestine of the specific microorganism fed with the objective of providing health benefits to the host animals (Fuller 1989). The most commonly used probiotics include species of *Lactobacillus*, *Enterococcus*, *Bifidobacterium*, and *Saccharomyces* (Alvarez-Olmos and Oberhelman 2001; Holzapfel et al. 2001; Rolfe 2000). Most studies involving probiotics have concentrated on improving intestinal health, particularly during the weaning period when the pig gut microflora undergoes dramatic change.

It is generally accepted that with careful attention to the criteria used to select the probiotic strain, there may be a place for probiotics in the prevention of enteric disease, but results to date are inconsistent. There are several criteria that potential probiotic strains must meet in order to be considered for use as a probiotic, including the ability to demonstrate predictable and measurable health benefits. The screening and selection of a probiotic includes testing *in vitro* or *in vivo* of the following criteria:

It must be nonpathogenic and proven safe.
It must have stability in an acid environment, in the presence of bile, and resistance to degradation by digestive enzymes.
It must adhere to gut epithelial tissue and be able to persist in the gastrointestinal tract of the host.

In addition, the microbials used as probiotics must retain viability and stability during commercial production, feed processing, storage, and delivery and must be cost effective.

The main mechanisms whereby probiotics exert protective or therapeutic effects are not fully understood, but several ways have been postulated. Probiotics produce antimicrobial substances such as organic acids, free fatty acids, ammonia, hydrogen peroxide, and bacteriocins (Alvarez-Olmos and Oberhelman 2001). In addition, probiotics may enhance specific and nonspecific host immunity (Kailasapathy and Chin 2000), and probiotics may prevent colonization of pathogenic microorganisms by competitive inhibition for microbial adhesion sites.

Inconsistent findings have been observed when probiotics have been used in trials to control pig disease or improve growth performance (Conway 1999). It is unlikely they can replace antibiotics in the control of disease, but they may have a place alongside other techniques for improving the health of the gut microflora and reducing the shedding of pathogens such as *Salmonella*.

There is considerable interest in the use of fermented liquid feed, and there appears to be an association between its use and a reduction in *Salmonella* prevalence (van der Wolf et al. 2001). A possible explanation for the beneficial effect of fermented liquid feed is that the reduced pH of the diet and the presence of large numbers of organic acid-producing bacteria in the feed have a positive effect on the gut microflora and create an environment unsuitable for *Salmonella* and other coliform bacteria.

Bacteriophages

Bacteriophages or phages are bacterial viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacterium to lyse (Sulakvelidze et al. 2001). From a clinical standpoint, phages appear to be innocuous to host animals, do not attack normal gut flora, and are extremely common in the environment. Recently, naturally occurring phages with activity against *Salmonella* species and *E. coli* were isolated from swine feces, and this may facilitate the development of a direct application to enhance food safety (Callaway et al. 2011). Phages appear to be specific to bacterial species or serovars. Albino et al. (2014) isolated specific autochthonous bacteriophages that were active against four *Salmonella typhimurium* serotypes (Abony, Enteritidis, Typhi, and Typhimurium but not Arizonae, Choleraesuis, Gallinarum, and Pullorum), reduced bacterial counts *in vitro*, and reduced prevalence of positive fecal samples among inoculated pigs.

Nutrients

There is a plentiful supply of physiologically active feed ingredients that can improve pig performance and health by modifying the environment of the digestive tract (Pettigrew 2003). Zinc oxide added to nursery rations at a level of 2500 ppm for 2 weeks will result in increased growth rate and reduced prevalence of diarrhea (Jensen-Waern et al. 1998). *In vitro* studies have shown that zinc has antimicrobial effects, but *in vivo* studies show no reduction in *E. coli* numbers and no change in the function of circulating neutrophils. There are concerns that high levels of zinc oxide will cause liver toxicity if fed longer than 3–4 weeks.

Likewise, copper sulfate at levels of up to 250 ppm has been added to pig feed to promote growth. However, the combination of zinc and copper does not result in an additive growth response (Hill et al. 2000). In the case of both copper and zinc, there are environmental concerns regarding their use because of the accumulations of these minerals in manure. The development of resistance is not specific to antimicrobials, and a transferrable copper resistance gene has been demonstrated in the fecal enterococci of swine in the United States.

The quest for alternatives to antibiotics in pig feed has caused interest in natural remedies, including herbs, spices, botanicals, and essential oils. These products may improve performance by improving feed palatability and by exerting antibacterial effects, but there needs to be further evidence of their effectiveness (Pettigrew 2003).

Organic acids (fumaric, formic, and lactic) are commonly added to feed or water to improve growth and reduce diarrhea during the post weaning period (Tsiloyiannis et al. 2001). Modes of action claimed for the growth-promoting effect of organic acids include decreased gastric pH, reduced coliform population, stimulated pancreatic exocrine secretion, increased pepsin activation, altered gut morphology, and improved intake and digestibility (Partanen 2001).

Response to acidification has been variable and may be attributed to feed and animal factors as well as differences in the properties of the various organic acids. Two problems that are associated with the use of high levels of organic acids are that the acids may have a negative effect on palatability and the feed is corrosive to cement and steel in swine housing (Canibe et al. 2001).

An alternative approach to altering the gut microflora is to feed nondigestible material that provides a substrate for beneficial bacteria such as lactic acid-producing bacteria. These products are often referred to as prebiotics. For a feed to be classified as a prebiotic, it must be neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract, must be a selective substrate for one or a limited number of potentially beneficial commensal bacteria, and must induce luminal or systemic effects that are beneficial to the host's health (Roberfroid 2001). Nondigestible oligosaccharides are the most common type of prebiotics, including fructo-oligosaccharides and mannan-oligosaccharides. In general, prebiotics are considered to provide small but positive improvements in growth rate. However, their role in providing health benefits, such as reducing *Salmonella* shedding, needs to be clarified.

Enzymes added to feed to encourage improved feed efficiency and, in some cases, potential health benefits are used widely. For example, feed can be supplemented with phytase to allow swine to digest plant phosphorus that is in the form of phytate. It has been hypothesized that the use of enzymes may allow the industry to utilize coarse feed particle size as a means of reducing both gastric ulcers and the prevalence of *Salmonella* while still maintaining acceptable feed conversion. There is a possibility that in-feed antimicrobials for growth promotion could be reduced through a combination of feeding manipulations, such as the use of various combinations of liquid feeds or coarse particle size, enzymes, probiotics, prebiotics, and acidifiers.

Antiparasitics

In modern confinement operations, there are few parasitic problems (Roepstorff and Jorsal 1989). Under conditions of good hygiene and management, the regular application of anthelmintics may be of little or no benefit (Roepstorff 1997). Ascariasis is generally the main concern, and strategic medication with a wide range of effective products can easily control this parasite.

External parasitism caused by mange mites and lice no longer needs to be a significant problem because of good husbandry practices and effective drugs, particularly the avermectins. Failure to control sarcoptic mange or lice infestation is generally due to a poor understanding of the epidemiology of the organisms and apathy on the part of the herdsman (Cargill et al. 1997). Antiparasitic products and their application are presented in Table 10.5.

Hormones

Oxytocin is widely used as an aid in stimulating parturition and milk letdown. Prostaglandin F_{2a} or a synthetic analogue can be used to induce parturition. Puberty can be induced in gilts by treatment with a single injection of 200 IU of human chorionic gonadotropin and 400 IU of

Table 10.5 Common swine anthelmintics and doses.

Product	Dosage
Dichlorvos	11.2–21.6 mg/kg body weight in feed with 1/3 of regular ration
Doramectin	300 µg/kg body weight IM
Fenbendazole	9 mg/kg body weight over 3–12 days via feed
Fenbendazole oral solution	2.2 mg/kg body weight for 3 days via drinking water
Ivermectin	300 µg/kg SQ or 100 µg/kg body weight for 7 days via feed
Levamisole hydrochloride liquid	8 mg/kg body weight in drinking water
Piperazine	275–440 mg/kg body weight in feed or water
Pyrantel tartrate	22 mg/kg of body weight as 1-day treatment or 96 g/t of feed as prophylactic dose

equine chorionic gonadotropin. The injection of follicle-stimulating hormone at weaning and an injection of luteinizing hormone approximately 72–80 hours later have been shown to induce a predictable ovulation (Barnabe et al. 2002) and can be used in artificial insemination programs where these hormones are licensed for this purpose. Estrus can be synchronized by administering a progestin for 14–18 days. The progestin inhibits follicular maturation until the progestin is withdrawn. Be aware that many of these represent extra-label uses that might be prohibited in some countries including the United States.

Triptorelin, a gonadotropin-releasing hormone (GnRH) agonist, applied as an intravaginal gel has been shown to be effective in advancing and synchronizing ovulation in weaned sows. While wean-to-estrus interval was not impacted, wean-to-ovulation interval was reduced in treated animals, and more sows ovulated 40 hours after treatment than controls (Knox et al. 2014). Further details are presented in Chapter 20.

Hormones are also used in certain countries for growth manipulation. Daily injection of porcine somatotropin (PST) strongly influences feed efficiency, growth, and carcass composition. Ractopamine, a phenethanolamine or β-agonist, is used as a feed additive in several countries. Its function is as a repartitioning agent causing improved feed efficiency and a lean carcass at slaughter.

Anti-inflammatory drugs

Anti-inflammatory drugs including ketoprofen, flunixin meglumine, dexamethasone, sodium salicylate, acetylsalicylic acid, indomethacin, and meloxicam have been evaluated in pigs as interventions for various clinical conditions with variable results that mostly suggest more investigation is needed. Meloxicam (Georgoulakis et al. 2006) was demonstrated to reduce the prevalence of clinical signs and the need for re-treatment in pigs with respiratory disease when used as an adjunct to antimicrobial treatment. Additionally, flunixin has an approved label indication for control of pyrexia associated with swine respiratory disease. Currently, there are two limitations to the use of most NSAIDs in swine: (1) pharmacodynamic information to inform selection of an effective dose, especially for analgesia, is lacking and (2) pharmacokinetic information in swine on the disposition of many formulations. A review of the pharmacokinetic information available for NSAIDs in swine indicates that elimination half-lives are generally very short, ranging from 0.19 (meloxicam) to 7.76 (flunixin meglumine) hours (Karriker 2014). A concern about the potential for NSAIDs to cause enteric lesions at higher doses coupled with a short half-life means that multiple doses per day may be necessary for pain relief.

References

Agerso H, Friis C, Haugegaard J. 1998. *J Vet Pharmacol Ther* 21:199–202.

Albino L, Rostagno M, Huńgaro H, et al. 2014. *Foodborne Pathog Dis* 11(8):602–609.

Alvarez-Olmos MI, Oberhelman RA. 2001. *Clin Infect Dis* 32:1567–1576.

Backstrom L, McDonald J, Collins MT, et al. 1994. *J Swine Health Prod* 2:11–14.

Barnabe RC, Viana CHC, Candini PH, et al. 2002. *Rev Brasileira Repro Anim* 26:177–179.

Bimazubute M, Cambier C, Baert K, et al. 2009. *J Vet Pharmacol Ther* 33:183–188.

Bimazubute M, Cambier C, Baert K, et al. 2010. *J Vet Pharmacol Ther* 34:176–183.

Boothe DM. 2006. Principles of antimicrobial therapy. *Vet Clin North Am Small Anim Pract* 36:1003–1047.

Callaway TR, Edrington TS, Brabban A, et al. 2011. *Foodborne Pathog Dis* 8:261–266.

Canibe N, Zteien SH, Øverland M, et al. 2001. *J Anim Sci* 79:2123–2133.

Carattoli A, Tosini F, Giles WP, et al. 2002. *Antimicrob Agents Chemother* 46:1269–1272.

Cargill CF, Pointon AM, Davies PR, et al. 1997. *Vet Parasitol* 70:191–200.

Center for Veterinary Medicine, US Food and Drug Administration. 2004. Department Guidance for Industry #144—Preapproval Information for Registration of New Veterinary Medicinal Products for Food-Producing Animals with Respect to Antimicrobial Resistance—VICH GL27, Final Guidance, April 27, 2004. USFDA CVM website.

Chernysheva LV, Friendship RM, Gyles CL, et al. 2004. *J Swine Health Prod* 12:119–122.

Conway PL. 1999. Specifically selected probiotics can improve health and performance of pigs. In Cranwell PD, ed. *Manipulating Pig Production VII*. Werribee: Australasian Pig Science Association, pp. 220–224.

Cornick NA. 2010. *Vet Microbiol* 143:417–419.

Crane J, Bryson W, Anderson Y, et al. 2006. *J Swine Health Prod* 14:302–306.

Davies P. 2010. *Foodborne Pathog Dis* 8:189–201.

Deckert A, Gow S, Rosengren L, et al. 2010. *Zoonoses Public Health* 57:71–84.

van Dijk AJ, Everts H, Nabuurs MJ, et al. 2001. *Livest Prod Sci* 68:263–274.

Dorr P, Nemechek M, Scheidt A, et al. 2009. *J Am Vet Med Assoc* 235:299–304.

Fard RM, Heuzenroeder MW, Barton MD. 2011. *Vet Microbiol* 148:276–282.

Fuller R. 1989. *J Appl Bacteriol* 66:365–378.
Georgoulakis IE, Petridou E, Filiousis G, et al. 2006. *J Swine Health Prod* 14:253–257.
Giguere S, Prescott J, Dowling P. 2015. *Antimicrobial Therapy in Veterinary Medicine*, 5th ed. Wiley Blackwell.
Health Canada. 2002. *Use of Antimicrobials in Food Animals in Canada: Impact on Resistance and Human Health*. Ottawa: Veterinary Drugs Directorate.
Hernandez E, Rey R, Puig M, et al. 2005. *Vet J* 170:237–242.
Hill GM, Cromwell GL, Crenshaw TD, et al. 2000. *J Anim Sci* 798:1010–1016.
Holzapfel WH, Haberer P, Geisen R, et al. 2001. *Am J Clin Nutr* 73:365–373S.
Jensen-Waern M, Melin L, Lindberg R, et al. 1998. *Res Vet Sci* 64:225–231.
Kailasapathy K, Chin J. 2000. *Immunol Cell Biol* 78:80–88.
Karriker L. 2014. Pharmacokinetics and efficacy of NSAIDS in swine. In Proceedings of the Twenty-second Annual Swine Disease Conference for Swine Practitioners, Ames Iowa.
Knox R, Taibl J, Breen S, et al. 2014. *Theriogenology* 82: 379–386.
Macedo N, Cheeran M, Rovira A, et al. 2017. *Vet Microbiol* 199: 91–99.
Marquardt RR, Li S. 2001. Control of diarrhea in young pigs using therapeutic antibodies. In Proceedings of the AD Leman Swine Conference, pp. 227–239.
Mason S, Baynes R, Almond G, et al. 2009. *J Anim Sci* 87:3179–3186.
Morrell JM, Wallgren M. 2011. *Anim Reprod Sci* 123:64–69.
Partanen K. 2001. Organic acids — Their efficacy and modes of action in pigs. In Piva A, Bach Knudsen KE, Lindberg JE, eds. *Gut Environment of Pigs*. Nottingham University Press, pp. 201–207.
Pettigrew JE. 2003. Alternative products: Are there any silver bullets? In Proceedings of the Annual Meeting on American Association of Swine Veterinarians, Orlando, pp. 439–441.
Pijpers A, Schoevers EJ, van Gogh H, et al. 1991. *J Anim Sci* 69:2947–2954.
Pomorska-Mól M, Czyżewska-Dors E, Kwit K, et al. 2015. *BMC Vet Res* 11:268.
Pomorska-Mól M, Kwit K, Wierzchosławski K, et al. 2016. *Vet Rec* 178(22):559.
Radke S. 2017. Update on ionophore toxicity and investigating feed-related issues. In Proceedings of the ISU James D. McKean Swine Disease Conference, November 2–3, 2017, pp. 110–114.
Rhouma M, Beaudry F, Letellier A. 2016. *Int J Antimicrob Agents* 48:119–126.
Roberfroid MB. 2001. *Am J Clin Nutr* 73:406–409S.
Roepstorff A. 1997. *Vet Parasitol* 73:139–151.
Roepstorff A, Jorsal SE. 1989. *Vet Parasitol* 33:231–239.
Rolfe RD. 2000. *J Nutr* 130:396–402S.
Sjölund M, Martín de la Fuente A, Fossum C, et al. 2009. *Vet Rec* 164:550–555.
Smith C. 2011. AWIC Special Reference Brief: Swine Anesthesia and Analgesia, 2000–2010. Unites States Department of Agriculture, Animal Welfare Information Center SRB 11-01.
Sparks J, Karriker L, Day D, et al. 2017. *J Vet Pharmacol Ther* 40(4):363–369.
Sulakvelidze A, Alavidze Z, Morris GJ. 2001. *Antimicrob Agents Chemother* 45:649–659.
Tantituvanont A, Yimprasert W, Werawatganone P, et al. 2009. *J Antimicrob Chemother* 63:369–373.
Tsiloyiannis VK, Kyriakis SC, Vlemmas J, et al. 2001. *Res Vet Sci* 70:287–293.
Walter D, Holck J, Sornsen S, et al. 2000. *J Swine Health Prod* 8:65–71.
Winokur PL, Vonstein DL, Hoffman LJ, et al. 2001. *Antimicrob Agents Chemother* 45:2716–2722.
van der Wolf PJ, Wolbers WB, Elbers ARW, et al. 2001. *Vet Microbiol* 78:205–219.
World Health Organization. 2000a. *Global Strategy for Containment of Antimicrobial Resistance*. Geneva, Switzerland. WHO website.
World Health Organization. 2000b. *Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food*. Geneva, Switzerland. WHO website.
World Health Organization (WHO). 2004. *Joint FAO/OIE/WHO 2nd Expert Workshop on Nonhuman Antimicrobial Usage and Antimicrobial Resistance: Management Options*. Oslo, Norway. WHO website.
World Organization for Animal Health (OIE). 2003. *Joint First FAO/OIE/WHO Expert Workshop on Nonhuman Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment*. Geneva, Switzerland. WHO website.
Zhao S, Qaiyumi S, Friedman S, et al. 2003. *J Clin Microbiol* 41:5366–5371.

11

Anesthesia and Surgical Procedures in Swine

David E. Anderson and Pierre Yves Mulon

Best practices for surgical procedures increasingly conflict with economic goals. The veterinarian must critically evaluate the economic benefit versus cost and prognosis of surgery. In most scenarios, services offered by the veterinarian are directed to the need of the enterprise. Surgery on an individual pig is not always cost efficient. However, pigs selected for genetic improvement, show pigs, pet pigs, etc. have individual value, and surgery may be performed with little regard to costs. Some spontaneous conditions such as inguinal and umbilical hernias, rectal prolapse, dystocia, and rectal stricture that can occur in large numbers of animals can be very costly and need to be investigated so that treatment and prevention solutions can be applied. In a commercial swine operation, the veterinarian is often a teacher, showing the manager and experienced personnel how to perform some minor surgical procedures during baby pig processing (castration, ear notching, teeth clipping, tail amputation) in a cost-effective fashion. It is the veterinarian's role to make sure that these procedures are done properly and humanely.

Among purebred pigs, pet pigs, or pigs used as animal models in biomedical research, the individual animal may be of high value, and surgery under conditions of idealized anesthesia and analgesia may be required. A veterinarian who is able to offer excellent surgical service to swine producers may have greater credibility as a herd consultant. The purpose of this chapter is to describe clinical swine anesthesia, routine surgical procedures done during baby pig processing, hernia repair, and some common surgical procedures of the digestive, urogenital, and musculoskeletal systems in swine.

Anesthesia

Minor surgical procedures (ear notching, teeth clipping, tail amputation, castration) in young swine often are performed without anesthetizing the animal. Performed skillfully, these surgical procedures are tolerated by young pigs without an anesthetic; however a recent study demonstrated that weight gain was greater at 3 and 6 days post surgery in piglets receiving local anesthesia prior to castration compared with non-anesthetized ones (Sturlini-Barticciotto et al. 2016). Sometimes economics has an influence on the use of an anesthetic. However, public scrutiny of management practices increasingly demands attention to pain and prevention of unnecessary distress. Management of swine anesthesia presents some difficulties. Swine resist mechanical restraint more and are usually more difficult to hold than other species. Strong assistance often is needed. When possible, adult swine should be withheld from all feed for 1–24 hours before general anesthesia, whereas piglets, which are prone to hypoglycemia, should be held from suckling for only 1–2 hours before anesthetic induction.

Readers are reminded that there are no FDA-approved drugs labeled for use in swine for the treatment of pain or for local or general anesthesia in the United States. The regulatory management of anesthesia drugs in pigs is specific to country and regions and beyond the scope of this chapter. The reader is encouraged to seek out knowledge of regulations for their jurisdiction and prioritize protection of the food chain.

Malignant hyperthermia or porcine stress syndrome is a heritable condition in swine that can be triggered in a susceptible pig by any stress and many injectable (acepromazine, ketamine, and succinylcholine) and inhalant anesthetics. Susceptible pigs are extremely muscular and usually have reduced subcutaneous fat. Halothane traditionally has been incriminated, but delayed onset of malignant hyperthermia can occur with exposure to isoflurane (Wedel et al. 1993). The clinical signs of malignant hyperthermia can be any of the following: a severe increase in body temperature, muscle rigidity, tachycardia, tachypnea, hypoxemia, cardiac arrhythmias, unstable blood pressure, and myoglobinuria. Death of an affected animal appears to be the result of peripheral

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

circulatory changes that are produced by severe acidosis, vasoconstriction, hyperkalemia, decreased cardiac output, and hypotension. The susceptibility to malignant hyperthermia is due to an autosomal recessive defect in the gene that codes for ryanodine receptor calcium channels in skeletal muscle (Rosenberg and Fletcher 1994). In response to a trigger, intracellular calcium rises abruptly, which causes muscle contracture and release of heat. Treatment of malignant hyperthermia is largely symptomatic. Early recognition is the key to successful treatment. Whenever malignant hyperthermia is suspected, volatile anesthetics should be discontinued. Aggressive cooling should be instituted using ice packs and alcohol baths. Dantrolene sodium is effective in the treatment of a malignant hyperthermia or as a prophylaxis when given before the anticipated trigger. Suggested doses for swine are 1–3 mg/kg IV for treatment and 5 mg/kg orally given prophylactically.

Anticholinergics (atropine and glycopyrrolate) are recommended before sedation and anesthetic techniques in pigs. Atropine sulfate (0.04 mg/kg IM) or glycopyrrolate (0.02 mg/kg IM) will decrease the risk of bradycardia, excessive salivation, bronchoconstriction, and excessive airway secretion. In healthy swine receiving light surgical anesthesia, administration of supportive fluids is not considered necessary, unless the animal was off feed and water for more than 24 hours.

Intravenous catheterization and support treatment

Long periods of general anesthesia are best managed with intravenous fluid support. In swine that are hypovolemic or showing other signs of shock, fluid therapy is essential preferably before or during anesthesia. Pigs have few superficial veins and arteries suitable for catheter placement and intravenous drug administration (Sakaguchi et al. 1996). In addition, variation in accessibility of these vessels exists among swine breeds. The auricular (ear) vein is the safest and most accessible vein (Figure 11.1). This vein is most easily catheterized along the caudal aspect of the dorsal surface of the ear. To inject or place a catheter in the ear vein, the pig can be restrained. The vein is held at the base of the ear by the fingers, forceps, or a rubber band to distend it. Rubbing the ear with alcohol and vigorous massage seem to create better visualization for needle insertion. Inserting a small catheter (20 gauge) will allow the administration of fluid or injection of additional anesthetic solution intravenously. The medial saphenous vein can be catheterized easily in the anesthetized or well-restrained pig (Figure 11.2). In pigs requiring maintenance of the catheter for intravenous therapy, the cranial superficial epigastric vein is, in our experience, the most easily managed (Figure 11.3).

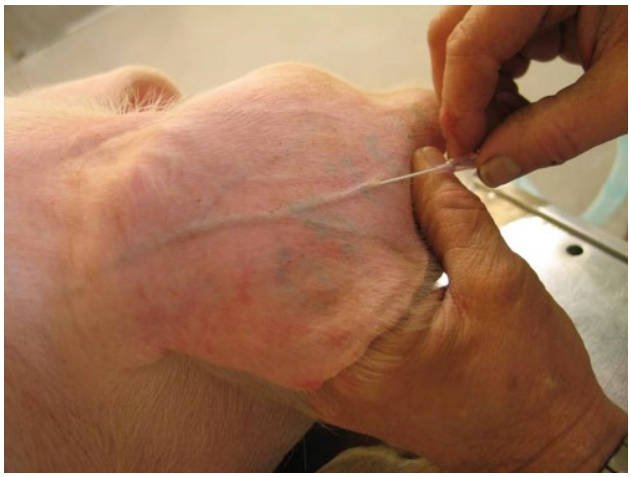


Figure 11.1 Intravenous catheterization of the ear vein (auricular vein) of a pig.

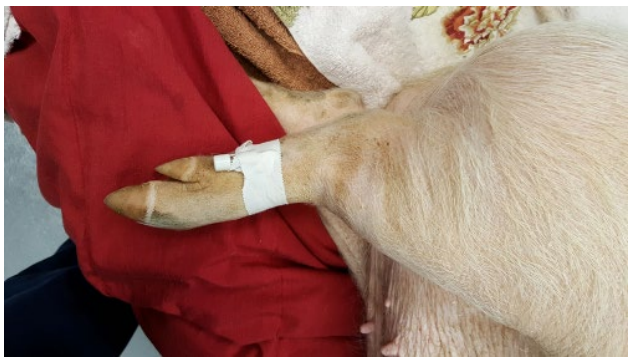


Figure 11.2 Intravenous catheterization of the medial saphenous vein in a pig.

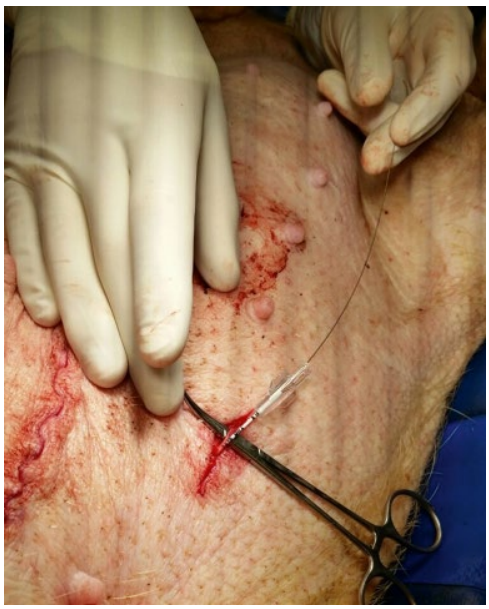


Figure 11.3 Placement of an IV catheter in the caudal superficial epigastric vein of a pig using a "cutdown" method for placement.

Alternatively, intramedullary cannulation should be considered when vascular access is vital, but an IV catheter has not been established. An 18 gauge cannula can easily be inserted into the greater tubercle of the humerus or via the trochanteric fossa of the femur for intramedullary infusions. Fluid and drug administration is easily done in immature pigs. Older pigs usually have fat and fibrosis of the medullary canal to limit administration rate.

In the authors' experience, fluid therapy using balanced electrolyte solution before and during anesthesia is essential during Cesarean section with dead piglets *in utero*. Pigs have a diffuse epitheliochorial placenta. Sow–fetal drug distribution is quite complete. Therefore, it must be assumed that any drug in the maternal circulation reaches the fetus rapidly in relatively high concentration. Because the fetal blood–brain barrier is extremely permeable, these drugs exert a profound anesthetic effect on the fetus. In addition, anesthesia tends to persist after delivery because of the neonate's poorly developed liver enzymes and renal function. Anesthetic agents should be chosen that would minimize fetal depression. General anesthesia will induce greater neonatal depression than regional anesthesia.

Tranquilization and sedation

Acepromazine

Acepromazine decreases spontaneous motor activity. Used alone, it usually provides only slight inconsistent sedation in swine. It predisposes to hypotension and hypothermia and should not be used in debilitated pigs. Recommended dose is 0.1–0.2 mg/kg (Table 11.1). Acepromazine has been reported to decrease the incidence of malignant hyperthermia (Moon and Smith 1996). It is useful when combined with ketamine or tiletamine–zolazepam (Table 11.2).

Benzodiazepines (diazepam and midazolam)

Diazepam (1–2 mg/kg IM; Table 11.1) can be used in combination with ketamine or xylazine (Table 11.2). Midazolam can be absorbed more rapidly and completely than diazepam because it is water soluble. A dose of 0.5 mg/kg IM of midazolam has been associated with a sufficient level of sedation with minimal alteration of the cardiovascular functions (Bustamante and Valverde 1997). They both ensure a smooth recovery and have a longer effect than xylazine. Association with alfaxalone at 5 mg/kg IM can be used to increase the muscle relaxation and sedation levels.

Intranasal administration of 0.2 mg/kg of midazolam produced a significant and reliable sedation with an onset of 3–4 minutes (Lacoste et al. 2000).

Azaperone

Azaperone is a neuroleptic agent and can be given to tranquilize or immobilize swine (Table 11.1). The degree of sedation is dose dependent and should not exceed 1 mg/kg in large boars, because priapism has been reported (Moon and Smith 1996). Azaperone must be given intramuscularly, because intravenous injection often results in excitation. Excessive salivation, hypothermia, sensitivity to noise, and hypotension have been seen in pigs receiving azaperone (Greene 1979). Azaperone is not an analgesic and often is used in combination with other drugs for surgical procedure. Deep tranquilization from azaperone should be obtained before ketamine is given. If azaperone is used alone for surgical procedures, local or regional anesthesia should be administered.

Alpha-2 receptor agonists

Swine are more resistant to alpha-2 agonists (xylazine, dexmedetomidine) compared with other meat-producing animals (Table 11.1). Sedation will result, but animals are aroused easily. Xylazine usually is used in combination with other drugs to produce good muscle relaxation and a smooth recovery. Vomiting has been seen following the use of xylazine in pigs with digestive disturbances. Medetomidine is a more potent alpha-2 agonist than xylazine (Sakaguchi et al. 1992). Medetomidine in

Table 11.1 Sedative agents for swine.

Drug	Dose	Route	Onset (min)	Duration (min)
Acepromazine	0.1–0.5 mg/kg	IM	20–30	30–60
Azaperone	1–2 mg/kg	IM	5–15	60–120
Diazepam	0.5–1 mg/kg	IM	—	—
Midazolam	0.1–0.5 mg/kg	IM	2–4	40–60
Xylazine	0.5–3 mg/kg	IM	5	10
Medetomidine	10 µg/kg	IM	—	—

Table 11.2 Combination of injectable anesthetic agents for short-term anesthesia in swine.

Drug	Dose	Route	Onset (min)	Duration (min)
Thiopental	10–20 mg/kg	IV	Immediate	2–10
Acepromazine Ketamine	0.4 mg/kg 15 mg/kg	IM IM	5 min	15–30
Acepromazine Ketamine Telazol	0.03 mg/kg 2.2 mg/kg 4.4 mg/kg	IM	2–4	40–50
Diazepam Ketamine	1–2 mg/kg 10–15 mg/kg	IM IM	10	20–40
Midazolam Ketamine	0.1–0.5 mg/kg 10–15 mg/kg	IM	5–10	20–40
Azaperone	2–8 mg/kg	IM	5–15	60–120
Xylazine	0.5–3 mg/kg	IM	5	10
Xylazine Ketamine	2 mg/kg 20 mg/kg	IM	7–10	20–40
Xylazine Ketamine Tramadol	2.5 mg/kg 25 mg/kg 5 mg/kg	IM	7–10	30–60
Xylazine Ketamine Telazol	4.4 mg/kg 2.2 mg/kg 4.4 mg/kg	IM	1–2	60
Xylazine Ketamine Midazolam	2 mg/kg 20 mg/kg 0.25 mg/kg	IM	5–10	70–100
Medetomidine Butorphanol Ketamine	80 µg/kg 200 µg/kg 2 mg/kg	IM	1–5	60–120
Medetomidine Butorphanol Ketamine	80 µg/kg 200 µg/kg 10 mg/kg	IM	1–5	75–120
Xylazine Butorphanol Ketamine	2 mg/kg 200 µg/kg 10 mg/kg	IM	1–5	60–120
Propofol Fentanyl	11 mg/kg/h 2.5 mg/kg q30 min	IV IV	Immediate	Continuous infusion

combination with atropine induces deeper sedation than xylazine, and its effects are enhanced by butorphanol. The anesthetic state is characterized by profound somatic analgesia, but visceral analgesia is poor. Combination of medetomidine, butorphanol, and ketamine induces excellent surgical anesthesia in pigs (Table 11.2). This anesthetic regimen can be partially reversed by atipamezole (240 µg/kg), a selective and potent alpha-2 antagonist.

Injectable anesthetics

Injectable agents are most appropriate for field use. With injectable agents, a minimum of equipment is needed, requiring only a small investment. The drugs can be transported easily to the animal, compared with inhalation anesthetics, which are more expensive and can be difficult to transport to a field situation.

Anesthesia can also be induced using IM drug protocols or gas anesthetics via facemask followed by catheterization for IV fluid therapy or drug administration. Intramuscular injections should be given with a 2 in. needle in the cervical muscles of an adult commercial pig to assure that the drug goes into the muscle and not in the fat. To obtain the maximal effect with the drug, the pig should be in a quiet environment if possible. Combinations of anesthetic agents administered in appropriate doses are often superior to any one agent (Table 11.2).

Barbiturates

Barbiturates as a group are poor analgesics. Thiopental is the most commonly used barbiturate. It is a potent central nervous system depressant and should be administered intravenously. The shorter-acting barbiturate thiopental (10–20 mg/kg) is used sometimes for induction before inhalation anesthesia or for very short procedures. A 2.5–5% solution should be used, and one-half of the calculated dose quickly injected. When the pig lies down, incremental amounts are injected until the desired plane of anesthesia is achieved. Apnea often is observed, and means of assisting ventilation should be readily available.

Ketamine

Ketamine induces rapid onset of unconsciousness (Thurmon 1986). The anesthetic state is characterized by somatic analgesia, but visceral analgesia is poor. Ketamine has been used as a major component of many regimens to induce chemical restraint or anesthesia in pigs. It can be given IM, IV, or intratesticularly. Intratesticularly, a combination of ketamine (6 mg/kg) and xylazine (2 mg/kg) has been used successfully for castration (Thurmon 1986). When ketamine is used alone, it exerts some undesirable effects, such as poor muscle relaxation and analgesia emergence delirium, tachycardia, and hypertension. It commonly is combined with a muscle relaxant or sedative such as acepromazine, diazepam, xylazine, or droperidol (Table 11.2).

Propofol

This short-acting hypnotic agent has recently been used for IV anesthesia in pigs (Martin-Cancho et al. 2004). One dosage regimen reported was 11 mg/kg body weight (BW)/h for abdominal surgery. This was done in combination with fentanyl (2.5 mg/kg IV q30min). Compared with pigs anesthetized with isoflurane, propofol-anesthetized pigs required significantly longer to recover consciousness.

Guaifenesin

Guaifenesin is a centrally acting muscle relaxant. Because it produces little analgesia, it should not be used alone. Intravenous infusion of guaifenesin combined with thiobarbiturates and with ketamine and xylazine has been used for induction and maintenance of anesthesia (Thurmon 1986). The authors recommend adding 500 mg of ketamine and 500 mg of xylazine to each 500 mL of 5% guaifenesin in 5% dextrose in water. The mixture is given rapidly in a catheter in the ear vein at a dose of 0.5–1 mL/kg for induction. Anesthesia is maintained by continuous infusion at a rate of 2.2 mg/kg/h of ketamine, which also equals 2.2 mL per kg per hour of the triple-drip mixture. At the end of the surgery, recovery time may be hastened by administration of yohimbine (0.125 mg/kg) or tolazoline (2.5–5 mg/kg) to reverse the effect of xylazine (Thurmon 1986)

Combination injectable anesthesia

Combinations of injectable drugs have been used to increase quality, duration, and analgesia of anesthesia when they can be administered safely. A recent study compared three regimens for maintaining anesthesia in pigs induced using azaperone (1 mg/kg IM) and ketamine (2.5 mg/kg IM). These pigs were divided into three groups: (1) etomidate (200 µg/kg IV) and midazolam (100 µg/kg IV), (2) ketamine (2 mg/kg IV) and midazolam (100 µg/kg IV), and (3) pentobarbital (15–20 mg/kg IV) (Clutton et al. 1997). Pentobarbitone provided the least satisfactory anesthesia because of profound respiratory depression, difficulty completing orotracheal intubation, and prolonged time to standing. Another study compared medetomidine–butorphanol–ketamine (MBK) (80–200 µg/kg–10 mg/kg, respectively, all IM) with xylazine–butorphanol–ketamine (XBK) (2 mg/kg–200 µg/kg–10 mg/kg, respectively, all IM) (Sakaguchi et al. 1996). MBK was found to provide longer and more satisfactory anesthesia as compared with XBK.

Telazol is a unique combination of a dissociative molecule (tiletamine) and a benzodiazepine (zolazepam) that provides some muscle relaxation and sedation and immobilizes swine (Moon and Smith 1996). It requires a smaller volume of injectable compared with ketamine. It frequently is combined with a third sedative such as xylazine or acepromazine to provide better muscle relaxation and an easier recovery (Table 11.2).

Reversal agents

On occasion, reversal of anesthesia may be necessary. Yohimbine is an alpha-receptor antagonist that is commonly used to reverse the effects of xylazine. However, the selective nature of this drug in the reversal of alpha-agonists can be difficult to apply when combination anesthesia has been employed. In a study on the effectiveness of yohimbine to speed recovery of pigs anesthetized using tiletamine + zolazepam + xylazine, pigs were shown to recover more quickly when yohimbine was administered (Kim et al. 2007). In that study, pigs were given a combination of either xylazine + zolazepam or xylazine + tiletamine. Pigs attained sternal recumbency in significantly less time (52 minutes) when yohimbine was administered compared with pigs that did not receive yohimbine (76 minutes). Opioid toxicity (fentanyl patch) can be observed.

Inhalation anesthetics

For debilitated swine, for surgical procedures lasting more than 30 minutes, for difficult procedures, or for valuable swine, an inhalation anesthetic provides a more controlled plane of anesthesia. Inhalation anesthetics can be administered to small pigs by an open or semi-open method; in larger swine, the semi-closed or closed system is preferred. Swine weighing up to 140 kg can be anesthetized with an anesthetic machine designed for small animals (Tranquilli 1986).

Selection of induction technique and anesthetic protocol depends on the size and special needs of the swine, preference of the veterinarians, and availability of drugs and equipment. Sedation is desirable to reduce the stress of physical restraint before induction of anesthesia (Table 11.1). Swine up to 100 kg can be restrained in a webbed stanchion. Large swine can be restrained in a crate with a head catch or by a snare. Induction of anesthesia can be accomplished with a rapid bolus of injectable agent (barbiturate), by rapid infusions of a combination of drugs (Table 11.2), or by using a facemask delivering a high concentration of anesthetic agent (5% isoflurane) (Figure 11.4). Tracheal intubation assures a patent airway for delivery of anesthetic and protects the airway from aspiration pneumonia. In the authors' experience, facial, pharyngeal, and laryngeal anatomy of the pig makes endotracheal intubation more challenging than in other meat-producing species. It is often difficult to open the jaws wide enough for good laryngeal exposure, and the pig has a small narrow larynx that deviates ventrally, creating a sharp angle from the pharynx to the tracheal opening. Also laryngeal spasms occur frequently and are induced easily. Occurrence of spasms can be reduced by achieving a sufficient depth of anesthesia prior to intubation or by spraying the larynx with lidocaine.

Following induction, the pig should be placed in sternal recumbency, and the jaws should be held open with small rope. Orotracheal intubation has been shown to be significantly easier when pigs are in sternal recumbency as compared with dorsal recumbency (Theisen et al. 2009). Pigs in sternal recumbency were intubated by experienced personnel in a mean of 17 seconds as compared with 58 seconds for pigs in dorsal recumbency. The tongue is pulled forward by an assistant. A laryngoscope and blades of different lengths are needed. For adult swine, the blade length must be at least 25 cm. The blade of the laryngoscope is placed at the base of the tongue, and downward pressure is applied until an unobstructed view of the larynx is provided (Figure 11.5).

Endotracheal tubes should be available in sizes from 3 to 20 mm outside diameter and lengths of 25–50 cm. A malleable metal rod with the first 5 cm bent at a 30° angle is placed inside the endotracheal tube to act as a guide. With the laryngeal opening visualized, the endotracheal tube with the stylet extending slightly beyond the tip is placed into the laryngeal opening. The endotracheal tube is pushed over the tip of the stylet and with a twisting motion is passed through the larynx into the trachea. The tracheal diameter is surprisingly small in the pig. A 50 kg pig often requires only a 7–9 mm tube, and a 10–14 mm tube often is adequate for adult sows (Tranquilli 1986). In some cases, orotracheal intubation may not be possible. The authors have used facemasks as the sole means of administration of oxygen and gas anesthesia for various surgical procedures when tracheal intubation could not be achieved. This practice is not encouraged because of the inability to protect the airways

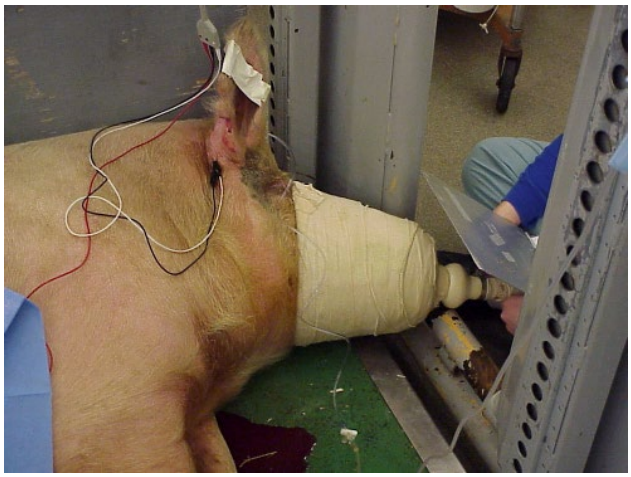


Figure 11.4 Facemask used for administration of oxygen, with or without inhalant gas anesthetic, during a Cesarean section in a sow.

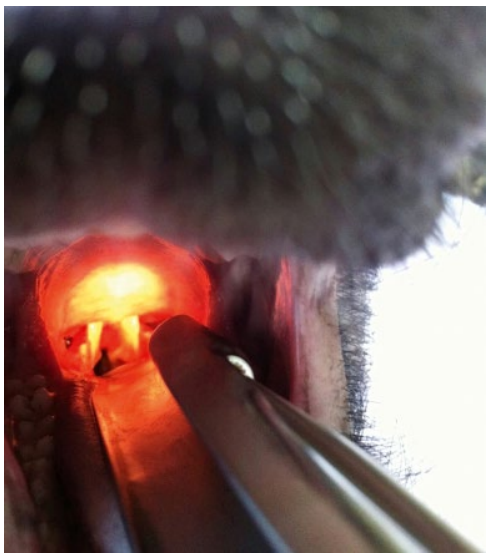


Figure 11.5 Use of a long blade laryngoscope to visualize the arytenoid cartilages during orotracheal intubation in a pig.

and the possibility of respiratory distress or obstruction if dorsal displacement of the soft palate occurs. However, facemask can be effective for short procedures (e.g. <60 minutes).

Safe maintenance of inhalation anesthesia requires knowledge of the signs associated with anesthetic depth and continual monitoring of the patient and anesthetic equipment. Routinely monitored signs should include pulse quality and rate, respiratory rate, color of mucous membranes, capillary refill time, blood pressure, and electrocardiogram. The body temperature should be evaluated regularly, and appropriate padding should be placed. The pulse can be palpated over the median auricular artery. Direct auscultation of the heart also should be done. The normal heart rate in swine ranges from 60 to 90 beats per minute and may vary greatly during inhalation anesthesia. During recovery from inhalation anesthesia, frequent and careful monitoring is necessary, because life-threatening complications can occur (Moon and Smith 1996). Recovery should be in a quiet place, and the pig should be placed in sternal recumbency as soon as possible. The endotracheal tube should be maintained until the pig is spontaneously moving its head or will not tolerate the tube. The pig should not be returned to the herd until it is fully awake.

Recently CO_2 anesthesia has been proposed as a tool to provide sufficient surgical anesthesia depth for castration in piglets aged less than a week. However this type of gas anesthesia did not inhibit all defensive movements in piglets undergoing surgical castration and should not be considered a sufficient tool for that aim (Langhoff et al. 2016).

Local anesthesia

The use of local anesthesia without additional chemical restraint is limited in swine. Pigs, even in the absence of pain, will resist physical restraint by continuing to struggle. In addition to chemical restraint, infiltration of 2% lidocaine around the surgical site will facilitate surgery involving the skin and superficial underlying tissues. Local infiltration of lidocaine is used commonly for surgical repair of umbilical and inguinal hernias and scirrhous cord removal.

Epidural regional anesthesia

Lumbosacral epidural anesthesia is the most commonly used form of regional analgesia in swine (Skarda 1996). Minimal equipment and expense are necessary to perform the procedure during epidural anesthesia. Compared with general anesthesia, the swine is in an awake state, so the risk of aspiration pneumonia is minimal. Local infiltration of lidocaine compared with epidural anesthesia has several disadvantages. Infiltration requires a larger amount of lidocaine and can retard wound healing and muscle relaxation, and analgesia is not as profound.

Lumbosacral epidural anesthesia is relatively easy to perform and greatly facilitates Cesarean section, repair of rectal, uterine or vaginal prolapses, repair of hernia, and surgery of the prepuce and penis or rear limbs (Skarda 1996). This should be avoided in patients that are in shock or toxemic because of sympathetic blockade and consequent depression of blood pressure (Skarda 1996). Also, general anesthesia may be more appropriate than regional anesthesia when the sow is very aggressive. Complications that may result from faulty techniques during lumbosacral epidural injection include cardiovascular and respiratory collapse after overdose or subarachnoid injection, meningitis associated with septic technique and tremor, and vomiting and convulsions after injection of the analgesic into the vertebral venous sinus.

The block can best be administered while the animal is standing and restrained with a hog snare, lariat, or head catch. Large hogs can be restrained by placing their heads in the head catch of a cattle chute. The site for injection for epidural anesthesia in the pig is the lumbosacral space. The conus medullaris of the cauda equina of the pig terminates in the region of the first or second sacral vertebra. The filum terminale terminates at the sixth or seventh coccygeal vertebra. Although the meninges extend beyond the lumbosacral articulation, there is only a very slight probability of entering the subarachnoid space. The lumbosacral space is on the midline and identified by drawing a line across the animal's back from tubes coxa to tuber coxa. This line will be just cranial to the point of the stifle joint (Skarda 1996). The line passes usually through the spinous process of the last lumbar vertebra. The injection site is usually 1–2 in. caudal to this transverse line. Clipping or shaving the hair, thoroughly scrubbing the site with a surgical soap, and applying a skin antiseptic prepare the site of injection. The location is infiltrated with a local anesthetic agent prior to needle insertion. A 6–8 cm 20 gauge needle is used for pigs up to 30 kg. A 10 cm 18 gauge needle is used for pigs between 35 and 90 kg, and a 12–16 cm gauge needle for pigs over 90 kg. The needle is inserted with the level directed cranially and at an angle of 10° caudal to perpendicular between the last lumbar and first sacral vertebrae. The needle penetrates the skin, backfat, muscle, and then the fibrous interarticular spinous ligament. The needle passes through a definite area of resistance as it encounters the ligament and a slight pop is felt as the needle passes into the epidural space and drops to the floor of the spinal canal. The lumbosacral space is large in the pig (1.5 × 2.5 cm) and allows for a relatively large margin of error (Skarda 1996). Aspiration should be attempted before injection of the anesthetic to ensure

that the subarachnoid space or a blood vessel has not been entered. Little resistance to injection will be encountered if the needle is located properly in the epidural space.

The anesthetic agent most commonly used is 2% lidocaine. The dose is calculated by either weight or length of the pig (Skarda 1996). Generally, a dose of 1 mL/9 kg of BW is adequate. Analgesia should be present within 10 minutes and last approximately 2 hours. A total dose of 20 mL must not be exceeded regardless of the weight. Four ml per 100 kg and 6 mL per 200 kg of BW are sufficient for standing castrations (Skarda 1996). Ten ml per 100 kg, 15 mL per 200 kg, and 20 mL per 300 kg have given satisfactory results for Cesarean section (Skarda 1996). If the pig is restrained in lateral recumbency, it is important that the head not be placed in extension. In swine with the head extended, the soft palate can occlude the airway, and the patient can suffocate (Benson 1986).

In 28–35 kg pigs, epidural injections of xylazine (2 mg/kg diluted in 5 mL of 0.9% NaCl solutions) at the lumbosacral intervertebral space induce immobilization, mild sedation, and regional anesthesia that extends from the anus to the umbilicus within 10 minutes and lasts at least 2 hours (Ko et al. 1992). The injection of a xylazine (1 mg/kg 10% solution) and lidocaine (10 mL, 2% solution) combination into the lumbosacral epidural space has produced excellent anesthesia for Cesarean in large sows (Ko et al. 1993). The forequarters in these sows were immobilized by IV injection of 0.003 mL of Telazol, mixture containing 50 mg of Telazol per mL, 50 mg of ketamine per mL, and 50 mg of xylazine per mL per kg of BW (Ko et al. 1993). In preparing the Telazol, ketamine, and xylazine combination, 2.5 mL of 10% ketamine (250 mg) and 2.5 mL of 10% xylazine (250 mg xylazine) were used as the diluent instead of sterile water. A mean of 3 mL of this combination was given per sow; the sows were quiet and immobilized for an average of 105 minutes (Ko et al. 1993). The sows were able to walk 12 hours after surgery, and the piglets were without signs of sedation or tranquilization. Intravenous tolazoline (2.2 mg/kg) partially reversed the Telazol-, ketamine-, and xylazine-induced sedation after surgery but did not antagonize the xylazine–lidocaine epidural effect (Ko et al. 1993). Epidural analgesia used in combination with general anesthesia allows a light plane of anesthesia with good muscle relaxation distal to the midthoracic region. Medetomidine (0.5 mg/kg diluted in 5 mL of 0.9% NaCl solution) injected epidurally in the lumbosacral space of swine produced sedation and decubitus but minimal analgesia caudal to the umbilicus. The onset of these effects occurred in 10 minutes, and they lasted for less than 30 minutes. Intravenous administration of atipamezole (0.2 mg/kg of BW) reversed epidurally administered detomidine-induced sedation and immobilization (Ko et al. 1992). Atipamezole had no effect on epidurally administered xylazine-induced sedation and immobilization (Ko et al. 1992).

Pain management

Nonsteroidal anti-inflammatory drugs

A recent study examined the effect of age on behavioral and physiological responses of piglets to tail docking and ear notching (Torrey et al. 2009). The authors found no age effect (1 day old vs. 3 days old) for suckling behavior, but piglets that had been processed had decreased passive transfer of immunoglobulin than sham-treated and control piglets. In a study of castration in 4- to 6-day-old piglets, local anesthesia was not found to be justified based on serum cortisol response (Zankl et al. 2007). A study examining the effect of nonsteroidal anti-inflammatory drugs found a beneficial effect in the use of meloxicam (Zöls et al. 2006). In that study, piglets were castrated between 4 and 6 days old. The piglets that received meloxicam before surgery had a markedly obtunded cortisol response similar to those that were control piglets. Another study examined the cortisol response in piglets (<7 days old) castrated with or without anesthesia and with or without the nonsteroidal anti-inflammatory drug meloxicam (Schulz et al. 2007). In that study, anesthesia was not found to have an effect on the cortisol response to castration, but piglets that received meloxicam did have significantly less cortisol response to being castrated. Thus, postoperative analgesia may be more beneficial than the use of transient anesthetics.

Administration of meloxicam to sows with the desire to use transmammary route of drug administration to the piglet via the milk recently has been demonstrated (Bates et al. 2014). Piglets had decreased blood cortisol and PGE2 concentration after piglet processing at 5 days of age.

Opioids

One study evaluated postoperative pain and compared transdermal fentanyl patches (25 and 50 µg/h) with buprenorphine (0.1 mg/kg, as needed) (Harvey-Clark et al. 2000). Assessment of analgesia was variable, but 50 µg/h transdermal patches placed on 26 kg pigs achieved blood concentrations similar to that considered to be in the analgesic range for humans.

Addition of butorphanol to meloxicam as a postoperative pain management for piglets undergoing tail docking and castration was associated with a lower plasma cortisol level than with meloxicam alone (Courboulay et al. 2014). Another study evaluated the use of isoflurane anesthetic gas for piglet castration at 14 days old

(Walker et al. 2004). In that study, piglets castrated under isoflurane gas had significantly fewer reactions to the surgical procedure, but stress hormone concentrations after castration were similar among groups.

Tramadol

Tramadol has recently been reported as a beneficial drug for combination anesthesia in pigs (Table 11.2). It is an analogue of codeine and has activity with opioid receptors. It acts as an agonist on μ-opioid receptors as well as stimulates the release of serotonin. As such, it is useful in mitigating moderate to severe pain. In combination with xylazine and ketamine in young piglets, tramadol increased the pain threshold of anesthetized piglets without having any adverse effects on duration of anesthesia or physiological parameters (Ajadi et al. 2009).

Mitigation of distress and/or pain will no doubt be a continued focal point for humane care of livestock. Research is required to provide objective assessment, maintain perspective without anthropomorphic bias, and establishment of meaningful guidelines. Nonsurgical methods of castration, such as immunization of boars against GnRH, may eliminate the necessity for surgical castration and thus eliminate many of these concerns (Thun et al. 2006).

Genitourinary surgery

Castration

Castration of male pigs is routinely performed with the intention to improve performance, feed conversion, and carcass traits (Kiley 1976). Also, management of castrated pigs through to finishing may be easier than for intact male pigs. With the onset of puberty, boar meat becomes tainted with an unpleasant odor and taste. However, recommendations for pig age at the time of castration are variable. Stress of castration was evaluated for pigs castrated at 1, 2, 4, 8, 16, and 24 days of age (White et al. 1995). This study indicated that pigs castrated after administration of lidocaine anesthetic subcutaneously and around the spermatic cords had lower heart rate and less vocalization than pigs castrated without local anesthesia. This effect was greatest for pigs castrated after 8 days old. Castration-associated behavioral changes were evaluated for pigs castrated at 1, 5, 10, 15, and 20 days old (McGlone et al. 1993). Castration caused reduced suckling, reduced standing, and increased lying time compared with intact male pigs at all ages. Pigs castrated at 14 days old were heavier at weaning and had a higher rate of gain compared with pigs castrated at 1 day old. Administration of aspirin or butorphanol failed to improve castration-associated reduction in feeding time and weight gain. Administration of lidocaine anesthesia prior to castration prevented castration-induced nursing behavior suppression (McGlone and Hellman 1988) in 2-week-old pigs. This effect was not observed for pigs castrated at 7 weeks old. Pigs castrated at 2 weeks old had less pronounced behavioral changes than pigs castrated at 7 weeks old. We recommend that baby pigs be castrated at 2 weeks old to minimize the stress of castration and maximize performance to weaning.

Castration of 14-day-old pigs is done by suspending the pig by the hind limbs while laying the pig across a smooth rail. The surgical site is wiped clean with alcohol or 2% tincture of iodine. If used, lidocaine anesthetic is injected subcutaneously (0.5 mL per site), overlying each testicle, and over each spermatic cord (0.5 mL per site) in the inguinal canal. A 1 cm long incision is made over each testicle, and the testicles are pulled from the scrotum. Hemorrhage is minimal at this age. Transfixation ligation of the spermatic cord is recommended for older pigs (see Castration of Older Pigs). Topical antiseptic ointment or spray may be applied at this time. Systemic antibiotics are usually not required, except when castrating older pigs. Castrated baby pigs are placed under a heat lamp in the farrowing crate for convalescence.

Castration of older pigs

Pigs are routinely castrated prior to or at 2 weeks old. However, veterinarians may be asked to castrate older pigs that are intended for show or mature boars that are no longer to be used for breeding. Castration of older pigs is best performed with the pig sedated or under general anesthesia (see ANESTHESIA section), but manual restraint and local anesthesia may be adequate for pigs weighing 50 kg or less (Becker 1986). The boar is restrained in lateral recumbency, and the surgical site aseptically prepared. A 4–6 cm incision is made overlying the testicle at the ventral aspect of the scrotum. The testicle should be removed with the vaginal tunic intact. Inguinal fat and soft tissue are stripped from the spermatic cord and evaluated for the presence of an inguinal hernia. The vaginal tunic and the spermatic cord are twisted until the cord is tightly compressed to the level of the external inguinal ring (Figure 11.6). Two transfixation ligatures (No. 1 chromic gut) are placed securing the vaginal tunic and spermatic cord to the medial aspect of the external inguinal ring. These sutures are intended to close the vaginal tunic and prevent the development of postoperative inguinal hernia. An emasculator may be used, but this method for orchiectomy does not result in closure of the vaginal tunic nor prevent inguinal hernia. Closure of the surgical wound should only be performed if asepsis has been maintained. Subcutaneous tissues may be sutured with No. 0 chromic gut in simple continuous pattern to reduce dead space and minimize

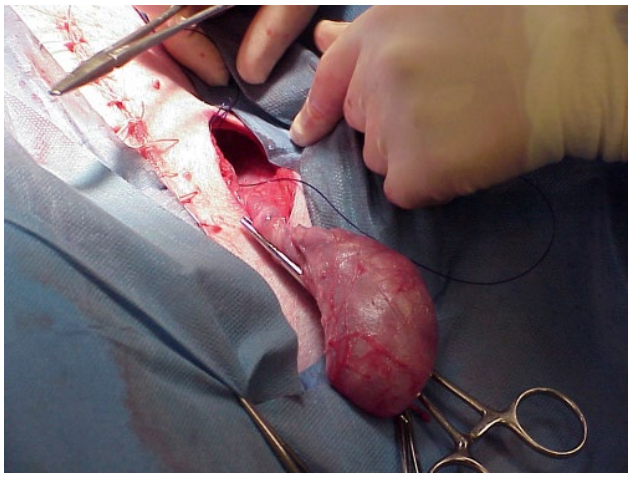


Figure 11.6 Inguinal castration using the twist technique to assist in closing the inguinal ring.

postoperative swelling. Skin sutures are placed in a Mayo (Ford) interlocking pattern. We prefer to administer antibiotics for 3 days, beginning the day of surgery, to reduce the incidence of postoperative infection. Also, the barrow should be kept in a clean, dry stall during this period.

Complications of castration

The most common complications following castration of pigs are hemorrhage, abscess, scirrhous cord, inguinal hernia, and seroma or hematoma formation. Fatal hemorrhagic shock has been reported after castration of 7-week-old pigs by a layperson (Libke 1967). The testicles had been pulled through a 10 cm incision and cut using a knife. Fatal hemorrhage occurred into the pelvic canal and abdomen; thus, the cause of death was not recognized until necropsy. This report emphasizes the need for routine necropsy examination of all deaths for which the cause is not apparent. Meat inspection of 131 pigs with post castration abscesses revealed that *Actinomyces pyogenes, alpha-hemolytic Streptococci, Streptococcus viridans, Staphylococcus aureus*, and *Pasteurella multocida* were the most common bacteria isolated (Százados 1985). Approximately 65% of the abscesses were monomicrobial, and 35% were polymicrobial infections. Evidence for bacteremia and septicemia was found in 28 and 11%, respectively. Of the 131 pigs inspected, 11% were judged to be unfit for human consumption. Bilateral hydronephrosis also has been reported as a complication of castration in a Hampshire pig castrated at 8 weeks old (McGavin and Schoneweis 1972). A ventral midline incision was used to remove both testicles, and tincture of iodine applied after castration. Infection of the soft tissues occurred, and the ensuing infection resulted in progressive occlusion of urethra at the level of the sigmoid flexure. Chronic resistance to urine outflow caused hydronephrosis, and the pig died 4 weeks after castration. This case illustrates the importance of adequate ventral drainage after castration.

Unilateral castration

Indications for removal of only one testicle include testicular trauma, hematoma, seroma, and orchitis or periorchitis (Becker 1986). The damaged testicle may cause enough swelling, heat, and pressure to reduce fertility. The boar is placed under general anesthesia, a 6 cm incision is made over the testicle starting at the most ventral aspect of the scrotum, and the testicle is removed by transfixation ligation and excision. The wound may be left open or closed. Strict asepsis and clean housing are required for closure of the wound to prevent abscess formation. We prefer to leave the wound open for second intention healing. Antiseptic ointment is placed in the defect, antibiotics are administered for 5–7 days, and daily hydrotherapy is used to minimize postoperative swelling. Affected boars may return to productive service 30–60 days after surgery.

Testicular abnormality (cryptorchidism, testicular atrophy, ectopic testicle)

Veterinarians may be presented with barrows demonstrating "boar-like" traits for removal of retained testicular tissues. The testicles of swine descend in the last 30 days of gestation and should be palpable at birth (Van Straaten et al. 1979). True cryptorchidism (testicle not descended at birth) is a common congenital defect in swine. A homozygous recessive trait involving two gene loci has been postulated based on a breeding trial of cryptorchid Duroc swine (Rothschild et al. 1988). Interestingly, the presence of cryptorchid piglets *in utero* has been associated with litter size and mortality (Dolf et al. 2008). In that study, litter size was found to increase with increasing number of cryptorchid piglets, and the number of stillborn piglets was greater in litters having a cryptorchid pig present. A progeny study of Lacombe and Yorkshire true cryptorchid boars resulted in 10.9 and 31.4 % of male progeny being cryptorchid (Fredeen and Newman 1968). Of boars with "late-onset" cryptorchidism (normal at birth but having only one testicle at 42 days old), 3.8% of male progeny were cryptorchid. Cryptorchid testicles are usually intra-abdominal and are usually found midway between the ipsilateral kidney and the inguinal ring (Thornton 1972). However, the affected testicle may be located within the inguinal canal and not readily palpable from either the inguinal region or the peritoneal cavity (Lachmayr 1966). Previous removal of the descended testis makes surgical removal of the retained testis more difficult because the incision is best made over the affected inguinal ring. Often,

determination of which testicle has been removed is difficult. Manual restraint and local anesthesia may be adequate for pigs less than 50 kg, but we prefer to perform cryptorchid surgery with the pig under general anesthesia. A 6 cm incision is made over the appropriate inguinal ring. Laparotomy may be performed by making a 4 cm incision 1–2 cm medial to the inguinal canal (parainguinal incision), or starting the incision at the cranial commissure of the external inguinal ring may enlarge the inguinal ring. The fingers of one hand are used to perform an exploration of the abdominal cavity starting at the pelvic brim and searching along the dorsal and lateral abdominal wall until the kidneys are encountered. For show pigs, we prefer to perform laparoscopic exploration and removal of abdominal testes because better cosmesis, fewer incisional complications, and more rapid incisional healing are achieved.

True cryptorchidism should be differentiated from testicular atrophy or degeneration ("late-onset" cryptorchidism) and ectopic testicular tissue. Pigs affected by testicular atrophy are reported to have palpably normal testicles at birth and weaning, but having only one testicle present at 42 days old (Fredeen and Newman 1968). Of 122 cryptorchids studied, 21 had "late-onset" cryptorchidism. At slaughter, only one testicle can be found, and, occasionally, a small mass of lymphoid tissue or epididymis is identified. Ectopic testicular tissue has been observed in numerous pigs at the time of slaughter (Todd et al. 1968). These tissues occur as smooth pink or tan nodules on the surface of the liver, spleen, mesentery, and other abdominal viscera. Initially, these masses may be interpreted as metastatic neoplasia, but histology reveals the presence of convoluted seminiferous tubules and interstitial cells. No evidence for neoplasia is seen. Ectopic testicular tissues may be found in castrated or intact male pigs.

Prolapsed penis

Penile and preputial prolapse has been seen after administration of neuroleptic drugs, but also may occur as a result of trauma to the penis or congenital defect. While prolapsed, the penis is at great risk of further injury. The penis and prepuce must be returned to their normal position as soon as possible after prolapse. Treatment of penile prolapse usually required that the boar be placed under general anesthesia. The penis is thoroughly cleaned with cold water, and a topical antiseptic ointment applied to the surface of the penis. If a penile wound is present, debridement may be done. Penile wounds typically are not sutured closed unless they have occurred recently (within 2–4 hours) because of the likelihood of formation of an abscess. The penis and prepuce are gently massaged until reduction into the sheath is completed. Use of hygroscopic agents (e.g. anhydrous glycerine) may help reduce the swelling by resolving edema. After the penis and prepuce have been repositioned, a purse-string suture may be used to prevent reoccurrence of the prolapse. The purse string should be removed in 5–7 days. If wounds or abrasions are present, daily preputial lavage or administration of systemic antibiotics and anti-inflammatory drugs is indicated. If wounds are not present, sexual rest should be enforced for at least 14 days. If wounds requiring treatment are present, sexual rest should be enforced for 30–60 days (depending on the severity of the wound). Reevaluation of the penile injury is advisable prior to use for mating.

Preputial diverticulum

Abnormalities of the preputial diverticulum may cause reproductive unsoundness. Preputial diverticulitis, diverticular ulcers, diverticular stones, urine retention, and penile deviation into the diverticulum may be found (Dutton et al. 1997; Tyler et al. 2000; Wieringa and Mouwen 1983). Preputial diverticulectomy may restore breeding soundness to affected boars. The boar is placed under general anesthesia and prepared for surgery. Any of three procedures for diverticulectomy may be performed: (1) Preputial diverticulectomy via the preputial orifice is done by passing forceps through the preputial orifice into one lobe of the bilobate diverticulum, gently everting the lobe out through the orifice, and repeating this procedure for the remaining lobe. After both lobes of the preputial diverticulum are everted, the diverticulum is excised. Suturing is not required for young boars, but the opening to the diverticulum may be sutured closed in adults. (2) A 6 cm incision is made overlying the lateral aspect of one lobe of the preputial diverticulum. The diverticulum is everted through the preputial orifice, excised, and sutured closed. (3) A 6 cm incision is made as above, but the diverticulum is dissected free from the surrounding soft tissues, excised, and sutured closed. For methods (2) and (3) above, extreme care must be taken not to perforate the diverticulum prior to removal because contamination will result in incisional infection. Flushing of the preputial diverticulum with antiseptic solutions before surgery is recommended to reduce this possibility. Also, filling the diverticulum with antiseptic solution or gauze pads before surgery makes identification of the diverticulum easier at the time of surgery.

Preputial prolapse

Prolapse of the prepuce may occur with penile prolapse or may result from preputial injury and swelling. If wounds to the prepuce are not present, the prepuce may be repositioned within the sheath, as described for penile prolapse, and a purse-string suture used to maintain

the reduction (Schoneweis 1971). Careful evaluation of the preputial swelling should be done to ensure that urination is possible. Preputial edema may be reduced by application of hygroscopic agents (anhydrous glycerine, saturated magnesium sulfate solution). A preputial retaining tube, constructed from rubber or polyurethane tubing, may be placed into the preputial space to prevent prolapse but allow exit of urine. Stay sutures are placed through the tubing and attached to the skin at the preputial orifice. Four sutures are placed 90° from each other suture to maintain the proper position of the tube. Alternatively, a 1.25 cm diameter Penrose drain may be sutured to the tip of the penis (No. 2-0 chromic gut suture) to ensure urine outflow. Often, the prolapsed prepuce has been traumatized, and surgical removal of the affected tissues is indicated. Preputial amputation may be performed, but the opening to the urethral diverticulum must be maintained. Alternatively, the preputial diverticulum may be removed at the time of surgery. The boar is placed under general anesthesia, the prepuce is pulled cranially until normal preputial epithelium is exposed, and stay sutures or crossed pins (7.6 cm 18 gauge needles) are placed through the exposed prepuce to prevent premature retraction into the sheath. The damaged tissues are amputated, and the two layers of the prepuce are sutured closed using an interrupted suture pattern (No. 2-0 chromic gut, PDS, or polyglycolic acid suture). After anastomosis, antiseptic ointment is placed on the prepuce, and it is replaced into the sheath. A purse-string suture is placed at the preputial orifice for 7–10 days, and sexual rest is enforced for 30–60 days. Systemic antibiotics should be administered perioperatively.

Vasectomy or epididymectomy

Vasectomy or epididymectomy is done to produce teaser boars used for heat detection in sows for artificial insemination or breeding to valuable boars or to promote onset of cyclicity in confined gilts (Becker 1986; Godke et al. 1979). For vasectomy, the boar is placed in dorsal recumbency under general anesthesia, and a 4 cm incision is made over each spermatic cord approximately 6 cm cranial to the ventral aspect of the scrotum. Each spermatic cord is elevated and incised, and the vas deferens is isolated. The vas deferens lies next to the spermatic artery and is firm and pale, and an arterial pulse is not present. A 3–4 cm segment of the vas deferens is excised, and each end is ligated. The incision through the tunic is sutured with No. 2-0 PDS or polyglycolic acid, and the skin is sutured with No. 0 polymerized caprolactam in a Mayo (Ford) interlocking suture pattern. An alternative technique for vasectomizing boars enables the surgery to be done with the boar in lateral recumbency (Althouse and Evans 1997a).

Epididymectomy may be done more quickly, more easily, and with similar results as vasectomy in boars (Althouse and Evans 1997b; Arkins et al. 1989). It is done by making a 2 cm incision in the scrotum overlying the tail of the epididymis. The tail and 1 cm of the body of the epididymis are isolated. Ligatures are placed between the testicle and the tail of the epididymis and around the exposed portion of the body of the epididymis. The epididymis is excised between these two ligatures. The skin is closed with No. 0 polymerized caprolactam in an interrupted pattern.

Persistent frenulum

The epithelial attachment of the penis and prepuce atrophies and these tissues separate between 4 and 6 months old in boars. Sexual maturity is achieved by 7–8 months old. Persistence of the frenulum attachment between the penis and prepuce beyond sexual maturity causes failure of breeding soundness and has been observed in boars (Roberts 1986). Surgical removal of the persistent frenulum is performed with the boar under general anesthesia or during a hand mating exercise. Resection of the tissue may be performed with scissors. Ligation is not required in most cases, and minimal bleeding is observed after excision. Sexual rest should be enforced from 7 to 10 days after surgery.

Vaginal prolapse

Vaginal prolapse can occur as a prepartum event but is uncommonly reported in pigs (Peek 1985). The cause of vaginal prolapse is unknown, but straining to urinate or defecate may be involved. Sows with lateral deviation of the bladder and difficulty urinating or with inflammation associated with cystitis and urethritis may develop vaginal prolapse because of straining. When the cause can be found, treatment should be aimed at resolving the initial lesion. The vagina is cleaned with cold water, hygroscopic agents (anhydrous glycerine, sugar, etc.) are applied, a towel is wrapped around the prolapsed portion, and constant gentle pressure is used to reduce the edema and swelling. The prolapse can usually be reduced in 15–20 minutes. The vagina should be cleansed, and topical antibiotic or antiseptic ointments are used to reduce the secondary bacterial vaginitis that invariably occurs. Administration of anti-inflammatory drugs may reduce straining and shorten convalescence. The bladder should be evaluated to ensure that it is in a normal position (see urinary bladder displacement). Often, partial rectal prolapse accompanies vaginal prolapse. The rectal prolapse should be treated appropriately (see rectal prolapse). A Buhner suture is placed around the vagina to prevent reoccurrence of the prolapse. The sow should be closely monitored, and the Buhner suture removed at the

first indication of farrowing. If excessive swelling of the soft tissues in the pelvic canal has occurred, a Cesarean section is indicated and should be performed early in the process of farrowing.

Bladder displacement (lateroflexed)

Displacement of the bladder occurs in multiparous sows in the latter stages of gestation (Greenwood 1989; Scott 1977). The bladder is displaced laterally and, occasionally, may become displaced caudally. When this occurs, urination is difficult. The displaced bladder may give the appearance of a vaginal prolapse when the sow is lying down. Affected sows may be seen straining because of the difficulty urinating, and this may lead to true vaginal prolapse. Decompression of the urinary bladder by cystocentesis or catheterization may allow permanent replacement of the bladder. When displacement recurs, an indwelling urinary catheter may be used to allow urination until after parturition. Ascending bacterial cystitis is a complication of the indwelling urinary catheter.

Urethral obstruction

Urethral obstruction has been observed in miniature pigs most commonly. Clinical signs include signs of abdominal pain, tail flagging, straining to urinate, blood in urine, decreased activity or restlessness, decreased appetite, and teeth grinding. Potential causes of urethral obstruction to be considered include urolithiasis, urethral polyps, and urethral stricture or trauma. Retrograde catheterization of the pelvic urethra and bladder is difficult because of the urethral recess and mucosal flap, which prevent passage of the catheter. Tube cystostomy with positive contrast urethrography was reported to be useful for diagnosis of obstruction of the distal urethra (Palmer et al. 1998). Urethral polyps were reported as a cause of urethral outflow obstruction in Vietnamese potbellied pigs (Helman et al. 1996). Surgical management of urethral outflow obstruction includes urethrotomy, cystotomy with normograde flushing, tube cystostomy, perineal urethrostomy, prepubic urethrostomy, and prepubic cystostomy. Prepubic cystostomy was successful in two Vietnamese pigs with urethral injury from castration or urethral stricture (Leon et al. 1997). In these two pigs, the pelvic urethra was exteriorized cranial to the brim of the pelvis with the urethra spatulated and the mucosa sutured to the skin. Both pigs maintained urinary continence. Urethroscopy with laser lithotripsy was used successfully in two potbellied pigs to alleviate urethral obstruction caused by uroliths (Halland et al. 2002).

Tube cystostomy procedure

Patients are placed in dorsal recumbency under general anesthesia. An 8–10 cm paramedian approach is made 4 cm lateral to the prepuce and extending from immediately caudal to the preputial orifice to 4 cm cranial to the brim of the pelvis. The bladder is exteriorized, and two stay sutures are placed near the apex. A cystotomy is performed near the apex, and suction and lavage is performed with saline used to evacuate urine and debris from the bladder. A gallstone scoop may be used to facilitate removal of stones if present. Particular attention is paid to suctioning the region of the trigone and urethral origin to remove debris, which may have migrated into the urethra during positioning for surgery. Normograde flushing is attempted to clear the urethra of debris. The cystotomy incision is then closed in two inverting layers using 0 or 2-0 absorbable monofilament suture materials. A purse-string suture is then pre-placed in the ventrolateral aspect of the bladder, near the apex on the same side as the celiotomy incision. A Foley catheter of size appropriate to the animal (range 12–18 F) is placed through a stab incision lateral to the paramedian body wall incision to enter the abdominal cavity. The end of the Foley catheter is then placed through a stab incision in the bladder within the purse-string suture. The purse-string suture is then tied tightly, and the catheter balloon inflated with saline. The bladder is pulled close to the body wall using tension on the Foley catheter, which was secured to the skin with a trapping suture pattern. The celiotomy incision is closed routinely using various suture materials, depending on surgeon preference.

Oophorectomy

Removal of the ovaries is rarely indicated in swine. However, oophorectomy may be requested to facilitate research or for pet pigs. For pet pigs, removal of the ovaries is easier, is faster, and has less risk of fatal hemorrhage than ovariohysterectomy (OVX). The blood vessels of the broad ligaments of the uterus are extensive and require ligation when OVX is chosen. Both ovaries may be removed from a paralumbar (flank), ventrolateral, paramedian, or ventral midline incision. We prefer to perform ovariectomy via a flank or ventral midline incision. Access to the abdomen is excellent with these incisions, and, in our experience, the risk of postoperative complications (incisional infection, hernia) is less. In either case, we prefer to use general anesthesia while performing the surgery. For ventral midline approach, the incision may be started immediately caudal to the umbilicus and extended caudally. For a paralumbar approach, the incision is started ventral to the transverse processes of the lumbar vertebrae, midway between the tuber coxae and the last rib. Each ovary is elevated through the incision, two hemostatic forceps are placed on the ovarian pedicle, two ligatures (No. 2-0 polyglactin 910) are placed proximal to the

first hemostat, and the pedicle is cut between the two hemostats and the ovary removed. Each ovarian artery must be observed for hemorrhage prior to closure. Closure of the ventral midline is done using No. 1 PDS or polyglactin 910 in an interrupted suture pattern. Chromic gut suture should not be used in the linea alba because of the increased risk of postoperative incisional hernia. The skin is closed with No. 2 polymerized caprolactam in a Mayo (Ford) interlocking suture pattern. Paralumbar incisions are closed in three layers (transversus abdominis m + peritoneum, internal + external abdominal oblique, skin). Ovariectomy, alone, may be performed in pet pigs that have not begun normal estrus cycles. Uterine atrophy is expected to occur after ovariectomy. We recommend OVX in sexually mature pigs because of the potential risk for pyometra in a uterus where the cervix has been open.

Uterus

Hysterectomy

Hysterectomy may be performed as part of Cesarean section and is discussed below. Elective hysterectomy is rarely done in swine. Hysterectomy has been reported for pet pigs having uterine neoplasia (Preissel et al. 2009). The authors have also performed hysterectomies on pet pigs because of chronic endometriosis. However, hysterectomy may be requested for research purposes or for pet pigs. When hysterectomy is performed for pet pigs, the ovaries also are removed. General anesthesia should be used during hysterectomy. The uterus may be removed via a flank, ventrolateral, paramedian, or ventral midline incision. We prefer to perform hysterectomy via either flank or ventral midline incision. The uterus is elevated through the incision, the ovaries are removed as described above, the broad ligament of the uterus is ligated using two to four overlapping simple interrupted sutures for mass ligation of the blood vessels, and transfixation ligatures are placed in the uterine body immediately cranial to the internal of the cervix. The uterus and ovaries are removed, and the incision is closed as described above. All sutured pedicles should be checked for adequate hemostasis prior to closure. Abnormalities have been found on 30.4% of 298 potbellied pigs undergoing ovariohysterectomies (unpublished data, Cypher, University of Tennessee 2017). Hemorrhage is the most common complication associated with this procedure. Recently, the authors have used minimally invasive surgery by laparoscopic OVX, and using a vessel-sealing device allows to minimize the risk the ligature slippage after the surgery (Figure 11.7).

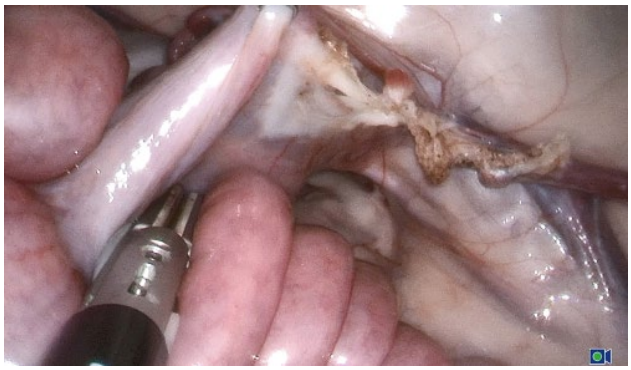


Figure 11.7 Laparoscopic image during a minimally invasive ovariohysterectomy in a pig.

Cesarean section: indication and decision analysis

Cesarean section is required when transcervical extraction of pigs from the uterus is not possible (complicated dystocia) and to obtain gnotobiotic or specific-pathogen-free (SPF) pigs. Cesarean section for gnotobiotic pigs is usually performed with the sow under general anesthesia and is discussed below. The most common reported causes of dystocia in swine are uterine inertia, small pelvic size, inadequate dilation of the birth canal, fetal-to-maternal disproportion, fetal malpresentation, and abnormalities of the birth canal (Titze 1977). Cesarean section for dystocia is usually chosen as a "last-resort" procedure for fetal extraction because of economic pressures. Therefore, the mortality rate among sows and gilts having Cesarean section is expected to be higher than for other species. This is not surprising because affected swine suffer extreme physical exhaustion, stress, and shock by the time the decision for Cesarean section is made. Interestingly, multiparous sows had a higher frequency of collapse (25.8%) prior to Cesarean section compared with primiparous pigs (16.4%) (Dimigen 1972). Owners and veterinarians may become reluctant to perform Cesarean section because of expense, previous experiences with fatalities, and the high rate of dead pigs delivered. It is our opinion that unnecessary delays in the decision for surgery are the principal cause of sow and baby pig mortality associated with Cesarean section. When the veterinarian is presented with a sow in dystocia, the decision as to whether the owner is willing to incur the costs of Cesarean section should be ascertained as early as possible during the initial examination. Other factors influencing the decision for Cesarean section include the cause of dystocia, how long the sow has been in labor, how long the owner has tried to manually extract the pigs, and how swollen or traumatized the sow's pelvic canal has become. Many owners are adept at extracting pigs, and their failure to successfully remove pigs may justify immediate Cesarean if the cause of dystocia is not

apparent. The most common indications for Cesarean section in pigs are undersized pelvic canal, inadequate cervical and soft tissue dilation, prolonged labor (including uterine inertia), fetal-to-maternal disproportion, and trauma to the birth canal (Titze 1977). In our experience, Cesarean section performed at the earliest indication has a high success rate for survival of the sow and a higher rate of live pigs obtained (Table 11.3).

Swine that are physically exhausted, stressed, or in shock must be stabilized prior to Cesarean section. Thirteen percent of sows in labor for less than 18 hours died or were salvaged compared with 30% of sows in labor for more than 18 hours (Dimigen 1972). Among sows necropsied after sudden death, retained fetuses and toxemia were found in approximately 10% (Sanford et al. 1994). Stabilization of the sow often is simple and readily achieved. We routinely place a 16 or 18 gauge 2 in. intravenous catheter in an ear vein. This catheter is sutured or glued in place, and intravenous fluids (0.9% saline or lactated Ringer's solution) are administered rapidly (initially 20–40 mL/kg of BW/h and then 4 mL/kg/h once stabilized) and continued for the duration of the surgery. The authors prefer to add dextrose (1.25% final solution) and calcium (1 mL/kg) to intravenous fluids *after* the patient has been stabilized. Further, the shock status of the sow may be improved by administration of dexamethasone (0.5–1.0 mg/kg IV) or flunixin meglumine (1 mg/kg IV). Because extensive manipulation of the intrauterine environment prior to Cesarean section increases the risk for postoperative septic peritonitis, we prefer to administer preoperative antibiotics (procaine penicillin G, 10,000 U/kg, IM; or oxytetracycline, 5–10 mg/kg, IM). In severely compromised sows, sedation (see anesthesia section) and local or regional anesthesia may be adequate for surgery. Intravenous fluids should be administered continuously. Epidural anesthesia (lumbosacral level) also may be useful. Overall, we prefer to perform general anesthesia rather than attempt Cesarean using physical restraint and epidural anesthesia. In our experience, this causes the least stress on the patient, surgeon, and assistants. The respiratory rate and heart rate should be monitored, and supportive therapy adjusted appropriately.

Surgical approach for cesarean section

Multiple surgical approaches have been described for Cesarean section. Selection of the surgical approach depends upon the preference of the surgeon, the condition of the patient, and means of restraint and anesthesia used for surgery. The most common approaches are paralumbar fossa, ventrolateral (horizontal low flank), ventral midline, paramedian, or paramammary (Figure 11.8) (Mather 1966; Turner and McIlwraith 1989). With a ventral or paramedian approach, movement by the sow must be prevented because of the risk for contamination of the incision. Also, the mammary veins must be carefully avoided or ligated to prevent excessive loss of blood during the procedure. In our experience, ventral and paramedian incisions have the highest risk for development of postoperative incisional infection (contamination on the floor and trauma from pigs searching for nipples). The authors advocate paramammary incisions for Cesarean section in sows. The paramammary incision is made parallel and ventral to the flank and lateral to the mammary chain (Figure 11.9) (Mather 1966). The sow is placed in lateral recumbency with the uppermost hind limb tied in adduction and extension. The incision is

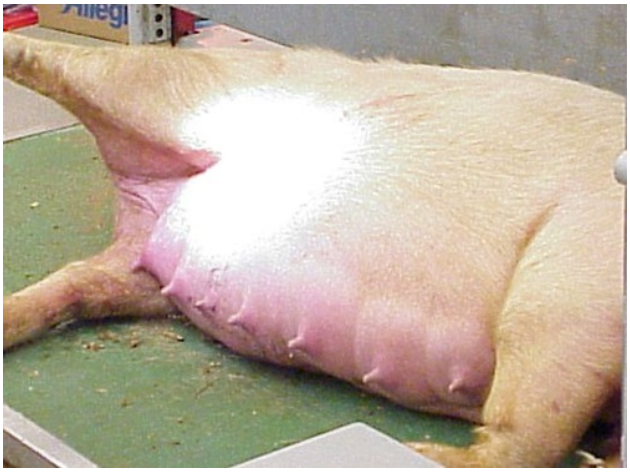


Figure 11.8 Sow positioned in left lateral recumbency with the right rear limb elevated and extended. This position is used to facilitate paramammary laparotomy.

Table 11.3 Outcome of Cesarean section in sows with respect to duration of labor.

Duration of labor	Number of sows	% sows having >50% live pigs	% sows having <50% live pigs	% sows having all dead pigs	% sows died or culled
<18 h	125	66.7	7.9	25.4	13.4
18–48 h	81	19.7	13.5	66.7	32.1
>48 h	21	0	4.7	95.3	28.5
Overall	227	43.7	9.7	46.6	21.5

Source: Adapted from Dimigen (1972).

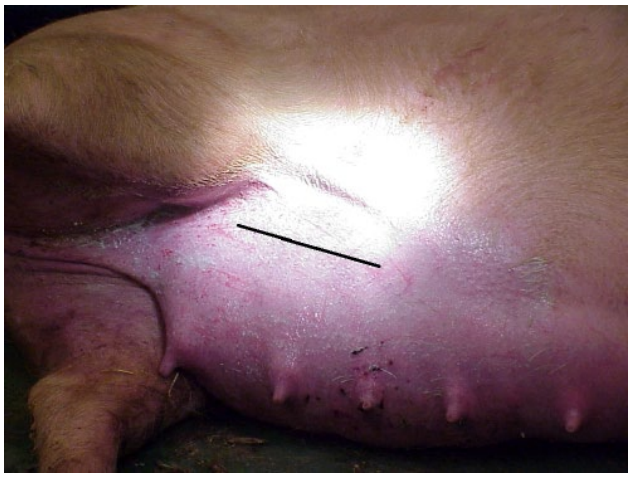


Figure 11.9 Location of the incision (black line) made for paramammary approach to the uterus.

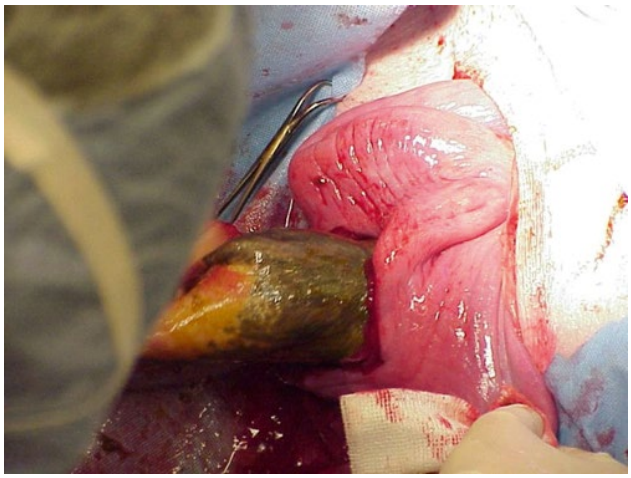


Figure 11.10 Exteriorization of the uterus during hysterotomy for fetal extraction during C-section via a paramammary laparotomy.

started approximately 10 cm cranial to the inguinal region and extended cranially for 15 cm. For paralumbar fossa incision, the sow is placed in lateral recumbency, and the incision is started cranial and ventral to the tuber coxae. The incision is extended ventrally to a point approximately 5 cm dorsal to the cranial skinfold of the flank. Paramammary and paralumbar incisions are relatively easy to perform, have little blood loss during surgery, and are less likely to become infected after surgery. Fewer fat deposits are encountered with paramammary incisions, and the meat portion of the flank is left undisturbed. The uterus can be easily exteriorized because of its proximity to the incision (Figure 11.10). After exteriorizing the closest uterine horn, a 6–8 cm long incision is made parallel with the uterine horn and as close to the bifurcation of the uterine horns as possible. All pigs may not be able to be removed from a single incision in the uterus.

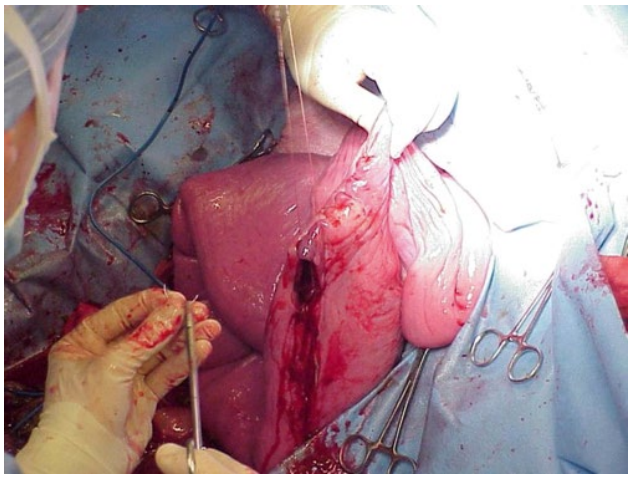


Figure 11.11 Hysterotomy closure after fetal extraction. A continuous inverting suture pattern should be done using absorbable suture material.

Closure of Cesarean section incision is based upon the conditions under which the surgery was performed. For a healthy uterus containing live or recently dead pigs, we use No. 1 chromic gut or No. 0 PDS or polyglycolic acid placed in a Cushing or Utrecht (modified Cushing) pattern for closure of the uterus (Figure 11.11). Some veterinarians have advocated hysterectomy when performing Cesarean section (Schoneweis 1971). This practice allows rapid removal of all pigs soon after entering the abdomen, ensures culling of the sow after the pigs have been weaned, and minimizes surgery time because an assistant does removal of individual pigs after removal of the uterus. The uterine arteries are ligated with No. 0 chromic gut, the broad ligaments are divided along the axis of the uterine horns, and the uterine body is ligated using rubber tubing. The rubber tubing may be secured to the uterine body using No. 1 chromic gut suture. Alternatively, sterile 1 cm cotton tape (umbilical tape) suture may be used to perform transfixation ligation of the uterine body. Then, the gravid uterus is removed. We urge caution with hysterectomy that precise hemostasis must be achieved prior to removal of the uterus. Ligation of abdominal bleeders after removal of the uterus is difficult, and life-threatening hemorrhage may occur if the uterine arteries are inadequately ligated. We close the transversus abdominis muscle and peritoneum together and the external abdominal oblique and internal abdominal oblique muscles together using No. 2 chromic gut or No. 1 PDS or polyglycolic acid placed in simple continuous pattern (Figure 11.12). For closure of ventral midline or paramedian incisions, we do not recommend the use of chromic gut because of the higher rate of postoperative hernia formation. We use No. 1 PDS or polyglycolic acid suture placed in simple interrupted or interrupted

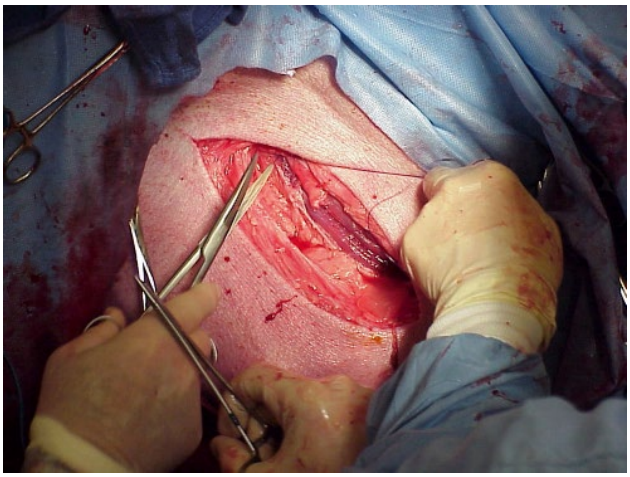


Figure 11.12 Closure of the abdominal wall of the paramammary incision after completion of laparotomy.

cruciate pattern. Skin is closed using No. 0 polymerized caprolactam in a Mayo (Ford) interlocking pattern. Alternatively, a subcuticular suture pattern may be placed to oppose the skin edges. This suture pattern eliminates the need for skin suture removal at a later date. The sow should remain confined for a minimum of 14 days after surgery.

Gnotobiotic pigs

The production of gnotobiotic or SPF pigs is an accepted model for scientific research. The selected sow should be placed under general anesthesia, and the surgery site aseptically prepared. Several methods have been described for obtaining gnotobiotic pigs including hysterectomy, closed hysterotomy (using a sterile chamber attached to the side of the sow through which surgery is performed), and open hysterotomy with germicidal trap. All methods are expected to have a baby pig mortality rate of less than 15%, except open hysterotomy performed with local anesthesia, which may have a 35% baby pig mortality rate (Miniats and Jol 1978; Tavernor et al. 1971). When a hysterectomy technique is selected, baby pig survival is better when the surgery is performed with the sow under general anesthesia rather than euthanasia of the sow prior to hysterectomy.

Uterine prolapse

Prolapse of the uterus is occasionally seen in sows during or up to several days after parturition. Excessive straining because of fetal malpositioning, fetal-to-maternal disproportion, or trauma with swelling and inflammation in the birth canal is thought to cause uterine prolapse. Prolapse of the entire uterus has the greatest potential for a life-threatening crisis because of profuse hemorrhage, but partial prolapse also may occur. The sow must be stabilized prior to attempts to replace the uterus into its normal position. If hemorrhage, hypovolemia, or shock (tachycardia, peripheral cyanosis) is present, the sow should be placed into a warm environment, IV catheter is placed into an auricular vein, and intravenous fluids are administered. Hypertonic saline may be administered rapidly (5–7 mL/kg BW, IV over 5–10 minutes) followed by isotonic crystalloid fluids (5–10 mL/kg/h). For replacement of the prolapse, the sow may be placed on an inclined floor or platform in sternal recumbency with the hindquarters elevated. Epidural anesthesia (administered at the lumbosacral space), sedation, or general anesthesia may be required to eliminate struggling, straining, and agitation of the sow. The uterus is thoroughly cleaned with cold water and assessed for the presence of lacerations and necrosis. Small lacerations may be cleaned, superficially debrided, and sutured closed (No. 0 chromic gut, simple continuous pattern). Hemorrhage may be stopped by ligating affected vessels or by performing en bloc tissue imbrication. Sutures may be placed over stents to increase the region of pressure to control hemorrhage. Then, hygroscopic agents (anhydrous glycerine, sugar, etc.) may be applied to the uterus to assist in reducing edema. The uterus is wrapped into a towel, and gentle pressure is applied, starting from the tip of the uterine horn and working toward the body of the uterus. After approximately 15 minutes, the edema should be sufficiently reduced to allow manipulation of the uterine horns. Each horn should be inverted starting with the tip and gradually reduced until the uterine body has been reached. Often, progress is impeded because of the extensive edema and swelling of the soft tissues of the pelvic canal. When this occurs, left paralumbar fossa laparotomy is indicated (Raleigh 1977). After appropriate preparation of the surgical site and surgeon, a 10 cm long, vertically oriented incision is made in the middle of the left paralumbar fossa. The left arm is passed into the peritoneal cavity and into the everted uterus. One of the uterine horns is grasped and pulled back into the peritoneal cavity. The right arm or an assistant helps by applying gentle pressure on the everted horn from the exterior. After the uterus has been repositioned, all remaining fetuses should be removed. The laparotomy incision should be closed in three layers (transversus abdominis m + peritoneum, internal + external abdominal oblique, skin). Chromic gut (No. 3) or a synthetic absorbable suture (polydioxanone, polyglactin 910, polyglycolic acid) is placed in simple continuous suture patterns in the muscle layers. Polymerized caprolactam (No. 2 braunamid) is placed in a Mayo (Ford) interlocking pattern in the skin. Antimicrobial and anti-inflammatory

medications are desirable, but strict attention should be paid to drug residues in the meat prior to slaughter. Finally, a Buhner suture should be placed around the vulva to prevent reoccurrence of the prolapse. The Buhner suture (6.4 mm wide sterile cotton tape) should be deeply placed at the junction of the labia and the skin of the perineum to recreate the function of the vestibular sphincter muscle. The Buhner suture may be removed in 7–10 days with minimal risk of prolapse. Oxytocin (20 units) is routinely administered to facilitate contraction and involution of the uterus and cervix. If prolapse reduction using laparotomy is not used as a "last-resort" treatment, sows should survive partial prolapse of the uterus (>75%), but complete prolapse carries a guarded prognosis (<50%).

Amputation of the uterus

Amputation of the uterus is indicated when excessive bleeding, extensive laceration, trauma, or necrosis of a uterine prolapse is found. The prognosis with uterine prolapse is guarded to poor for survival, and affected sows must be provided with supportive care as soon as possible (shelter, IV fluids via ear vein catheter, etc.). Focal lacerations and bleeders can be repaired, and the uterus replaced into the abdomen. When severe injury has occurred, amputation of the uterus provides the best option for salvage of the sow. Prior to amputation, the uterus should be closely inspected to ensure that the bladder or small intestine is not entrapped. Hypovolemic or hemorrhagic shock may be present and should be addressed during the course of treatment. If the uterus is swollen, it should be elevated above the pig to encourage drainage of venous congestion. We recommend placing towels around the uterus so that pressure may be applied without further trauma to the wall of the uterus. Hygroscopic agents (anhydrous glycerine, granular sugar, etc.) may be used to help resolve edema of the uterine tissues. After venous congestion has been reduced, amputation is more easily performed. Transfixation ligatures are placed around the circumference of the uterus. Heavy suture material (0.5 cm sterile cotton tape, No. 3 polymerized caprolactam) is used because the thickness of the uterus requires extreme tension to completely occlude the uterine arteries. Stay sutures or cross pins (using 15 cm long 18 gauge needles) are placed in the vital uterus, and the prolapsed portion is amputated. Then, any bleeders are ligated with No. 1 chromic gut before the remaining tissues are released and placed back into the pelvic canal. A Buhner suture or purse-string suture should be placed into the labia at the level of the vestibular sphincter to prevent prolapse of the remaining tissues. Affected sows are salvaged as soon as possible or after weaning of the litter.

Mastectomy

Mastitis caused by *Actinomyces suis* may cause formation of abscesses, granulomas, and mammary fistulas (Bollwahn and Meermeier 1989). The swellings may become large and problematic for the sow. Surgical removal of the mammae is indicated for return of the sow to production soundness. Sows with at least 12 intact mammary glands and that are not in the first week or last 4 weeks of gestation are suitable candidates for surgery. The sow is placed under general anesthesia, and the affected mammary gland prepared for surgery. An elliptical incision is made approximately 1 cm from the base of the swelling so that enough tissue remains to allow closure of the tissues with minimal tension. A combination of sharp and blunt dissection is used to extirpate the gland, granuloma, and abscesses. The cranial superficial epigastric vein (subcutaneous abdominal vein) should not be compromised, but hemostasis is essential. Using 2-0 chromic gut ligatures of transected blood vessels ensures hemostasis. The wound is closed in three layers: deep subcutaneous, superficial subcutaneous, and skin. Each subcutaneous tissue layer is sutured with a simple continuous suture pattern (No. 0 chromic gut, No. 2-0 PDS, or polyglycolic acid). Each suture is anchored to the deeper tissue layer in an attempt to close all dead space, thus minimizing the formation of postoperative seroma, hematoma, and abscess. Administration of perioperative antibiotics is indicated.

Abdominal surgery

Umbilical hernia

Umbilical hernia is a development defect of pigs that has recently been found to have a genetic cause (Ding et al. 2009). An umbilical hernia is a discontinuity of the abdominal wall at the umbilicus with protrusion of abdominal content into a hernia sac formed by the skin and surrounding connective tissue (Figure 11.13). In swine herds, the frequency of umbilical hernias ranges from 0.4 to 1.2% and varies with breed and sex (Searcy-Bernal et al. 1994). In addition to heredity, the etiology of umbilical hernia may be navel infection and umbilical abscess. After the umbilical cord is cut at birth, iodine should be applied to decrease the likelihood of infection. Pigs with umbilical hernias may suffer from growth retardation and may die from intestinal strangulation. In one study, pigs sired by American Spotted and Duroc boars were more likely to develop hernia than those sired by Yorkshire, and umbilical hernias often were detected in pigs between 9 and 14 weeks of age (Searcy-Bernal et al. 1994). One possible reason for the recognition of the condition at that age may be the rapid growth of pigs,

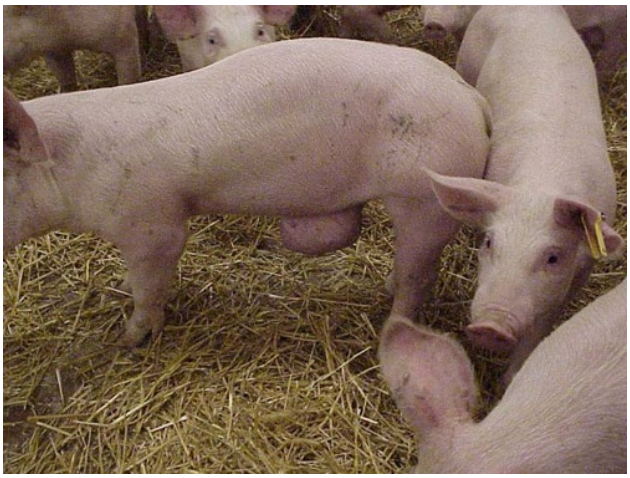


Figure 11.13 Clinical appearance of an umbilical hernia in a young pig.

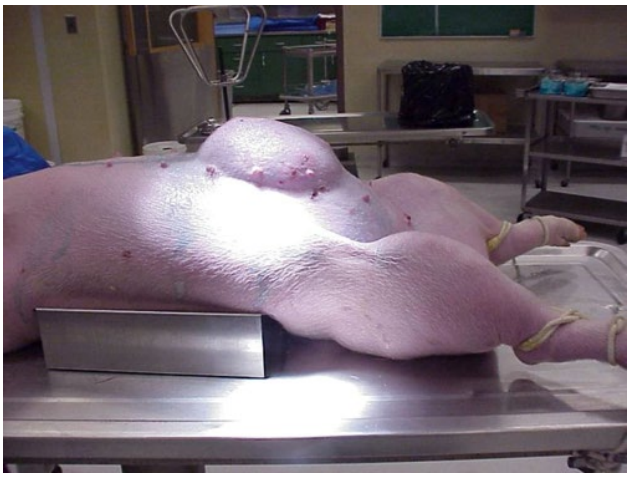


Figure 11.14 Positioning of a young pig in preparation for surgical treatment of umbilical hernia.

combined with increased weight of the abdominal contents, leading to a hernia of significant size. Females were at an increased risk of developing umbilical hernia. As with many other swine surgical conditions, the cost of treatment may preclude surgical correction. In that case, pigs should be consigned to an early slaughter soon (within 1 month) after detection of the hernia and before evisceration or intestinal strangulation or fistula occurs. A case of intestinal umbilical fistula has been described in a 30 kg castrated male pig (Lewis 1973). The risk of intestinal incarceration and strangulation is more frequent with an umbilical hernia of small dimension, (i.e. hernia, ring smaller than 8 cm). However, a pig with an umbilical hernia often will be discounted when it goes to slaughter.

Reduced growth rate in untreated pigs with umbilical hernias may encourage surgical correction of the defect. However, whether surgical correction of umbilical hernia will restore the growth potential is unknown. In purebred, show animals, and pigs kept as pets, surgical correction often is indicated. Herniorrhaphy should be performed early in life. Following anesthesia, the pig is restrained in dorsal recumbency in a V-shaped trough (Figure 11.14). The surgical area then is cleaned and prepared for surgery. If surgical correction is performed on a male, the prepuce, preputial diverticulum, and penis should be reflected posteriorly or to one side. The hernia sac then is isolated, and dissection is performed to the hernia ring. The hernia sac with an abscess, if present, should be removed, and the edges of the ring freshened. If intestinal contents are adhered to the hernia sac, the adhesions are separated, bowel viability is assessed, and if judged acceptable, the bowel is replaced in the abdomen. If intestinal viability is compromised, resection and anastomosis of viable intestine should be performed. If no infection is present, the hernia sac also can be inverted into the abdomen. The abdominal defect then is closed using an overlapping or simple continuous pattern. The prepuce, preputial diverticulum, and penis then are repositioned and sutured to the abdominal muscle with absorbable suture material. The skin then is sutured using a simple interrupted pattern of nonabsorbable suture material. For surgical correction of umbilical hernia in the female, an elliptical incision is made around the hernia sac, and the excess skin is discarded. With a combination of sharp and blunt dissection, the hernia sac then is cut and removed, and the abdominal muscle closed as in the male. The subcutaneous tissue and skin then are closed. Systemic antibiotic should be administered for 5 days, and the skin suture removed in 10 days.

Inguinal or scrotal hernia

Inguinal hernia and scrotal hernia are variants of a defect in which intestines or other abdominal organs pass into the inguinal canal (Figure 11.15). The hernia develops when there is an abnormally large and patent vaginal orifice through which the vaginal process and peritoneal cavity communicate. Scrotal hernia is the more exaggerated form of the defect in that the organs protrude into the scrotum (Vogt and Ellersieck 1990). These hernias are common in swine and have been the most common defect observed in swine (Vogt and Ellersieck 1990). The frequency of scrotal hernia among the porcine population varied between 0 and 15.7% with a realistic number of about 1% (Vogt and Ellersieck 1990). The development of these hernias seems to be genetically influenced (Ding et al. 2009). One study indicated that the variation associated with anatomic structures relevant to scrotal hernia is influenced polygenically. In that study, the heritabilities of susceptibility to scrotal hernia development were estimated to be

Figure 11.15 Clinical appearance of a right-sided inguinal hernia in a young pig.

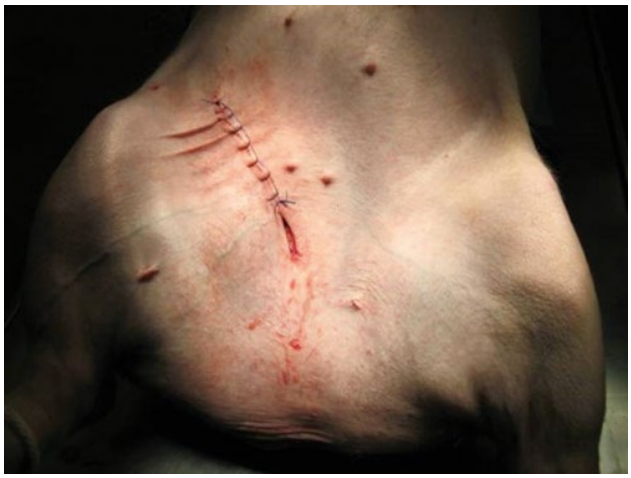


Figure 11.16 Location of parainguinal incision for reduction and closure of a unilateral inguinal hernia in a young pig.

0.29, 0.34, and 0.34 in Duroc-, Landrace-, and Yorkshire-sired pig groups, respectively (Vogt and Ellersieck 1990). Inguinal and scrotal hernias need to be differentiated from hydrocele, scirrhous cord, and hematoma of the testicle. Taking a good history (e.g. a pig that has been castrated before is more likely to have a scirrhous cord) and direct manipulation often will give the diagnosis. If necessary, ultrasonography and needle aspiration can be used. Inguinal hernias often are encountered at the time of castration. Some of these hernias will reduce spontaneously but recur later. With chronic inguinal hernia, intestinal incarceration and strangulation may be observed.

Surgical repair of an inguinal or scrotal hernia is easier if done before the pig is castrated. With the pig restrained in dorsal recumbency and its rear quarters elevated, the inguinal and scrotal area is thoroughly cleaned and prepared for surgery. An oblique incision is made over the affected external inguinal ring. Once through the skin, the subcutaneous tissue is dissected bluntly. Also by blunt dissection, the tunica vaginalis is isolated. The tunica vaginalis should be kept intact, because this will keep the intestine contained. While external pressure is put on the scrotum, the tunics are gently pulled free from their scrotal attachment. The entire hernia sac is removed through the inguinal incision. The tunic and testicle then are twisted to force the intestines into the peritoneal cavity. The tunics and spermatic cord are transfixated as close to the inguinal ring as possible. The tunic and cord are cut, and the inguinal ring is closed with interrupted or horizontal mattress suture. The herniorrhaphy is then checked by applying external pressure on the abdomen. The skin then is closed using absorbable sutures (Figure 11.16). The authors always recommend checking the other side for possible bilateral herniation before performing a castration. If the surgery was done to repair a large hernia in which marked serum accumulation in the scrotum is expected, an incision in the most ventral aspect of the scrotum should be performed via the inguinal incision before suturing to provide ventral drainage. If intestinal adhesion and incarceration are observed during surgical correction of a scrotal hernia, the vaginal tunic should be opened and the intestine is dissected free or an intestinal resection and end-to-end anastomosis are performed. If an inguinal hernia occurs after castration, one needs to clean and lavage the herniated bowel, enlarge the inguinal ring and replace the prolapsed intestine (if it is judged to still be viable), and then suture the inguinal ring.

Visceral torsion or volvulus

In one series of cases, acute abdominal accidents were characterized clinically by sudden death and were observed more commonly in dry sows (Morin et al. 1984). It was proposed that feeding dry sows in large breeding units once a day or every other day might be an important provoking factor. This feeding method often will make sows ingest large quantity of feed and water rapidly. In swine with gastric torsion, death was preceded sometimes by a short period of anorexia, abdominal distension, shortness of breath, cyanosis, and salivation. At necropsy, clockwise torsions were more common than counterclockwise. The torsions were about the longitudinal axis of the organ, and the stomach was distended severely with fluid, gas, and food (Morin et al.

1984). The spleen had rotated with the stomach in some sows; affected spleens were congested severely, and some had ruptured, causing hemoperitoneum. Torsion of the liver also was seen occasionally (Morin et al. 1984). In eight sows, intestinal volvulus was observed, and it was more common in younger sows. In four sows, the entire small intestine was included in the volvulus, the posterior half of the small intestine in one, the small intestine and colon in one, and the cecum and colon in the last one (Morin et al. 1984).

Intestinal obstruction

In swine with intestinal obstructions from intussusceptions or foreign bodies, clinical signs observed may include depression, vomiting, abdominal distension, and decrease in the amount of feces sometimes with blood and mucus in it. These two conditions are diagnosed rarely in live animals. If the condition is diagnosed early, a ventral midline celiotomy and an enterotomy are performed for the foreign body obstruction, and an intestinal resection and anastomosis are done for the intussusception. Spiral colon obstruction was diagnosed in an 8-year-old potbellied pig with depression, inappetence, and abdominal distension (Gallardo et al. 2003). Exploratory laparotomy found a stricture at the proximal centripetal loop of the spiral colon and megacolon proximal to the stricture. A side-to-side colonic anastomosis was performed, and the pig returned to normal after surgery. A 7-month-old female potbellied pig was diagnosed with idiopathic megacolon based on abdominal palpation, abdominal radiographs, and exploratory surgery (Bassett et al. 1999). A subtotal colectomy was performed, and an ileocolonic anastomosis performed. The pig survived and, after a period of diarrhea, returned to normal stool.

Gastric ulcers

Gastric ulcers are common conditions of the gastrointestinal tract of the pig. Clinical signs are pale mucous membrane (anemia) and dark tarry feces. In valuable pigs, sometimes gastrotomy can be the best treatment option. With the animal in dorsal recumbency, an incision is made on the ventral midline starting at the xiphoid cartilages. The stomach is isolated from the rest of the abdomen, and the serosal surface is evaluated for changes in color and appearance that would indicate an ulcer. A gastrotomy then is done, and the stomach contents are removed. If an ulcer is found, it can be surgically dissected, and the edges electrocoagulated or ligated with some suture material. The wall of the stomach then is closed with a double row of suture material using an inverting pattern. If multiple bleeding ulcers are present, the prognosis is poor even with surgery.

Atresia ani and rectal stricture

Atresia ani occurs more frequently in the pig than any other species and is possibly the most important cause of intestinal obstruction. This congenital defect is transmitted genetically, but may occur spontaneously as well. Recently, atresia ani was reported in a piglet that was a clone derived by somatic cell nuclear transfer from a miniature pig that did not have atresia ani (Lee et al. 2005). The diagnosis is made by an absence of anal opening, abdominal distension, slower growth rate, and vomiting. Because the pigs vomit, the diagnosis of atresia ani is sometimes not made until 3–4 weeks of age. In the female piglet, a fistula may occur between the rectum and vagina so that the feces may be voided through the vulva. Surgical treatment of atresia ani is necessary for survival. Following anesthesia, a circular piece of the skin is excised below the tail over the bulging rectum. Feces usually are discharged immediately. If the rectum is not present at the skin opening, pelvic dissection may be necessary. Depending on the location of the rectum, or if atresia of the rectum is also present, surgical correction may not be possible. In these extreme cases, a celiotomy and colostomy may be necessary but rarely justifiable economically. Following correction of atresia ani or recti, the pigs should be fed until they reach slaughter weight. Pigs with rectal stricture often show similar clinical signs as pigs with atresia ani, except that they have an anus and are generally older. In one series of cases, pigs with rectal stricture were 16–18 weeks of age (Saunders 1974). These pigs after being affected for 2 weeks were suffering from weight loss compared with their herd mates, no feces were passed, and the abdomen continued to distend. These pigs were slaughtered or killed by other pigs (Saunders 1974). Most cases of rectal stricture are the results of a rectal prolapse that has constricted after repair causing an obstruction. At necropsy, these pigs show a distended cecum and colon. The rectum usually is occluded for 3–5 cm by a band of fibrous tissue. It is speculated that inflammation of the rectal mucosa leads to rectal scar formation with subsequent stenosis and eventually possible complete obstruction (Saunders 1974). Pigs with rectal stricture may respond to celiotomy with colostomy or ileocutaneous anastomosis. Ileocutaneous anastomosis has been successfully performed in pigs as young as 10 days old (Anderson et al. 2000).

Rectal prolapse and rectal amputation

Rectal prolapse is a common occurrence in swine. Prolapse of the rectal mucosa occurs following straining to defecate. The mucosa rapidly becomes edematous and often shows bleeding lesions. Many factors have been associated with the development of rectal prolapse including genetics, a birth weight of less than 1 kg, being male, diarrhea, coughing, short tails, autumn and winter piling because of chilling, chronic water shortage, certain antibiotics, zearalenone toxicosis, and a diet containing excess lysine (20% more than required) (Amass et al. 1995). Diagnosis of rectal prolapse is not difficult, but care should be taken that the prolapse does not contain other organs.

The simplest procedure for rectal prolapse is reduction by gentle massage and retention by application of a purse-string suture pattern using umbilical tape (Borobia-Belsue 2006). The suture is passed in and out through the skin around the anal opening at a distance of 1 cm from the anus. A one-finger opening should be left when tying the purse string. The suture usually is left in place for 5 days. This should be done only if the rectal mucosa is viable and no laceration is present on close inspection. If the mucosa is too necrotic to replace, correction of the prolapse can be approached in different ways (Vonderfecht 1978). One technique is a surgical amputation. For this surgery, required instruments are hemostats, blade, scissors, thumb forceps, two 18 gauge needles 3 or 4 in. long, suture material, and a small-diameter rubber tube. Following anesthesia, the tube is inserted in the rectum until 2 or 3 in. protrude. The tube is fixed in the rectum by inserting the two needles through the rectum at right angles to each other so that they pass through the rectum and tube and emerge from the opposite side. The dissection is started about a centimeter from the mucocutaneous border where the mucosa is still healthy, and the entire circumference of the exposed mucosa of the rectum is cut down to the serosa of the inner wall. Hemorrhaging is usually minor and controlled with gauge until all the layers have been dissected and the dorsal artery of the rectum is cut. Once the dissection is completed around the prolapse, the rectum is held in place because it is attached to the rubber tube with needles. Instead of using tubing and needles, one could use forceps applied at two or three places as the prolapsed rectum is amputated to prevent telescoping into the animal's body (Kjar 1976). To suture the ends of the rectum together, the authors suggest using size 0 absorbable suture material in an inside-out continuous pattern. After the simple continuous pattern has been placed around the rectum, the needles then are pulled from the tube, and the tube is removed from the rectum. The rectum then automatically retracts into place. An alternative method of rectal amputation is to use a prolapse ring, PVC tubing, syringe case, or corrugated tube (Douglas 1985). The ring or tubing is placed in the rectum, and the halfway point on the tube needs to be inserted as far as the anal sphincter. A ligature or rubber band then is applied over the prolapse as near as possible to the anus. The ligature or rubber band must be tight enough to disrupt blood supply to the prolapse. Feces may go through the tube or may block the tube. Usually, the necrotic prolapse falls in 5–7 days with the implant in place, and then fecal production returns to normal.

Three possible complications seen with rectal prolapse are bladder retroversion, eventration of the small intestine, and rectal stricture (Peyton et al. 1980). In a 1-month-old castrated pig, eventration of the small bowel was seen concurrently with a rectal prolapse. The rectal prolapse was 5 cm long, edematous, and purple black. A small tear was found in the rectum in the pelvic area, and eventration of small intestine was observed. It was speculated that the prolapse was of long duration, allowing necrosis to occur. This provided a friable area, and during straining to defecate, the small intestine perforated this necrotic area (Peyton et al. 1980). Surgical correction of small intestine eventration in pig is usually not economically feasible. If treatment is requested, preoperative medical management is often necessary, because these pigs are often in shock. Under general anesthesia, the intestine then is examined and most likely will have to be resected. A ventral midline incision then is made to occlude the lumen of the intestine that remained within the abdominal cavity, and the portion of the intestine that is going to the rectal laceration is resected close to the rectum. The viable end of intestine then is exteriorized through the ventral midline incision, and an end-to-end anastomosis performed (Peyton et al. 1980). Bladder retroversion with rectal prolapse has been observed in a sow 2 days after normal farrowing (Greenwood 1989). The sow had a grapefruit-sized rectal prolapse with protrusion and tension of the perineal area. The bladder was drained by passage of a polypropylene catheter. One week postpartum, the prolapsed rectum was amputated. The sow reared nine piglets to 6 weeks of age and was sent for slaughter 1 week after weaning the piglets (Greenwood 1989).

Musculoskeletal surgery

Musculoskeletal injuries, particularly lameness, are increasingly recognized in swine. Lameness represents a significant animal welfare issue and is justification for concentrated research into cause and prevention of these disorders. A recent Bayesian analysis of leg disorders in finisher herds identified the need for accurate data collection and analysis (Jensen et al. 2009). In that study,

three classes of leg abnormalities were examined: physical injury, inherited defects, and infectious agents. In a survey of musculoskeletal disorders in dead sows in Denmark, 72% of sows had a classification of locomotor abnormality (Kirk et al. 2005). In that study, the most common locomotor disorders were septic arthritis (24%), most commonly caused by *Arcanobacterium pyogenes*, and fractures (16%), most commonly of the proximal humerus or femur. Interestingly, that study also revealed that disorders of the hoof and sole are common. Risk factor analysis is critical in the prevention of limb injuries. The following focuses on treatment of pigs having such injuries.

Septic arthritis

Septic arthritis may be caused by bacteremia, direct inoculation of bacteria into the joint, or extension of a local infection into the joint. Septic arthritis caused by direct inoculation or local extension is treated by wound management, joint lavage, and systemic antibiotic therapy. Infected joints may require daily, or every other day, lavage for 7–10 days or until granulation tissue has covered the wound. Sterile isotonic electrolyte solutions (0.9 % saline, lactated Ringer's solution) are lavaged through the joint by inserting an 18 or 14 gauge needle into the joint, injecting the solution under pressure into the joint, and inserting a second needle into the joint with as much separation as possible between the two needles. Approximately 500 mL of the solution is flushed through the joint. After lavage, antibiotics may be instilled directly into the joint to achieve maximal local antibiotic concentration. Success of treatment is assessed by improvement in lameness and wound appearance.

Digit amputation

Digit amputation is indicated when severe foot abscesses or septic arthritis of the interphalangeal joints has caused unmanageable damage to a single digit. These injuries are most commonly caused by wounds from trauma on concrete flooring or metal side panels. The decision for amputation should not be delayed. If the infection has extended to the fetlock or more proximal on the limb, digit amputation will not be curative. Also, the soundness of the opposite digit should be assessed to determine if the pig would be able to ambulate on the remaining digit after amputation.

After induction of general anesthesia, the affected digit is cleaned and prepared for surgery. A tourniquet is placed proximal to the surgery site to prevent extensive hemorrhage during surgery. A circumferential incision is made through the skin and soft tissues at a 45° angle to the coronary band starting at the axial aspect of the digit and continuing proximally to the abaxial surface. The skin is reflected proximal to the site for amputation, and a sterile obstetrical wire is used to amputate the digit. The third phalanx and a portion of the second phalanx are removed by this procedure. The remainder of the second phalanx also should be removed. The remaining tissues are debrided and cleaned thoroughly, and the skin is opposed over the wound. A sufficient opening is left to allow drainage, or a Penrose drain is placed into the wound. The foot is placed in a padded bandage for 7–10 days. Then, the foot is cleaned daily with water until the wound is healed. Perioperative antibiotics and anti-inflammatory drugs are indicated.

Ankylosis of the proximal or distal interphalangeal joint

Septic arthritis of the proximal or distal interphalangeal joint is an indication for digit amputation. However, the lateral claw of the hind limb is important to normal ambulation and breeding activity. Salvage of the digit by facilitated ankylosis is an option to preserve normal ambulation. The affected pig is placed under general anesthesia, and the digit prepared for surgery. A 1 cm incision is made into the affected joint. Placing a 3.75 cm long needle into the joint by inserting the needle immediately proximal to the coronary band and inserting the needle distally approaches the distal interphalangeal joint. The proximal interphalangeal joint may be located by palpation or insertion of the needle in the mid-pastern region. After the arthrotomy has been made, a 4 or 6 mm diameter drill bit is used to destroy the articular surfaces of the joint. Curettes are used to debride the joint and remove all infected subchondral bone. A distinct difference in texture and hardness will be noted between the necrotic (gritty and irregular) and healthy (smooth and hard) bone. Thorough curettage of all infected bone is critical to establishing effective joint ankylosis. The tissues are extensively lavaged with normal saline, and antibiotics are administered for 10–14 days. Strict confinement for 6–8 weeks is needed for ankylosis to occur. A cast extending from the ground to the carpus or hock will hasten convalescence.

Fracture repair

Swine with fracture of long bones are often salvaged because economic considerations preclude treatment. However, veterinarians may be asked to treat fractures in swine of potential value for genetic improvement. Treatment of fractures can be rewarding, and Vaughan reported clinical experiences with fracture fixation in commercial swine (Vaughan 1966). Fractures were associated with breeding injury (two pigs), injury on concrete flooring (three pigs), and fighting injury (one pig) and were of unknown cause in five pigs. The most common

fractures treated were tibia and fibula (five pigs), femur (three pigs), humerus (two pigs), and tibiotarsal joint luxation with fracture of the fibula (two pigs). Affected pigs weighed between 64 and 168 kg and were 6 months to 2 years old. The fractures of the tibia and fibula were treated by an open reduction and internal fixation by using a bone plate and a full limb cast (three pigs) or by using a full limb cast alone (two pigs). Fracture of the femur was treated by application of a bone plate (three pigs). Humerus fracture was treated by confinement (one pig) or by application of a bone plate (one pig). Tibiotarsal joint luxation with fracture of the fibula was treated by application of a bone plate and use of a full limb cast (two pigs). Of these 12 pigs, 10 returned to normal production use and 2 were salvaged: 1 pig with tibiotarsal joint luxation developed *Escherichia coli* osteomyelitis, and 1 pig with humeral fracture repaired by internal fixation suffered permanent radial nerve damage.

Surgical repair of articular fracture of the humeral condyles have been reported for miniature pigs (Payne et al. 1995). The medial humeral condyle was most commonly fractured, but "Y"-type fractures and supracondylar fractures of the humerus have been found in some miniature pigs. Fractures were repaired using lag screw and Kirschner wire fixation. Five pigs were reexamined 2 months after surgery, and all were walking sound at that time.

The risk of suffering fracture of the femur has been associated with dietary deficiencies of calcium in market pigs (Hejazi and Danyluk 2009). These fracture result in significant economic losses, especially when they occur in valuable breeding stock. Femoral fractures were diagnosed in 20 pigs over a 6-month period (Rousseaux et al. 1981). Nutritional analysis revealed inadequate calcium and phosphorus (both in absolute concentration and in calcium-to-phosphorus ratio) in the feed. Affected pigs were approximately 20 weeks old and weighed between 80 and 90 kg. Pigs walked with a stilted gait and arched back. Necropsy found separation of the proximal femoral epiphysis from the femoral neck. After the correction of dietary calcium and phosphorus, clinical evidence of femoral fracture was not observed in any additional pigs. Femoral, pelvic, and vertebral fractures have been found in pigs after accidental electrical shock (Bildfell et al. 1991). Multiple trauma injuries and fractures associated with nutritional deficiency are poor candidates for surgical repair. Fracture of the greater trochanter of the femur also has been identified as cause of lameness in pigs (Blowey 1992, 1994). A simple oblique fracture of the mid-diaphysis of the femur was successfully repaired in a 10-month-old 150 kg Berkshire boar using a bone plate (Grisel and Huber 1996). The boar returned to normal breeding 190 days after surgery.

Canine tooth (tusk) removal and resection

Removal of the canine teeth of adult boars is challenging because of the long dental root embedded in the mandible. Canine tooth extraction is done with the boar under general anesthesia. The gingiva and periosteum of the mandible are reflected laterally and ventrally using a periosteal elevator. Then the lateral alveolar plate of the tooth alveolus is resected following the course of the tooth root. After the periapical region of the alveolus has been reached, a periodontal elevator is used to disrupt the periodontal membrane around the circumference of the tooth, and the tooth is removed. The alveolus is debrided, rinsed, and sutured closed. Alternatively, the alveolus can be left open to heal by second intention healing.

Resection of the mandibular canine teeth is an easy and rapid method of preventing tusk injuries to personnel and other pigs. The boar is placed under general anesthesia, and obstetrical wire is placed around the tooth. The wire is used to saw through the tooth approximately 3 mm above the gingival margin to prevent exposure of the pulp cavity. This procedure is repeated every 6–12 months as needed to restrict growth of the tusks.

References

Ajadi A, Olusa T, Smith O, et al. 2009. *Vet Anaesth Analg* 36(6):562–566.

Althouse G, Evans L. 1997a. *J Am Vet Med Assoc* 210:675–677.

Althouse G, Evans L. 1997b. *J Am Vet Med Assoc* 2310(5):678–680.

Amass S, Schinckel A, Clark L. 1995. *Vet Rec* 137:519–520.

Anderson D, Kim J-H, Hancock J, et al. 2000. *Lab Anim Sci* 39:26–28.

Arkins S, Thomson L, Giles J, et al. 1989. *J Anim Sci* 67(1):15–19.

Bassett J, Mann E, Constantinescu G, et al. 1999. *J Am Vet Med Assoc* 215:1640–1643.

Bates J, Karriker L, Stock M, et al. 2014. *PLoS One* 9(12).

Becker H. 1986. Castration, inguinal hernia repair, and vasectomy in boars. In Morrow DA, ed. *Current Therapy in Theriogenology 2*. Philadelphia: WB Saunders, pp. 985–987.

Benson G. 1986. Anesthetic management of ruminants and swine with selected pathophysiologic alteration. *Vet Clin North Am Food Anim Pract* 2(3):677–692.
Bildfell R, Carnat B, Lister D. 1991. *J Vet Diagn Invest* 3:364–367.
Blowey R. 1992. *Vet Rec* 131:312–315.
Blowey R. 1994. *Vet Rec* 134:601–603.
Bollwahn W, Meermeier D. 1989. *Berl Munch Tierarztl Wochenschr* 102(7):223–227.
Borobia-Belsue J. 2006. *Vet Rec* 158(1):380.
Bustamante R, Valverde A. 1997. *Can J Vet Res* 61:246–250.
Clutton R, Blissitt K, Bradley A, et al. 1997. *Vet Rec* 141:140–146.
Courboulay V, Gillardeau M, Salaun M, et al. 2014. Effects of piglet age on some indicators assessing pain at tail docking, with or without analgesia. In *International Conference on the Assessment of Animal Welfare at Farm and Group Level (WAFL 2014)*. Clermont-Ferrand, France: Wageningen Academic Publishers.
Dimigen J. 1972. *Deutsche Tierarztl Wochenschr* 79:235–237.
Ding N, Mao H, Guo Y, et al. 2009. *J Anim Sci* 87(8):2469–2474.
Dolf G, Gaillard C, Schelling C, et al. 2008. *J Anim Sci* 86(10):2480–2485.
Douglas R. 1985. *Vet Rec* 117:129.
Dutton D, Lawhorn B, Hooper RN. 1997. *J Am Vet Med Assoc* 211:598–599.
Fredeen HT, Newman JA. 1968. *Can J Anim Sci* 48(3):275–284.
Gallardo M, Lawhorn D, Taylor T, et al. 2003. *J Am Vet Med Assoc* 222:1408–1412.
Godke R, Lambeth V, Kreider J, et al. 1979. *Vet Med Small Anim Clin* 74(7):1027–1029.
Greene C. 1979. Animal anesthesia. *London Lab Anim*:187–197.
Greenwood J. 1989. *Vet Rec* 125 (15):405–406.
Grisel G, Huber M. 1996. *J Am Vet Med Assoc* 209:1608–1610.
Halland S, House J, George L. 2002. *J Am Vet Med Assoc* 220:1831–1834.
Harvey-Clark C, Gilespie K, Riggs K. 2000 *Lab Anim* 34:386–398.
Hejazi R, Danyluk A. 2009. *Can Vet* 50:516–518.
Helman R, Hooper R, Lawhorn D, et al. 1996. *J Vet Diagn Invest* 8:137–140.
Jensen T, Kristensen A, Toft N, et al. 2009. *Prev Vet Med* 89(3–4):237–248.
Kiley M. 1976. *Br Vet J* 132:323–331.
Kim M, Park C, Jun M, et al. 2007. *Vet Rec* 161(18):620–624.
Kirk R, Svensmark B, Ellegaard L, et al. 2005. *J Vet Med A Physiol Pathol Clin Med* 52(8):423–428.
Kjar H. 1976. *JAVMA* 168:229–230.
Ko J, Thurmon J, Benson G, et al. 1992. *J Vet Anaesth* 19:56–60.
Ko J, Thurmon J, Benson G, et al. 1993. *Vet Med Food Anim Pract* May:466–472.
Lachmayr V. 1966. *Wien Tierarztl Monatsschr* 53:474–478.
Lacoste L, Bousquet S, Ingrand P, et al. 2000. *Lab Anim* 34:29–35.
Langhoff R, Auer U, Maneng J, et al. 2016. *Berl Munch Tierarztl Wochenschr* 129(7–8):282–289.
Lee G, Kim H, Lee S, et al. 2005. *J Vet Sci* 6(3):243–245.
Leon J, Gill M, Cornick-Seahorn J,et al. 1997. *J Am Vet Med Assoc* 210:366–368.
Lewis A. 1973. *Vet Rec* 93:286.
Libke KG. 1967. *Vet Med Sm Anim Clin* 62:551–554.
Martin-Cancho M, Carrasco-Jimenez M, Lima J, et al. 2004. *Am J Vet Res* 65:409–416.
Mather EC. 1966. *Vet Med Sm Anim Clin* 61:890–891.
McGavin M, Schoneweis D. 1972. *Cornell Vet* 62:359–363.
McGlone J, Hellman J. 1988. *J Anim Sci* 66:3049–3058.
McGlone J, Nicholson R, Hellman J, et al. 1993. *J Anim Sci* 71:1441–1446.
Miniats P, Jol D. 1978. *Can J Comp Med* 42:428–437.
Moon P, Smith L. 1996. *Vet Clin North Am* 12(3):663–691.
Morin M, Sauvageau R, Phaneuf J, et al. 1984. *Can Vet J* 25:440–442.
Palmer J, Dykes N, Love K, et al. 1998. *Vet Rediol Ultrasound* 39:175–180.
Payne J, Braun W, Anderson D, et al. 1995. *J Am Vet Med Assoc* 206:59–62.
Peek I. 1985. *Vet Rec* 116(1):26.
Peyton L, Colahan P, Jann H, et al. 1980. *VM-SAC* August:1297–1330.
Preissel A, Brugger N, Stassen T, et al. 2009. *Schweiz Arch Tierrheilkd* 151(5):229–232.
Raleigh J. 1977. *Vet Rec* 100:89–90.
Roberts S. 1986. Infertility in male animals. In Roberts SJ (ed.) *Veterinary Obstetrics and Genital Diseases Theriogenology*, 3rd ed. Ann Arbor: Edwards Brothers, pp. 752–893.
Rosenberg H, Fletcher J. 1994. *Ann Acad Med Singapore* 23 (Suppl):84S–97S.
Rothschild M, Christian L, Blanchard W. 1988. *J Hered* 79:313–314.
Rousseaux C, Gill I, Payne-Crosten A. 1981. *Austral Vet J* 57:508–510.
Sakaguchi M, Nishimura R, Sasaki N. 1992. *J Vet Med Sci* 54:1183–1185.
Sakaguchi M, Nishimura R, Sasaki N, et al. 1996. *Am J Vet Res* 57:529–534.
Sanford S, Josephson G, Rehmtulla A. 1994. *Can Vet J* 35:388.
Saunders C. 1974. *Vet Rec* 94:61.
Schoneweis D. 1971. *J Am Vet Med Assoc* 158:1410–1411.
Schulz C, Ritzmann M, Palzer A, et al. 2007. *Berl Munch Tierarztl Wochenschr* 120(5–6):177–182.

Scott W. 1977. *Vet Rec* 101(12):249–250.
Searcy-Bernal R, Gardner I, Hird D. 1994. *JAVMA* 204:1660–1663.
Skarda R. 1996. Local and regional anesthesia in ruminants and swine. Anesthesia update. *Vet Clin North Am* 12(3):579–626.
Sturlini-Barticciotto L, Loureiro Luna S, Rodolfo de Sá Lorena S, et al. 2016. *Medicina Veterinaria* 37(3):1307–1316.
Százados I. 1985. *Acta Vet Hung* 33:177–184.
Tavernor W, Trexler P, Vaughan L, et al. 1971. *Vet Rec* 88:10–14.
Theisen M, Maas M, Hartlage M, et al. 2009. *Lab Anim* 43(1):96–101.
Thornton H. 1972. *Vet Rec* 90:217.
Thun R, Gajewski Z, Janett F. 2006. *J Physiol Pharmacol* 57 Suppl 8:189–194.
Thurmon J. 1986. Injectable anesthetic agents and techniques in ruminants and swine. *Anesthesia Vet Clin North Am Food Anim Pract* 2 (3):567–592.
Titze K. 1977. *Deutsche Tierarztl Wochenschr* 84:135–138.
Todd G, Nelson L, Migaki G. 1968. *Cornell Vet* 48:614–619.
Torrey S, Devillers N, Lessard M, et al. 2009. *J Anim Sci* 87(5):1778–1786.
Tranquilli W. 1986. Techniques of inhalation anesthesia in ruminants and swine. Anesthesia. *Vet Clin North Am, Food Anim Pract* 2(3):593–619.
Turner A, McIlwraith C. 1989. Cesarean section in the sow. In Turner AS, McIlwraith CW, eds. *Techniques in Large Animal Surgery* 2nd ed. Philadelphia: Lea & Febiger, 358–359.
Tyler J, Waver D, Shore M, et al. 2000. *Vet Rec* 147:225.
Van Straaten H, Colenbrander B, Wensing C. 1979. *Int J Fertil* 24:74–75.
Vaughan L. 1966. *Vet Rec* 79:2–8.
Vogt D, Ellersieck M. 1990. *Am J Vet Res* 51:1501–1503.
Vonderfecht H. 1978. Amputation of rectal prolapse in swine. *Agri Prac (VM/SAC)* February:201–206.
Walker B, Jaggin N, Doherr M, et al. 2004. *J Vet Med A* 51:150–154.
Wedel D, Gammel S, Milde J, et al. 1993. *Anesthesiology* 78:1138–1144.
White R, DeShazer J, Tressler C, et al. 1995. *J Anim Sci* 73:381–386.
Wieringa W, Mouwen JMVM. 1983. *Tijdschr Diergeneeskd* 108:751–760.
Zankl A, Ritzmann M, Zöls S, et al. 2007. *Dtsch Tierarztl Wochenschr* 114(11):418–422.
Zöls S, Ritzmann M, Heinritzi K. 2006. *Berl Munch Tierarztl Wochenschr* 119(5–6):193–196.

12

Preharvest Food Safety, Zoonotic Diseases, and the Human Health Interface

Julie Funk and Elizabeth Wagstrom

Introduction

The ultimate purpose of rearing domestic swine is to supply wholesome and nutritious protein to humans. Although this high-quality protein has a positive impact on human nutrition, there is some risk for negative human health impacts. These impacts can occur via consumption of pork products, by occupational exposure to swine, or through environmental exposures. In this chapter we give an overview of potential negative human health risks associated with foodborne, direct contact, or environmental exposures associated with pigs.

Foodborne disease risks associated with pork

Physical hazards

Physical foodborne hazards are foreign objects that can cause injury to those that consume food products. Physical hazards associated with pork originate from two primary sources: preharvest practices on the farm, primarily from the use of hypodermic needles, and postharvest hazards associated with processing and packaging environments. Reports in the literature are scarce regarding the frequency and health implications of physical hazards. The scope of the problem can be estimated from records of meat and poultry recalls and reports from passive surveillance based on US Department of Agriculture Food Safety and Inspection Service (USDA FSIS) consumer complaint reporting systems. A review of recalls from 1994 to 2002 indicated that physical hazards accounted for 15% of total meat and poultry recalls (Teratanavat and Hooker 2004). Recent FSIS recall summaries published by the agency indicate that physical hazards may be becoming less common; from 2011 to 2015, extraneous materials (as physical hazards are characterized by FSIS) were responsible for 45 recalls, which was 8.9% of total meat and poultry recalls for that period. Of the 45, 11 were of pork products or mixed product containing pork – 7 for plastic material, 3 for metal material, and 1 as foreign material. Plastic contamination in most cases could be linked to a postharvest source; metal could not always be clearly linked to a pre- or postharvest source. Based on data from the US Food and Drug Administration (FDA) complaint recording system, the most common human illnesses/injuries associated with physical hazards are laceration or abrasion of the peri-oral area, gastrointestinal distress, and damage to teeth or dental prosthetics.

Preharvest control of physical hazards

The primary concern for physical hazard risk preharvest in swine is the risk of broken hypodermic needles and needle fragments in carcasses. Many factors contribute to the risk of broken hypodermic needles (Hoff and Sundberg 1999). The strength of a hypodermic needle contributes to the risk of breakage. Strength is determined by the length, gauge, hub material, and manufacturer. Studies indicate that hypodermic needles are very resilient to breakage under conditions of static load. Needles that were bent and restraightened were at greater risk for breakage. Needles that were reshaped twice post bending event had a 96.7% failure rate. Simulations of animal movement during injection impacted needle failure rates. Additionally, the location of the injection as well as animal movement impacts the needle puncture strength.

Appropriate restraint, needle selection, and injection techniques are critical to avoiding broken needle events in swine. Producer and worker education is a key intervention for prevention, and development of standard operating procedures for when a broken needle event occurs (including permanent identification of suspect animals and communication with the processor) is a best practice for control of physical hazards in pork. While education on broken needle avoidance has long been

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

included in the Pork Quality Assurance Plus® (PQA Plus®), in 2015 audit points on utilizing detectable needles and needle standard operating procedures were included in the Common Swine Industry Audit (https://lms.pork.org/CommonIndustryAudit).

Detection during processing is a second line of defense to prevent physical hazards in pork. Common methods for detecting/removing physical hazards during processing include physical separation using filters or sieves and magnets (not efficacious for whole meat products) and detection using X-ray. Needles have varying ability for detection by magnets and X-ray during processing depending on the metal alloys used as well as the needle orientation while being screened by the X-ray. A recent study on needle characteristics compared X-ray detection with detection from magnetic-based detectors and reported overall detection by X-ray of 62.5% compared with 21.4% for magnetic-based detection (Hoff 2016).

One way to decrease the risk of hypodermic needles as physical hazards is to remove them from the production system environment entirely. Needleless injection systems have been used in swine for control of physical hazards (Chase et al. 2008).

Chemical hazards

Chemical hazards are toxic substances and any other compounds that may render food unsafe for human consumption. At the preharvest level, primary concerns for control of chemical hazards involve veterinary drugs, pesticides, and environmental contaminants. Other potential preharvest chemical risks involve adulteration of animal feed for the purposes of economic fraud or intentional disruption of the food supply.

Codex Alimentarius represents the global food standard setting body, which establishes standards for residue limits in food, including meats. In the United States, the FDA is responsible for approval of all drugs for use in animals and animal feeds. The FDA sets the standards for tolerance determination of chemical hazards, while FSIS conducts the surveillance and detection of chemical residues in meats. The FDA is in charge of enforcement regarding chemical residues.

Control of chemical residues is managed by prohibition of use or, for chemicals approved for use in animals, determination of maximum residue limits (MRL), which are managed via withdrawal time periods prior to harvest. Codex Alimentarius standards may differ from those of their domestic regulations regarding chemical residues. This has significant implications for international trade of pork, a critical component for economic success of pork production, as pork destined to be exported to other countries may need to adopt different withdrawal periods in order to meet the standards of the importing country. It is important to be aware of these differences and implement on-farm practices that will meet export requirements. In the United States, label indications do not indicate these withdrawal differences. These differing withdrawal periods can be accessed via consultation with pharmaceutical manufacturers or referring to the National Pork Board chart of export MRL withdrawal times (https://www.pork.org/food-safety/maximum-residue-limit/).

Extra-label drug use

In the United States, the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) allows for veterinarians to prescribe some veterinary drugs, under specific clinical conditions, in ways that are not compliant with the label indication (extra-label drug use [ELDU]). While the availability of ELDU is important for animal health and welfare from a chemical hazard safety standpoint, this contributes challenges to establishment of appropriate withdrawal periods to avoid violative residues. In the case of ELDU, the sole burden of avoiding residue violations rests with the prescribing veterinarian. The AMDUCA compliance guide suggests veterinarians to establish extended withdrawal periods when ELDU occurs (Anonymous 1994). Resources for a decision-based algorithm for ELDU and determination for the duration of the withdrawal period for ELDU can be accessed via the Food Animal Residue Avoidance and Depletion Program (http://www.farad.org/).

Veterinary drug residues in pork

FSIS conducts three separate, but interrelated, residue sampling programs (Anonymous 2016): (1) scheduled sampling, which consists of the random sampling of tissue from healthy appearing food animals for the purpose of surveillance, (2) targeted sampling at the production or compound class level, and (3) targeted sampling at the herd/flock or compound class level. Scheduled sampling data serve as a baseline level for chemical residue exposure and can be used to identify marketed animals with violative levels of residues. Targeted sampling may be inspector generated when they suspect that animals may have violative levels of residues or generated by FSIS in response to information about misuse of animal drugs or exposure to environmental chemicals, as well as in response to Tier 1 scheduled sampling analytical results. Targeted herd sampling encompasses targeted testing at the herd or regional level when FSIS feels that it is necessary to test animals from the same herd or region to determine the level of chemical to which the livestock have been exposed.

In the United States, swine residue violation rates are very rare (Anonymous 2015a). Among swine production classes in the United States (market pigs, boars/stags, sows, and roaster pigs) in 2014, no violative residues from scheduled sampling were detected. Violative

residue rates for targeted sampling were 0.09, 0.0, 0.43, and 0.34% for market pigs, boars/stags, sows, and roaster pigs, respectively. The majority of the violations were for penicillin in sows, which followed deployment by FSIS of the more sensitive multi-residue test. These data suggest that at least in the United States, veterinary drug residues are well controlled in swine production. Continued vigilance on the part of veterinarians and producers to continue to ensure this level of food safety is necessary to protect public health.

Dioxins

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are collectively referred to as dioxins due to their similar structures, their actions in biological systems, and their chemical properties. They are ubiquitous environmental contaminants that bioaccumulate in the food chain. They are often considered an industrial contaminant since they are formed as by-products of chlorine-containing manufacturing, but incineration and forest fires are significant contributors to dioxin loads. The majority of human exposure to dioxins is estimated to be from consumption of animal and fish products, especially fats (Liem and Furst 2000). Animals are exposed via air deposition of dioxin on forage and, to a lesser extent, soils (Huwe 2002).

Dioxin contamination of animal feeds has occurred sporadically around the globe including feed components contaminated by pentachlorophenol-treated wood and bakery waste contaminated during the drying process in Europe (Huwe et al. 2009). In the United States, ball clay used as an anticaking agent in poultry and catfish feed and mineral mix formulated with a smelting by-product used in swine feeds have also been identified as dioxin-containing feed contaminants (Huwe et al. 2009). Dioxin contamination of swine feed in Ireland prompted the recall of 2 months of production of pork products (Dixon 2009) and euthanasia of animals. In Germany, dioxin contamination of poultry and swine feed resulted in more than 4700 farms being closed, destruction of chickens and eggs, and the loss of exports of poultry and pork (Kupferschmidt 2011).

A single toxic equivalency value (TEQ), which is the sum of individual dioxin-like compounds, is used for intake assessments or residue determinations. The USDA has conducted surveys of commodities since the mid-1990s. There has been a decreasing trend in TEQs from the mid-1990s to 2013; although the significant decrease for pork was from the mid-1990s to 2002, TEQs declined to a lesser extent from 2002 to 2013 (Anonymous 2015b). The levels were similar to levels published from surveys in Europe and Asia (Huwe et al. 2009) and fall below the European Union regulation for accepted limits of dioxins in meats of 1–3 pg of TEQ per gram of lipid (Huwe 2002).

Biological hazards

Biological hazards are those foodborne hazards caused by bacteria, viruses, parasites, and other infectious agents. The following represent the most common biological hazards associated with pork consumption.

Salmonella

Salmonellosis is a major foodborne disease threat to public health in the United States and around the world. More than 1.2 million illnesses are associated with *Salmonella* infections in the United States annually (Scallan et al. 2011) *Salmonella* in humans most commonly results in gastrointestinal symptoms, such as diarrhea, abdominal cramping, and vomiting. The immunocompromised, young, and elderly are at greater risk for severe disease.

It has long been recognized that swine as well as many other species can be subclinical carriers of *Salmonella*. In essence all vertebrates and many invertebrates have been identified to be able to be colonized with *Salmonella*. *Salmonella* is considered to be ubiquitous in the environment and can survive indefinitely, particularly in farm environments (McLaren and Wray 1991; Plym-Forshell and Eskebo 1996). Its wide host distribution and indefinite environmental survival provide significant challenges for on-farm control. In the United States, the National Animal Health Monitoring Service (NAHMS) reported that in its Swine 2006 survey (Anonymous 2009a), the prevalence of *Salmonella*-positive production sites was 52.6% and the prevalence of positive pigs was 7.2%.

Salmonella is the only foodborne pathogen currently utilized by FSIS for process control during swine harvest. The passage of the Hazard Analysis Critical Control Point (HACCP)/Pathogen Reduction Act (Anonymous 1996) mandated the monitoring of contamination of carcasses for "generic" *Escherichia coli* as an indicator of contamination with gastrointestinal contents as well as testing for *Salmonella*. Recent data from FSIS monitoring indicate that the proportion of positive swine carcasses that are *Salmonella* positive was 1.66% in 2011, as compared with 8.7% in 1988, prior to implementation of the HACCP/Pathogen Control Act (Anonymous 2011). This indicates significant improvements in control of contamination at harvest and processing.

In the United States, control of *Salmonella* is primarily focused at the processing phase. Sweden since 1961 (Anonymous 1995) and Denmark as of 1993 (Mousing et al. 1997) have established *Salmonella* monitoring and control plans. These plans include routine herd surveillance as well as interventions at processing. There are mandatory intervention requirements on farms if they are identified as having high *Salmonella* prevalence. These interventions can include changes in hygienic

practices, feeding protocols, and pig flow (i.e. moving from continuous to all-in/all-out pig flow). Additionally, farms identified as high prevalence are penalized at harvest with decreased sales values, and product may be required to go into cooked final products. Additionally, high prevalence herds may be harvested at different facilities or as the final groups harvested at the end of the day or shift to prevent cross-contamination.

Despite a multitude of investigations, there is a lack of strong evidence for cost-effective preharvest (on-farm) interventions for *Salmonella* in swine. Review of the literature (Funk and Gebreyes 2004) suggests that a multitude of risk factors are associated with the *Salmonella* status of farms, including hygiene practices, production flows, feed form, and season. There is evidence that efforts at the farm level may be lost through reinfection during lairage at harvest (Hurd et al. 2002). There is an absence of studies that have evaluated *Salmonella* control measures in controlled on-farm trials. Systematic reviews of the literature suggest that vaccination (Denagamage et al. 2007) and feed form (O'Connor et al. 2008) may be important for *Salmonella* control. Unfortunately, both studies concluded that the quality of the reports was insufficient to strongly support these interventions. Mathematical models of *Salmonella* control strategies suggest that the greatest impact on *Salmonella* contamination of carcasses comes from interventions during harvest and processing (Alban and Stärk 2005; Goldbach and Alban 2006). In order to understand the role and efficacy of preharvest *Salmonella* interventions, on-farm controlled clinical trials are needed to evaluate these proposed interventions.

Campylobacter

Campylobacteriosis is considered one of the most common bacterial causes of foodborne disease in the United States (Mead et al. 1999) and in the developed world. Clinical symptoms in humans include gastrointestinal-related symptoms: bloody diarrhea, nausea, fever, headache, and abdominal cramps. Most human cases of campylobacteriosis are self-limiting, therefore requiring no antimicrobial treatment. Chronic sequelae are also possible, most notably Guillain–Barré syndrome. In the United States, most human clinical disease is caused by *Campylobacter jejuni*, although a small percentage is attributed to *Campylobacter coli* (Horrocks et al. 2009).

Campylobacter have been found in the intestinal tract of domestic and wild mammals, poultry, and wild birds and in untreated water (Horrocks et al. 2009). Swine are most commonly infected with *C. coli* (Horrocks et al. 2009). Most infected swine are subclinical. Pigs are thought to be colonized soon after birth, and the prevalence of *Campylobacter* reported in pigs is high. A recent review reported farm prevalence of 100% and individual pig prevalence ranging from 25 to 100%.

Campylobacter contamination of retail pork products has been reported to be low. The US FDA conducts a survey of retail meats and in 2008 reported that 0.3% of pork chops were *Campylobacter* positive (Anonymous 2008). Given the low rate of isolation, the USDA terminated sampling pork chops for *Campylobacter* after 2009.

Control strategies to prevent *Campylobacter* contamination of pork products focus on minimizing fecal contamination of carcasses during slaughter. Blast chilling of carcasses has been shown to reduce *Campylobacter* on swine carcasses better than traditional carcass cooling (Thakur and Gebreyes 2005).

Yersinia enterocolitica

The public health burden of *Yersinia enterocolitica* is estimated at 116,716 annually in the United States (Scallan et al. 2011). It is believed that most *Y. enterocolitica* infections are asymptomatic or at least mild enough to go unreported. The main clinical symptoms are self-limiting fever with diarrhea and/or vomiting. *Y. enterocolitica* clinical illness can also mimic appendicitis, which has resulted in unnecessary surgical interventions.

Yersinia enterocolitica has been recovered from several livestock species, but swine are the only species that have been consistently linked to human infections and are considered the primary reservoir for pathogenic *Y. enterocolitica* (Christensen 1980; Schiemann 1989; Toma and Deidrick 1975).

There have been few studies in the US swine population regarding *Y. enterocolitica*. Estimates of *Y. enterocolitica* prevalence in pigs at harvest are approximately 25%. The farm-level prevalence in the United States is likely high. Funk et al. (1998) found that 92% of market lots had at least one pig contaminated with *Y. enterocolitica*. In a study by Bowman et al. (2007), of the 2349 pigs sampled on 8 US swine farms, 120 (5.1%) tested positive for *Y. enterocolitica*, and of those, 42.5% harbored a virulence gene associated with the ability to cause human illness. At least one positive animal was found on seven of the eight farms. On all positive farms, there was a consistent trend of increasing prevalence as pigs mature, with market-ready pigs and sows having the highest prevalence.

Yersinia enterocolitica is a cold-loving (psychotropic) organism that can grow at refrigeration temperatures. It has the highest prevalence in swine during cooler seasons in temperate regions (Fukushima et al. 1983; Toma and Deidrick 1975; Tsubokura et al. 1976) and is more prevalent in regions with cooler climates (Fredriksson-Ahomaa and Korkeala 2003; Smego et al. 1999). Human disease is also more common during cooler months, but whether this is a factor of its psychotropic nature or human behaviors is not known (Ray et al. 2004).

Specific control measures for *Y. enterocolitica* have been primarily directed at control of contamination of carcasses with fecal contamination. The use of a plastic bag to seal the rectum during slaughter greatly reduced *Y. enterocolitica* contamination of carcasses (Nesbakken et al. 1994).

Shiga toxin-producing *escherichia coli*

Shiga toxin-producing *E. coli* (STEC) represents an estimated prevalence of 265,232 illnesses annually (Scallan et al. 2011) in the United States. Of these 96,534 are estimated to be caused by serogroup O157. Acute symptoms of STEC include severe stomach cramps, diarrhea (sometimes bloody), and vomiting. Of those diagnosed with STEC infection, 5–10% develop a potentially life-threatening complication known as hemolytic uremic syndrome (HUS).

Concerns regarding non-O157 STEC are important for pork production as current evidence suggests that pigs are more likely to be infected with non-O157 STEC as compared with the O157 serogroup.

STEC (stx_{2e} toxin producing) have long been known to be the etiological agent associated with edema disease in swine that cause gastrointestinal and central nervous signs in pigs post weaning. There is a lack of clarity in the literature regarding the role of stx_{2e} STEC strains from swine for human clinical disease (Beutin et al. 2008; Sonntag et al. 2005; Werber et al. 2008). Recent cross-sectional studies in the US swine herd have isolated STEC strains that have the potential to cause human illness (Fratamico et al. 2004). In the study by Fratamico et al. (2004), 31.8% of pigs were STEC positive. Of particular interest was the fact that all isolates were non-O157 serotypes. In fact, a total of 29 STEC serotypes harboring stx_1, stx_2, or stx_{2e} were identified. Tseng et al. (2015) in a descriptive study on one commercial swine farm found that 65% of finishing swine shed STEC at least once and that there was significant diversity of serotypes, with 19 different serotypes identified.

Epidemiologically, pork products are infrequently associated with foodborne illness as compared with food products contaminated with ruminant feces (Bettelheim 2007). Nonetheless, outbreaks and illnesses have been associated with pork products (Bettelheim 2007; Conedera et al. 2007; Honish et al. 2017). Further research is needed to assess pork's contribution to the burden of foodborne illness from STEC.

Trichinella spiralis

Trichinella spiralis is a nematode parasite that encysts in the tissues of a broad range of wild and domestic animals. Transmission is via the consumption of these tissue cysts in raw or undercooked meat products derived from infected animals. While there are eight species of *Trichinella*, only *T. spiralis* is considered important in domestic livestock. Pigs have traditionally been considered the domestic livestock most likely to be infected with *Trichinella* due to past practices such as feeding of uncooked meat containing waste products and outdoor access to wildlife carcasses. These risk factors are largely eliminated in modern confinement production systems.

Symptoms and clinical signs of trichinellosis in humans include eosinophilia, abdominal pain, fever, periorbital edema, myalgia, and, rarely, death (Kennedy et al. 2009). In the United States, reported trichinellosis cases have fallen from 393 during 1947–1952 to 15 annual case compatible reports in the period from 2008 to 2012 (Wilson et al. 2015). This decline in human cases has mirrored the decline in prevalence of *Trichinella* in commercial pork products that has resulted from the move to confinement rearing of pigs (Gamble and Bush 1999). The majority of trichinellosis cases in the United States are currently linked to consumption of wild game, especially bear (Wilson et al. 2015).

Cooking, curing, and freezing are used to inactivate *T. spiralis* in pork. *Trichinella* larvae can be killed at temperatures as low as 55 °C; however, uneven heat distribution should be taken into account during the cooking process to assure that all portions of the meat reach the proper temperature. Freezing remains effective in killing *Trichinella* in pork since freeze-resistant species of *Trichinella* in the United States have low infectivity and persistence in pigs (Kapel and Gamble 2000).

In the United States the NAHMS conducts a statistically based survey of pork production every 5–6 years. Since 1990 serology for *Trichinella* has been conducted on a portion of animals in the survey. The seroprevalence has fallen from 0.16% in 1990 to 0.013% in 1995, and no positive samples identified in the 2000 and 2006 surveys. This virtual elimination of *Trichinella* in the commercial pig industry has been accompanied by an increase in the number of production sites reporting adherence to good production practices that reduce the risk of *Trichinella* infection (Hill, personal communication). However, the move to non-confinement production systems may signal a potential increasing risk for human cases of trichinellosis. Gebreyes et al. (2008) reported 2 positive samples from 324 animals tested in niche-market, outdoor, antibiotic-free herds compared with 0 positive samples from 292 animals sampled in conventional US herds.

While individual carcass testing has historically been utilized by governments to assure freedom from *Trichinella*, international standard setting agencies for animal health and food safety (OIE and Codex Alimentarius, Anonymous 2015c) have published criteria to develop and maintain negligible risk compartments through consideration of a combination of historical data, serology, and audits of premises supplying animals.

Toxoplasma gondii

While felines are the definitive host of the protozoan parasite *Toxoplasma gondii*, a wide range of species have been shown to be susceptible to infection by *T. gondii*. Humans can become infected with *T. gondii* through three potential routes: exposure to oocysts shed in cat feces, consumption of under- or uncooked meat from infected animals, or congenitally when infection occurs in a susceptible pregnant woman. *T. gondii* is a public health concern, and in the United States it is estimated that 500–5000 newborns are born congenitally infected each year and that 1.26 million people are affected by ocular toxoplasmosis (Jones et al. 2009). Scallan et al. (2011) estimated that *T. gondii* was the second leading cause of foodborne illness deaths in the United States and the third leading cause of foodborne illness hospitalizations. This is largely due to reactivation of bradyzoites in immunocompromised patients.

Pigs become infected with *T. gondii* when they consume feedstuffs or other substances contaminated with cat feces containing infective oocysts. They can also be infected by consuming carcasses of rodents and other wildlife that have *T. gondii* muscle cysts or through cannibalism of infected swine carcasses. Traditionally, pork has been considered a source of human infection due to waste feeding and other dietary habits of swine that are largely eliminated by modern pork production methods.

Nationwide surveys of the US swine herd are conducted every 5–6 years by the USDA as part of NAHMS. In 1990 only sows were tested and 20% were seropositive, in 1995 15% of sows and 3.2% of finishers were seropositive, in 2000 6% of sows and 0.9% of finishers were seropositive, and in 2006, when only finishers were sampled, 2.6% were seropositive (Hill et al. 2009). It should be noted that the serological method changed in 2006 from the MAT to an ELISA that makes direct comparison of these results difficult. In 2012, utilizing the same laboratory methods as were used in 2006, seropositivity in finishers was 0.8% (Bush, personal communication). The NAHMS 2012 results are similar to what was observed by McKean et al. (2003) in 2002 who found a 0.75% seroprevalence in Midwestern market swine and Gebreyes et al. (2008) who found a 1.1% seroprevalence in confined pigs in three states.

The NAHMS surveys also gathered information on production practices that were considered to be potential risk factors for *Toxoplasma*, and analysis of that data found that farms where pigs were not housed in total confinement were 7.7 times more likely to be positive than total confinement farms (Hill et al. 2009). Additional risk factors included not using baits/poisons/traps for rodent control and certain carcass disposal techniques (Hill et al. 2009). Similar findings were reported by Gebreyes et al. (2008) with extensively raised pigs almost seven times more likely to be seropositive than pigs in confined herds. Breeding sites in NAHMS 2012 that utilized cats for rodent control were almost 70% positive compared with 39.1% for sites that did not use cats (Bush, personal communication).

It is uncertain what percentage of human infection is due to exposure to cat feces versus consumption of infected meat. A cross-sectional seroprevalence study compared Seventh-Day Adventist (non-meat eaters) with a control group (meat eaters) in one region of Maryland and found that seroprevalence was approximately 50% less for the non-meat-eating group (Roghmann et al. 1999). However, concerns with that study include that the seroprevalence of the control group (31%) was approximately twice the national average seroprevalence in the United States, while the non-meat-eating group had a similar seroprevalence to the national average (Jones et al. 2003). Additionally, the control group was recruited from commercial fishermen, seafood processing plant workers, and visitors to a seafood festival. This is important since bivalves such as oysters and clams can concentrate oocysts and consumption of raw oysters, clams, or mussels has been shown to be a risk factor for *T. gondii* seropositivity (Jones et al. 2009).

Retail meat sampled from 28 major geographical areas across the United States found that viable tissue cysts were isolated from 0.38% of pork samples and 0.57% of pork samples had antibodies to *T. gondii*. A case-control study of US patients with recent *T. gondii* infections identified eating raw ground beef, rare lamb, or locally produced cured, dried, or smoked meat, working with meat, drinking unpasteurized goat's milk, and having three or more kittens as significant risk factors (Jones et al. 2009). Consumption of pork was not a significant risk factor in that study; however, the move to niche pork from non-confined production systems may increase the risk of exposure to *T. gondii* in the United States.

Taenia solium

Taenia solium is commonly called the pork tapeworm, although the definitive host is actually humans. Swine are the natural intermediate host for the parasite. Humans acquire taeniosis by eating undercooked pork that contains cysticerci, the larval form of *T. solium*. The adult worm is found in the intestine of infected humans, and infective eggs are regularly shed in the feces. In endemic areas inadequate sanitation allows pigs access to human feces containing infective *Taenia* eggs. Upon ingestion the eggs hatch in the pig's intestine and migrate throughout the body preferentially encysting in the striated or cardiac muscle (Acha and Szyfres 2003a).

The main public health concern arises when humans ingest the eggs shed in the feces of infected humans. This can occur through fecal contamination of water or food

or autoinoculation. In this case, the *Taenia* larva migrates throughout the human as it would in the pig and forms cysticerci. In humans, the principal site of migration is the central nervous system, resulting in neurocysticercosis (NCC) (Carabin et al. 2005a).

The World Health Organization has designated cysticercosis a "major neglected disease" (Savioli and Daumerie 2010). NCC is reported to be one of the main causes of preventable epilepsy in low income countries. Coyle et al. (2012) estimate up to 7.65 million people have epilepsy due to NCC in Latin America, India, China, and Africa. Carabin et al. (2005b) estimated that the costs for medical care and lost productivity due to NCC in India were $634 million annually. It should be noted that more than 90% of Indians are either Muslim or Hindu and do not consume pork. However, the inadequate sanitation results in person-to-person transmission and allows for access of pigs to human feces completing the parasite life cycle.

In the United States cysticerci are identified in very few hog carcasses. In 1998, only 1 carcass of more than 75 million inspected by the USDA was condemned due to visible cysticerci. Although infection in pigs is rare, it is estimated that 18,584 hospitalizations for NCC with associated hospital charges totaling >$908 million occurred in the United States between 2003 and 2012 (O'Neal and Flecker 2015). Nearly 75% of the hospitalized patients were Hispanic, a growing immigrant population from areas of endemic cysticercosis.

Non-foodborne zoonoses

Many zoonotic diseases are a risk from direct contact with swine. Although many of the previously discussed foodborne diseases can be transmitted via direct contact, the following are those that are primarily transmitted via methods that do not include a food vector.

Methicillin-resistant *Staphylococcus aureus* (MRSA)

Staphylococcus aureus is a common organism found on the skin and mucous membranes of animals and humans. It can function as an opportunistic pathogen causing superficial and/or invasive infections. A subset of *S. aureus* are resistant to methicillin with this resistance encoded by the *mec*A gene, which codes for penicillin-binding protein 2a that confers resistance to all beta-lactam antimicrobials (Kluytmans 2010). Approximately 20–30% of healthy people in developed countries harbor *S. aureus* in the nasal cavity (Sivaraman et al. 2009), and it was estimated in the United States that 18.6% of people are colonized with *S. aureus* and 1.5% with methicillin-resistant *Staphylococcus aureus* (MRSA) (Gorowitz et al. 2008).

MRSA emerged as a health-care-associated infection in the 1960s, and in the late 1990s community-acquired MRSA was recognized (Naimi et al. 2003). In 2004 a new MRSA strain was identified in Holland that was resistant to digestion with the restriction endonuclease *Sma*1 when typing with PFGE was attempted and was associated with contact with pigs (Voss et al. 2005). This strain belongs to the multilocus sequence type ST398. MRSA ST398 is usually resistant to tetracycline and generally is resistant to a narrower range of other antimicrobials than most health-care-associated MRSA strains. MRSA ST398 has been referred to as the livestock-associated strain and has been found in several other food and companion animal species. Since the discovery of MRSA in Dutch swine herds, numerous studies have been conducted, and the presence of MRSA in pigs, pork producers, and veterinarians has been reported globally. This has led to the recognition that additional MLST sequence types of *S. aureus* are associated with pigs, particularly ST9 in Asia and ST5 in North America (Chuang and Huang 2015; Sun et al. 2015).

The European Food Safety Authority (EFSA) conducted a Europe-wide survey of swine farms in 2008, and 17 of 24 member states identified positive farms with a prevalence of 22.8% (EFSA 2009a). Positive farms that raised breeding stock were identified in 12 of 24 member states with a prevalence of 14% (range 0–46%), and 16 of 24 member states had MRSA-positive farms raising market hogs with a prevalence of 26.9% (range 0–51%) (EFSA 2009b). ST398 was the predominant type isolated with a mean of 1.4% of isolates being non-ST398 (EFSA 2009b). The first North American reports of MRSA in pigs were in 2008 when Khanna et al. (2008) reported that 9 of 20 farms and 24.9% of pigs were positive with the predominate spa type fitting into the clonal complex 398. In the United States, one published paper reported high levels of colonization in pigs (70%) within one production system, while another production system was negative for MRSA ST398 (Smith et al. 2009c). Subsequent studies in the United States have documented the presence of ST398, ST5, and ST9 MRSA in US swine, but current data indicate herd prevalence on the order of 10% or less (Smith et al. 2013; Sun et al. 2015).

Risk factors for MRSA-positive status of swine/swine farms include animal source, herd size, age/time in herd, transport and lairage, and perhaps others (Broens et al. 2009, 2010). The role of antimicrobial use in MRSA status is uncertain, with some studies suggesting an association and others not demonstrating a significant association (Broens et al. 2010). It has been suggested that due to the tetracycline resistance observed in ST398, tetracycline use contributes to its presence in swine herds. Aarestrup et al. (2009) analyzed methicillin-sensitive *Staphylococcus*

aureus (MSSA) ST398 and MRSA ST398 isolates from Danish swine. They found that a similar percentage of both MRSA and MSSA were resistant to tetracycline; however, 74% of MRSA ST398 were resistant to zinc chloride, while none of the MSSA ST398 showed zinc resistance. They concluded that use of non-antibiotic zinc compounds to prevent post weaning diarrhea in piglets may contribute to the emergence of MRSA in Danish swine herds.

Swine workers were sampled in several studies, and those working in facilities with positive pigs have higher than expected rates of nasal colonization, although there is little evidence to indicate that they have higher than expected rates of MRSA clinical disease. Veterinarians that work with all species of animals also have been reported to have higher rates of colonization with MRSA than the general public. Invasive illness with ST398 MRSA has been reported, albeit rarely, and published studies often make it difficult to determine if human cases are nasal carriers or indeed have clinical infection. It has been concluded in Europe that the potential for humans colonized with ST398 to contribute to the spread of MRSA in hospitals is less than for the healthcare-associated MRSA strains.

MRSA ST398, as well as other strains of MRSA, has been isolated from pork and other meats globally (de Boer et al. 2009; Kluytmans 2010; van Loo et al. 2007; Weese et al. 2010a). Public health agencies in the United States and Europe have not found evidence that contaminated meats contribute to an increased risk of MRSA (Centers for Disease Control and Prevention 2008). Weese et al. (2010a) quantified the amount of bacteria in positive samples purchased at retail stores throughout Canada and found that 37% of positive samples were below the detection threshold for quantification and <100 CFU per gram were found in most quantifiable samples.

Streptococcus suis

Streptococcus suis is common in domestic swine worldwide. Clinical signs in pigs include central nervous system signs (head tilt and paddling), arthritis, and polyserositis. A recent review and meta-analysis (van Samkar et al. 2015) identified 913 patients with *S. suis* meningitis included in 24 studies between 1980 and 2015. The mean age was 49 years and 82% of patients were male. Sixty-one percent had exposure to pigs or pork. Fever, headache, and neck stiffness were the most common symptoms, and 78 of 384 patients (20%) had a skin injury in the presence of pig/pork contact. The case fatality rate was 2.9%, and hearing loss was a common sequel occurring in 259 of 489 patients (53%).

In 2005 over 200 human cases of *S. suis* were reported in China. Human cases were largely associated with butchering and handling uncooked meat products from clinically ill animals (Gottschalk and Segura 2007; Yu et al. 2006). Twenty-eight percent of the Chinese cases of 2005 suffered a toxic shock-like syndrome (Yu et al. 2006). Investigation of the Chinese outbreak revealed that most cases had close contact with sick or dead pigs and many were involved in the slaughter or butchering of the animals (Gottschalk and Segura 2007; Yu et al. 2006). Furthermore, the investigation also identified that the human cases were caused by one clone of *S. suis* (Gottschalk and Segura 2007). A pathogenicity island has been found in the genome of isolates from the Chinese cases, but its exact role and function in *S. suis* virulence are poorly understood (Chen et al. 2007).

Clostridium difficile

The role of *Clostridium difficile* as a zoonotic agent from pigs is unknown. *C. difficile* is a gram-positive spore-forming anaerobe that is an important cause of enteric disease in humans. It is one of the most commonly diagnosed causes of hospital- and antibiotic-associated diarrhea in people and is emerging as a community-associated pathogen. *C. difficile* has also been isolated from numerous animal species (Gould and Limbago 2010; Jhung et al. 2008), meats (Norman et al. 2009; Songer et al. 2007; Weese et al. 2009), vegetables (Bakri 2009), hospital, barn and household environments (Baverud et al. 2003; Weese 2010), water (al Saif and Brazier 1996), pets (Borriello et al. 1983; O'Neill et al. 1993; Weese et al. 2010b), and healthy humans (McDonald et al. 2007; Rupnik 2010).

There are various schemes utilized to type *C. difficile* including PCR ribotyping, PFGE, and toxinotyping, and strains are often described using classifications from more than one of the different methods to improve discrimination. There are two strains of *C. difficile* that are of special interest in the apparently changing epidemiology of *C. difficile* in humans: a toxinotype III strain designated 027/NAP1 (NAP1) and a toxinotype V strain designated ribotype O78. NAP1 has emerged as an important hospital-acquired infection with increased incidence and severity that is thought to be influenced by the use of fluoroquinolone antimicrobials (McDonald et al. 2005). The second strain, ribotype 078, is an important strain associated with the increased recognition of community-associated *C. difficile* infection. Both of these strains, as well as others, have also been isolated from animal and food sources. The overlap of strain types between humans, animals, and meat has led some to hypothesize that animals may be an important source of *C. difficile* human infections. However, there are three possible explanations for this overlap that must be considered: (1) a common source causing infections in both humans and animals, (2) human-to-animal transmission, or (3) animal-to-human transmission.

Clostridium difficile colitis is not uncommonly diagnosed in neonatal pigs (Songer 2004), although diagnosis is complicated as results of commercial toxin tests in feces often poorly correlate with culture results (Rupnik 2010; Songer 2004). Several studies have examined the prevalence of *C. difficile* shedding among pigs in North America and globally. Methodological differences in sampling and isolation make comparison of apparent prevalence between studies impractical. If fecal shedding is considered as a source for contamination of pork that could potentially serve as a public health threat, it is important to note that prevalence of fecal shedding in piglets is higher than in adult swine. Weese et al. (2010c) noted that there was a significant decrease in colonization over time with 74% of piglets on day 2, 56% on day 7, 40% on day 30, 23% on day 44, and 3.7% shedding on day 62. This is similar to what Norman et al. (2009) observed with 50% of nursing pigs and only 3.9% of grower/finisher pigs with positive fecal cultures as well as Gebreyes (2009) who found 74.5% of pre-weaned piglets, 0.45% of nursery pigs, and 0% of finishing pigs to be shedding. The majority of the isolates in these studies were toxinotype V although other types were also isolated.

Studies of retail meats, including pork, have demonstrated the presence of *C. difficile*. Techniques vary between studies, so it is difficult to compare the prevalence between studies. Most of the studies also used enhancement steps that make enumeration of *C. difficile* spores in these products impossible. Weese et al. (2009) found that 71% of 14 ground pork samples were positive by enrichment culture only. Of the 4 that were positive by direct culture, 20 spores/g were present in 3, while 60 spores/g were present in 1. This would indicate that while *C. difficile* contamination of meat is not uncommon, levels of contamination are low. The infectious dose of *C. difficile* in either healthy or compromised humans is not known.

Influenza A viruses

Influenza A virus is an RNA virus, and its genome is composed of 8 separate segments that encode up to 11 proteins. Influenza is a common pathogen in numerous species, and interspecies transmission has been documented. Interspecies transmission provides the virus an opportunity to reassort, or exchange gene segments, when two or more strains infect a cell at the same time. The 2009 pandemic H1N1 virus was called a "quadruple-reassortant" virus because it contained neuraminidase (NA) and matrix (M) gene segments from Eurasian swine influenza viruses combined with triple-reassortant proteins of North American swine influenza viruses human-origin polymerase B1 (PB1), avian-origin polymerase B2 (PB2) and polymerase A (PA), and classical swine-origin hemagglutinin (HA), nucleoprotein (NP), and nonstructural (NS) genes. The evolutionary analysis of the 2009 pandemic H1N1 shows that the generation of this strain was not likely a recent event (Smith et al. 2009a). In fact, in order to facilitate human-to-human spread, it probably adapted to the human host through secondary reassortments in humans (Ding et al. 2009). Smith et al. (2009b) raised the possibility that the 1918, 1957, and 1968 pandemic strains were generated through a series of multiple reassortment events and emerged over a period of years prior to the pandemic recognition. They also noted that each of these strains was produced by reassortment of a previously circulating human virus and at least one virus of animal origin (avian or mammalian) (Smith et al. 2009b).

There were a total of 37 civilian human cases of zoonotic swine influenza virus infection (now referred to as variant influenza A virus) reported between 1958 and 2005 (Meyers et al. 2007; Van Reeth 2007). A majority of these infections were with classical swine H1N1 viruses, and the total does not include the Fort Dix cases in 1976 that resulted in 1 death and up to 230 infected soldiers (Meyers et al. 2007; Van Reeth 2007). Since 2005, over 400 cases of variant influenza A infections in humans have been reported in the United States (Anonymous 2017b). Persons identified as infected with the variant influenza A virus typically had close contact with pigs, and 8 of 11 cases had contact with pigs and often were children (Anonymous 2017b). Antibodies to swine influenza may be present in up to 23% of humans with occupational exposure to pigs, although the presence of antibodies is also associated with other factors such as age and previous influenza vaccination (Van Reeth 2007). It is not clear if the seropositivity of this population relates to exposure or clinical illness.

Hepatitis E virus

Hepatitis E virus (HEV) is the leading cause of enterically transmitted sporadic non-A and non-B hepatitis in tropical and subtropical countries. Inadequate sanitation had led waterborne HEV epidemics in these countries (Meng 2000). Mortality due to HEV infection is usually low (<1%) except in pregnant women where mortality in Asia and Africa approached 20%. While human cases of HEV are only sporadically reported in the United States and other industrialized countries, serosurveys indicate a larger proportion of the population has been exposed to the virus (Meng et al. 2002). Swine workers in the United States and Taiwan have been shown to be at increased risk of seroconversion, but history of clinical disease among these workers is largely lacking (Hsieh et al. 1999; Meng et al. 2002; Withers et al. 2002). Those working with young pigs on farms are at higher risk of infection than workers (Geng et al. 2011).

Meng et al. (1997) first reported an HEV from swine that was closely related to, but distinct from, human HEV isolates. An HEV was subsequently identified in swine in Taiwan that was distinct from the US swine

strain but similar to the human strains in Taiwan (Hsieh et al. 1999). Generally, HEV strains identified are genetically related, and molecular studies of human and swine HEV isolates globally found that swine and human isolates from the same geographic region are more similar to each other than they are to swine or human HEV isolates from other regions (Clemente-Casares et al. 2003; Meng 2003). Swine HEV appears to be ubiquitous with incidence of swine HEV antibodies in swine herds globally varying by geographical region and age of animals sampled, ranging from 4.1 to 79% (Smith 2001). Anti-HEV antibodies have also been identified in rodents, chickens, dogs, cattle, sheep, and goats (Meng 2000).

In 2003 in Japan, the first direct evidence of zoonotic transmission of HEV was documented in two separate incidents involving human consumption of undercooked pork liver and raw sika deer meat (Yazaki et al. 2003). HEV RNA was identified in 1.9% of raw pork livers in Japanese grocery stores and in 14 of 127 pork livers in US grocery stores (Feagins et al. 2007; Yazaki et al. 2003). While sporadic cases such as those in Japan and France provide strong evidence that HEV is zoonotic, it is uncertain what the contribution of swine HEV is to the human burden of illness due to HEV. In a study of young health adults at a university in the United States anti-HEV seroprevalence was 6.27% with odds of a subject who answered they "always" or "occasionally" consumed undercooked meat being positive for anti-HEV antibodies were 12.9 times the odds of those who answered "never" (Cossaboom et al 2016). The same study identified HEV RNA in 3 of 12 packages of chitterlings purchased in grocery stores in Southwest Virginia but not in the other 116 packages of non-liver pork products. Figatellu (raw pork liver sausage) has been implicated as the source of sporadic cases of HEV in France, and HEV-3 RNA has been recovered from supermarket-purchased figatelli samples, as well as anti-HEV antibodies detected in 7 of 13 humans consuming raw figatelli (Colson et al. 2010).

Meng et al. (1999) surveyed swine in four countries, two with a high prevalence of human HEV cases and two with a low prevalence of human cases. They found that swine HEV was endemic in all four countries regardless of the prevalence of human cases. Human exposure (as measured by antibody response) to HEV in both developed and developing countries is not uncommon, while clinical illness is rare in developed countries.

Japanese encephalitis

Even though Japanese encephalitis virus (JEV) in humans usually results in unapparent infections, it is the most frequent cause of mosquito-borne encephalitis globally (Oya and Kurance 2007; van den Hurk et al. 2009; Weaver and Reisen 2010). The distribution of JEV has expanded throughout East, Southeast, and South Asia and into the South Pacific (Oya and Kurance 2007; van den Hurk et al. 2009) and has been associated with increases in human population, irrigated rice production, and pig production.

Wading birds are considered the primary endemic hosts of JEV; however, pigs are important in the JEV transmission cycle as they are the only known mammals to fulfill the criteria as an amplifying host for the virus (van den Hurk et al. 2009). High levels of JEV infection in pigs often precede human epidemics (Acha and Szyfres 2003b), and sentinel pigs provide a useful system for estimating JEV risk in humans (Oya and Kurance 2007). In some regions of Asia, increasing pig populations have been linked to increasing human infections, but in Japan that trend was reversed. During the last 40 years, the number of pigs produced in Japan has increased, while the number of farms has decreased. As more pigs are raised in modern facilities that are segregated from residential areas, human cases of JEV have declined, demonstrating that pig farming can be maintained without increasing the transmission of JEV to humans (Oya and Kurance 2007). An Australian study (van den Hurk et al. 2008) determined that relocating domestic pigs did not prevent virus in mosquito populations.

Nipah virus

Nipah virus (NV) was first identified in 1999 in Malaysia during an outbreak of respiratory illness and encephalitis in pigs accompanied by an often fatal encephalitis in people with close contact with pigs as well as abattoir workers (Tee et al. 2008) and military personnel involved in culling infected herds (Ali et al. 2001). There were also reports of human-to-human transmission of NV among health-care workers (Tee et al. 2008). NV has also been diagnosed as the cause of fatal encephalitis cases in people without close contact with pigs in Bangladesh during the winters of 2001, 2003, and 2004 (Bellini et al. 2005).

NV belongs to the genus *Henipavirus*, in the family *Paramyxoviridae*, and is closely related to the Hendra virus of equines (Bellini et al. 2005). Fruit-eating bats (*Pteropus* and *Chiroptera* species) are considered the natural reservoir for NV, and humans are infected either through contact with an intermediate host such as pigs or the exposure to infected bats, material/foods contaminated by bats, or direct human-to-human transmission (Bellini et al. 2005; Tee et al. 2008). The implicated host species of bats is found across Southeast and South Asia (Tee et al. 2008). Outbreaks in pigs have not been observed since the 1998–1999 outbreak in Malaysia.

Reston ebolavirus

Reston ebolavirus (REBOV) is the only member of the filovirus family that is thought to be pathogenic in Asian monkeys but not in African monkeys or humans (Morikawa et al. 2007). Other filoviruses are associated with acute fatal hemorrhagic disease of humans or nonhuman primates.

In July 2008 REBOV was identified in swine diagnostic samples from the Philippines that were being tested at the USDA Foreign Animal Disease Diagnostic Laboratory. These samples were associated with clinically affected animals thought to be affected with highly pathogenic porcine reproductive and respiratory syndrome virus (PRRSV). REBOV was only found in samples that were also positive for PRRSV. Six humans of 141 with swine exposure in the Philippines had positive IgG titers to REBOV, but no human illness was detected (Barrette et al. 2009). There was no evidence of any involvement of swine in the 2014–2016 Ebola virus outbreak (caused by *Zaire ebolavirus*) in West Africa.

Antimicrobial resistance

Antimicrobial resistance (AR) in bacterial pathogens is a global public health concern. It is clear that human consumption of antimicrobials provides selection pressure for AR infections in humans. The scientific and political debate is focused on the contribution of animal antimicrobial use to the burden of AR infections in humans.

Evidence of the contribution of antimicrobial use in animals to AR infections in humans includes foodborne pathogens (e.g. multidrug-resistant (MDR) *Salmonella* Newport and fluoroquinolone-resistant *C. jejuni*) as well as emergence of community-acquired commensal infections associated with poultry and swine (e.g. vancomycin-resistant *Enterococcus* spp.) (Aarestrup et al. 2000). Because AR genes can be horizontally transmitted between bacteria, nonpathogenic bacteria may serve as a reservoir of AR. AR bacteria zoonoses have led to the ban of subtherapeutic antimicrobial use in the European Union (Casewell et al. 2003) and the removal of specific uses of fluoroquinolones in chickens in the United States (Department of Health and Human Services, US Food and Drug Administration 2005). In 2017, the US FDA implemented the changes outlined in Guidance #213 that eliminated the use of medically important antimicrobials for growth promotion and required a veterinary order or prescription for the remaining uses of those same antimicrobials (Anonymous 2017a).

The evidence regarding the impact of antimicrobial use in animals and the ultimate impact on human health is mixed. For example, in Denmark, the removal of avoparcin as a growth promotant for poultry and swine has decreased the prevalence of vancomycin-resistant *Enterococcus* (Anonymous 2009c). Yet, there was increased use of antimicrobials for therapeutic purposes in swine following the ban and certainly mixed results regarding AR changes in some pathogens as a result (e.g. *Salmonella*). A systematic review (Young et al. 2009) comparing organic production with antimicrobial-using production systems found that there was an association with antimicrobial use and fluoroquinolone resistance in *Campylobacter* from poultry and generally increased AR in bacteria isolated from other species, yet AR bacteria were also isolated from organically produced animals and animal products. Unintended consequences to food safety as a result of impaired animal health from subtherapeutic antimicrobial removal have been demonstrated (Singer et al. 2007). The use of macrolides in food animals has been suggested to present an extremely low probability of human treatment failure (Hurd et al. 2004).

A best practice for control of emergence of AR bacteria for both human and animal health is the judicious use of antimicrobials. Judicious use guidelines for antimicrobials have been developed (Anonymous 2009d).

Certification programs

There are certification programs within the United States that focus on production practices that will help to assure safe and wholesome pork products. All of these programs have some level of third-party oversight that certifies the education of, or adherence to, the production practices.

Pork Quality Assurance Plus

The Pork Quality Assurance (PQA) program was originally introduced in 1989 to address concerns over violative drug residues in pork. Subsequent revisions widened the scope of the program to address a broader range of physical, chemical, and microbiological hazards. The PQA Plus, an expanded version of the original PQA, was introduced by the Pork Checkoff in 2007. PQA Plus was developed as a continuous improvement program, retaining the original focus with added emphasis on the responsible use of antimicrobials in pork production, animal caretaker training, and animal care and well-being. Currently the PQA Plus program also addresses workplace safety, environment, and community. PQA Plus has been well accepted by the US meat packers, and most of them require either certification or site assessment status of the producers who supply them with pigs.

Safe Feed/Safe Food

The American Feed Industry Association (AFIA) established the Safe Feed/Safe Food Certification Program, which now operates independently of AFIA. The Safe Feed/Safe Food Certification Program is a voluntary, third-party-certified initiative aimed at feed mills and feed- and ingredient-related facilities in the United States and Canada. The program has established standards addressing documentation, training, facility planning and control, manufacturing and processing, monitoring devices, infrastructure, ingredient purchasing, traceability, and control of nonconforming products.

References

Aarestrup FM, Agerso Y, Gerner-Smidt P, et al. 2000. *Diagn Microbiol Infect Dis* 37:127–137.

Aarestrup FM, Cavaco L, Hasman H. 2009. *Vet Microbiol* 142:455–457.

Acha PN, Szyfres B. 2003a. Cysticercosis. In *Zoonoses and Communicable Diseases Common to Man and Animals*, Vol. III. Washington, DC: Pan American Health Organization, pp. 166–175.

Acha PN, Szyfres B. 2003b. Japanese encephalitis. In *Zoonoses and Communicable Diseases Common to Man and Animals*. Washington, DC: Pan American Health Organization, pp. 172–179.

Alban L, Stärk KDC. 2005. *Prev Vet Med* 68:63–79.

Ali R, Mounts AW, Parashar UD, et al. 2001. *Emerg Infect Dis* 7:759–761.

Anonymous. 1994. Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). Title 21 Code of Federal Regulations, Pt. 530. 1994 ed. Downloaded on January 2, 2017. http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/ActsRulesRegulations/ucm085377.htm.

Anonymous. 1995. Swedish *Salmonella* control programmes for live animals, eggs, and meat, 1995. Commission Decision of 23rd of February, 1995. 95/50/EC. 2-23-1995. Ref Type: Statute

Anonymous. 1996. Hazard Analysis Critical Control Point/Pathogen Reduction Act. Federal Register 61, 38805–38855.

Anonymous. 2008. Public Health Risk-Based Inspection System for Processing and Slaughter: Appendix D, Data Sources. USDA Food Safety and Inspection Service National Advisory Committee on Meat & Poultry Inspection. Downloaded on December 21, 2010. http://www.fsis.usda.gov/OPPDE/NACMPI/Feb2008/Processing_Appendix_D_041808.pdf.

Anonymous. 2009a. *Salmonella* on U.S. Swine Sites—Prevalence and Antimicrobial Susceptibility. USDA APHIS VS Info Sheet N536.0109. Downloaded on December 21, 2010. http://www.aphis.usda.gov/animal_health/nahms/swine/index.shtml.

Anonymous. 2009b. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 States, United States, 2008. MMWR 58:333–337.

Anonymous. 2009c. Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. DANMAP 2009—Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark, Copenhagen, Denmark.

Anonymous. 2009d. American Association of Swine Veterinarians Basic Guidelines of Judicious Therapeutic Use of Antimicrobials in Pork Production. Downloaded on December 21, 2010. http://www.avma.org/issues/policy/jtua_swine.asp.

Anonymous. 2011. The Nationwide Microbiological Baseline Data Collection Program: Market Hogs Survey August 2010—August 2011. Downloaded on June 12, 2017. https://www.fsis.usda.gov/wps/wcm/connect/d5c7c1d6-09b5-4dcc-93ae-f3e67ff045bb/Baseline_Data_Market_Hogs_2010-2011.pdf?MOD=AJPERES.

Anonymous. 2015a. National residue program for meat, poultry and egg products. FY 2014 Residue Sample Results. Downloaded January 6, 2017. https://www.fsis.usda.gov/wps/wcm/connect/2428086b-f8ec-46ed-8531-a45d10bfef6f/2014-Red-Book.pdf?MOD=AJPERES.

Anonymous. 2015b. Dioxin FY2013 Survey: Dioxins and dioxin-like compounds in the U.S. domestic meat and poultry supply. Downloaded on January 6, 2017. https://www.fsis.usda.gov/wps/wcm/connect/da1d623d-3005-4116-bef7-2a61d1ebd543/Dioxin-Report-FY2013.pdf?MOD=AJPERES.

Anonymous. 2015c. Guidelines for the control of Trichinella spp. In meat of suidae. CAC/GL 86-2015. Adopted in 2015. Downloaded on June 12, 2017. www.fao.org/input/download/standards/13896/CXG_086e_2015.pdf.

Anonymous. 2016. National residue program for meat, poultry and egg products. 2016 Residue Sampling Plans, USDA, FSIS. Downloaded January 6, 2107. https://www.fsis.usda.gov/wps/wcm/connect/04d78d46-c519-428c-a856-fe6416ae9e18/2016-Blue-Book.pdf?MOD=AJPERES.

Anonymous. 2017a. FDA announces implementation of GFI #213, outlines continuing efforts to address antimicrobial resistance. Downloaded January 31, 2017. http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm535154.htm.

Anonymous. 2017b. Reported infections with variant influenza viruses in the United States since 2005. Downloaded April 24, 2017. https://www.cdc.gov/flu/swineflu/variant-cases-us.htm.

Bakri M. 2009. *Emerg Infect Dis* 15:817–818.

Barrette RW, Metwally SA, Rowland JM, et al. 2009. *Science* 325:204–206.

Baverud V, Gustafsson A, Franklin A, et al. 2003. *Equine Vet J* 35:465–471.

Bellini WJ, Harcourt BH, Bowden N. 2005. *J Neuroviorol* 11:481–487.

Bettelheim KA. 2007. *Crit Rev Microbiol* 33(1):67–87.

Beutin L, Kruger U, Krause G, et al. 2008. *Appl Environ Microbiol* 74(15):4806–4816.

Borriello S, Honour P, Turner T, et al. 1983. *J Clin Pathol* 36:84–87.

Bowman AS, Glendening C, Wittum TE, et al. 2007. *J Food Prot* 70(1):11–16.

Broens EM, Graat EA, van der Wolf PJ, et al. 2009. Transmission of MRSA ST398 during transport of pigs from farm to slaughter house and during time spent in lairages at the slaughterhouse. In Proceedings of American Society Microbiology Conference on Methicillin-resistant *Staphylococcus* in Animals: Veterinary and Public Health Implications, p. 16.

Broens EM, Graat EAM, van der Wolf PJ, et al. 2010. Prevalence study and risk factor analysis on MRSA in

sow-herds. In Proceedings of the International Pig Veterinary Society Congress, p. 120.
Carabin H, Budke CM, Cowan LD, et al. 2005a. *Trends Parasitol* 21:327–333.
Carabin H, Cowen LD, Willingham AL, et al. 2005b. The monetary impact of Taenia solium cysticercosis in four countries. Presentation – American Public Health Association. Philadelphia, PA.
Casewell M, Friis C, Marco E, et al. 2003. *J Antimicrobial Chemother* 52:159–161.
Cossaboom C, Heffron C, Cao D, et al. 2016. *J Med Virol* 88(9):1641–1645.
Centers for Disease Control and Prevention. 2008. Letter to the U.S. House of Representatives Agricultural Committee.
Chase CCL, Daniels CS, Garcia R, et al. 2008. *J Swine Health Prod* 16:254–261.
Chen C, Tan J, Dong W, et al. 2007. *PLoS One* 2:e315
Christensen, SG. 1980. *J Appl Bacteriol* 48:377–382.
Chuang YY, Huang Yc. 2015. *Int J Antimicrob Agents* 45(4):334–340.
Clemente-Casares P, Pina S, Buti M, et al. 2003. *Emerg Infect Dis* 9(4):448–454.
Colson P, Borentain P, Queyriaux B, et al. 2010. *J Infect Dis* 202(6):825–834.
Conedera G, Mattiazzi E, Russo F, et al. 2007. *Epidemiol Infect* 135:311–314.
Coyle CM, Mahanty S, Zunt JR, et al. 2012. *PLoS Negl Trop Dis* 6(5):e1500.
De Boer E, Zwartkruis-Nahuis JTM, Huijsdens XW, et al. 2009. *Int J Food Microbiol* 134:52–56.
Denagamage TN, O'Connor AM, Sargeant JM, et al. 2007. *Foodborne Pathog Dis* 4:539–549.
Department of Health and Human Services, US Food and Drug Administration. 2005. Final Decision of the Commissioner, Docket No. 2000N-I 57 1. Withdrawal of approval of the new animal drug application for enrofloxacin in poultry.
Ding N, Wu N, Xu Q, et al. 2009. *Virus Genes* 39(3):293–300.
Dixon B. 2009. *Curr Biol* 19:R3–R4.
EFSA. 2009a. Joint scientific report of ECDC, EFSA and EMEA on meticillin resistant Staphylococcus aureus (MRSA) in livestock, companion animals and foods. EFSA-Q-2009-00612 301,1-10 and EMEA/CVMP/SAGAM/62464/2009.
European Food Safety Authority. 2009b. Analysis of the baseline survey on the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008, Part A: MRSA prevalence estimates. *EFSA J* 7:1376–1377.
Feagins AR, Opriessing T, Guenette DK, et al. 2007. *J Gen Virol* 88:912–917.
Fratamico PM, Bagi LK, Bush EJ, et al. 2004. *Appl Environ Microbiol* 70:7173–7178.
Fredriksson-Ahomaa M, Korkeala H. 2003. *Clin Microbiol Rev* 16:220–229.
Fukushima H, Nakamura R, Ito Y, et al. 1983. *Vet Microbiol* 8:469–483.
Funk J, Gebreyes WA. 2004. *J Swine Health Prod* 12:246–251.
Funk JA, Troutt HF, Isaacson RE, et al. 1998. *J Food Prot* 61(6):677–682.
Gamble HR, Bush E. 1999. *Vet Parasitol* 80:303–310.
Gebreyes W. 2009. Epidemiology, Toxino- and Geno-typing of *Clostridium difficile* in swine at farm, slaughter and retail. National Pork Board Research Reports. Downloaded December 21, 2010. www.pork.org on.
Gebreyes WA, Bahnson PB, Funk JA, et al. 2008. *Foodborne Path Dis* 5:199–203.
Geng J, Wang L, Wang X, et al. 2011. *J Viral Hepat* 18(10):e583–e590.
Goldbach SG, Alban L. 2006. *Prev Vet Med* 77:1–14.
Gorowitz RJ, Kruszon-Moran D, McAllister SK, et al. 2008. *J Infect Dis* 197(9):1226–1234.
Gottschalk M, Segura M. 2007. Lessons from China's *Streptococcus suis* outbreak: The risk to humans. In Proceedings of American Association of Swine Veterinarians Annual Meeting.
Gould LH, Limbago B. 2010. *Clin Infect Dis* 51:577–582.
Hill DE, Haley C, Wagner B, et al. 2009 *Zoonoses Public Health* 57:53–59.
Hoff SJ. 2016. Evaluating strength, sharpness, and detection of swine-use hypodermic needles – NPB#13-055. http://research.pork.org/FileLibrary/ResearchDocuments/13-055-HOFF-ISU.pdf.
Hoff SJ, Sundberg P. 1999. Breakage and deformation characteristics of Japanese and U.S, manufactured hypodermic needles under static and dynamic loading. In Proceedings of the American Association of Swine Practitioners, pp. 217–227.
Honish L, Punja N, Nunn S, et al. 2017. *Can Commun Dis Rep* 43(1):21–24.
Horrocks SM, Anderson RC, Nisbet DJ, et al. 2009. *Anaerobe* 15:18–25.
Hsieh SY, Meng JJ, Wu YH, et al. 1999. *J Clin Microbiol* 37:3828–3834.
Hurd HS, McKean JD, Griffith RW, et al. 2002. *Appl Environ Microbiol* 68:2376–2381.
Hurd HS, Doores S, Hayes D, et al. 2004. *J Food Prot* 67:980–992.
Huwe J. 2002. *J Agric Food Chem* 50:1739–1750.
Huwe J, Pagan-Rodriguez D, Abdelmajid N, et al. 2009. *J Agric Food Chem* 57:11194–11200.
Jhung MA, Thompson Ad, Killgore GE, et al. 2008. *Emerg Infect Dis* 14:1039–1045.
Jones JL, Kruszon-Moran D, Wilson M. 2003. *Emerg Infect Dis* 9:1371–1374.
Jones JL, Dargelas V, Robert J, et al. 2009. *Clin Infect Dis* 49:878–884.
Kapel CM, Gamble HR. 2000. *Int J Parasitol* 30:215–221.
Kennedy ED, Hall RL, Montgomery SP, et al. 2009. *MMWR Surveill Summ* 58:1–7.
Khanna T, Friendship R, Deway C, et al. 2008. *Vet Microbiol* 122:366–372.
Kluytmans JAJW. 2010. *Clin Microbiol Infect* 16:11–15.
Kupferschmidt K. 2011. *CMAJ* 183(4):E221–E222.
Liem AKD, Furst P, Rappe C. 2000. *Food Addit Contam* 17:241–259.

McDonald LC, Killgore GE, Thompson A, et al. 2005. *N Engl J Med* 353(23):2433–2441.
McDonald LC, Coignard B, Dubberke E, et al. 2007. *Infect Control Hosp Epidemiol* 28:140–145.
McKean JD, Beary J, Brockus S, et al. 2003. Determination of Toxoplasma gondii antibody prevalence in midwest market swine. In Proceedings of 5th International Symposium on Epidemiol Control Foodborne Path in Pork, pp. 223–224.
McLaren IM, Wray C. 1991. *Vet Rec* 129:461–462.
Mead PS, Slutsker L, Dietz V, et al. 1999. *Emerg Infect Dis* 5:607–625.
Meng XJ. 2000. *J Hepatol* 33:842–845.
Meng XJ. 2003. *Curr Top Microbiol Immunol* 278:185–215.
Meng XJ, Purcell RH, Halbur PG, et al. 1997. *Proc Natl Acad Sci* 94:9860–9865.
Meng XJ, Dea S, Engle RE, et al. 1999. *J Med Virol* 59:297–302.
Meng XJ, Wiseman B, Elvinger F, et al. 2002. *J Clin Microbiol* 10:117–122.
Meyers KP, Olsen CW, Gray GC. 2007. *Clin Infect Dis* 44:1084–1088.
Morikawa S, Saijo M, Kuran I. 2007. *Comp Immunol Microbiol Infect Dis* 30:391–398.
Mousing J, Jensen PT, Halgaard C, et al. 1997. *Prev Vet Med* 29:247–261.
Naimi TS, LeDell KH, Como-Sabitti K, et al. 2003. *JAMA* 290:2976–2984.
Nesbakken, T, Nerbrink E, Rotterud O, et al. 1994. *Int J Food Microbiol* 23:197.
Norman KN, Harvey RB, Scott HM, et al. 2009. *Anaerobe* 15:256–260.
O'Connor AM, Denagamage T, Sargeant JM, et al. 2008. *Prev Vet Med* 87:213–228.
O'Neal SE, Flecker RH. 2015. *Emerg Infect Dis* 21(6):969–976.
O'Neill G, Admas JE, Bowman RA, et al. 1993. *Epidemiol Infect* 111:257–264.
Oya A, Kurance I. 2007. *J Travel Med* 14:259–268.
Plym-Forshell L, Eskebo I. 1996. *Acta Vet Scand* 37:127–131.
Ray SM, Ahuja SD, Blake PA, et al. 2004. *Clin Infect Dis* 38(Suppl 3):S181–S189.
Roghmann MC, Faulkner CT, Lefkowitz A, et al. 1999. *Am J Trop Med Hyg* 60(5):790–792.
Rupnik M. 2010. *Clin Infect Dis* 51:583–584.
al Saif N, Brazier JS. 1996. *J Med Microbiol* 45:133–137.
van Samkar A, Brouwer MC, Schultsz S, et al. 2015. *PLoS Negl Trop Dis* 9(10):e0004191.
Savioli LS, Daumerie D. 2010. *First SHO Report on Neglected Tropical Diseases: Working to Overcome the Global Impact of Neglected Tropic Diseases*. Geneva: World Health Organization, pp. 1–169.
Scallan E, Hoekstra RM, Angulo FJ, et al. 2011. *Emerg Infect Dis* 17(1):7–15.
Schiemann, DA. 1989. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. In Doyle MP, ed. *Foodborne Bacterial Pathogens*. New York: M. Dekker, pp. 601–672.
Singer RS, Cox Jr LA, Dickson JS, et al. 2007. *Prev Vet Med* 79:186–203.
Sivaraman K, Venkataraman N, Cole AM. 2009. *Future Microbiol* 4(8):999–1008.
Smego RA, Frean J, Koornhof HJ. 1999. *Eur J Clin Microbiol Infect Dis* 18:1–15.
Smith JL. 2001. *J Food Prot* 64:372–380.
Smith FJ, Vijaykrishna D, Bahl J, et al. 2009a. *Nature* 459:1122–1125.
Smith GJD, Bahl J, Vijaykrishna D, et al. 2009b. *PNAS* 106:11709–11712.
Smith TC, Male MJ, Harper AL, et al. 2009c. *PLoS One* 4(1):e4258.
Smith TC, Gebreyes WA, Abley MJ, et al. 2013. *PLoS One* 8(5):e63704.
Songer JG. 2004. *Anim Health Res Rev* 5:321–326.
Songer JG, Trinh HT, Killgore GE, et al. 2007. *Emerg Infect Dis* 15(5):819–831.
Sonntag AK, Bielaszewska M, Mellmann A, et al. 2005. *Appl Environ Microbiol* 71(12):8855–8863.
Sun J, Yang M, Sreevatsan S, et al. 2015. *PLoS One* 10(11):e0143670.
Tee KK, Takebe Y, Kamarulzaman A. 2008. *Int J Infect Dis* 13:307–318.
Teratanavat R, Hooker NH. 2004. *Food Control* 15:359–367.
Thakur S, Gebreyes WA. 2005. *J Food Prot* 68:2402–2410.
Toma S, Deidrick VR. 1975. *J Clin Microbiol* 2:478–481.
Tseng M, Fratamico PM, Bagi L, et al. 2015. *Epidemiol Infect* 143(3):505–514.
Tsubokura M, Fukuda T, Otsuki K, et al. 1976. *Nippon Juigaku Zasshi* 38:1–6.
Van den Hurk AF, Ritchie SA, Johansen CA, et al. 2008. *Emerg Infect Dis* 12(11):1736–1738.
Van den Hurk AF, Ritchie SA, Mackenzie JS. 2009. *Annu Rev Entomol* 54:17–35.
Van Loo IHM, Diedren BMW, Savelkoul PHM, et al. 2007. Methicillin-resistant *Staphylococcus aureus* in meat products, the Netherlands. *Emerg Infect Dis* 13:1753–1755.
Van Reeth K. 2007. *Vet Res* 38:243–260.
Voss A, Loeffen F, Bakker J, et al. 2005. *Emerg Infect Dis* 11:1965–1966.
Weaver SC, Reisen WK. 2010. *Antiviral Res* 85:328–345.
Weese JS. 2010. *Clin Microbiol Infect* 16:3–10.
Weese JS, Avery BP, Rousseau J, et al. 2009. *Appl Environ Mircrobiol* 75:5009–5011.
Weese, JS, Wakeford R, Reid-Smith R, et al. 2010a. *Anaerobe* 16(5):501–504.
Weese JS, Finley R, Reid-Smith RR, et al. 2010b. *Epidemiol Infect* 138:1100–1104.
Weese JS, Avery BP, Reid-Smith RJ. 2010c. *Lett Appl Microbiol* 51:338–342.
Werber D, Beutin L, Pichner R, et al. 2008. *Emerg Infect Dis* 14(11):1803–1806.
Wilson NO, Hall RL, Montgomery SP, et al. 2015. *MMWR* 64(SS01):1–8.
Withers MR, Correa MT, Morrow M, et al. 2002. *Am J Trop Med Hyg* 66:384–388.
Yazaki Y, Mizuo H, Takahashi M, et al. 2003. *J Gen Virol* 84:2351–2357.
Young I, Rajić A, Wilhelm BJ, et al. 2009. *Epidemiol Infect* 137(9):1217–1232.
Yu H, Jing H, Chen Z, et al. 2006. *Emerg Infect Dis* 12:914–920.

13

Special Considerations for Show and Pet Pigs

Amy L. Woods, Valarie V. Tynes, and Kristie Mozzachio

Show pigs

Size and scope of the show pig industry

The show pig sector is often overlooked by many in the swine industry since the overall numbers of animals are much fewer than the commercial swine production industry. However, the show pig industry is a significant business when the number of people and amount of money invested are considered. It is difficult to estimate the exact size and monetary value of the US show pig industry.

In February 2017, there were nearly 31,500 youth certified in the National Pork Board's (NPB) Youth Pork Quality Assurance program (NPB, personal communication, February 28, 2017). All of these youth are in the swine exhibition industry, as many shows require this certification for kids to show and sell their projects. There are nearly 14,000 youth involved as members of the National Junior Swine Association (NJSA), the youth sector of the National Swine Registry (NSR), which represents youth involved in showing Duroc, Hampshire, Landrace, and Yorkshire breeds at national shows (NJSA, personal communication, February 28, 2017). In addition, there are over 4200 youth members of Team Purebred, which represents youth exhibiting Berkshire, Chester White, Poland, Spotted, Hereford, or Tamworth breeds at national shows (Team Purebred, personal communication, March 1, 2017). These membership numbers have grown tremendously over the past few years, as more people become involved in the show pig industry. As seen in Figure 13.1, the number of pigs exhibited at these select national shows has grown tremendously over the past few years.

Ninety-nine percent of the NSR senior members are involved in production of animals for exhibition (NSR, personal communication, February 28, 2017). It is estimated that over 1 million pigs are raised for exhibition, or approximately 1% of the total swine industry in the United States (Warren Beeler, personal communication, February 28, 2017). However, that 1% has a big economic impact when considering the dollars spent not only on pigs, but specialized show feeds and supplements, show supplies, show pig semen, bedding, facilities, and travel costs for various shows. It has been estimated that this 1% of the swine population is a half-billion-dollar industry (Warren Beeler, personal communication, February 28, 2017).

With the growth of the show pig industry, a considerable "niche" market has developed for veterinary products and services. Because of the unique nature of show pigs, an awareness of some of the special considerations of the industry will allow practitioners to better address the needs of the pigs and the clients. There are different types of show pig clients – breeders that produce their own pigs to show and sell for exhibition projects. There are also those that purchase young pigs (typically purchased around 2 months of age) and only raise pigs to market size for exhibition. Some clients may be casual show pig producers with a goal of just having a project to show at a local fair. Others are extremely serious about the show pig industry and spend considerable time, money, and attention on their show pigs. Another type of show pig clients is the boar stud owners that market semen specifically to the show pig customer.

To be able to better serve show pig clients, a veterinarian should understand the show pig industry. First of all, it is important to realize that these individual animals can be extremely valuable – up to tens of thousands of dollars. Secondly, these animals are bred and raised primarily for exhibition, not necessarily for pork production. Thirdly, the same pigs may frequently be shown at various locations during a show season. The show season will vary depending on the geographical area of the United States, but typically lasts around 3 months. Many of the more serious show pig clients will participate in a jackpot show circuit, in which the pigs earn points and money for the placing they receive at numerous shows.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

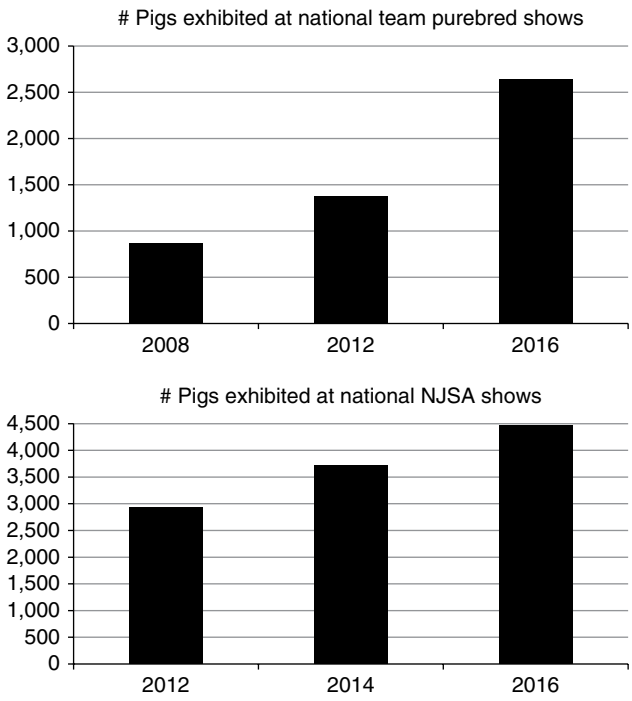


Figure 13.1 The recent growth of the show pig industry can be observed in these examples of the total numbers of pigs exhibited at these select national shows annually (NJSA and Team Purebred, personal communication, March 1, 2017).

Therefore, these pigs may be traveling to multiple shows per month throughout the show season. A survey conducted in 2014 of Midwest swine exhibitors indicated that an individual may exhibit swine at up to 50 shows in a single year (Bliss et al. 2017). At the culmination of the show season, pigs may be exhibited at a terminal show and go directly to harvest. Alternatively, many animals (particularly breeding animals) return home or are sold to another farm after the show season. This show schedule creates unique biosecurity and health concerns.

Biosecurity

As previously mentioned, show pigs are exposed to many other pigs from various sources when they are sold at auction and exhibited at shows. Figure 13.2 depicts the major events and opportunities for disease exposure in the life of a show pig. Some of the larger shows, such as Houston Livestock Show and World Pork Expo, will have 2000–3000 head of pigs on-site for a single show (NSR and Houston Livestock Show, personal communication, April 1, 2017). There is obviously a lot of opportunity for disease transmission in these situations.

When selecting a show pig to purchase, often health status or source is not considered. Pigs are purchased according to their pedigree and appearance. Veterinarians need to advise clients on proper biosecurity measures and the risks involved when comingling pigs from various sources. Prophylactic medication may also occur when purchasing these pigs and mixing them together for the first time, as respiratory and enteric disease is common in these situations. Veterinarians must also educate clients about taking proper biosecurity measures when bringing animals back home after a show. A survey conducted after the 2002 Indiana State Fair (at the end of the Indiana show season) indicated that nearly half of the pigs exhibited went back home or to another farm after the fair (Amass et al. 2004). Often there are no isolation or testing procedures in place for these animals returning from a show.

Veterinarians must be aware of the state and federal regulations regarding movement, identification, and exhibition of swine in their area since show pigs travel frequently, often crossing state lines. Veterinarians may also be hired by specific pig sales and shows to ensure compliance of all participants with any state and federal regulations regarding identification, certificates of veterinary inspection, and animal health. Since show pigs are primarily identified by ear notches, veterinarians must be proficient in reading standard ear notches to meet these regulations.

Zoonotic implications

Zoonotic disease potential is important in commercial pork production, but with show pigs there is increased risk due to the closer interaction between show pigs and their caretakers. Those caretakers are also children, which are more likely immunocompromised than adults working in commercial swine operations. In addition, members of the public can closely interface with animals at local fairs and exhibitions. Shiga-toxigenic *Escherichia coli* O157:H7 has been demonstrated to be present in cattle, pig, sheep, and goat feces at public fairs (Keen et al. 2006), and influenza A virus (IAV) infections have been documented in people with no swine exposure except for visiting a local fair in Wisconsin (Wells et al. 1991) and Ohio (Vincent et al. 2009). An outbreak of IAV at fairs occurred in Indiana and Ohio in 2012, resulting in numerous cases of IAV-positive pigs and humans (Boman et al. 2014). Bliss et al. (2016) found that IAV-positive pigs entered agricultural fairs in Indiana and Ohio at 5 out of the 9 (55%) fairs tested. Of those with IAV-positive pigs, prevalence ranged from 0.2 to 10.3% of incoming pigs testing positive via virus isolation on snout wipe samples. This virus can then spread rapidly through other pigs at the fair due to the close penning proximity and contacts with fomites such as scales, taggers, wash pens, etc.

Veterinarians must be available to quickly identify and treat and/or recommend removal of sick animals from a fair to minimize the zoonotic disease transmission risk. Veterinarians should also educate clients as well as the

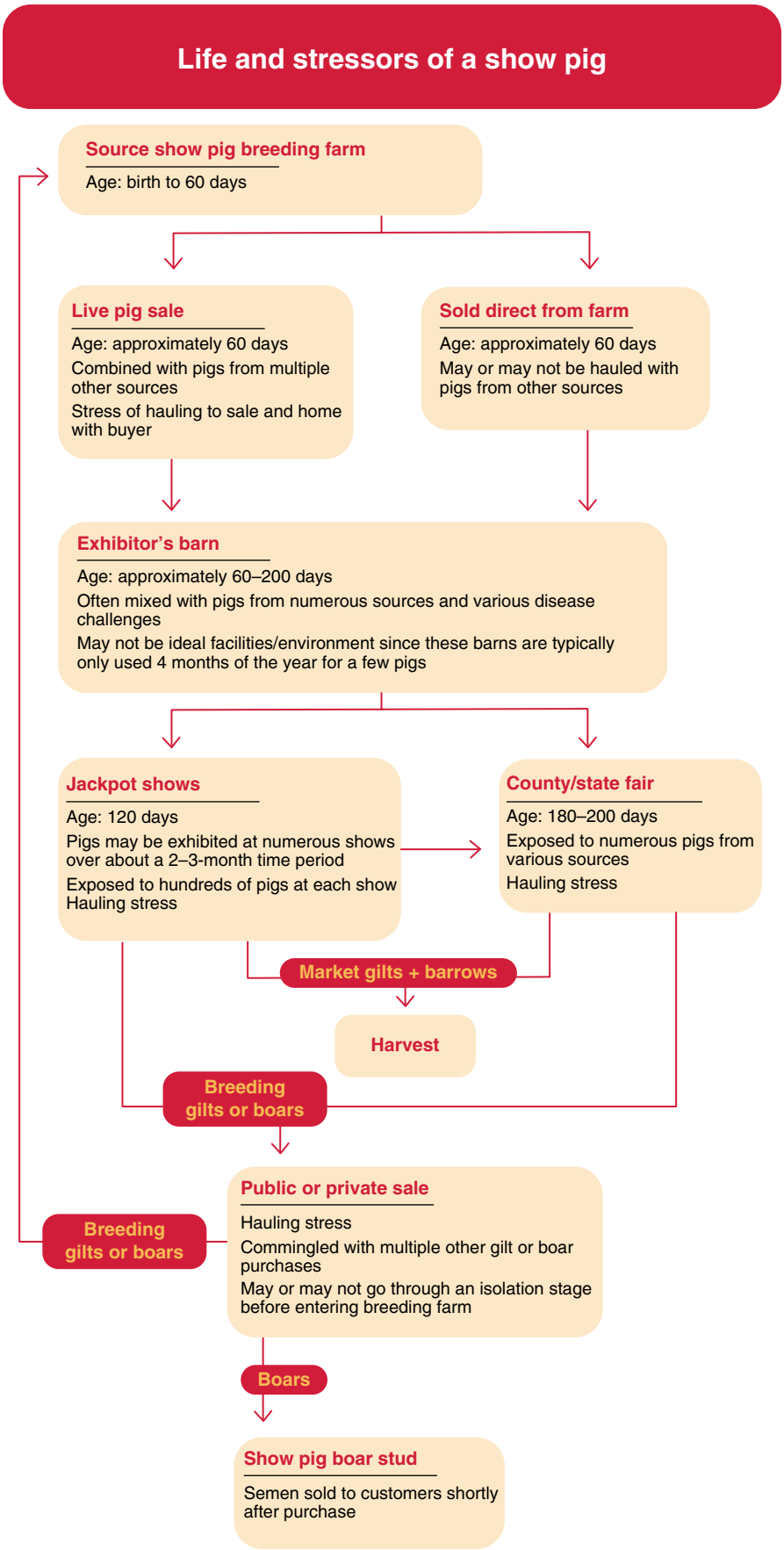


Figure 13.2 Timeline of major events and disease exposure of show pigs.

public about these zoonotic risks and may be expected to advise fair officials in the event of a zoonotic disease.

General health issues

Vaccination and health protocols

Veterinarians need to be involved with formulating a vaccination protocol for show pig clients. With most commercial farms, herd epidemiological information such as serology or diagnostics can be used to customize a vaccination and health management program. However, this is typically not practical when working with show pigs due to the small herd size. With federal changes mandating Veterinary Feed Directives (VFD) for many common feed-grade antibiotics, many show feed manufacturers have removed all antibiotics from their show feeds. Therefore, many small show pig clients will not have medicated feed for their pigs at any stage. This makes vaccination programs even more important at the breeder level to prevent common diseases such as ileitis or mycoplasma pneumonia.

Show pig health protocols must also include regular treatments for both internal and external parasites, as both are extremely common in show pigs. The risk of infestation with mange is higher in show pig herds due to the wide exposure to other animals at sales and shows. Show pigs are often housed and/or exercised on dirt lots or older barns where pigs have been present for many years, therefore increasing the exposure risk for internal parasites. It is common to utilize a rotation of antiparasitic products, such as fenbendazole, ivermectin, or doramectin throughout the growing period. Ensure that all show pig breeding herds are also routinely treated with antiparasitic products.

Individual animal medicine

Unlike commercial herds, where veterinarians are focused on population medicine, show pig veterinarians often focus on individual animal problems. Clients may call their veterinarian to examine a single sick, injured, or lame animal. Common problems such as pneumonia or scours should be treated the same as these issues are treated in a commercial farm. Lameness in show pigs is extremely common since they are exercised vigorously and are often very heavy muscled. Common lameness problems result from osteochondrosis (OCD) and osteoarthritis (OA), joint infections, hoof cracks, pad bruising, and traumatic injuries. Infectious causes of lameness in show pigs can often be related to *Mycoplasma hyosynoviae* or erysipelas. Lameness that would be of no consequence to a commercial pig can easily render a show pig unfit for their intended use. Most show pig clients are willing to invest in the best possible treatment as a first choice since they have a significant investment in the animal. Oral joint supplements containing glucosamine and chondroitin or hyaluronic acid are used extensively in show pigs to promote joint health and minimize problems from OCD and OA, although there is no efficacy data available for this practice. These supplements are often fed for several weeks leading up to a show or when an animal may start to show clinical signs of joint stiffness. In some cases, show pig owners will seek alternative therapies, such as chiropractic care, acupuncture, or laser therapy to address lameness. This is an opportunity for veterinarians that practice these alternative medicines.

In the past couple of years, there has been an increased prevalence of *Senecavirus A* (SVA) in the US swine industry, and the show pig industry has been affected with this virus as well. SVA typically presents in show pigs after they come home from a show, where they were exposed to numerous other pigs. It is the author's opinion that there are also many cases of SVA in show pigs that go undiagnosed due to the client's fear of reporting clinical signs to their veterinarian that may result in a subsequent foreign animal disease investigation. Veterinarians must encourage clients to always report any unusual clinical signs in their animals.

A common problem for outdoor show pig operations is sunburn. If burned severely enough, these pigs will have scabs and scarring across the back. In the acute stages, they will appear to have a neurological deficit, exhibiting an unusual gait in the rear legs induced by the pain across the back. Sunburns are often treated with over-the-counter aloe products and addition of shade in the pen. Many show pig producers prevent sunburn by applying a sunscreen to light-colored pigs before taking them outside. Veterinarians need to be aware of the use of these types of non-Food and Drug Administration (FDA)-approved topical treatments used by show pig producers.

Veterinarians are also asked to perform a variety of surgical procedures on show pigs. These procedures are not economically justifiable on most commercial farms. Examples of commonly requested surgical procedures for show pigs include the following:

1) Castration – Many show pigs remain boar prospects until an older age and require anesthesia for castration; or clients will grow the pig as a boar to maximize growth and muscling and not castrate the pig until 30–40 days before exhibition.
2) Scrotal hernia repair.
3) Abdominal hernias – May often also have abscess present and persistent umbilical structures.
4) Cryptorchidectomies – May be requested by breeder before castration if only one testicle is descended or requested by exhibitor after their pig starts exhibiting boar behaviors.
5) Abscess removal – Surgical cosmetic removal of abscesses resulting from castration, injections, etc.

6) Scirrhous cord removal – Due to post castration scarring when the vaginal tunic is not completely removed at castration.
7) Preputial diverticulectomies – Performed on boars or barrows to eliminate urine pooling, as many clients prefer not to manually express the contents routinely; also performed on boars that get their penis "balled up" in the diverticulum and do not extend their penis properly.
8) Penis problems – Examination and repair of a persistent frenulum or trauma; helpful to use 10 in. curved Bozeman uterine forceps to exteriorize the penis of an anesthetized boar.
9) Extra dewclaw amputation – Some breeds will not allow animals with extra dewclaws to be registered as purebreds, so the decision to perform this surgery may have ethical implications.
10) Rectal prolapse – Often after cool environmental conditions and piling of pigs, coughing or scouring, and gastrointestinal parasites – all conditions that are common when show pig prospects are purchased and brought home with pigs from other sources.

Anesthesia options and techniques for these surgical procedures can be found in Chapter 11.

Breeding management

Veterinarians are often consulted for assistance and advice on artificial insemination techniques and estrous management in show pig herds. Unlike commercial farms, show sows and gilts are typically bred to a specific boar based on the compatibility of phenotypes or predicted phenotypic outcome of the individual cross. Semen is purchased from boar studs that specialize in show pig semen, and doses can be very costly. Show pig boar stud biosecurity is not as stringent as most commercial boar studs, so clients need to be aware that there is a higher risk of disease transmission through semen. Boars in these studs have often been comingled at large shows before being purchased and brought into the stud, so disease risk is just as high as for all other show pigs.

Due to the timing of the show season, show pig producers desire to breed animals to farrow at very specific times. Veterinarians may need to aid and educate on heat synchronization options or breed and abort programs. In addition, many show pig herds are small and do not have a boar present to assist in heat detection and stimulation during breeding.

Farrowing management

Obstetrical difficulties are often more common in show animals for several reasons. Firstly, show pig producers tend to farrow more gilts in order to keep up with the most current genetic trends in the show industry. Secondly, there is often a disparity between the phenotypes of gilts that are successful in the show ring and the breeding herd. Thirdly, birth weights may be heavier due to lower litter size and/or the genetic type. Fourthly, show herd sows are often over-conditioned. The combination of these factors results in an increased rate of dystocia. Since many show pig producers are not as experienced at assisting sows with difficult dystocia as commercial producers and the animals have greater monetary value, veterinarians are called to provide obstetrical care. In extreme dystocia cases, Cesarean section may be indicated. Surgical techniques, as well as epidural and general anesthesia options, can be found in Chapter 11.

Nutrition

Nutritional aspects of the show pig industry are much different than the commercial industry since show pigs are fed to achieve a desired appearance and weight range for exhibition rather than for growth performance or efficient gain. Show pigs are typically fed much higher protein and fat diets than comparable commercial pigs. A wide variety of supplements and feedstuffs are utilized in show pig diets. Some of these include human foods or feedstuffs typically utilized by other species. Efficacy and safety of these supplements are generally not available. The desired appearance of successful show pigs tends to change every few years, and subsequently, the composition of feedstuffs and supplements also changes to produce the desired appearance. One common supplement that deserves special mention is ractopamine HCL, a repartitioning agent that promotes lean tissue production. Veterinarians can play an important role in educating clients on feeding the proper rate of this supplement in small batches of feed, as this supplement can easily be abused and fed at illegally high levels.

Veterinarians must be aware of some of these nontraditional feedstuffs and supplements, since there may occasionally be some health issues related to their use. For example, the increased fat in show pig diets has been known to result in steatitis in the inguinal and abdominal area. The desire to enter a pig in a specific weight class may result in withholding water or use of a diuretic, which can cause dehydration. It has also been common for show pig producers to feed altrenogest for increased feed consumption in not only gilts but also barrows and boars. This is not approved label use of this product and can negatively impact semen quality of boars (Kluber et al. 1985).

Education

The show pig veterinarian must be heavily involved in education of clients. Most show pig producers lack the

experience and knowledge of those producers in the commercial industry. Veterinarians must not only educate these producers on their individual pig projects but also on the swine industry and pork production. Veterinarians must be diligent in educating these clients about proper pharmaceutical use, administration, and withdrawal times since many pharmaceutical products are used in show pigs.

In addition to reaching individual clients, veterinarians are also asked to speak to 4-H or FFA groups about general pig care, health, and disease prevention. There are also opportunities to be involved in formal educational programs for show pig producers, such as the NPB Pork Quality Assurance Plus (PQA Plus) or Youth PQA Plus training sessions.

Ethics

Any veterinarian working with show pig producers must determine their own set of ethical standards concerning the procedures and recommendations they provide. As with many competitive industries, there are clients who will do anything to win and may ask their veterinarian to provide a service that is contradictory to good judgment or ethics. Practitioners must also be mindful of how their recommendations might affect all pork producers and consumers. Some categories of ethical dilemmas that veterinarians may face include altering animal identification, illegal drug use, and surgical procedures that alter conformation, among many others.

Drug use and testing in show pigs

Veterinarians working with show pigs must also understand drug testing regulations and procedures for various shows. Each show sets their own regulations regarding drug use, and this may vary greatly. Most shows will prohibit pharmaceutical products not approved by the appropriate state or federal agencies, which include the FDA in the United States. Others may also prohibit the use of any "performance-enhancing products," such as NSAIDS or corticosteroids, since they may mask a lameness problem. Even when these products are used according to label directions and meet legal withdraw times, pigs may be disqualified from shows for their use. Common substances such as caffeine may also be prohibited at certain shows. Some shows enforce a zero-tolerance policy on any drug residues found in an animal. Veterinarians and exhibitors must know the rules and regulations for a show before administering any treatments to a show pig. A veterinarian can prescribe treatment for an animal following all regulatory guidelines including established tissue withdraw times and still have that animal disqualified from a zero-tolerance show due to the extremely sensitive testing methods utilized. In addition to the extreme test sensitivity, most shows test urine samples, and the correlation between urine and tissue levels is not well established for all drugs.

Many shows will test animals for banned substances. Most of this testing is conducted on urine samples, although some shows may choose to test serum. For terminal shows, carcasses will often be detained, and multiple tissue samples (muscle, liver, retina, etc.) will be tested. Urine testing allows labs to test for not only the parent drug but also metabolites of that drug. Some of those metabolites can be detected in urine for much longer than in serum and may not be indicative of what drugs have been administered to the animal more recently. Most labs now test samples via liquid chromatography with mass spectrometry (LC-MS). LC-MS protocols often allow a lab to screen for multiple drugs simultaneously and detect very low concentrations of drug residues. Suspicious samples are re-extracted and analyzed with tandem mass spectrometry (MS/MS) for confirmation. Numerous drug classes are tested for, including but not limited to NSAIDs, corticosteroids, anabolic steroids, tranquilizers, sedatives, stimulants, antihistamines, antipsychotics, and muscle relaxers. It is not uncommon to find flunixin, dexamethasone, caffeine, theophylline, and theobromine to test positive in show pigs (Travis May, personal communication, February 21, 2017).

All practitioners working with exhibition swine must be aware of these drug testing policies and use extreme caution when treating or recommending treatment for any animal that will be attending a show with a drug testing policy. One sometimes must decide between treating an animal in the best welfare of the animal and the risk of getting an animal disqualified at a drug test.

Miniature pet pigs

While their diminutive size makes them more appropriate as pets, owners often need to be reminded that miniature pigs are still members of the family Suidae and share similar behaviors, environmental needs, diseases, and parasites. They are anatomically and physiologically much like the larger commercial varieties of swine, and, with a few exceptions, treatment will be similar.

Behavior and training

Aggression in the pet pig is a significant concern and results in the abandonment or re-homing of many pigs (Lord and Wittum 1997; Tynes et al. 2007). For that reason, pet owners should be counseled about the potential for problems as soon as possible after they acquire the pig. Most owners of pet pigs know little about the normal behavior of the pig, so they may miss

the initial signs of aggression. These signs may include lip smacking or chomping, stiffening and staring with piloerection of the hair along the back, and "head swiping." The head swipe is often, but not always, performed with a slightly open mouth and is a bite threat, probably often made with no intention to bite. It is a warning that says the pig wants you to stop doing what you are doing. If the warning goes unheeded, the behavior will likely escalate to an actual bite. No form of aggression directed toward humans, regardless of how mild, should be ignored in the hope that the problem will get better on its own. As is the case with most behavior problems, aggression rarely decreases without treatment, and it usually worsens with time if left untreated. Pet pigs usually begin displaying aggressive behavior toward humans as they mature socially (6 months to 3 years), and typically, the first victims of aggression will be visitors to the home (Tynes et al. 2007). This is believed to be a normal response to an unfamiliar individual, similar to how pigs behave when meeting unfamiliar conspecifics. Aggression toward familiar people within the home also commonly begins around the time of social maturity and appears to be a form of dominance aggression. The reasons for the behavior remain unclear, but it should be noted that pigs are animals that commonly use aggression among themselves in order to establish rank and ensure access to valued resources. When pigs become aggressive to their owners, it is usually in the context of resources such as resting places. If the owner's response is to punish the pig, aggressive behavior may escalate, due to the pig becoming fearful of the owner and using aggression in an attempt to stop what it perceives as a frightening threat. While many responses to this behavior have been described by pet owners and trainers, it is recommended that pig aggression be treated similarly to dog aggression; the animal should be taught that humans are the leaders of the group. Consistent, predictable reward-based interactions should be emphasized, and punishment avoided so that the pig learns to trust the owner. This is safest and easiest to accomplish by conditioning the pig to a harness and leash at a very early age; harnesses made specifically for miniature pigs are recommended and will fit better and be easier to use than harnesses intended for dogs. The pig should then be taught to respond to a simple verbal cue, such as "sit," before being given something that it desires. It is relatively simple to teach the pig to sit using a food lure, just as you would a dog. It should then be asked to sit before being fed, brushed, petted, invited onto furniture, allowed outside, etc.

The pig should always be wearing its harness when visitors are expected, and a responsible adult should have control of the leash whenever visitors arrive at the home. Once a visitor has been in the home for a few minutes, they can be allowed (if they desire to interact with the pig) to ask the pig to sit and reward it with small pieces of fruit, vegetables, dry cereals, or other food treats when it complies. Alternatively, the owners can simply be advised to separate their pig from all visitors by confining it to another room or pen whenever visitors arrive at the home. Avoidance of stimuli that lead to aggression is always the first and most important approach to problem behaviors. Notably, aggression directed toward humans seems to be a problem that occurs primarily in single-pig households (Tynes et al. 2007). As pigs are herd animals, the addition of a second pig can be an alternative method to alter undesirable behavior or, better yet, to avoid it altogether if another pig is introduced early. Many pig shelters and rescue groups require people to adopt two pigs at the same time, and this may prove to be an excellent method for decreasing the incidence of aggression. Additionally, it has been noted by some that a significant amount of outdoor exploration can improve aggressive tendencies.

Restraint

Physical

Just as with larger swine, miniature pigs resist firm restraint by struggling and vocalizing. Superficial examination and vaccination is possible using physical restraint, but for more thorough examinations, tusk trimming, and nail (hoof) trimming, chemical restraint is often necessary. Most owners of miniature pigs consider their pigs a pet and will be unhappy if their pig is treated as a farm animal. For that reason, most will prefer to pay for chemical restraint rather than see (or hear) their pig restrained while struggling and squealing. In addition, miniature pigs have a smaller, less stable cardiovascular system than large swine and can be stressed to the point of collapse. Due to their propensity to joint dislocation, they should never be lifted by their legs. Nose snares are rarely useful as the miniature pig may simply panic and thrash about; they do not reliably back away from the snare, and injuries have been reported with snare use.

To lift the small or medium-sized pet pig, place one arm in front of the front limbs and the other arm behind the rear limbs. Hold the pig firmly to your chest and move it as quickly and smoothly as possible to an examination table. Once the pig is on the table, do not try to firmly restrict its movements; it should simply be corralled with the arms. A rubber mat or other nonslip surface will greatly decrease the pig's distress and help keep it calm. If the person restraining the pig will scratch it firmly on the neck, flank, belly, or inner thigh, this will also help to keep the pig quiet and calm. Continuously feeding the pig small bites of food (if anesthesia is not planned) will typically keep it occupied and allow for examination and vaccination.

Larger pigs can be lifted by two people using a version of the "fireman's carry"; approaching the pig from each side, each individual places one arm in front of the pig's forelegs and the other arm behind the pig's rear legs. The two people then grasp elbows and lift the pig, squeezing it between them. The pig can then quickly be placed on an examination table.

Hammock-like slings with a hole cut for each of the pig's feet have also been used for pet pigs. The sling can be very useful for aiding restraint in some larger and less docile pigs. Many pigs will allow nail and teeth trimming while sitting in the sling.

Chemical

Achieving predictable results with injectable agents can be challenging in the miniature pig due to their thick layer of body fat. A 1½ or 2 in. needle may be required to deposit the injection in the muscle of adult pigs. The preferred site for injection of anesthetic agents is the semimembranosus or semitendinosus muscles. Injection into the gluteal muscles may lead to lameness. The commonly used dissociative anesthetic agents have been associated with prolonged and violent recovery and bizarre behavioral changes after recovery. However, when inhalation anesthesia is not available, dexmedetomidine (40 mcg/kg), butorphanol (0.3 mg/kg), and midazolam (0.3 mg/kg) combined in one syringe and administered IM have been found to be safe and reliable while offering a smooth recovery. In addition the effects of dexmedetomidine can be reversed with atipamezole to speed recovery.

Inhalation anesthesia has also been shown to be safe and effective and offers a quick and relatively predictable recovery. Most pigs can be easily masked down by including nitrous oxide with oxygen for 30–60 seconds initially and then very slowly turning on the anesthetic gas (isoflurane). Once the pig visibly relaxes, the nitrous should be discontinued.

Malignant hyperthermia has been rarely documented in the miniature pig and appears to be uncommon. The prevalence of the porcine stress syndrome (PSS) gene that predisposes a pig to malignant hyperthermia is currently unknown in the miniature pet pig population, although it is known to exist.

Miniature pigs should be monitored carefully during recovery from anesthesia as complications such as laryngeal edema, hypothermia, and cardiovascular compromise are not uncommon.

Vaccinations

In spite of the fact that many pet pigs will never be exposed to other swine, some vaccinations are still important to ensure their continued good health as well as prevent the potential spread of zoonotic diseases and diseases of economic interest to the commercial swine industry. No single vaccination protocol will be best for all pet pigs, but all of them should be routinely vaccinated against erysipelas (*Erysipelothrix rhusiopathiae*) as the organism is soilborne and has zoonotic potential. Swine are capable of contracting rabies although confirmed reports of rabid swine are quite small compared with other domestic mammals. Rabies has been reported in three miniature pet pigs between 1997 and 2003 (DuVernoy et al. 2008). Extra-label use of this vaccine should also be considered as a safety measure, in part because it may protect the pig from being euthanized by a local public health department in case of a bite to a human. Other vaccinations will depend upon the pig's age, prior vaccination status, the environment, and geographical area in which it resides as well as potential exposure to other pigs.

Parasites

Pet pigs are subject to the same parasites that can infect larger swine. Sarcoptic mange (*Sarcoptes scabiei*) is one of the more commonly seen problems. Miniature pigs often exhibit abundant dark crusty exudate in their external ear canals, but this appears to be normal, and ear mites (*Otodectes cynotis*) are rarely, if ever, found within the discharge. However, *S. scabiei* mites are often present in ear swabs of infested pigs.

Internal parasites such as roundworms, whipworms, and threadworms are less common in pigs acquired from reputable breeders, and large worm burdens are rare, as is clinical illness associated with endoparasites. All pet pigs should nevertheless have fecal flotations performed at 8–12 weeks of age. After reaching adulthood, fecal exams are less critical if the pig is maintained in a clean environment and is not exposed to environments where other swine have been. If parasites are identified on a fecal exam, the pet pig can be treated with the same anthelmintics at the same dosages as larger swine.

Teeth trimming

All miniature pigs have four permanent canine teeth that erupt at about 5–7 months and, in males, grow continuously. Even the tusks of castrated males can reach a dangerous length, although they usually grow more slowly than in intact males. The best-behaved pigs can do damage to furnishings and accidently harm humans with these long, sharp teeth, so regular occasional trimming may be needed. Anesthesia makes the procedure safer and easier for all involved. Obstetrical wire, high speed dental tools, and Dremel™ tools have all been used successfully. Crushing instruments should be avoided as they can fracture the tooth longitudinally causing pain and possibly infection. The teeth should be cut only as short as possible necessary for safety while avoiding the

pulp cavity, the length of which can vary between individuals. A sanding blade on a Dremel™ tool can be used to smooth the tooth so that no sharp edges remain. Removal of the canine teeth is extremely difficult and not recommended unless necessary due to fracture or infection.

Hoof trimming

Pet pigs that live in the home and get minimal exercise on rough surfaces usually have hooves that require regular trimming. Overgrown hooves hyperextend the joints of the legs and, along with obesity, are a common cause of lameness in pet pigs. Routine trimming is usually required and will be most easily performed when the pig is anesthetized. However, many pigs will tolerate the procedure while being restrained in a sling or held on their backs. Trimming is easily accomplished using trimmers, hoof nippers, and rasps as you might use for goats or horses. In some cases, especially where the hooves have become severely overgrown, the sensitive laminar tissue (the "quick") will be extended. Cutting deeply into the quick can cause lameness that lasts for several days and should be avoided when possible. After trimming, a Dremel™ tool can be used to smooth the nails and remove any rough edges.

Common surgical procedures

All female pigs not intended for breeding should be ovariohysterectomized. A high rate of reproductive tract neoplasia has been noted (Mozzachio et al. 2004) in pet pigs over 5 years of age. While these tumors are not usually malignant, they can reach extreme sizes, leading to discomfort, occasional colic-like signs, cardiovascular compromise, and death. Removal of the tract after the tumors have become very large is more difficult and places the patient at higher risk.

Ovariohysterectomy is easier for the surgeon and less stressful for the pig if performed prior to the pig reaching a weight of about 25 pounds. In a smaller animal, the immature reproductive tract can be more difficult to access within the deep ("potbellied") abdominal cavity, and in a heavier patient, the larger amount of subcutaneous and intra-abdominal body fat may make accessing exteriorizing the tract difficult. This can result in excessive bleeding and increase the risk of dehiscence. Obese patients are a greater anesthetic risk as well. A ventral midline approach is recommended. The use of a spay hook should be avoided as it can be damaging, leading to excessive bleeding within the fat, and is generally unnecessary. Closure of the skin using an absorbable suture and a buried subcuticular pattern and burying the knot alleviates the necessity of a later struggle to remove sutures.

All boars intended to be kept as pets should be castrated, preferably before 12 weeks of age. Intact boars are less likely to make good pets due to their unpredictable behavior and strong, objectionable odor. While early castration greatly reduces odor and leads to a decrease in size of the preputial diverticulum, odor and drainage can still occur in some pigs. Preputial diverticulectomy as described by Lawhorn et al. (1994) can greatly increases pet owner satisfaction in one author's experience. The procedure should be considered an option in all pet pigs castrated later than 2–3 years of age or any time that odor becomes an issue for the pet owner. Castration should be performed through a pre-scrotal incision using a technique similar to that used in the dog. The vas deferens and associated blood vessels should be ligated and excised. Removing the cremaster muscle, tunic, and extraneous subcutaneous tissues decreases dead space and reduces the chance of seroma formation. Closing the skin with absorbable sutures using a buried subcuticular pattern eliminates the need for the later restraint and struggle associated with suture removal.

Cryptorchidism and inguinal hernias are commonly occasionally seen in miniature pigs. Inguinal rings should be examined at the time of castration and surgically closed if patent.

Common disease problems and therapeutics

Physical exam on the pet pig should be conducted similarly to a physical exam on any animal. One of the noted differences between the miniature pigs and the larger commercial breeds of swine is that their normal resting rectal body temperature is likely to be lower, with temperatures as low as 37.6 °C (99.7 °F) reported in one study (Lord et al. 1999).

While subject to all of the same diseases that occur in other swine, illness is uncommon in the pet pig that is properly vaccinated, fed, and housed. However, health problems may include:

1) Obesity – Obesity is common in the pet due to lack of exercise and improper feeding. Many pet owners think obesity is a normal condition for all pigs, often purposely feeding to achieve over-conditioning; they are unaware of the numerous associated health problems associated with obesity in the pig. Obesity contributes to chronic lameness and blindness secondary to excessive fat accumulation around the eyes and places strain on the heart and lungs. Pet owners must be instructed as to the importance of feeding pet pigs a commercial diet made especially for miniature pigs. In addition, placing food in food dispensing toys (hollow balls or plastic jugs with holes cut in them), or simply broadcasting the ration over a clean, grassy area of the yard, requires that the pig expend more calories acquiring its food.

2) Arthritis – Miniature pigs have been reported to live as long as 23 years, with an average life span of 15–18 years. One of the more prevalent health problems associated with aging in the pig is OA. This is often secondary to chronic obesity and/or overgrown hooves but is also common with normal conformation. Ongoing lameness that eventually becomes nonresponsive to anti-inflammatories and painkillers is a common cause for euthanasia in the geriatric pig.
3) Dental disease – While pigs develop impressive amounts of dental tartar as they age, severe periodontal disease is uncommon. Regular teeth cleaning similar to that for a dog may be beneficial for some individuals. The most common dental problem seen in geriatric pigs is tusk root abscessation in males. This may present as chin or jaw abscesses that recur after initial treatment. Radiographs are diagnostic, often revealing extensive bone lysis. Successful treatment will require tusk removal.
4) Uterine neoplasia – See "Common Surgical Procedures" above.

Regulatory issues

Many pigs have been abandoned by their owners after they discover that zoning regulations prohibit the keeping of a pig in their community. In the eyes of many legal entities, a miniature pig is a farm animal and subject to the same federal rules and regulations governing the maintenance and movement of other swine. While most owners will deny the possibility of their pet pig ever entering the food chain, it has been reported (Lord and Wittum 1997; Mozzachio pers. obs.), and for this reason, caution must be used when administering or prescribing medications for the pet pig. Drugs illegal for use in food animals must be avoided, and discharge instructions should include mention of drug withdrawal times.

Acknowledgments

The authors gratefully acknowledge the assistance of Bruce Lawhorn, DVM, in the preparation of the pet pig section of this chapter.

References

Amass SF, Schneider JL, Kenyon SJ. 2004. *J Swine Health Prod* 12(6):282–284.
Bliss N, Nelson SW, Nolting JM, et al. 2016. *Zoonosis Public Health* 63:477–485.
Bliss N, Stull JW, Moeller SJ, et al. 2017. *J Am Vet Med Assoc* 251(6):706–713.
Boman AS, Nelson SW, Page SL, et al. 2014. *Emerg Infect Dis* 209:1472–1480.
DuVernoy TS, Mitchell KC, Myers RA, et al. 2008. *Zoonoses Public Health* 55:431–435.
Keen JE, Wittum TE, Dunn JR, et al. 2006. *Emerg Infect Dis* 12(5):780–786.
Kluber E, Pollmann D, Davis D, et al. 1985. *J Anim Sci* 61:1441–1447.
Lawhorn BL, Jarrett PD, Lackey GF, et al. 1994. *J Am Vet Med Assoc* 205:92–96.
Lord LK, Wittum TE. 1997. *J Am Vet Med Assoc* 211:562–563.
Lord LK, Wittum TE, Anderson DE, et al. 1999. *J Am Vet Med Assoc* 215:342–344.
Mozzachio K, Linder K, Dixon D. 2004. *Toxicol Pathol* 32:402–407.
Tynes V, Hart B, Bain M. 2007. *J Am Vet Med Assoc* 230:385–389.
Vincent A, Swenson S, Lager K, et al. 2009. *Vet Microbiol* 137(1–2):51–59.
Wells D, Hopfensperger D, Arden N, et al. 1991. *J Am Med Assoc* 265(4):478–481.

Section II

Body Systems

14

Cardiovascular and Hematopoietic Systems

Nicholas A. Robinson and Alan T. Loynachan

Introduction

The cardiovascular and hematopoietic systems are essential components that conjointly aid in the distribution of oxygen, nutrients, minerals, proteins, and cellular constituents to the body systems. Disease or functional disruption of either system can have detrimental effects. This chapter will briefly highlight normal porcine anatomy and physiology and the pathology of frequently encountered porcine cardiovascular and hematopoietic diseases.

Anatomy and physiology

A thorough understanding of anatomy and physiology is essential for the appropriate detection and evaluation of porcine cardiovascular abnormalities. Further review of veterinary references, such as the *Textbook of Veterinary Anatomy* (Dyce et al. 2010) and *Duke's Physiology of Domestic Animals* (Reece et al. 2015), may be beneficial.

Hematopoietic system

There is approximately 56–69 mL of blood per kilogram of body weight in the pig (Fox et al. 1984). Blood is composed of both acellular and cellular components. Acellular constituents (water, minerals, electrolytes, gases, acid–base regulatory ions, proteins, lipids, and carbohydrates) are regulated by other body systems and are introduced into the cardiovascular system for tissue distribution and excretion. Both direct and indirect alterations of acellular biomarkers can be important indicators of locomotor, digestive, respiratory, urogenital, endocrine, cardiovascular, or nervous disease. Reference intervals for porcine acellular biochemical analytes can be found in Table 14.1.

The hematopoietic system generates the cellular components of the blood, which include lymphoid (lymphocytes) and myeloid (nonlymphoid leukocytes and erythrocytes) elements. Hematopoiesis is primarily localized to the bone marrow of adult animals but regularly occurs in extramedullary tissues in fetal and neonatal animals. Bone marrow is not frequently evaluated in swine, but myeloid to erythroid reference ratios of 1.77–2 : 1 are reported (Jain 1986; Sanderson and Phillips 1981). Porcine hematological parameters fluctuate based on age, breed, sex, diet, stage of gestation, lactation, management practices, and season. As a result, normal complete blood count (CBC) reference intervals are quite broad (Table 14.2). Friendship et al. (1984) and *Schalm's Veterinary Hematology* (Thorn 2010) are useful citations for reference intervals based on age, sex, gender, and other confounding factors.

Porcine erythrocytes typically circulate for 86 days before removal from the circulatory system. Morphologically, porcine erythrocytes average 6 µm in diameter, lack basophilic stippling, and commonly exhibit poikilocytosis (Brockus 2011; Thrall 2004). Immature forms of red blood cells (RBCs), nucleated RBCs, and reticulocytes normally circulate in healthy pigs but are found in higher proportions in suckling animals (Brockus 2011).

The structure and function of porcine leukocytes and platelets are similar to those seen in other domestic mammals. *Schalm's Veterinary Hematology* (Thorn 2010) is a useful reference for morphological and functional characteristics of pig leukocytes, particularly for age, castration, pregnancy, and parturition status-related differences.

Cardiovascular system

Anatomically, the heart is located in the mediastinum and extends from the second to fifth ribs and is essentially a one-way pump composed of four chambers: The low-pressure side consists of the right atrium and ventricle carrying blood to the lungs for oxygenation. The high-pressure side consists of the left atrium and ventricle carrying blood to the systemic circulation for metabolic

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 14.1 Porcine clinical biochemistry reference intervals.

Biochemical analyte	Reference interval	Unit
Aspartate aminotransferase (AST)	0–125	U/L
Alanine aminotransferase (ALT)	0–103	U/L
Alkaline phosphatase (ALP)	0–300	U/L
γ-Glutamyl transferase (GGT)	0–82	U/L
Glutamate dehydrogenase (GD)	0–8	U/L
Creatine kinase (CK)	0–10,101	U/L
Lactate dehydrogenase (LDH)	0–1,893	U/L
Total protein	49–67	g/L
Albumin	19–29	g/L
Globulins	28–41	g/L
Albumin/globulin ratio	0.52–0.95	—
Urea	1.7–4.5	mmol/L
Creatinine	88–130	μmol/L
Total bilirubin	0.0–1.0	μmol/L
Cholesterol	2.0–4.2	mmol/L
Triglycerides	0.3–2.7	mmol/L
Free fatty acids	0.0–1.0	mmol/L
Glucose	4.3–8.6	mmol/L
Inorganic phosphate	2.8–4.3	mmol/L
Calcium	2.5–3.1	mmol/L
Magnesium	0.9–1.2	mmol/L
Sodium	143.0–156.0	mmol/L
Potassium	4.8–7.8	mmol/L
Sodium/potassium ratio	19.4–28.8	—
Chloride	99.5–112.3	mmol/L
Iron	9–54	μmol/L

Source: Adapted from Klem et al. (2010). Reproduced with permission of John Wiley and Sons.

Table 14.2 Porcine hematological reference intervals.

Hematology analyte	Reference interval	Unit
Red blood cells (RBCs)	6.4–8.4	$\times 10^{12}$/L
Hemoglobin concentration (HGB)	105–135	g/L
Hematocrit (HCT)	0.34–0.44	%
Mean corpuscular volume (MCV)	49–59	fL
Mean corpuscular hemoglobin concentration (MCHC)	287–325	g/L
Erythrocyte distribution width (RDW)	15–24	%
Platelets	211–887	$\times 10^{9}$/L
White blood cells (WBCs)	15.6–38.9	$\times 10^{9}$/L
Neutrophils	3.0–17.4	$\times 10^{9}$/L
Lymphocytes	7.7–20.4	$\times 10^{9}$/L
Monocytes	0.6–3.4	$\times 10^{9}$/L
Eosinophils	0.1–2.3	$\times 10^{9}$/L
Basophils	0.1–0.3	$\times 10^{9}$/L
Large unstained cells (LUCs)	0.1–1.4	$\times 10^{9}$/L

Source: Adapted from Klem et al. (2010). Reproduced with permission of John Wiley and Sons.

use. A series of heart valves (atrioventricular [AV] and semilunar) work to enable the one-way flow by restricting the backflow of blood into the preceding chamber. Arteries and arterioles carry blood to the peripheral tissues where oxygen exchange occurs at the capillary level. Deoxygenated blood then returns to the heart by a series of venules and veins. Fluid that escapes from the blood returns to the vascular system by lymphatic vessels.

The pericardium is a thin fibroserous sac that completely surrounds the heart. It acts to reduce motion within the thoracic cavity but at the same time allows almost frictionless movement within the sac. 1–2 mL of clear serous, lightly viscous fluid is normally located within the pericardium. The myocardium is located beneath the epicardium and is composed of specialized striated muscle, which generates the force necessary to pump blood. The endocardium lines the inner layer of the heart including valves and is composed of vascular endothelium with subjacent supporting collagen, elastin, and small caliber blood vessels. Specialized electrical conduction (Purkinje) fibers are distributed throughout the heart but are most prominent in the subendocardium. Conduction fibers coordinate the rhythmic depolarization of the cardiomyocytes, which results in muscular contraction. Cardiac electrical impulse is influenced by the autonomic nervous system and is generated by the pacemaker sinoatrial (SA) node. The SA node chronologically and subsequently transmits electrical impulse to the AV node, bundle of His, right and left bundle branches, and Purkinje fibers, which ultimately stimulate cardiomyocytes and result in the synchronized contraction of the heart.

Postmortem examination of the heart

The heart should be visualized in a consistent and methodical manner so that the pericardium, myocardium, mural and valvular endocardium, and great vessels are examined. All lesions identified should be grossly described and placed in fixative for microscopic examination by a pathologist. The thoracic cavity is carefully opened so that the lungs and heart can be visualized and the pericardial sac is examined and incised *in situ* before any organs are removed. Normal epicardium and pericardium are smooth and glistening due to the presence of a small amount of serous fluid. The pericardium is then examined for thickening, adhesions, the presence of

fibrin, and the volume and nature of the pericardial fluid. Swabs, fluid samples, and tissue samples should be taken if abnormalities are evident.

Next, the heart can be removed from the pluck and examined for abnormal shape, size, and color unless congenital defects of the great vessels (e.g. PDA) or thrombosis (particularly pulmonary artery) is suspected. Rigor mortis occurs rapidly in the heart compared with skeletal muscle and may give the heart a very firm or even rigid appearance. Prior to the onset of rigor mortis, the heart can appear flabby or enlarged as the chambers may dilate antemortem due to myocardial disease or secondarily to postmortem autolysis. Heart chambers that dilate prior to death frequently have endocardial fibrosis or thickening that appears as diffuse whitening. Histopathology should be performed on these sections to definitively rule out underlying myocardial disease.

A common method used to ensure consistency when examining the heart is to view myocardial structures in the direction of normal blood flow. The right atrium should be opened from the caudal vena cava to the auricle allowing a view of the atrial endocardium and tricuspid valve. The next cut should be a "U shape" through the tricuspid annulus and beneath it the valve, down along the septum to the apex of the right ventricle, and up along the septum of the attached free wall through the outflow tract to the pulmonic valve and pulmonary artery. The left atrium should be incised from the pulmonary vein return to the auricle allowing the same view as for the right atrium. The next incision should extend through the mitral valve and left ventricular free wall to the apex of the left ventricle. The aortic valve and outflow tract can be visualized after the mitral valve and aortic annulus is incised (with one incision). Clotted blood should be removed, the heart rinsed in water, and the endocardium observed for thickened regions, pallor, hemorrhage, and valvular changes. Incision through the ventricular free walls, atria, and septum can aid in visualizing myocardial lesions that do not extend to the epicardium or endocardium. Sampling of the papillary muscles located at the base of the chordae tendineae for histopathology is recommended as these muscles are highly active and susceptible to myocardial damage.

Cardiac pathology

Cardiac output is negatively influenced by conduction abnormalities, heart malformations, disease in noncardiovascular organs, and epicardial, myocardial, or endocardial lesions. The speed at which heart disease develops depends on the underlying etiology and the location of the abnormality or diseased tissue; this determines if the sequel is sudden unexpected death or the development of peripheral lesions secondary to congestive heart failure.

Congenital anomalies

Porcine congenital cardiovascular malformations have been reported to occur at an incidence of 0.49–14.6% (Salsbury 1970; Wang 1978) in conventionally raised swine. Malformations may have minimal pathological effects or result in fatal cardiovascular compromise.

Hsu and Du (1982) identified 122 cardiac anomalies in a study of 1906 crossbred and purebred pigs. This study identified anomalies in pigs ranging from 1 day to 4 years of age but found the highest incidence of malformations in animals 29–110 days old. Both single and multiple anomalies were frequently identified within individual pigs. Common porcine cardiovascular anomalies are listed in Table 14.3.

Diseases of the pericardium

Hemorrhage, transudate, modified transudate, or exudate can accumulate within the pericardium of swine. Increased pericardial fluid is capable of applying external pressure to the heart, resulting in decreased diastolic filling of the chambers and venous congestion (cardiac tamponade). While both acute and chronic congestive heart failure can ensue if the material is not removed, in most pigs, cardiac tamponade is usually fatal.

Hemopericardium

Hemopericardium is the expansion of the pericardial cavity with blood. This condition commonly develops from traumatic insult to the epicardium or major blood vessels within the pericardium, including bleeding needles. Idiopathic rupture of an atrium, coronary artery, or

Table 14.3 Porcine congenital cardiovascular anomalies.

Anomaly
Cardiac position
Ectopia cordis
Shunting
Atresia, ostium atrioventriculare
Atrial septal defect
Atrioventricular canal defect
Patent ductus arteriosus
Persistent truncus arteriosus
Ventricular septal defect
Valvular
Endocardiosis of the left atrioventricular valve
Subaortic stenosis
Tricuspid dysplasia
Vascular
Aortic coarctation
Right aortic arch

the aorta is occasionally diagnosed in swine. Similar cardiovascular ruptures have been reproduced in experimental models of porcine copper deficiency (Shields et al. 1962).

Hydropericardium

Expansion of the pericardium with clear to yellowish watery transudate or modified transudate is termed hydropericardium. This condition most commonly develops due to nonspecific insult to the vascular endothelium with subsequent fluid and fibrin loss into the pericardial cavity. Hydropericardium, in swine, has been associated with edema disease, mulberry heart disease (MHD), cachexia, hypoalbuminemia, and congestive heart failure. Wispy fibrin strands in pericardial fluid can be from modified transudate and should not be overinterpreted as fibrinous pericarditis.

Pericarditis

Pericarditis is the expansion of the pericardial cavity with inflammatory exudate and is one of the most common causes of pericardial disease in swine. Exudate can be fibrinous, purulent, or fibrinopurulent based on the amount of fibrin, number of inflammatory cells, and the presence or absence of pyogenic bacteria. Pericarditis can arise from hematogenous dissemination of bacteria or lymphatic extension of inflammation from adjacent tissues such as the lungs or pleura. Grossly, the pericardium is typically expanded with fibrin that gives the pericardial surface a roughened either "ground-glass" or "shaggy" appearance. Granulation tissue will replace the fibrin if mesothelial damage is sufficient or the exudate is not promptly removed. Chronic constrictive pericarditis can lead to cardiac dysfunction and result in congestive heart failure. Causes of porcine epicarditis and pericarditis can be found in Table 14.4.

Table 14.4 Infectious etiologic agents of inflammatory heart disease.

Lesion	Reference chapter
Endocarditis	
Erysipelothrix rhusiopathiae	53
Streptococcus suis, Streptococcus spp.	62
Trueperella pyogenes	64
Myocarditis	
Bacteria associated with septicemia	
Encephalomyocarditis virus	40
Foot-and-mouth disease virus	40
Porcine circovirus 2	30
PRRSV	41
Pseudorabies/Aujeszky's virus	35
Swine vesicular disease virus	40
Pericarditis and epicarditis	
Actinobacillus sp.	48
Haemophilus parasuis	55
Mycoplasma hyorhinis	61
S. suis, Streptococcus spp.	62
Other septic bacteria	

Diseases of the myocardium

Myocarditis

Inflammation of the myocardium can develop from hematogenous distribution of pathogens or extension of inflammatory lesions from the adjacent pericardium or endocardium. A number of bacterial and viral etiologies can induce porcine myocarditis (Table 14.4). Although less common, parasitic stages of *Toxoplasma gondii*, *Trichinella spiralis*, *Taenia solium*, and *Taenia saginata* may infest the myocardium and produce nodular or cystic lesions. While these intermediate parasitic stages do not often cause overt inflammation unless the cysts rupture, they do pose significant public health risks in locations where offal is consumed. Inflammation within the myocardium, regardless of cause, can alter cardiovascular function by inducing myocardial degeneration and necrosis, altering electrical conduction, and disrupting myocardial contraction. These factors can result in acute death associated with dysrhythmia or contribute to chronic heart failure.

Myocardial degeneration and necrosis

Porcine myocardial degeneration and necrosis can develop from a number of primary insults to the heart or secondarily from systemic disease. Primary causes in swine include ionophore, gossypol, or injectable iron toxicosis and nutritional cardiomyopathy. Myocardial necrosis may also develop secondarily from hypertrophic or dilated cardiomyopathy, fever, anemia, disseminated intravascular coagulation (DIC), toxemia, neurological lesions (brain–heart syndrome), porcine stress syndrome, systemic infections, or myocardial inflammation. The wide array of potential causes of myocardial degeneration and necrosis frequently make identification of a definitive etiology difficult.

Mulberry heart disease

MHD is a diagnosis historically associated with vitamin E and/or selenium deficiency in swine. Affected animals are usually found dead with hemorrhagic foci within the walls of the heart. The name was applied because of the distinct transmural hemorrhages that resemble a mulberry.

MHD occurs sporadically in young rapidly growing swine. Disease incidence is generally low, although

epidemics have been reported (Moir and Masters 1979). Clinical signs are uncommon as the disease primarily manifests as sudden death in well-conditioned animals. On rare occasion, affected animals may develop weakness, cyanosis, slight icterus, subcutaneous edema, tachycardia, and increased serum muscle enzymes (Gudmundson 1976). Death is thought to be associated with dysrhythmia that develops secondary to myocardial damage. Diagnosis of MHD must be confirmed by necropsy and histopathology.

To date, the definitive pathophysiological mechanism behind MHD development remains elusive. Current theory suggests that disease development is associated with the lack of balance between free radical development and scavenging, that is, uncompensated metabolic oxidative stress. Free radicals are highly reactive molecules generated during normal oxidative metabolism. Cells ordinarily neutralize free radicals with antioxidant scavengers such as superoxide dismutase, glutathione peroxidase, and vitamins E and C. Selenium is historically included in this list due to its essential involvement in glutathione peroxidase activity. Unscavenged free radicals are capable of inducing cellular damage by reacting with cellular proteins, membrane lipids, and nucleic acids. Deficiency of free radical scavengers can result in severe cellular injury and death.

Historically, vitamin E and/or selenium deficiency was thought to be essential component of disease development. This theory was developed based on the experimental reproduction of MHD in swine deficient of one or both of these constituents (Grant 1961); however, inconsistencies in identifying deficient levels of vitamin E, selenium, or glutathione peroxidase activity in animals with MHD are reported (Nielsen et al. 1989; Pallarés et al. 2002; Rice and Kennedy 1989), suggesting MHD has other risk factors. Current hypotheses suggest that animals that die of MHD lack a sufficient balance between free radicals and free radical scavengers, which predispose animals to oxidative damage. Associated predisposing factors associated with MHD include stressors of various types, rapid growth rate, increased iron tissue concentrations (Korpela 1990), increased calcium and decreased magnesium concentrations (Korpela 1991), and diets containing corn oil (Nolan et al. 1995). It has also been hypothesized that there may be genetic predispositions to free radical damage, altered vitamin E metabolism, or decreased vitamin E bioavailability influenced by polyunsaturated fatty acids or excess vitamin A.

Gross pathology findings typically include hydropericardium, pulmonary edema, excess pleural fluid, and epicardial and transmural cardiac hemorrhage (Figure 14.1). More specifically, the pericardium is often distended by a large amount of cloudy to straw-colored fluid that contains fibrin strands not firmly attached to serosal surfaces. Multifocal-to-coalescing ecchymotic hemorrhages are evident in the epicardium and myocardium and occasionally extend to the endocardium (Figure 14.2). Regions of myocardial necrosis may or may not be grossly evident.

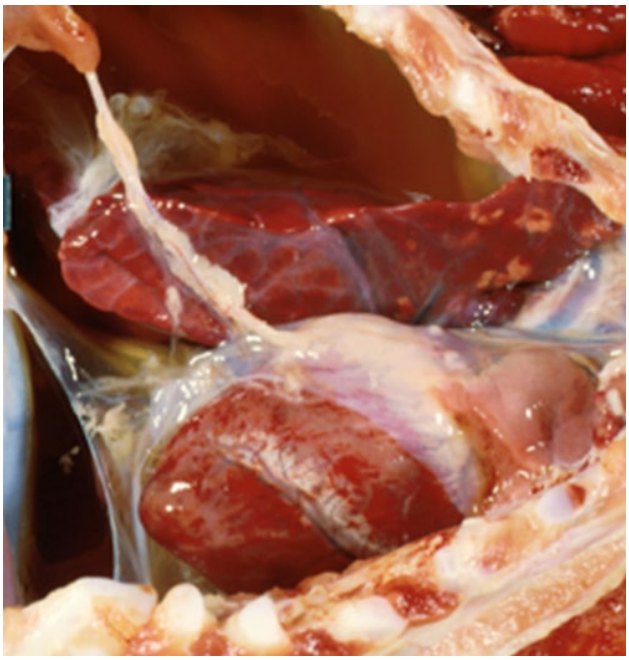


Figure 14.1 Hemorrhage visible on epicardium with excess pericardial and pleural fluid containing loose fibrin strands. Mulberry heart disease. *Source:* Veterinary Diagnostic Laboratory, Iowa State University.

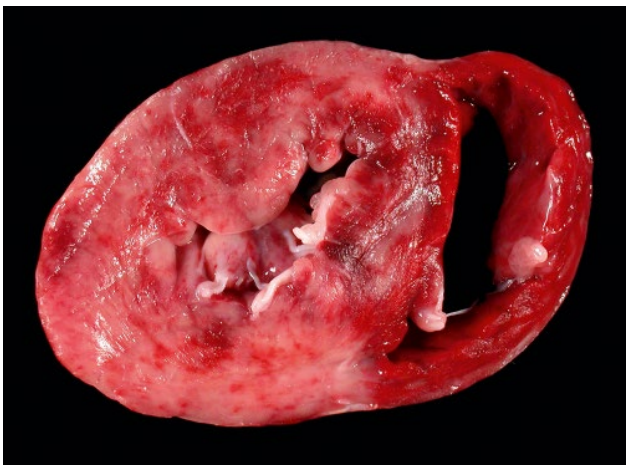


Figure 14.2 Hemorrhage and necrosis extends into the myocardium. Mulberry heart disease. *Source:* Marie Culhane, University of Minnesota.

Histological hallmarks of MHD include interstitial hemorrhage, subserosal edema, and variable amounts of myofiber degeneration, necrosis, and mineralization. Histological lesions can vary based on chronicity of the lesions. In cases of acute death, hemorrhagic lesions may

predominate, and degenerative and necrotic lesions may be minimal or absent. Hemorrhage with myocardial degeneration, necrosis, and mineralization is typically present in subacute cases. Microscopic lesions consistent with dietary microangiopathy/MHD may also be found in arterioles and capillaries of the heart, kidneys, liver, stomach, intestine, mesentery, skeletal muscle, and skin. These systemic vascular lesions can vary in severity from nonspecific endothelial hypertrophy, capillary microthrombosis, or fibrinoid necrosis. Animals that die of MHD may also have lesions consistent with acute heart failure. Less common is centrilobular hepatocellular necrosis (hepatosis dietetica), with skeletal muscle degeneration and necrosis (white muscle disease) rarer still.

A diagnosis of MHD is typically made based on the presence of typical gross and microscopic lesions. Low tissue (i.e. liver) concentrations of vitamin E and/or selenium can support a diagnosis.

Treatment of affected animals is usually not plausible due to the lack of distinct clinical signs and rapidity of death. Van Vleet et al. (1973) suggested that parenteral injection of all pigs aged 1–4 months with commercially available selenium–vitamin E products may be beneficial in herds experiencing increased death losses due to MHD. Prevention generally relies on increased levels of vitamin E via injection, water, or feed supplementation. Evaluation of feed constituents for excess oxidants may be warranted. Supplemental vitamin E should be favored over supplemental selenium in feed since the latter is more associated with hepatosis dietetica and can cause toxicity at relatively low doses.

Diseases of the endocardium

A majority of endocardial lesions in swine involve the heart valves. Disrupted valve function negatively impacts hemodynamics, decreases cardiac efficiency, and may result in cardiac hypertrophy or chamber dilation. Sequelae include endocardial mural fibrosis, thromboembolism, or even chordae tendineae rupture.

Valvular endocarditis is a commonly acquired lesion of the porcine endocardium associated with a variety of bacteria (Table 14.4), with *Streptococcus suis* probably the most common. Sporadic epidemics with *Erysipelothrix rhusiopathiae* are reported. Fungal and parasitic causes are rare. The mitral valve is most commonly affected, followed in frequency by the aortic, tricuspid, and pulmonic valves. Regardless of the affected valve, postmortem findings typically consist of one or more irregular friable reddish-gray to yellow nodules that extend from the valve leaflets and may involve the adjacent mural endocardium. This lesion is typically referred to as "vegetative valvular endocarditis" based on the floret-like appearance of the inflammatory mass. Microscopically, acute lesions are composed of ulcerated valvular endothelium covered by concentric layers of fibrin, bacterial colonies, and variable numbers of inflammatory cells. Granulation tissue may cover the valves in chronic lesions. Valvular dysfunction can be auscultated as murmurs, and affected animals may die from heart failure. Tissue infarction can also result when vegetative nodules break free from the endocardium and form thromboemboli.

Valvular cysts can be found in swine and usually contain either blood (hemocyst) or yellow serous fluid (serous cysts). Both types may be congenital or acquired and have little clinical significance.

Diseases of the conduction system

Diseases of the conduction system are sparsely reported in conventionally grown swine. Abnormalities may result in myocardial dysrhythmia, which can alter the systematic contraction of the heart, disrupt circulatory hemodynamics, and result in acute death. A majority of conduction system diseases arise secondary to cardiac, central nervous system (CNS), or pulmonary disease; drugs (anesthetic agents); or systemic alterations such as hypo- and hyperthermia, anemia, shock, sepsis, excitement, and electrolyte imbalances (hyperkalemia, hypocalcemia). These diseases may induce ectopic impulses that result in premature complexes, tachycardia, flutter, and fibrillation or result in conduction alterations such as nodal arrest, conduction blocks, and pre-excitation. Electrocardiography is required for the diagnosis of conduction system diseases, which explains the lack of epidemiological data associated with these entities in swine.

Compensatory mechanisms

The heart is incapable of generating new cardiac muscle to cope with increased workloads or repair damaged myofibers. Consequently, the cardiovascular system utilizes compensatory mechanisms to temporarily increase cardiac output and meet systemic metabolic demands. Cardiac dilation, myocardial hypertrophy, and tachycardia are compensatory changes of the heart that increase cardiac output. Neurohormonal mechanisms also modify the vascular system by increasing vascular resistance, promoting vasoconstriction, and increasing the systemic blood volume.

Heart failure

Congestive heart failure ensues when the heart can no longer compensate to meet systemic metabolic needs. Mechanistically, heart failure can be characterized as a decrease in cardiac output and/or decrease in venous return of the blood. Heart failure may result from impaired myocardial function (cardiomyopathies, decreased myofiber contractibility, lack of distensibility, and dysrhythmia) or an increase in cardiac demand (cor

pulmonale, pressure overload, and volume overload). Heart failure may be characterized as left or right sided depending on the location of diseased tissue and underlying mechanisms. Left-sided heart failure results in stasis of blood and fluid in the lungs. This pathologically manifests as pulmonary congestion and edema and can become clinically evident as dyspnea and coughing. Pathological findings associated with right-sided heart failure include systemic congestion, ascites, edema, and tissue hypoxia.

Vascular pathology

Vascular pathology can arise from a magnitude of different etiologies and result in abnormal hemodynamics, fluid loss from the vascular compartment, hemolytic anemia (HA), and tissue ischemia.

Vascular rupture and aneurism

Iatrogenic laceration of large blood vessels is a common side effect associated with blood sampling in swine. Lacerations, as well as other causes of vascular rupture, can lead to hypovolemic shock and death.

Copper is an essential element utilized by lysyl oxidase to cross-link collagen and elastin in blood vessel walls. Copper-deficient swine are predisposed to arterial aneurisms due to the lack of sufficient vascular integrity (Coulson and Carnes 1963). Affected vessels may bulge, dilate, and rupture.

Vascular degeneration and necrosis

Atherosclerosis is a degenerative disease that naturally occurs in aged pigs fed high-cholesterol diets. It develops slowly over time and commonly affects larger arteries. The disease is characterized by the plaque-like narrowing of vascular lumens, which alter hemodynamics, predispose to thrombus formation, and can result in vascular occlusion and tissue infarction. Microscopically, vessel walls are expanded by lipid deposited in smooth muscle cells, aggregates of lipid-laden macrophages, and fibrous connective tissue. Due to similarities in the mechanism and development of human atherosclerosis, the pig has become a useful model to study the disease in man.

Vascular mineralization due to vitamin D poisoning is occasionally observed in swine and usually associated with accidental feed overdoses. Vitamin D toxicosis results in hypercalcemia and/or hyperphosphatemia. Increased serum concentrations of calcium or phosphate predispose to vascular and soft tissue mineralization, which hinders vascular elasticity and organ function.

Fibrinoid necrosis develops secondary to endothelial damage with subsequent fibrin and serum protein deposition into vessel walls. Edema disease, mercury toxicosis, and MHD are three diseases associated with fibrinoid necrosis in swine.

Toxic ergot alkaloids, produced by *Claviceps purpurea*, can also produce marked vasoconstriction by stimulating adrenergic nerves in the vascular smooth muscle. This results in endothelial necrosis and vascular thrombosis that may provoke infarction of limbs and extremities.

Vascular thrombosis

Thrombosis is characterized by intravascular coagulation and can be induced by endothelial damage, hypercoagulable disorders, and abnormal blood flow (Virchow's triad). If severe, it can completely occlude the vascular lumen and result in tissue infarction. Arteritis, regardless of the cause, can provoke endothelial cell damage and result in vascular thrombosis. DIC is a common cause of vascular thrombosis in swine. Thrombosis due to DIC can affect arterioles and capillaries in all organ systems. Septicemia, endotoxemia, viral infection, hemolysis, shock, and massive regions of tissue necrosis predispose to DIC development.

Vascular inflammation

Vasculitis is a collective term to indicate inflammation of the arteries, veins, or lymph vessels. Inflammation of the vessels can occur from primary insult by infectious agents, drug reactions, immune-mediated mechanisms or by expansion of inflammatory processes from adjacent tissues. Inflamed blood vessels have increased permeability and are predisposed to thrombosis. Infectious etiologies associated with porcine vasculitis are listed in Table 14.5.

Table 14.5 Infectious causes of porcine vasculitis.

Classification	Reference chapter
Bacterial	
Actinobacillus sp.	48
Erysipelothrix rhusiopathiae	53
Escherichia coli	52
Haemophilus parasuis	54
Leptospira interrogans	55
Salmonella sp.	59
Streptococcus suis	61
Viral	
African swine fever virus	25
Classical swine fever virus	39
Ovine herpesvirus 2	35
Porcine circovirus 2	30
PRRSV	41

Edema

Edema is the abnormal collection of transudate in the interstitium and/or intercellular spaces due to fluid loss from the vascular system. Edema can develop from increased vascular permeability, increased intravascular hydrostatic pressure, decreased intravascular osmotic pressure, or decreased lymphatic drainage.

Increased vascular permeability is a common cause of localized edema in swine and occurs secondarily to vascular damage, inflammation, type 1 hypersensitivity reactions, neovascularization, endotoxin, and DIC.

Increased vascular volume may result in generalized or localized edema by increasing the intravascular hydrostatic pressure. This mechanism of edema is commonly seen in congestive heart failure due to venous congestion and compensatory fluid retention mechanisms.

Intravascular osmotic pressure is maintained by plasma proteins such as albumin. Plasma protein loss reduces intravascular colloidal osmotic pressure and leads to increased fluid transudation into the interstitium. Causes of hypoproteinemia can include liver disease, malnutrition, and protein-losing enteropathies (PLE) and nephropathies (PLN).

Interstitial fluid is normally removed by regional lymphatic vessels. Lymphatic blockage by thrombosis, compression, or inflammation can reduce the effective drainage of the interstitium and result in localized edema.

Diseases of the body cavities

The pericardial, peritoneal, and pleural cavities normally contain small amounts of serous fluid. Normal cavity fluid should be colorless to yellow tinged and translucent to slightly turbid and contain less than 2.5 g/dL of protein and 5000 nucleated cells/μL (Rakich and Latimer 2011).

Characterization of cavity fluids can aid in the identification of ongoing disease processes. An increase in fluid volume and turbidity is abnormal and may result from increased cellularity, increased protein and lipid concentrations, fibrin, bacterial colonization, or ruptured organs. Abnormal fluid can be classified as transudate, modified transudate, or exudate. Characteristics of these fluids are presented in Table 14.6. Transudates commonly develop from decreased plasma osmotic pressure associated with hypoalbuminemia. Modified transudates are less diagnostic and can be associated with increased hydrostatic pressure and increased vascular permeability or secondarily result from heart or liver disease. Exudates commonly result from increased vascular permeability associated with inflammatory processes.

Table 14.6 Body fluid classifications and parameters.

	Fluid type		
	Transudate	**Modified transudate**	**Exudate**
Cellularity (cells/μL)	<1500	1000–7000	>5000
Color	Colorless	Variable	Yellow to tan
Protein (g/dL)	<2.5	2.5–5.0	>2.5
Turbidity	Clear	Variable	Cloudy to opaque

Shock

Shock is a rapidly progressive disturbance of hemodynamics and cellular metabolism. It can be characterized as hypovolemic, cardiogenic, or vasogenic based on the underlying mechanism. Despite the mechanism, it concludes with vascular hypotension, tissue hypoperfusion, cellular hypoxia, and acidosis and may lead to death.

Hypovolemic shock develops from a significant decrease in blood volume, which results in decreased vascular pressure. Blood volume can be decreased by the direct loss of whole blood (i.e. hemorrhage) or fluid components (i.e. dehydration) from the vascular system.

Cardiogenic shock is characterized by the heart's inability to efficiently pump blood. Lesions within the endocardium, myocardium, or pericardium may hinder efficiency and result in shock by decreasing stroke volume and cardiac output.

Vasogenic shock arises from peripheral vasodilation. Inadequate vascular tone leads to pooling of blood, reduction of circulating blood, and tissue hypoperfusion. Vasogenic shock is commonly identified in pigs and can result from trauma, stress, anaphylaxis, sepsis, endotoxemia, or insult to the CNS.

Diseases of the hemopoietic system

Anemia

Anemia is defined as a decrease in the hematocrit level, hemoglobin concentration, or RBC mass. A number of classification systems exist that categorize anemia based on physical properties (erythrocyte size and hemoglobin concentration), bone marrow response (regenerative vs. nonregenerative), or the underlying pathological processes (defective erythropoiesis, hemorrhage, and hemolysis). Clinical signs may be variable dependent on the severity of the anemia and the underlying mechanism. Anemia can clinically manifest as mucous membrane

Table 14.7 Causes of porcine anemia.

Classification
Defective erythropoiesis
Anemia of chronic disease
Nutritional deficiencies (iron, copper, vitamin B_{12})
Viral infections (PRRSV, porcine circovirus 2)
Hemolytic
Autoimmune: neonatal isoerythrolysis
Erythrocyte fragmentation
Disseminated intravascular coagulation, vasculitis
Erythrocyte parasitism: *Mycoplasma suis* (*Eperythrozoon suis*)
Hemorrhagic
Hemorrhagic enteropathies, dysentery
Gastric ulceration
Hemorrhagic bowel syndromes/volvulus
Parasitisms
External: lice (*Haematopinus suis*)
Internal: worms (*Trichuris, Strongyloides)*
Clotting deficiency (navel bleeding, vitamin K deficiency)
Thrombocytopenia

pallor, weakness, tachycardia, and/or tachypnea. Potential causes of porcine anemia are listed in Table 14.7.

Causes of porcine anemia

Defective erythropoiesis

Defective erythropoiesis is an important cause of porcine anemia and can develop due to nutritional deficiencies, chronic disease, or infectious agents. Suckling piglets are particularly susceptible to iron deficiency anemia (IDA) due to rapid growth, a low iron storage capacity, and a lack of sufficient dietary intake. Piglets require approximately 7 mg of iron daily and only obtain half of that requirement through milk. Modern indoor husbandry practices have reduced the amount of iron naturally obtained by piglets from the soil and have increased the need for neonatal iron supplementation. Affected piglets classically appear normal at birth but become unthrifty and pale and develop edema of the lungs, muscles, and connective tissues at approximately 1–3 weeks of age. IDA is characterized by erythrocyte microcytosis and hypochromasia. Bone marrow may be dark red and hyperplastic, and exhibit an erythroid shift, but may become hypoplastic with chronic deficiency.

Copper promotes iron absorption and is essential for the incorporation of iron into hemoglobin (Lee et al. 1968). Copper deficiency thus can result in a lack of iron availability and predispose to IDA.

Anemia of inflammatory disease (AID) can occur with multiple infectious, inflammatory, or neoplastic processes. Mechanistically, AID develops secondarily to the impact of inflammatory cytokines on iron availability, erythropoietin production, and the bone marrow's response to erythropoietin. Erythrocytes are typically normocytic and normochromic, but microcytosis and hypochromia may be evident. The primary disease process commonly dominates the clinical assessment, and anemia typically resolves once the primary disease has been alleviated. Certain viruses also play a direct role in the development of anemia. Porcine reproductive and respiratory syndrome virus can experimentally induce anemia in pigs (Halbur et al. 2002). The exact mechanism of anemia is not known, but the virus has been postulated to directly or indirectly disrupt erythropoiesis.

Hemolytic anemia

HA develops from the premature destruction of erythrocytes. In swine, it has been associated with immune-mediated mechanisms, erythrocyte parasitism, and erythrocyte fragmentation. Clinically affected animals may develop icterus and hemoglobinuria.

Neonatal isoerythrolysis is an immune-mediated disease of pigs and other animals. HA develops through the passive acquirement of maternal antibodies that have been primed against fetal erythrocytes. Once introduced into the neonatal circulatory system, material antibodies bind to piglet erythrocytes and activate the complement cascade. Complement lyses erythrocytes and releases hemoglobin into the plasma. Acquired immune-mediated anemia is rare but can result from haptens or autoimmune phenomena.

Hemotropic *Mycoplasma suis*, formerly known as *Eperythrozoon suis*, is capable of attaching to and infecting porcine erythrocytes. Extravascular hemolysis develops secondary to the removal of infected erythrocytes from the circulatory system.

Microangiopathic anemia results from the removal of injured erythrocytes from the circulatory system. Erythrocyte membranes may become fragmented by fibrin when passing through damaged blood vessels. Vasculitis, DIC, and vascular neoplasia are potential causes of microangiopathic anemia in swine.

Hemorrhagic anemia

Hemorrhagic anemia (HeA) frequently develops secondarily to an acute or chronic blood loss event. External evidence of hemorrhage is usually evident in cases of trauma or umbilical hemorrhage, but may be externally inconspicuous in cases of gastric ulceration, hemorrhagic bowel syndrome, enterocolitis, external or internal

parasitism, and thrombocytopenia. Clinical signs may or may not be present based on the amount of blood loss, chronicity of disease, and distribution of lesions. A regenerative response is evident within days of blood loss, but may become nonregenerative in chronic cases due to iron deficiency.

Hemoglobin disorders

Pathological alterations of hemoglobin reduce the erythrocyte's ability to carry oxygen to the tissues. Carboxyhemoglobinemia and methemoglobinemia are recognized hemoglobin disorders in swine. Both conditions typically result in cyanosis and anoxia.

Carbon monoxide poisoning is a potentially fatal disease that manifests in pigs housed indoors with defective heaters and improper ventilation. Carbon monoxide is a colorless, odorless, and tasteless gas generated from the partial or incomplete oxidation of carbon-based fuels. Carbon monoxide has a higher binding affinity to hemoglobin (approximately >200 times greater), in comparison with oxygen, and blocks the oxygen-carrying capacity of hemoglobin. Affected animals have bright cherry red blood as a result of the formation of carboxyhemoglobin. Animals that do not die can develop CNS disorders as a result of anoxia.

Swine exposed to excessive amounts of oxidizing agents, such as nitrate and nitrite, are at increased risk of developing methemoglobinemia. Normally, the iron component of hemoglobin must be in the reduced ferrous state to effectively bind to oxygen and form oxyhemoglobin, which transports oxygen to the tissues. Methemoglobinemia develops when ferrous hemoglobin is oxidized to methemoglobin and can no longer transport oxygen. Blood of affected animals classically turns a distinctive dark chocolate brown color.

Coagulation disorders

Bleeding disorders are fairly uncommon in swine but can develop due to maternal isoimmunization (thrombocytopenic purpura) (Nordstoga 1965) or consumption of anticoagulant compounds such as warfarin. Pathological lesions are restricted to multiorgan hemorrhage. Diagnosis is by identifying sow-derived isoantibodies to piglet platelets or the presence of anticoagulants in body tissues.

Cardiovascular and hematopoietic neoplasia

Naturally occurring neoplasia is infrequently diagnosed in swine due to the short life span of production animals. Porcine tumors do not frequently cause clinical signs and are typically found as incidental lesions in diagnostic specimens or in carcasses at slaughter. Tumors that are most frequently identified in the cardiovascular and hematopoietic systems of swine include lymphoma, hemangiosarcoma, hemangioma, and cardiac rhabdomyoma.

Lymphoma is the most commonly diagnosed and economically important neoplasia in swine. It develops from the neoplastic proliferation of T or B lymphocytes and most frequently develops in pigs less than 1 year of age. A number of factors, such as C-type viruses and hereditary genetic components have been associated with the development of lymphoma. Multicentric, thymic, and leukemic forms have been identified with the former being the most common. Porcine multicentric lymphoma is frequently of B-cell origin and when occurs most commonly does so in the spleen, liver, kidney, intestine, and bone marrow. Conversely, thymic lymphoma develops in the cranial mediastinum and is of T-cell origin. Leukemia is rarely identified in swine but can occur in late stages of either thymic or multicentric lymphoma.

Tumors of the porcine cardiovascular system are rare. Over an 11-year period, Fisher and Olander (1978) identified five vascular tumors in tissues and necropsy samples submitted to Purdue University. These tumors were diagnosed as cutaneous and testicular hemangiosarcomas and cutaneous and meningeal hemangiomas, respectively.

While cardiac tumors are rare, congenital cardiac rhabdomyoma is the only tumor identified in the porcine heart with any frequency. This tumor consists of nonneoplastic nodular proliferations of dysplastic myocardial fibers (Omar 1969). Given the misnomer, alternative names have been suggested such as porcine cardiac rhabdomyomatosis and purkinjeoma. Affected hearts may have well-demarcated white myocardial nodules that impede into the heart chambers. Most of these tumors are identified as incidental lesions at necropsy and are not thought to cause significant pathology.

References

Brockus CW. 2011. Erythrocytes. In Latimer KS, ed. *Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology*. Chichester, West Sussex, UK; Ames, IA: Wiley, pp. 3–43.

Coulson WF, Carnes WH. 1963. *Am J Pathol* 43:945–954.

Dyce KM, Sack WO, Wensing CJG. 2010. *Textbook of Veterinary Anatomy*. St. Louis, MO: Saunders Elsevier.

Fisher LF, Olander HJ. 1978. *J Comp Pathol* 88:505–517.

Fox JG, Cohen BJ, Loew FM. 1984. *Laboratory Animal Medicine*. Orlando, FL: Academic Press.
Friendship RM, Lumsden JH, McMillan I, et al. 1984. *Can J Comp Med* 48:390–393.
Grant CA. 1961. *Acta Vet Scand* 2(Suppl 3):1–107.
Gudmundson J. 1976. *Can Vet J* 17:45–47.
Halbur PG, Pallarés FJ, Rathje JA, et al. 2002. *Vet Rec* 151:344–348.
Hsu FS, Du SJ. 1982. *Vet Pathol* 19:676–686.
Jain NC. 1986. *Veterinary Hematology*. Philadelphia: Lea & Febiger, pp. 240–255.
Klem TB, Bleken E, Morberg H, et al. 2010. *Vet Clin Pathol* 39:221–226.
Korpela H. 1990. *Ann Nutr Metab* 34:193–197.
Korpela H. 1991. *J Am Coll Nutr* 10:127–131.
Lee GR, Nacht S, Lukens JN, et al. 1968. *J Clin Invest* 47:2058–2069.
Moir DC, Masters HG. 1979. *Aust Vet J* 55:360–364.
Nielsen TK, Wolstrup C, Schirmer AL, et al. 1989. *Vet Rec* 124:535–537.
Nolan MR, Kennedy DG, Blanchflower WJ, et al. 1995. *Int J Vitam Nutr Res* 65:181–186.
Nordstoga K. 1965. *Pathol Vet* 2:601–610.
Omar AR. 1969. *Pathol Vet* 6:469–474.
Pallarés FJ, Yaeger MJ, Janke BH, et al. 2002. *J Vet Diagn Invest* 14:412–414.
Rakich PM, Latimer KS. 2011. Cytology. In Latimer KS, ed. *Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology*. Chichester, West Sussex, UK; Ames, IA: Wiley, pp. 331–364.
Reece WO, Erickson HH, Goff JP, et al. 2015. *Duke's Physiology of Domestic Animals*. Ames, IA: Wiley-Blackwell, John Wiley & Sons Inc.
Rice DA, Kennedy S. 1989. *Am J Vet Res* 50:2101–2104.
Salsbury DL. 1970. *Vet Med Small Anim Clin* 65:479–481.
Sanderson JH, Phillips CE. 1981. Pigs. In *An Atlas of Laboratory Animal Hematology*. New York: Oxford University Press, pp. 432–469.
Shields GS, Coulson WF, Kimball DA, et al. 1962. *Am J Pathol* 41:603–621.
Thorn CE. 2010. Hematology of the pig. In Weiss DJ, Wardrop KJ, eds. *Schalm's Veterinary Hematology*. Ames IA: Wiley-Blackwell, pp. 843–851.
Thrall MA. 2004. Erythrocyte morphology. In Troy DB, ed. *Veterinary Hematology and Clinical Chemistry*. Philadelphia: Lippincott Williams & Wilkins.
Van Vleet JF, Meyer KB, Olander HJ. 1973. *J Am Vet Med Assoc* 163:452–456.
Wang FI. 1978. Pathological study on cardiac diseases in swine. MS thesis, National Taiwan University, Taipei.

15

Digestive System

Jill R. Thomson and Robert M. Friendship

Introduction

Conditions affecting the gastrointestinal tract (GIT) occur in pigs of all ages. Enteric diseases substantially compromise production efficiencies and profitability of pig production globally. Some diseases, such as highly virulent porcine epidemic diarrhea (PED), can result in epidemics of high piglet mortality and significant economic hardship to farmers. Such events gain global media coverage, stimulating international alerts and precautionary measures. However, recurring endemic enteric diseases have the greatest long-term impact on health and productivity globally. In addition to direct economic and productivity losses, there are concerns regarding animal welfare and the rate of antimicrobial use for endemic diseases. The latter fuels public health concerns regarding antimicrobial resistance, which sometimes results in legislated requirements to reduce antimicrobial use in pigs. Hence, there are ongoing and global efforts to seek innovative ways to prevent enteric disease expression, thereby limiting the need for antimicrobial use.

The complexity of the sciences, risk factors, and perceptions involved in our understanding of intestinal function that affect health and productivity cannot be overstated; the interface of anatomy, physiology, diet, endemic microflora, pathogens, and immune mechanisms allows for nearly infinite permutations of situations and outcomes. Fortunately, ever-improving quantitative molecular methods allow researchers to better understand the influence of the intestinal microbiota and explore how flora can be modulated through diet or other methods to optimize intestinal health, immunity, and the overall productivity of the pig.

Current research efforts increasingly acknowledge the role of intestinal flora in mitigating disease expression and addresses public expectations over food safety and antimicrobial issues. Successful control of enteric diseases requires a holistic view that considers factors such as nutrition, stress, immunity, intestinal microbiota, and management practices in the expression of disease. Research into these wider issues provides a foundation for future practical advances in enteric disease control and is briefly reviewed in the following sections. Thereafter there is an overview of the enteric system, with many of the specific diseases covered in depth in other chapters.

Anatomic and histologic features

Genetic selection has dramatically changed many characteristics of swine over the years; however, the gut anatomy apparently remains unchanged. Genetic selection does have some promise in selection for disease resistance for specific pathogens, one example being the development of swine genotypes lacking specific receptors, therefore resistant to F18 and F4 (K88) *Escherichia coli*.

Gut maturation occurs rapidly after birth in response to factors such as oxygenation, enteral nutrient presentation, development of microflora, and hormones such as cortisol and epidermal growth factor. Neonatal hypoxia is associated with intestinal dysfunction and an increased incidence in neonatal enterocolitis (Cohen et al. 1991; Powell et al. 1999). At birth, the sudden increase in blood arterial oxygen is a vital factor in gut development. Common occurrences such as delayed or prolonged parturition and congenital or periparturient pulmonary compromise or lung infections could be initiating factors for neonatal diarrhea. After birth, the small intestine undergoes a rapid growth in response to enteral presentation of nutrients (Burrin et al. 2000). This is stimulated by a wide range of factors such as hormones and growth factors (Sangild 2001). Good intake of colostrum and milk in the early stages of life is important for rapid gut growth, passive immunity, and piglet vigor.

Piglets are born with fully erupted "milk" or "needle" teeth. The majority of deciduous premolars erupt

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

between the first and fifth week of life (Tucker and Widowski 2009) followed by other deciduous teeth that are replaced by permanent teeth over time. The age at which the premolars erupt was found to affect piglets in different ways. Piglets that were less than 17 days of age were inhibited from feeding, presumably due to the associated discomfort. Piglets that were 21 days of age or older were actually attracted to feed, possibly getting comfort from the mechanical effects of feed against the gums (Tucker et al. 2010). The problems of weaning at very young ages could be compounded by anorexia associated with discomfort of tooth eruption.

Digestive enzymes are produced primarily by the pancreas and enterocytes. The production of enzymes by the pancreas, such as amylases and lipases, is under nervous and hormonal control and increases substantially during the first 6 weeks after birth (Pluske 2001). During the first 3–4 weeks of life, the fetal enterocytes that have high endocytotic activity are replaced by adult-type enterocytes devoid of such activity. The process occurs in a proximal-to-distal direction in the intestine and is an important part of intestinal maturation (Baintner 1986). Changes in enterocyte generation influence the expression of brush border enzymes. Lactase activity is high in the newborn pig and steadily decreases to become minimal after weaning, whereas sucrase and maltase activities are low in the newborn but increase after weaning (Pluske et al. 1997). Intestinal maturation may be hastened in the unweaned pig by stimulating additional milk intake (Pluske et al. 1996a,b) or feeding kidney bean lectin (*Phaseolus vulgaris*) (Biernat et al. 2001; Rådberg et al. 2001). This dietary means of promoting rapid gut maturation could be of value in reducing or preventing post weaning diarrhea problems in pigs in the future.

Weaning is associated with adverse effects, such as abrupt withdrawal of sow's milk, low and variable feed intake, growth stasis, and compromised integrity of the small intestinal epithelium. Changes in gut morphology at weaning include reduced villus height and increased crypt depth (Hampson 1986; Kelly et al. 1991). These adverse effects can be diet dependent and diet independent (Boudry et al. 2004; McCracken et al. 1995). Villus length reduces by 30–40% at days 4–7 post weaning but can be restored by 14 days post weaning (Verdonk et al. 2001a). Additionally, a reduction in the length of microvilli occurs by 3–7 days after weaning (Cera et al. 1988).

There are important impacts of voluntary feed intake and diet composition on mucosal architecture (Makkink et al. 1994; Pluske et al. 1996b). Low feed intakes or a period of starvation post weaning reduces the villus height throughout the small intestine particularly in the proximal jejunum. Moreover, feed ingredients that contain anti-nutritional factors such as antigenic proteins, protease inhibitors, or some types of (harmful) lectins and tannins or are poorly digestible can induce changes in gut morphology as well and can influence the rate of recovery. To minimize the changes, diets high in milk products, highly digestible ingredients, and low levels of anti-nutritional factors are often used.

Raw cereals and legume seeds adversely affect gut mucosa, especially in young piglets. Feeding properly heat-processed (e.g. cooked, extruded, micronized, pelleted) legume seeds and cereals can improve post weaning growth (Lawlor et al. 2001). The temperature of the feed presented to newly weaned pigs significantly influenced feed consumption and growth performance when mash was fed at a temperature of 34 °C as compared with 14 °C (Reiners et al. 2008).

Anorexia and other stress-related factors at weaning impair the normal barrier function, which increases mucosal permeability by adversely affecting the tight junctions between cells (McCracken et al. 1999). This increases over the first 4 days after weaning, which can contribute to the appearance of dietary toxins and endotoxins in the bloodstream and to local inflammation (Verdonk et al. 2001b). Early weaning heightens this effect (Smith et al. 2010). Recent studies suggest that these changes can extend beyond 1–2 weeks post weaning depending upon the degree of stress experienced (Moeser 2016).

The dietary changes at weaning and the resultant villous atrophy and crypt hyperplasia lead to decreased digestive and absorptive capacities in the intestine (Pluske et al. 1997; Rådberg et al. 2001). If severe, this can result in a malabsorptive form of diarrhea due to electrolytes, nutrient solutes, and osmotically associated water. Other factors of importance include poor feed consumption, inflammation in response to bacterial metabolites, rotaviruses, and hypersensitivity to antigenic components of the diet (Kelly 1990). These physiological changes can result in alterations in the numbers, types, and distribution of the enteric bacterial flora that can increase likelihood for enteropathogenic bacteria to proliferate, resulting in serious post weaning enteropathies such as colibacillosis. The morphological and functional changes in the small intestine of the newly weaned pig have been reviewed by Pluske (2001).

Transport-associated stress of pigs has been shown to lower the intestinal pH and result in increased intestinal permeability. Permeability is the highest immediately after transportation and decreases after 2–3 hours of rest (van der Meulen et al. 2001). Translocation of bacteria and/or endotoxins from the gut into the systemic circulation is possible due to increased permeability (Berg 1999; Zucker and Krüger 1998). This might explain the increase in disease problems seen after transportation (Berends et al. 1996). Similarly, in growing pigs, heat stress has been found to reduce the intestinal barrier

function and mucosal immunity emphasizing the need for effective thermoregulation of the pig's environment to maintain optimal gut health (Pearce et al. 2013).

Digestive physiology

The intestinal mucosa receives nutrients from two sources – the diet (brush border membrane) and the systemic circulation (basolateral membrane). The gut tissues have their own particular nutrient requirements for growth and function. The gastrointestinal tissues in the very young pig utilize up to 50% of the dietary intake of key amino acid such as lysine, glutamine, leucine, and threonine (Burrin et al. 2001). A substantial proportion of amino acid and glucose needs are derived from the arterial circulation rather than from direct dietary sources, while some enteric fermentation products such as butyric and lactic acid serve as nutrients and metabolic regulators of gastrointestinal tissues as well (Burrin et al. 2001). The amino acids are utilized in many ways, such as the formation of secretary mucins (Stoll et al. 1998), biosynthesis of other amino acids (Stoll et al. 1999), glutathione (Reeds et al. 1997), and nucleic acids (Perez and Reeds 1998).

Nutrient supply for the neonate is derived solely from colostrum and milk. Additionally, colostrum and milk contain a large number of biologically active peptides that have important functions in regulating growth and differentiation of intestinal tissues. Targeted expression of key genes for production of milk peptides holds potential for the future (Kelly and Coutts 1997). On low protein diets, the amino acid requirements of the gut remain relatively high and are preferentially met, which limits the systemic availability of amino acids for lean tissue growth (Ebner et al. 1994).

Dietary carbohydrates are composed of sugars, oligosaccharides, starch, and non-starch polysaccharides (NSPs), these being vital dietary components in addition to protein and fat. The enzyme activity responsible for carbohydrate degradation adapts according to the age of the pig and dietary composition. In the young pig there is efficient pre-cecal digestion of lactose during suckling and sucrose and starch after weaning. Carbohydrates that do not get digested in the small intestine are fermented in the large intestine by a diverse population of anaerobic bacteria.

Most of the starches used in pig diets are highly digestible, with up to 98% of digestion occurring in the small intestine of pigs with mature digestive systems (Bach Knudsen and Canibe 2000; Glitsø et al. 1998). NSPs (fiber) are present in cereals, such as barley, wheat, oats, and rye, and legume seeds, such as soybeans, peas, and faba beans. Although there is some digestion of NSPs in the small intestine, the major site of NSP degradation is in the large intestine. Passage of ingesta through the large intestine generally takes from 20 to 40 hours, allowing time for bacterial degradation. Fermentation of carbohydrates and NSPs in the large intestine results in the production of short-chain fatty acids, mainly acetate, propionate, and butyrate, and the gases H_2, CO_2, and CH_4. Studies using naturally occurring fermentable carbohydrates to promote fermentation and gut health have shown beneficial results (Aumiller et al. 2015; Jha and Berrocoso 2015). They also increase production of short-chain fatty acids, which are rapidly absorbed from the large intestine and may provide up to 24% of the maintenance energy requirements for growing pigs (Yen et al. 1991) and potentially even more for adults. The total tract digestibility of NSPs is influenced by a number of complex factors, such as the source of NSPs, the level of inclusion in the diet, the solubility, the degree of lignification, the age and weight of the animal, the transit time, and the microbial composition (Bach Knudsen and Jørgensen 2001).

Large volumes of fermentable substrate arriving in the large intestine can cause osmotic overload, with resulting diarrhea. This may result from excessive intake or malabsorption of carbohydrate in the small intestine. The increased volatile fatty acid (VFA) production can overwhelm the colonic buffering capacity, causing reduced pH and increased numbers of lactic acid-producing bacteria. As lactic acid is absorbed at a slower rate than VFAs, further acidification occurs, with the resulting influx of water and solute from tissue into the lumen. Diarrhea results from this process if acidification is excessive. Adult animals are more capable of degrading fiber than growing pigs due to the greater intestinal volume and slower transit times. Adaptation to dietary changes in terms of digestibility of NSP residues is considered to take 3–5 weeks (Longland et al. 1993).

In weaned pigs, the use of enzyme combinations has been found to increase the apparent ileal digestibility of feeds based on hull-less barley, which have high beta-glucan concentrations (Yin et al. 2001). Due to the improved apparent ileal digestibility, there was also a reduction in hindgut fermentation. Similarly, the addition of certain enzymes to wheat-based diets of growing pigs has been found to have beneficial effects (Hazzledine and Partridge 1996). There is increasing interest in the role of dietary factors, especially NSP and feed processing methods in so-called nonspecific colitis of pigs (Strachan et al. 2002; Thomson et al. 2004). This condition is thought to be an important precursor to other forms of colitis in pigs although the pathogenesis of this diet-associated form of colitis is poorly understood.

Immunology

For the first 24–48 hours postpartum, the pig intestine is capable of absorbing macromolecules, including immunoglobulins by pinocytosis, providing the neonate

with passive immunity from colostrum (Weström et al. 1984). This process commences prepartum and may form part of prenatal nutritional programming of the GIT (Guilloteau et al. 2010); however, the major absorptive function occurs postnatally (Sangild et al. 1999). This is a specific maturational process that is timed to maximize immunoglobulin uptake shortly after birth. Piglets born prematurely have a lower capacity for protein absorption than piglets born at full term (Sangild et al. 1997); therefore fetal maturity is an important factor in successful immunoglobulin uptake from the colostrum.

The intestinal immune system of the young pig is very immature, and its slow development may result in increased susceptibility to disease (Stokes et al. 2001). Intestinal lymphoid tissue is present in the form of mesenteric lymph nodes, intestinal Peyer's patches, and lymphocytes distributed through the mucosal lamina propria and intraepithelial sites. In the jejunum there are between 11 and 26 discrete Peyer's patches containing multiple lymphoid follicles (B lymphocytes), separated by T cells. Plasma cells containing IgM, IgG, and IgA are present in the subepithelial lymphoid dome and between the follicles (Brown and Bourne 1976). The dome region contains dendritic-type cells that express high levels of MHC II antigens. Microfold cells (M cells) that are thought to absorb luminal antigens occur in the overlying lymphoepithelium (Gebert et al. 1994).

In the mature pig the intestinal lamina propria is heavily populated with lymphocytes. Plasma cells and B cells predominate in the crypt area, whereas T cells are found mainly in the villi, $CD8^+$ cells occur in the subepithelial sites, and $CD4^+$ cells occur in association with capillary plexuses in the lamina propria (Vega-Lopez et al. 1993). The majority of intraepithelial lymphocytes express CD2, but in mature pigs a high proportion also express CD8 (Stokes et al. 2001). The general immune status of piglets can be influenced by factors such as weaning age, and photoperiod allowed for piglets after weaning. Niekamp et al. (2007) found benefits to piglet immunity from weaning at 28 days as compared with 14 or 21 days and from a daily photoperiod allowance of 16 hours as compared with 8 hours.

Functionally the intestinal immune mechanisms play a complex role in preventing and controlling harmful intestinal infections while tolerating many dietary antigens and nonharmful antigens from the intestinal flora. The surface epithelium serves as an effective barrier, providing it remains intact. For the neonatal piglet, exposure to new antigens is critical for the proper development of the gut mucosal immune system, which is characterized mainly by the establishment of IgA response (Levast et al. 2014). Significant quantities of dietary protein are absorbed across the intestinal mucosa (Telemo et al. 1991; Wilson et al. 1989).

So-called intestinal tolerance to dietary proteins has been demonstrated in the pig, whereby immune responses to the dietary proteins are regulated to prevent inflammatory reaction and tissue damage associated with foreign protein absorption (Bailey et al. 1993). The interaction between the different components of the intestinal immune system is complex, and the basis of inflammation and apoptosis versus downregulation of immune responses is the subject of ongoing research.

Development of the intestinal immune system occurs in response to antigen exposure. Full development of lymphoid tissue can take 7–9 weeks and can be delayed by early weaning at 3–4 weeks of age, a practice common in most modern pig-producing countries (Vega-Lopez et al. 1995). This, among many other factors, predisposes to post weaning diarrhea associated with enterotoxigenic *E. coli* (ETEC) or other pathogens. There is increasing interest in the role of glutamine in intestinal immune function. Glutamine is an important nitrogen source for enterocytes and plays a key role in maintaining mucosal cell integrity and gut barrier function (den Hond et al. 1999). Key functions of lymphocytes are dependent on glutamine provision (Graham et al. 2000); glutamine depletion results in immunosuppression, whereas glutamine administration has been found to have significant beneficial effects on the gut mucosal structure and intestinal immune function of piglets after weaning (Pierzynowski et al. 2001). Enhanced intestinal immune function of piglets at weaning by feeding nucleotides for 2–4 weeks after weaning was found to have an immune-enhancing effect on piglets through improving T-cell–mediated responses (Cameron et al. 2001).

The use of dietary immunomodulators including yeast extracts, plant extracts, and animal by-products in weaned pigs has been reviewed by Gallois and Oswald (2008). To date the most promising results have been obtained with the use of spray-dried animal plasma, particularly porcine plasma. A number of studies in which pigs were orally challenged with pathogenic *E. coli* have shown that pigs fed spray-dried porcine plasma (SDPP) had less adverse clinical signs and growth effects (Bosi et al. 2004; Niewold et al. 2007; Torrallardona et al. 2007; Yi et al. 2005). In addition to providing specific antibody protection, nonspecific binding of the plasma molecules to intestinal receptors for *E. coli* is also thought to occur in pigs fed SDPP. Starter diets containing 5% or more SDPP fed for 14 days after weaning were found to reduce intestinal barrier dysfunctions associated with weaning stress and increase feed intake and growth rate (Peace et al. 2011; Pujols et al. 2016).

The role of conjugated linoleic acid (CLA) in the prevention of bacterial-induced colonic inflammation in the pig was studied by using a swine dysentery challenge model (Hontecillas et al. 2002). Feeding CLA-supplemented diets for 7 or 10 weeks pre-challenge prevented clinical

signs and lesions of swine dysentery. Despite colonization by *Brachyspira hyodysenteriae*, the CLA-supplemented pigs maintained cytokine profiles and lymphocyte subset distributions in keeping with non-challenged control pigs. This suggests that CLA modulates the pig's immune effector mechanisms preventing the normal host response to infection, instead of targeting the bacterial agent. Finding cost-effective methods of enhancing immune responses that promote tolerance of intestinal pathogens in weaned and grower pigs under commercial conditions would bring significant benefits to the pig industry.

Gut flora

The intestinal flora of the pig is extremely complex and diverse, making it difficult to study quantitatively and qualitatively, but this is an area of increasing interest in relation to maintaining nutritional, physiological, and immunological functions (Lee and Mazmanian 2010, Brestoff and Artis 2013). Our knowledge of the gastrointestinal microbiota has dramatically changed with the development of new molecular tools to describe taxonomic compositions and distributions. The increasing realization that the microbial ecosystem of the gut strongly influences health and performance has sparked interest in this area. Isaacson and Kim (2012) have reviewed studies of the microbiome of the GIT of pigs and described the impact of diet changes, stress, and disease. In summary, they report that the majority of bacteria in the pig intestine belong to two phyla: *Firmicutes* and *Bacteroidetes*. There are distinct differences in the microbiome from region to region. For example, in the ileum, there are a high proportion of bacteria from the phylum *Proteobacterium*. Most of the studies reviewed by Isaacson and Kim (2012) are descriptive in nature, and there remains a need for research that investigates how the composition of the gut microbiome contributes to the health of the pig.

Marked changes occur in the gut environment (for example, pH and organic acids) and microbial activity along the GIT of pigs (Bach Knudsen et al. 1991, 1993). Differences in the diet composition can impose further changes and could affect the diversity of the gut microflora. In experiments involving diets containing different levels of soluble and insoluble NSPs, a medium to high content of NSP resulted in higher microbial diversity in the small intestine and rectum (Högberg et al. 2001, 2004). The high NSP diets induced a higher proportion of propionic acid, and the low NSP diets induced a higher proportion of acetic acid, suggesting that the balance of the microbial flora was altered accordingly by differences in the dietary carbohydrate composition. The ratio of soluble to insoluble NSP also influenced the coliform diversity in the large intestine. A higher proportion of soluble NSP increased the coliform diversity, reflecting a more balanced microbiota compared with that generated by the diets that were higher in insoluble NSP. In recent years, many studies have shown how the intestinal microbiota can be manipulated through dietary changes aimed to promote "beneficial" bacteria, such as use of a low fat/high fiber diet (Heinritz et al. 2016). It is a noteworthy reminder that the microbiota in the gut lumen is distinct from the bacteria that adhere to the mucosa. Levesque et al. (2012) showed that ileal mucosa-associated bacteria, but not the bacteria in the digesta, were influenced by nutrition and the use of antibiotics.

The quantity and quality of protein fed to pigs can also have an impact on the microbiota, particularly if there is an imbalance between fermentable carbohydrate and potentially fermentable protein in the large intestine (Bikker et al. 2006; Piva et al. 2006). Microbial protein digestion in the large intestine may result in increased levels of potentially toxic substances, e.g. ammonia, amines, and phenols, that can contribute to the adverse effects of enteropathogenic processes (Bikker et al. 2006; Nyachoti et al. 2006).

The potential value of prebiotics (compounds other than dietary nutrients that act as substrates for populations of beneficial microbial organisms in the gut) and probiotics (preparations containing beneficial bacteria) has been the subject of many studies in relation to gut health and prevention of enteric infections. Prebiotics achieve their beneficial effects in two ways. First, compounds such as fructo-oligosaccharides can be fermented by favorable bacteria (e.g. *Bifidobacteria* and lactobacilli), giving them competitive advantage (Bindels et al. 2015; Nemcová et al. 1999). Second, mannose-containing compounds added to the diet result in binding with pathogenic bacteria such as *E. coli* and *Salmonella* that may have mannose-specific lectins in fimbriae. This reduces attachment of pathogenic bacteria to receptor sites on gut mucosal cells (McDonald et al. 2002). Inulin is a natural polymer of fructose extracted from chicory that is considered to have prebiotic properties (Gibson and Roberfroid 1995; Roberfroid et al. 1998). Digestion of inulin in weaned pigs is mainly microbial and takes place in the large intestine where it modifies the profile of fermentation metabolites, resulting in an increase in N-valerate and propionate and a decrease in acetate and ammonia (Rossi et al. 1997). The short-chain fatty acids including butyrate stimulate the growth of *Bifidobacteria* and lactobacilli. Butyrate regulates processes associated with proliferation and differentiation of colonic enterocytes as well as apoptosis, thereby having a direct effect on colonic health (Tako et al. 2008). Using an *in vitro* adhesion assay for *E. coli*, 5% inulin was found to partially inhibit adhesion of F4-positive *E. coli* to the small intestinal villi. The study also suggested that inulin might have immunomodulatory effects by boosting IgA and

IgM antibodies in pigs exposed to foreign proteins (Rossi et al. 2001). In newly weaned pigs, addition of fructo-oligosaccharide and/or sugar beet pulp to the diet tended to increase the number of intestinal *Bifidobacterium* species and reduce the level of *E. coli*, although there was little difference in the incidence of diarrhea (Kleingebbink et al. 2001). The population of *Bifidobacterium* species is variable in piglets and constitutes less than 1% of the total bacterial population (Mikkelsen and Jensen 2001), so research to determine the full spectrum of beneficial bacteria in the microbiota will aid understanding greatly in years to come (Bindels et al. 2015). Certain plant metabolites may interact with short-chain fatty acids to create inhibitory conditions for pathogens such as *E. coli* 0157 (Duncan et al. 1998).

Probiotics, known as direct-fed microbials in the livestock industry, work on the principle of competitive exclusion of pathogenic bacteria and have been used successfully to control *Yersinia* infection in pigs (Asplund et al. 1996). Feeding probiotic bacteria, especially *Lactobacillus* and *Bifidobacterium*, may help with controlling enteric infections pre- and post weaning when the resident microflora are not yet stable (Hou et al. 2015; Pieper et al. 2010). They can enhance immune responses during infection (Naqid et al. 2015) and promote weight gain (Konstantinov et al. 2008). Probiotics may also inhibit adherence of enteropathogenic *E. coli* and other gram-negative bacteria to enterocytes through occupying receptor sites (Mack et al. 1999; Spencer and Chesson 1994). This has potential for improved control of a wide range of enteric infections, particularly those of zoonotic importance such as *Salmonella* and *Campylobacter*. However, the efficacy of probiotics is reported to be inconsistent, requiring continued application, with timing and correct dosing both playing a major role in their success (Fouhse et al. 2016). The use of probiotics is subject to regulatory control in many countries to ensure their safety and efficacy.

Dietary interventions

Following the European Union ban on the use of the majority of antibiotic growth promoters, alternative measures to control the number and activity of intestinal bacteria have been explored. These include changes in management, feeding, and hygiene practices; the use of products such as probiotics, prebiotics, enzymes, herbs and plant extracts, prefermented feeds, and organic acids (de Lange et al. 2010; Thomke and Elwinger 1998); selective breeding for resistance; improving the pig's immune response through the use of vaccines, cytokines, and other immunomodulatory agents; organic acids; inorganic chemicals such as zinc oxide; and the use of specific bacteriophages or bacteriocins (de Lange et al. 2010; Gallois and Oswald 2008). Different cereals (wheat-based vs. barley-based diets), methods of feed processing (mash vs. pellets), and factors including feed particle size have all been explored (Jansman 2016). Weaned pigs were found to have better uniformity of the mucosal microbiota when fed a barley-based diet as compared with wheat (Levesque et al. 2014). Furthermore, feeding barley-based diets with large particle sizes as a mash has been found to reduce shedding of *Salmonella* species, reduce intestinal *E.coli* numbers, and increase beneficial butyric and propionic acid levels in the cecum and colon (Pieper et al. 2012; Longpré et al. 2016). However, despite such benefits, in general, growth performance was found to be poorer on such diets, so they are commercially disadvantageous.

Dietary fiber and cereals

Different forms of fiber in the diet can influence the composition and metabolic activity of the large intestinal microflora in pigs (Bach Knudsen et al. 1991; Jensen and Jørgensen 1994; Reid and Hillman 1999). Little is known of mechanisms by which the commensal colonic microflora interacts with pathogenic bacteria; hence the basis of dietary control of infectious enteric diseases through manipulating the microbiota is not understood. Ways in which diet could influence pathogenic enteric infections include changing the amount or balance of substrates available for the organism at particular sites, influencing the viscosity, accessibility to receptor sites, and/or intestinal motility. For example, different cereal types and particle sizes were shown to alter epithelial cell proliferation and lectin binding patterns of the epithelium of the large intestine of pigs (Brunsgaard 1998).

The diet can also influence intestinal function. Components in boiled rice inhibit secretion in the small intestine and therefore reduce the magnitude of secretory diarrhea due to pathogens such as ETEC (Mathews et al. 1999).

One of the best-recognized examples of dietary effects on enteric pathology is gastric ulceration (complete discussion below), in which ulceration of the pars esophagea occurs, particularly in growing and finishing pigs. Such lesions can be associated with reduced growth rates (Ayles et al. 1996a) but more importantly can be a cause of gastric hemorrhage and perforation leading to acute illness and death. Many studies have demonstrated a strong association between finely ground high wheat diet and gastric ulceration (Accioly et al. 1998).

Early studies on the influence of diet in post weaning colibacillosis suggested that inclusion of fiber sources reduced the severity and incidence of diarrhea (Bertschinger et al. 1978; Bolduan et al. 1988). Later studies have compared the effects of feeding different post weaning diets in experimental *E. coli* challenge

models. Conclusions from research by different groups are inconsistent, revealing that this area is still poorly understood. For example, studies by McDonald et al. (1999, 2001) showed that diets supplemented with NSP increased the number of *E. coli* in piglet intestines and concluded that these diets were "provocative" in terms of causing diarrhea. Conversely, Wellock et al. (2008) found that inclusion of soluble NSP in the diet was beneficial to piglets challenged with ETEC. The occurrence of diarrhea was significantly reduced; there were lower cecal digesta pH and an increased *Lactobacillus* to coliform ratio when compared with pigs fed diets containing insoluble NSP. Clearly, this is a complex area where the quantity, nature, viscosity, and transport time of the small intestine contents influence the proliferation rate of intestinal microbes. This affects the rate of intestinal motility, fermentation processes, and ultimately the health of the newly weaned piglet. In studies comparing wheat- and barley-based diets in weaned pigs, better uniformity of the mucosal microbiota was found with barley-based diets (Levesque et al. 2014). *Salmonella* persistence and shedding can be reduced significantly through dietary changes, notably by feeding barley-based diets as a meal; however this is not practical for many feeding systems, and it can be disadvantageous economically.

The most work has been done on the influence of diet to manipulate expression of *Brachyspira* infections through altering the fermentation processes or the microbiota in the large intestine or a combination of both. Whereas a highly digestible cooked rice diet was found to be protective for swine dysentery in some studies (Siba et al. 1996), it was not in others (Kirkwood et al. 2000; Lindecrona et al. 2003). Feeding the cooked rice diet to pigs already affected with swine dysentery did not reduce the duration or severity of disease (Durmic et al. 2000). In porcine colonic spirochetosis, pigs on the cooked rice diet developed infection later and to a lesser extent than pigs on wheat-based diet (Hampson et al. 2000; Lindecrona et al. 2004). In a study of different cereal types, feeding steam-flaked maize or sorghum reduced the incidence of swine dysentery (Pluske et al. 1996a). Soluble NSP and resistant starch were identified as important factors promoting large intestinal fermentation and bacterial colonization, whereas with the addition of a source of mainly insoluble NSP (oat chaff), the diet remained protective (Pluske et al. 1998). Addition of enzymes to wheat-based diets and the use of heat extrusion to increase digestibility of starch in the small intestine were tested in terms of their potential protective effects in swine dysentery, but neither processes prevented colonization (Durmic et al. 2000). Likewise the use of a sorghum-based diet was tested as sorghum is inherently low in soluble NSPs, but this diet was not protective against swine dysentery (Durmic 2000). However, the grind size of diets was important with significantly more pigs developing swine dysentery with coarsely ground wheat or sorghum than with these grains finely ground (Hampson et al. 2001). In a comparative study using a barley/triticale-based diet supplemented with either rapeseed cake or dried chicory roots and sweet lupins, the latter diet was found to be completely protective when pigs were challenged with *B. hyodysenteriae* (Thomsen et al. 2007). The colonic microflora of those pigs had higher proportions of *Bifidobacteria* and *Megasphaera* species that might have inhibited colonization by *B. hyodysenteriae* (Mølbak et al. 2007). *In vitro* studies have shown that growth of *B. hyodysenteriae* can be inhibited by at least four different bacteria isolated from the pig GIT (Klose et al. 2010), but it remains to be seen if this effect can be achieved *in vivo*. Dietary factors that have been found to promote the onset of swine dysentery are high levels of soybean meal (Jacobson et al. 2004) and diets containing 30% distillers dried grains with solubles (Wilberts et al. 2014). The latter causes significant changes in the microbiome, reducing numbers of *Lactobacillus* species and increasing *Prevotella* species (Burrough et al. 2015).

Carriage of *Oesophagostomum dentatum* in pigs is enhanced by diets rich in insoluble fiber (Petkevicius et al. 1997), whereas colonization by *Trichuris suis* did not appear to be significantly influenced by diets that differed in terms of their carbohydrate properties (Thomsen et al. 2006). Addition of chicory roots and sweet lupins to the diet did not prevent colonization by *T. suis* in pigs that were challenged experimentally (Thomsen et al. 2007).

Dietary protein

The protein levels in commercial rations for newly weaned pigs are usually high in order to promote optimal growth rates. However, in early comparative studies using high (21%) and low (13%) protein diets, Prohaszka and Baron (1980) showed that the high protein diet, especially when the protein is of reduced digestibility, predisposed to post weaning colibacillosis. These findings have been supported by a number of subsequent studies in piglets with naturally acquired infections and also in experimental challenge studies using ETEC, as reviewed by Pieper et al. (2016). The role of bacterial fermentation of proteins in the hindgut as a potential risk factor for diarrhea in pigs has been demonstrated (Jha and Berrocoso 2016; Rist et al. 2013). The formation of toxic metabolites is believed to have adverse effects on the colonic epithelial cells, arising from the proliferation of proteolytic bacteria. The benefits of reducing protein level in weaners reared under large-scale commercial conditions require careful assessment. However, reduction in dietary protein (to less than 18%) or a total daily intake of less than 60 g could be beneficial in units where

repeated problems of post weaning diarrhea due to ETEC result in piglet losses (Heo et al. 2012, 2015).

Organic acids, inorganic compounds, and fatty acids

Alternatives to antibiotic inclusion in weaner diets include the use of organic acids such as potassium diformate (Roth et al. 1998). Addition of 1.8% potassium diformate to a piglet starter ration decreased the counts of total anaerobic bacteria, lactic acid bacteria, yeasts, and coliforms in the digesta from the stomach, distal small intestine, cecum, and midcolon over a period of 4 weeks after weaning (Canibe et al. 2001). The apparent antimicrobial effects were attributed to the protons and anions formed from formic acid passing through the bacterial cell wall. These have a disruptive effect on protein synthesis and inhibit bacterial enzymes, thereby reducing bacterial replication (Partanen and Mroz 1999). Other studies agree that coliform counts in the stomach and proximal colon decrease when levels of formic acid increase (Gabert et al. 1995; Kirchgessner et al. 1992) or when potassium diformate is used (Février et al. 2001).

Supplementation of weaner diets with organic acids or other salts has been shown to reduce the incidence of post weaning diarrhea and improve growth performance of piglets (Sutton et al. 1991). When the coliform inhibitory effects of various organic acids were compared, the results going from most effective to least effective were benzoic, fumaric, lactic, butyric, formic, and propionic acid. By far the greatest increase in the use of organic acids in pig rations has occurred in response to *Salmonella* control programs in European countries. Clinical trials have shown that including formic acid or lactic acid in the rations of growing and finishing pigs significantly reduces the seroprevalence to *Salmonella* when tested by "meat juice" ELISA at slaughter age (Creuz et al. 2007; Dahl 2008). A similar effect can be achieved by supplying organic acids to pigs via the drinking water (van der Wolf et al. 2001). The impact of these organic acids on other enteric organisms has not been closely studied. However, it seems probable that they lead to a general reduction in coliform numbers that could have additional benefits if other pathogens are prevalent.

Feed supplemented with 2500 ppm zinc oxide has been shown to reduce post weaning diarrhea and improve growth rates through limiting proliferation of enteropathogenic *E.coli* (Melin et al. 2001). However, some countries have legislation that bans its use or limits the maximum permissible level of zinc inclusion in pig diets. Mounting environmental concerns mean that the use of zinc oxide to control post weaning diarrhea is likely to be widely phased out in the coming years.

Phytogenics

Phytogenic feed additives comprise a wide variety of herbs, spices, and products derived from these materials (including essential oils) that have been shown to have antimicrobial properties *in vitro*. Generally the phenolic components of these products tend to be most active and appear to act by increasing the permeability of bacterial membranes (Burt 2004). A variety of essential oils have been tested against *Salmonella* Typhimurium and *E. coli* F4 (Si et al. 2006a), and although they retained their antibacterial activity in the presence of pig cecal content (Si et al. 2006b), pig challenge studies showed poor efficacy. One explanation for the lack of efficacy *in vivo* has been put forward by Michiels et al. (2008), who demonstrated that certain essential oils absorbed in the stomach of pigs therefore may not be available to act on bacteria in the small intestine.

Fermented liquid feeds

Liquid feeding can improve the feed intake, growth, feed conversion, and health of weaned pigs (Brooks et al. 1996). However, steeping feed in water promotes bacterial proliferation in the feed, which reduces the quality of the feed and presents health risks. Feeding fermented liquid feed has been used as a means of controlling enteric infections through acidification. As part of the diet for newly weaned pigs, fermented liquid feed has beneficial effects on the villus height and ratio of villus height to crypt depth in the proximal jejunum (Scholten et al. 1999). The mechanism is uncertain, but it could be due to a lower pH, increased levels of organic acids, and an altered microbiological status. The use of fermented liquid feed in newly weaned piglets caused a significant reduction in the coliform population in the terminal ileum, cecum, and colon compared with piglets fed dried feed (Jensen and Mikkelsen 1998; Moran et al. 2001). Feed was prepared by inoculating the diet with lactic acid bacteria (*Lactobacillus plantarum*) and steeping it for 5 days at 25 °C before feeding. Fermentation prevents bacterial colonization and spoilage of the liquid diet by enteropathogens and other spoilage bacteria. The fermentation temperature is important in relation to bacterial survival. *E. coli* was eliminated more effectively at 37 °C than 20 °C (Beal et al. 2001). There were also strain differences in terms of *E. coli* survival in fermented liquid feed, with F4 (K88) being the most resistant to killing by fermentation (Beal et al. 2001). The effect of temperature has implications for the management of fermented liquid feed systems. Cold-shock proteins help *E. coli* survive at lower temperature (Phadtare et al. 1999). Prefermented diets not only lower the acidity of the diet but also reduce the soluble NSP content of the diet (Hampson et al. 2001).

Piglets showed a significant preference for freshly prepared liquid feed over fermented liquid feed when given

the choice (Demeckova et al. 2001). To prevent spoilage, chlorine dioxide added at 300 ppm was found to eliminate coliforms from liquid feeds for young piglets without adversely affecting palatability or growth performance (Demeckova et al. 2001). Chlorine dioxide is a strong oxidizing agent with broad antimicrobial spectrum, being active against bacteria and viruses (Junli et al. 1997). Addition of chlorine dioxide to freshly prepared wet feed did not significantly alter the intake when compared with nonsanitized freshly prepared wet feed. Chlorine dioxide is reported to kill *E. coli* through loss of permeability control of the outer bacterial cell membrane (Berg et al. 1986).

Enzymes

The use of feed enzymes for swine has made great progress over the past decade. Their value in promoting growth and improving feed efficiency has been well recognized. Feed enzymes also affect the microbiome and thus impact gut health (Kiarie et al. 2013). Phytase is the most commonly used feed enzyme, but carbohydrase, proteases, and lipases are also used (Adeola and Cowieson 2011). It has been observed that supplementing weaner pig diets with phytase results in increased *Bifidobacterium* and *Clostridium* numbers in the ileum (Wang and Lei 2011). The potential for developing enzymes to aid in the control of enteric diseases including post weaning *E. coli* diarrhea, swine dysentery, and salmonellosis is reviewed by Kiarie et al. (2013). These authors emphasize that research is needed in this area in order to develop strategies to alter host–microbiome–diet interactions in favor of the host, but feed enzymes could have a role to play.

Rehydration of diarrheic piglets

Oral rehydration fluids are used in piglets with acute diarrhea especially during outbreaks of ETEC and rotavirus infections. Studies in rats and clinical studies in children have shown that reducing the osmolality of oral rehydration solutions has beneficial effects on the course of diarrhea and the clinical outcome (Thillainayagam et al. 1998). Using an experimental pig model, Kiers et al. (2001a) demonstrated that solutions with low osmolality promoted intestinal fluid absorption. However, ETEC infection resulted in a decrease in net fluid absorption independent of osmolality, as compared with that of unaffected tissue.

In an experimental model, mold-fermented soybean products were found to be beneficial in maintaining fluid balance during post weaning ETEC infection through preventing fluid loss (Kiers et al. 2001b). The mechanism is uncertain. It might interfere with the attachment of *E. coli* to epithelial cells or modulate the effects of toxin in the intestine.

Regional diseases and pathology of the digestive system

The oral cavity

There are several recognized congenital defects affecting the oral cavity. Cleft palate (palatoschisis) is a multifactorial developmental abnormality. Cleft palate in piglets has occurred with feeding poisonous plants such as poison hemlock (*Conium maculatum*) or wild tree tobacco (*Nicotiana glauca*) to sows or gilts in early pregnancy (Keeler and Crowe 1983; Panter et al. 1985). Likewise, accidental contamination of sow feed with seeds of *Crotalaria retusa* during pregnancy has resulted in palatoschisis in piglets (Hooper and Scanlan 1977). Brachygnathia superior (shortness of maxillae) is an inherited condition that is progressive and may be confused with progressive atrophic rhinitis; it is a normal characteristic for some breeds. Hypertrophy of the tongue is a rare congenital anomaly in pigs that interferes with normal suckling behavior. Prominent erectile marginal papillae are a normal feature of the lateral margins of the tongue of newborns. Epitheliogenesis imperfecta can affect the gingiva and tongue and is seen as irregular, well-demarcated red areas that are devoid of epithelial tissue.

Oral lesions arising from traumatic damage are relatively common. Clipping the teeth of baby pigs exposes the dental pulp. If the dental pulp becomes infected, pulpitis, dental abscesses, and osteomyelitis can ensue. Gingivitis and periodontal inflammation is usually associated with poor teeth clipping technique, resulting in damage to the gingival epithelium. Stomatitis and tooth root abscesses may follow. *Fusobacterium necrophorum* is a common isolate from such lesions. Pigs have a diverticulum of the pharynx in the posterior wall immediately above the esophagus. Barley awns and other fibrous materials can lodge there and penetrate the pharynx, causing pharyngeal cellulitis, which is usually only seen in young pigs. Stomatitis can also be caused by irritating chemicals, caustic substances, toxic compounds, and physical burns. Blistering and erosion of the snout epithelium can arise due to sunburn or vesicle-forming viruses. Oral "thrush" or candidiasis (*Candida* sp.) is occasionally seen in piglets, presenting as pale plaque-like lesions on the tongue, hard palate, or pharynx. In rare cases they can extend further, affecting the esophagus and stomach. Factors such as frequent antibiotic administration or intercurrent diseases such as porcine reproductive and respiratory syndrome (PRRS) virus infection can promote the onset of candidiasis.

A number of important infectious diseases show lesions on the snout and oral tissues. These are primarily the viral vesicular diseases, including foot-and-mouth disease, swine vesicular disease, vesicular stomatitis, and

Senecavirus A. Lesions include blanching of the epithelium, vesicles, erosions, and epithelial flaps. Sunburn and perhaps other viruses (i.e. parvoviruses, picornaviruses, Aujeszky's disease virus) can occasionally cause lesions on the snout resembling vesicular diseases.

Ulcerative glossitis and stomatitis have been reported in piglets with exudative epidermitis. Piglets may also develop ulcers on the dorsum of the tongue and occasionally on the hard palate associated with *Staphylococcus hyicus* infection (Andrews 1979). Oral erosions and ulcers can also be seen in piglets with congenital swinepox. *Actinobacillus lignieresii* can cause swelling and inflammation of the tongue, with nodule and ulcer formation. Soft tissues of the pharynx and neck can also be affected. Of the parasitic infections, cysticercosis and *Trichinella spiralis* can affect the tongue and muscles of mastication. In grazing pigs, *Gongylonema* species has been found in the mucosa of the tongue where they cause mild, localized inflammation (Zinter and Migaki 1970).

The tonsils have a strategic role in immune surveillance of the oropharynx (Horter et al. 2003). A host of bacterial agents, including many types of *Streptococcus suis* and *Pasteurella* species, are frequently carried in the tonsils (Torremorrell et al. 1998). Crypt inflammation and lymphoid hyperplasia are associated with bacterial infections. Necrotizing tonsillitis occurs with pseudorabies, the tonsils being the site of primary virus replication (Terpstra and Wensvoort 1988). Tonsillitis is also a feature of swine vesicular disease. Hemorrhagic necrotizing tonsillitis can occur in pigs with anthrax. In pigs clinically infected with porcine circovirus type 2 (PCV2), the lymphoid tissue of the tonsils is involuted (Chae 2004). The altered status of the tonsillar lymphoid tissue in particular is thought to facilitate bacterial infections of the tonsils, with an increased likelihood of bacteremia.

There are few reported problems concerning the salivary glands in pigs, but sialoadenitis occurs in vitamin A deficiency (Uzal et al. 2016). The interlobular ducts of the salivary glands undergo squamous metaplasia, leading to salivary stasis, secondary infection, and purulent inflammation. This results in pronounced swelling of salivary glands. Epithelial degeneration of salivary ducts is seen in swine vesicular disease.

The esophagus

Conditions affecting the esophagus are uncommon but include hyperkeratosis, parakeratosis, mycotic infection, obstructions, and traumatic lesions. Hyperkeratosis and thickening of the epithelium are associated with vitamin A deficiency or chlorinated naphthalene toxicity. Parakeratosis of the esophagus occurs in pigs with cutaneous parakeratosis due to zinc deficiency. Parakeratotic thickening of the epithelium of the distal esophagus, with basal hyperplasia of the epithelium, is commonly seen in pigs with ulceration of the pars esophagea of the stomach. Reflux esophagitis is recognized in some pigs with ulceration of the pars esophagea. The gastric secretion has corrosive effects on the squamous epithelium, resulting in mucosal erosion, ulceration, and inflammation. Some cases develop annular scarring as part of the healing process, leading to stenosis and muscular hypertrophy of the distal esophagus. As mentioned previously, mycotic esophagitis caused by *Candida albicans* can occur in suckling piglets and weaners that are immunocompromised or have microflora disruption.

Obstruction and/or perforation of the esophagus is associated with ingestion of large objects such as stones, potatoes, apples, or corncobs. Perforation can result from ingestion of sharp objects such as fence wire or nails. Inflammation and subsequent stricture are sequelae that affect the esophagus at the site of perforation. Such conditions lead to dysphagia and distension of the esophagus cranial to the site of obstruction or stricture. Encephalitis affecting the medulla oblongata and/or the nuclei or tracts of the cranial nerves involved in swallowing (V, IX, X, XII) also results in dysphagia. Such conditions are very rare in the pig. Nematode parasites (*Gongylonema* species) occasionally occur in the esophageal mucosa leaving serpentine-shaped tracts. However, these parasites do not appear to have any adverse effects in pigs.

The stomach: gastric ulceration

Conditions affecting the stomach are mainly physical or functional in nature, the most important being gastroesophageal ulceration affecting the pars esophagea. Abattoir surveys demonstrate that the prevalence of stomach lesions, including parakeratosis, erosions, and ulcers, often approaches 90%, depending on the feeding and husbandry practices (Driesen et al. 1987). There is great herd-to-herd variation in prevalence and severity. Ulceration of the pars esophagea can affect any age of pig, but the highest rate of ulceration occurs in pigs 3–6 months of age. Mortality due to gastric ulceration among grower/finisher pigs has been reported to be about 1–2% on some farms, with much higher levels occurring sporadically (Deen 1993; Melnichouk 2002). Sows at the time of parturition are also a relatively high-risk group. Examination of culled sows revealed 60% with stomach lesions and 10–15% with ulcerations (Hessing et al. 1992; O'Sullivan et al. 1996). Frequently, sow stomachs have extensive scar tissue, indicating previous severe ulcerative episodes. Gastric ulceration has been reported as a common cause of sow mortality (Chagnon et al. 1991; Sanford et al. 1994).

Lesions associated with ulceration of the pars esophagea rarely extend into the contiguous esophagus or the glandular region of the stomach. Ulcerations and erosions of the pars esophagea may involve only a small portion or all

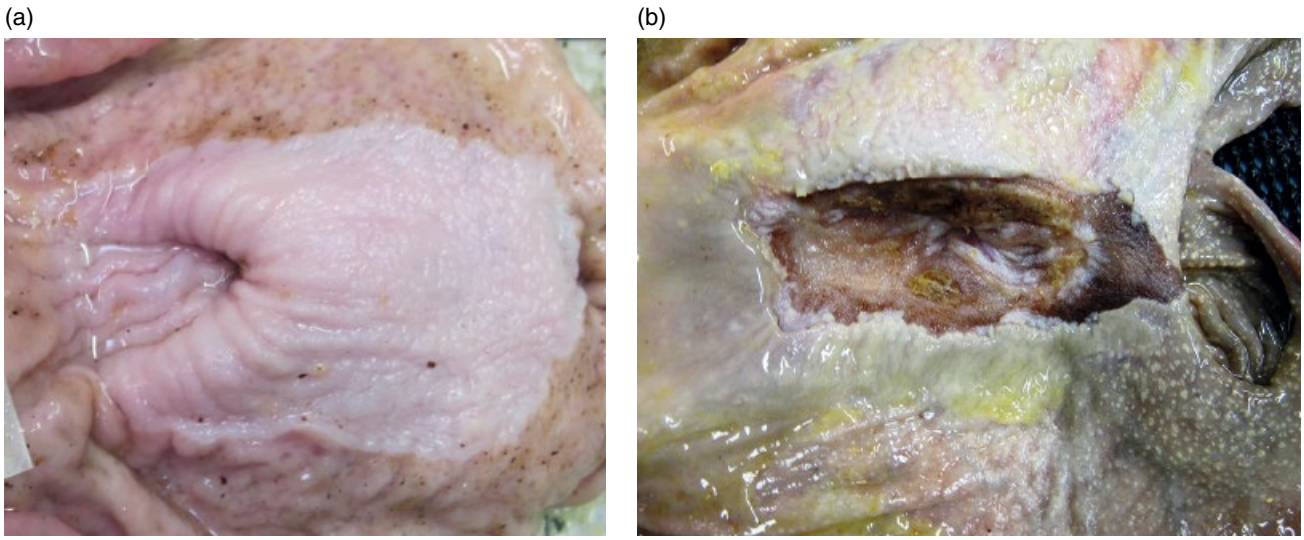


Figure 15.1 Pars esophagea of the stomach. (a) Normal appearance. (b) Extensive ulceration of the squamous epithelial tissue.

of the gastric squamous mucosa. The most common site for ulceration is at the junction of the pars esophagea and the cardiac mucosa (Penny and Hill 1973). The normal epithelium of the pars esophagea is smooth, white, and glistening and is easily distinguished from the surrounding glandular mucosa (Figure 15.1a). It is believed that lesions usually progress from hyperkeratotic parakeratosis that causes a thickened, rough appearance to fissuring and peeling that result in erosion and eventually ulceration (Figure 15.1b).

Frequently, the hyperplastic epithelium of affected pars esophagea is greenish yellow as a result of bile staining, particularly when the surface is rough and thickened due to parakeratosis. This type of corrugated surface may flake and peel off readily. When ulceration involves the entire pars esophagea, the lesion has a punched-out or crater-like appearance with elevated ridges at the margin of the circular ulcer. The floor of the ulcer may be so smooth that it is misinterpreted as normal tissue (Uzal et al. 2016).

Gross evaluation of stomach lesions is commonly incorporated into an abattoir surveillance program to monitor disease status. To examine the pars esophagea, the stomach should be incised along the greater curvature and inverted. Emptying the contents and rinsing the stomach before examination will improve the accuracy of the inspection. Morphological scoring guides have been published (Kopinski and Mckenzie 2007).

Histologically, the lesion is the result of thickening and parakeratosis, with nucleated cells present on the mucosal surface. Rete pegs and proprial papillae are elongated, and neutrophils and eosinophils are often present at the tips of the proprial papillae. Epithelial separation and erosion usually occur beneath a band of cells with cytoplasmic pallor and nuclear degeneration (Embaye et al. 1990). Ulcers of the pars esophagea usually involve only the submucosa, but they may advance to the muscularis externa and occasionally to the serosa.

In the case of a pig that has died acutely of gastric ulceration, postmortem diagnosis is usually straightforward. The carcass is typically pale but in good body condition. The stomach may contain clotted or unclotted blood and fibrinous exudate enclosing a variable amount of food. Blood may be found in the intestine as well but is generally dark and seen as melena in colon. A blood clot may be adhered to the surface of the ulcer, which is generally deep and extensive in the case of a fatal hemorrhagic episode. Clinical signs tend to reflect the degree of blood loss associated with the gastric lesion. Frequently, a pig that had appeared to be healthy only a few hours earlier is found dead, and the carcass is extremely pale. If blood loss occurs more slowly, signs associated with anemia will be apparent, such as paleness, lethargy, weakness, increased respiratory rate, and anorexia. In addition, black tarry feces may be noted, and some pigs show signs of abdominal pain by grinding their teeth and arching their backs. Vomiting may occur. Rectal temperatures of affected pigs are often below normal. It is important to remember that not all pigs that die from gastric ulcers will have clotted or fresh blood present in the stomach. To differentiate gastric ulcers from other causes of anemia or hemorrhagic enteric diseases, especially porcine hemorrhagic enteropathies, one must always examine the pars esophagea grossly for evidence of ulceration.

Sporadic outbreaks of gastric ulceration tend to occur among grower/finisher pigs, and frequently when one pig has suddenly died, careful observation will reveal others in the group that are showing signs of anemia. When ulceration occurs without significant blood loss,

animals will generally appear to be in normal health. Evidence that subclinical ulceration reduces growth rate is inconsistent. Various researchers have attempted to correlate severity of stomach lesions at slaughter with growth performance during the grower/finisher phase. Certain studies have found no relationship (Backstrom et al. 1981; Pocock et al. 1969), whereas Elbers et al. (1995) observed a 50–75 g/day decrease in growth rate for pigs with ulcers versus pigs with normal stomachs. This last result is in agreement with findings of a trial that followed pigs using endoscopic examination to measure gastric lesions (Ayles et al. 1996b).

Ulcers can occur rapidly and heal quickly, making it difficult to relate lesions at slaughter with performance during the grower/finisher stage. Evidence of past ulceration can sometimes be seen as cicatrization or fibrosis with a reduction in size of the pars esophagea. In extreme cases, the pars esophagea is entirely destroyed, and stenosis of the esophagus at its entry to the stomach occurs. Pigs afflicted with stenosis are observed to vomit shortly after eating but are hungry and therefore will immediately resume eating. These pigs tend to grow more slowly than pen mates despite maintaining good appetites.

The exact cause or causes of gastric ulceration are not completely understood, but many of the risk factors are well known, relating to the fluidity of the stomach content and the speed of passage of ingesta through the stomach. Generally, factors that increase the firmness of the stomach content help prevent gastric lesions, and factors that cause increased fluidity of the contents increase the risk of ulcers (Nielsen and Ingvartsen 2000).

Fine particle size of feed has been shown to increase the prevalence of gastric lesions (Ayles et al. 1996b; Wondra et al. 1995a). In addition, pelleting of feed may also increase the likelihood of ulcers developing (Potkins and Lawrence 1989). The prevalence and severity of ulcers vary with the cereal component of the diet. Generally, oats and barley appear to have a sparing effect (Reese et al. 1966), and corn and wheat tend to be more ulcerogenic (Smith and Edwards 1996).

The method by which the grain is processed affects the prevalence of ulcers. Grain that is ground using a hammer mill tends to be more ulcerogenic than if a roller mill is used (Nielsen and Ingvartsen 2000; Wondra et al. 1995b). Feed particle size is affected by grain component, milling procedure, and processing. Grains such as wheat are more likely to shatter during grinding and result in finer particle size compared with oats or barley. If grain is processed using a roller mill, there is less chance of the grain kernel shattering and creating "fines."

In addition, the pelleting process causes a further decrease in particle size. Nielsen and Ingvartsen (2000) showed that, in general, barley and rolling prevented stomach lesions, while wheat grinding and pelleting increased the prevalence and severity of ulcers. The overall effect of a feed with very fine particle size is that the stomach content is fluid and the emptying time is relatively rapid (Regina et al. 1999), and as a result, the pH gradient between the neutral proximal part of the stomach and the acidic distal region is lost.

The method of feeding may be as important as feed processing and composition. A major risk factor of ulcer development is an interruption of feed intake. Fasting of pigs has been a consistent method of experimentally producing gastric lesions (Lawrence et al. 1998; Pocock et al. 1968). Various workers have noted dramatic increases in ulcer severity and prevalence at slaughter in pigs examined after a 24 hour holdover period compared with pigs from the same herds slaughtered on the day of arrival at the abattoir (Davies et al. 1994; Lawrence et al. 1998). However, at least one study documented no association between a 24 hour feed withdrawal and an increased prevalence or severity of ulcers (Eisemann et al. 2002). Presumably, there are interactions between factors affecting stomach emptying, such as feed particle size and the effect of feed withdrawal that can explain the differences in findings between these studies.

Interruption of normal feed intake commonly occurs on almost all farms because of mechanical problems or human error. Periods of transition in feeding patterns, such as when gilts are transferred to the breeding herd from a finishing barn or when sows approach the time of parturition, should be viewed as high risk for ulcerogenic events. Hot weather resulting in dramatic reduction in feed intake is associated with gastric ulcer outbreaks (Deen 1993).

It is likely that factors such as acute infectious disease and season influence ulcer development in a similar manner to management practices that interrupt feed intake. Acute respiratory disease is associated with an increased likelihood of gastric ulceration (Dionissopoulos et al. 2001). In addition to respiratory disease causing inappetence, it is possible that increased levels of histamine as a result of infection could play a role, in that histamine is a powerful stimulant of gastric acid secretion. Injection of histamine has been shown to experimentally produce ulceration of the pars esophagea (Hedde et al. 1985). Experimental infection of gnotobiotic pigs with various viral pathogens, including PRRS virus, does not result in gastric ulceration; however, experimental infection of concurrent PRRS virus and PCV2 in Cesarean-derived colostrum-deprived pigs has caused gastric lesions (Harms et al. 2001).

Research clearly demonstrates that ulceration of the pars esophagea is not mediated by glucocorticoids (Zamora et al. 1980). Chronic elevation of corticosteroids in response to stressful conditions has been shown under controlled trials to not result in an increase in ulcer prevalence or severity (Jensen et al. 1996). Genetic susceptibility may play a role in ulcer development.

Researchers have reported a link between fast growth rate and/or low backfat and a high prevalence of gastric ulcers (Berruecos and Robinson 1972). It has also been reported that injection of swine with porcine somatotropin causes an increase in prevalence and severity of ulcers (Smith and Kasson 1991).

There has been considerable interest in finding an infectious cause of porcine gastric ulcers similar to the situation in humans. *Helicobacter*-like organisms have been identified in the glandular region of the stomach (Mendes et al. 1990) and appear to be widespread in the pig population with possibly more than half of all market hogs infected (Foss et al. 2013; Hellemans et al. 2007; Szeredi et al. 2005). Isolates of these tightly coiled spiral bacteria have been successfully cultured and given the name *Helicobacter suis* (Baele et al. 2008). Whereas some workers have observed a correlation between the presence of these bacteria in the pyloric region and the prevalence and severity of lesions in the pars esophagea (Barbosa et al. 1995; Queiroz et al. 1996), other researchers have not (Magras et al. 1999; Melnichouk et al. 1999; Szeredi et al. 2005). Bacterial colonization of the glandular stomach causing gastritis and the development of ulcers in the pars esophagea region have been observed in experimental challenge studies (Haesebrouck et al. 2009; Krakowka et al. 2005). It has been hypothesized that because *H. suis* are found in the antrum in close contact to hydrochloric acid-producing cells, gastritis due to *Helicobacter* infection may result in parietal cell stimulation causing hyperacidity and indirectly damage the unprotected epithelium of the pars esophagea or modifying the stomach microbiome. Recently, a novel *Fusobacterium* sp. was demonstrated to colonize the pars esophagea in *Helicobacter*-infected pigs and was found in higher numbers in pigs with ulceration, leading researchers to speculate that this novel *Fusobacterium* sp. may play a role in creating the ulcer lesion (De Witte et al. 2016).

Many of the factors associated with an increased risk of ulcer development are closely tied to economic competitiveness such as the use of finely ground feed and fast-growing, lean genetics. Therefore, steps to reduce the prevalence of gastric ulcers need to be carefully balanced between economic considerations and welfare concerns. Treatment for the most part is prohibitively expensive, laborious, and often unsuccessful. In addition, early diagnosis of gastric ulcer disease is difficult. Therefore, prevention of stomach lesions is generally considered the most appropriate approach to handling this problem. Many causative factors and complex interactions of nutrition, environment, and management contribute to the expression of this disease, but a coordinated effort by feed providers, owners, production personnel, and herd veterinarians can result in feed preparation standards and management that limit gastric ulceration as a swine production problem without reducing animal performance. The use of roller mills instead of grinding feed appears to be one of the best methods of reducing ulcers (Nielsen and Ingvartsen 2000). Feeding practices must be carefully monitored. An interruption of feed intake appears to be a major contributing factor to ulcer formation. Blocked feeders or waterers, heat stress, unpalatable feed, or the presence of vomitoxin in the feed can lead to ulcer problems. Good management practices can minimize the occurrence and influence of these factors.

Various attempts have been made to incorporate protective substances in swine feeds to prevent ulcer development. Increasing levels of antioxidants such as vitamin E and selenium beyond physiological requirements does not appear to be useful (Davies 1993). There is some evidence to suggest that the inclusion of S-methylmethionine sulfonium chloride (sometimes referred to as vitamin U) reduces the prevalence and severity of ulcers, but in general the slight ulcer score improvement may not be economically justified (Kopinski et al. 2007). Alfalfa has been used at levels of up to 9%, because it is high in vitamins E and K, as well as a source of increased fiber. Alfalfa was not effective in reducing the level of severity of ulcers in pigs treated with porcine somatotropin (McLaughlin et al. 1997). The addition of sunflower hulls to diets was shown to be effective in reducing lesions (Dirkzwager et al. 1998).

Products that reduce gastric emptying have been examined and found somewhat effective, at least in an experimental setting. Melatonin has been reported to slow gut motility and when included at levels as low as 2.5 g/ton results in less ulcer development (Ayles et al. 1996a). Similarly, diets containing sodium polyacrylate are retained for longer periods of time in the stomach of swine, and gastric ulceration is reduced (Yamaguchi et al. 1981). There may be circumstances in which various therapeutic agents are useful for treatment or prevention, but because of the many contributing factors and the interactions of these factors, it is unlikely that a single pharmaceutical product or management technique will be found that completely prevents gastric ulceration caused by various combinations of factors in different herds.

The stomach: other gastric conditions

Gastric over-distension can occur in adult pigs, especially in sows, but the cause is uncertain. It is thought to be associated with excessive intake of finely ground grain and water, resulting in excessive fermentation and gaseous distension.

Gastric volvulus is thought to be due to rapid intake of large quantities of feed and water followed by physical activity in a competitive group situation. Clockwise

torsion is apparently most common, although torsion can occur in either direction about the long axis of the stomach. The spleen is often involved and becomes extremely enlarged due to blood engorgement (Morin et al. 1984). The stomach becomes massively distended with gas and fluid, and intense mucosal congestion develops. This condition is rapidly fatal.

Gastric foreign bodies such as stones are frequently found in stomachs of outdoor sows that are maintained on stony ground. Stone chewing is a regular activity, and swallowing of the stones is thought to be accidental. Large quantities of gastric stones have been recorded in some sows, limiting the capacity of feed intake and resulting in loss of body condition.

Gastric venous infarction occurs in pigs, usually in association with bacteremia such as salmonellosis, erysipelas, or Glässer's disease or with toxemia. The lesion can also be seen in classical swine fever (CSF) (Elbers et al. 2003). The mucosa in the fundic area becomes reddish black in color, and caseous mucosal necrosis may be evident. Thrombosis of capillaries and venules in the mucosa and submucosa with fibrin plugging is the cause of infarction. Multifocal areas of gastric infarction have also been recorded in cases of porcine dermatitis and nephropathy syndrome (PDNS), due to fibrinoid vasculitis of capillaries.

Edema of the stomach wall is a characteristic change in edema disease caused by specific strains of *E. coli*. Edema affects the submucosa, particularly on the greater curvature of the stomach. Other conditions causing gastric edema include hypoproteinemia, arsenic toxicity, and portal hypertension. In these conditions, edematous changes are less pronounced than those of edema disease.

Gastritis in pigs is most commonly associated with ulceration of the pars esophagea and the inflammatory sequelae affecting tissue in the cardiac zone of the stomach, as previously mentioned. Candidiasis of the pars esophagea may occur in association with pre-ulcerative epithelial hyperplasia and parakeratosis. Gastritis could occur due to accidental intake of toxic compounds – such as arsenic, thallium, formalin, bronopol, and phosphatic fertilizers – and by the toxic principles in bitterweed (*Hymenoxys odorata*) or the blister beetle (*Epicauta* sp.). In commercial farming, such conditions are very rare and should be easy to exclude. Mycotic gastritis is occasionally seen in piglets, usually in association with repeated use of antibiotics. Lesions present as multifocal yellowish plaques on the gastric mucosa, with intense congestion of the peripheral gastric mucosa. Fungal hyphae colonize the mucosa and invade tissue and capillaries, resulting in thrombosis. Agents are usually zygomycetes such as *Rhizopus*, *Absidia*, or *Mucor* species. *Aspergillus* species involvement is rare (Mahanta and Chaudhury 1985).

Parasitic gastritis is now rare in large commercial pig farms but can be problematic in organic units, small holdings, and backyard piggeries, in instances where anthelmintics are seldom used. Affected pigs show poor body condition due to chronic gastritis. Of the parasites that can cause gastritis, *Hyostrongylus rubidus* is of most importance because it is associated with poor growth rates in young stock or loss of body condition in adult pigs.

Other parasites that can cause gastritis in heavy infections include *Ascarops* sp. and *Physocephalus* sp. These parasites are common in many parts of the world, in feral pigs and those kept under extensive conditions with access to grazing and foraging conditions. *Simondsia* sp. are found in Europe, Asia, and Australia and are associated with nodular gastritis in pigs. The parasite *Gnathostoma* sp. occurs in Asia. It invades the mucosa and undergoes development within inflammatory cysts in the submucosa. In heavy infestations this parasite can cause thickening of the stomach wall.

The intestinal tract

Congenital defects

Atresia ani is the most common congenital defect of the intestinal tract of pigs, and it is believed to be hereditary (Norrish and Rennie 1968). It arises due to failure of perforation of the membrane separating endodermal hindgut from ectodermal anal membrane. Evident at birth, the defect can be corrected by minor surgery unless atresia of the rectum is also present. Persistent Meckel's diverticulum is a rare anomaly in which there is persistence of the omphalomesenteric duct. It presents as a tube of intestinal tissue similar to ileum that branches from the intestine to the umbilicus. Occasionally, it can be involved in abdominal catastrophes such as intestinal strangulation.

Intestinal displacements

Intestinal displacement and obstruction are common in pigs, and a number of different conditions arise.

Rectal prolapse and rectal stricture

Rectal prolapse is relatively common and an important condition affecting growing pigs and adults that gives rise to welfare and management problems. The rectum is held in place by a complex matrix of fascia, collagen fibers, muscles, and ligaments, and rectal prolapse will occur if the support mechanism is either overcome by pressure or is weakened for some reason. Pressure on the support mechanism may be brought about by straining (proctitis, urethritis, constipation, coughing, and farrowing) or by physical pressure (excessive slope on the floor or increase in abdominal pressure for any reason).

Brockman et al. (2004) produced rectal prolapse in ten anesthetized 49–74 kg pigs by insufflating the abdominal cavity with water at pressures of 222–343 mmHg (mean 292 mmHg).

Pigs of all ages can be affected by rectal prolapses, and outbreaks do occur and can be prolonged. The incidence has been reported as 1–10% (Kjar 1976), 0.7–4.7% (Garden 1988), and as high as 10–15% (Becker and Van der Leek 1988). Perfumo et al. (2002) found that rectal prolapse was responsible for 7.7% of deaths that occurred in pigs from weaning to market. Daniel (1975) noted that rectal prolapse in sows could occur in all sizes of units and that the incidence varied from 0.5 to 1%.

Rectal prolapse is sometimes associated with enterocolitis caused by viral, bacterial, parasitic, or mycotic infection (Pfeifer 1984; Straw 1987). In cases where the inflammation is severe and irritation of the rectum occurs, tenesmus results, and rectal prolapse may be a sequel. At the recto-anal junction, the rectal mucosa changes abruptly to stratified squamous epithelium of the anus. This junction is often the site of inflammatory changes in growing pigs when examined microscopically (JT, personal observations). Intense inflammatory changes at this site could lead to irritation-related straining and rectal prolapse. In older swine, urethritis and vaginitis from any cause may lead to straining, which may in turn lead to prolapse of the rectum or vagina or both.

Sudden changes in the diet (e.g. from meal to whey) may lead to occasional cases of rectal prolapse. Constipation caused by chronic water shortage or low fiber diet may result in straining and rectal prolapse. Other nutrition-related associations that have been reported include high lysine levels (Amass et al. 1995) and lupin bean meal toxicosis with megacolon observed as well (Casper et al. 1991).

Injury to the rectum or urethra from service by the boar may also lead to tenesmus and prolapse. In addition, gradual weakening of the pelvic diaphragm may arise as sows age or during pregnancy as the abdominal contents become heaviest. Rupture of one or more of the supporting structures may then occur, with prolapse of either rectum or vagina or both as a sequel. Rectal prolapses were recorded in sows housed in inappropriate gestation crates but not in group-housed sows in the same unit (Papatsiros et al. 2012).

Guise and Penny (1990) noted that rectal prolapses occurred when pigs were transported at high stocking density.

It is generally agreed that rectal prolapse occurs more commonly during winter months, and there is some evidence to support this (Gardner et al. 1988; Kjar 1976; Prange et al. 1987; Wilson 1984). It has been suggested that cold weather causes pigs to pile, thus increasing the likelihood of prolapses. Rectal prolapse is a common sequel to vulvovaginitis caused by mycotoxicosis (see Chapter 69).

Muirhead (1989) noted that a behavioral aberration, anal nuzzling, in recently weaned pigs led to a rectal prolapse problem (4–6% incidence). Improving the climatic environment prevented further cases from arising. Another outbreak associated with anal nuzzling was reported by van Sambraus (1979).

When pigs cough, the rectal mucosa often protrudes temporarily. As with piling, it has been suggested that coughing may precipitate rectal prolapse, but there are no objective data to support this hypothesis. In another study, the prevalence of rectal prolapse was dramatically reduced from 4.7 to 0.7% when weaners (30–35 kg) were placed in a strawed yard for 3 weeks between being held in the second-stage flat decks and the fully slatted finishing accommodation (Garden 1985). Occasionally, severe intestinal prolapse can accompany vaginal prolapse, via laceration to the vaginal fornix.

For sporadic cases of rectal prolapse, it was not considered worthwhile to implement any specific control or preventive measures apart from treatment and noting risk factors. It was common practice to isolate feeder pigs with rectal prolapse to allow the prolapse to resolve naturally in 10–14 days. However, this practice is not good welfare, and a simple nonsurgical amputation technique such as described by Douglas (1985) should be considered. Many surgical procedures for treating rectal prolapses have been described (Daniel 1975; Grosse-Beilage and Grosse-Beilage 1994; Ivascu et al. 1976; Kjar 1976; Kolden 1994; Moore 1989; Schon 1985; Vonderfecht 1978).

Some prolapses resolve spontaneously, but more commonly the tissue becomes traumatized or is removed by pen mates, followed by scar formation as part of the healing process. This can result in rectal stricture (Becker and Van der Leek 1988; Häni and Scholl 1976; Jensen 1989; Prange et al. 1987; Von Muller et al. 1980) and progressive obstruction leading to a marked distension of the colon known as megacolon. In a study of 25 pigs with rectal prolapses that were allowed to heal naturally without treatment, Smith (1980) noted that three developed complete rectal stricture and died and the remainder grew normally, but in every case there was evidence of partial rectal stricture at slaughter. Treatment of rectal stricture is rarely cost effective, but a surgical technique has been described (Boyd et al. 1988). Visibly affected pigs should be euthanized at an early stage for welfare reasons.

Wilcock and Olander (1977a) noted cases of rectal stricture preceded by enteric disease, and ulcerative proctitis from which *Salmonella enterica* Typhimurium was isolated was a possible precursor. In later studies Wilcock and Olander (1977b) produced rectal strictures experimentally by injecting chlorpromazine into the cranial hemorrhoidal artery and suggested that rectal prolapses may be a sequel to ischemic proctitis induced by thrombosis associated with salmonellosis.

Intestinal obstruction, impaction, and hernia

Intestinal impaction and obstruction can occur in a variety of situations, for example, deaths have occurred in piglets maintained on wood shavings or other fibrous materials such as peat due to impaction of the ileum or colon with such materials. Sand impactions of the colon have caused deaths in gilts newly arrived in outdoor breeding units with sandy soil, having been raised in slatted buildings. On occasions, heavy infestations of *Ascaris suum* have been found to cause small intestinal impaction in grower pigs.

Herniation of the intestine is most commonly associated with a patent umbilicus. This occurs when weakened supporting muscles around the navel or umbilical stump impede umbilical closure. Genetic and environmental factors can contribute to this (Searcy-Bernard et al. 1994; Zhao et al. 2009). Suggested environmental factors include excessive stretching of the umbilical cord during farrowing, placing navel clips too close to the skin, and neonatal bacterial infections of the umbilicus. Close inspection of the lesion often reveals evidence of chronic bacterial infection in growing pigs. Small lesions are of little consequence unless expanded by trauma or infections. The larger, pendulous lesions are regularly traumatized, with increased risk of adhesions, particularly when the peritoneum is breached. Intestinal strangulation within the hernia can follow unless the defect is corrected surgically.

Hernias via the inguinal and vaginal rings into the scrotal sac can also occur. Should the gut become incarcerated within the hernia site, life-threatening intestinal infarction occurs. The wisdom of keeping pigs with umbilical or scrotal hernias through to finishing is questionable for welfare and economic reasons. Though there is little specific data on this, one study showed a 15% mortality rate for affected pigs in the finisher and significantly lower growth rates than unaffected pigs (Straw et al. 2009). Most of the pigs that survive to reach slaughter weight are subject to condemnation for peritonitis (Keeliside 2006).

Intestinal torsion (volvulus) and hemorrhagic bowel syndromes

Torsion of the long axis of the mesentery is a common condition in pigs and leads to rapid death. The torsion may involve small intestine or both small and large intestines (Figure 15.2a). Often, the stomach and duodenum are unaffected with an abrupt transition to the affected congested intestine (Figure 15.2b). Rotation is usually counterclockwise when viewed from the ventrocaudal aspect and may be a full 360° twist or a partial twist sufficient to disrupt blood flow. Torsion is associated with pigs having disruptions of feed intake leading to engorgement coupled with making sudden unpredictable movements, such as sudden deceleration combined with abrupt changes in direction, particularly when the gut is filled. Feed or water engorgement following out-of-feed events, sorting, or commingling are considered risk factors, as well as the gaseous dilatation of intestines in pigs fed a highly fermentable ration. Once torsion occurs, pigs rapidly develop distension of the abdomen. The intestinal loops, apart from the first 20–30 cm of duodenum, become very turgid and reddish black in color. The lumen is distended with gas and contains dark red, watery fluid. The mesenteric vasculature is extremely engorged due to obstruction to the venous return.

In pigs with "intestinal hemorrhage syndrome" (also called "hemorrhagic bowel syndrome," "porcine intestinal

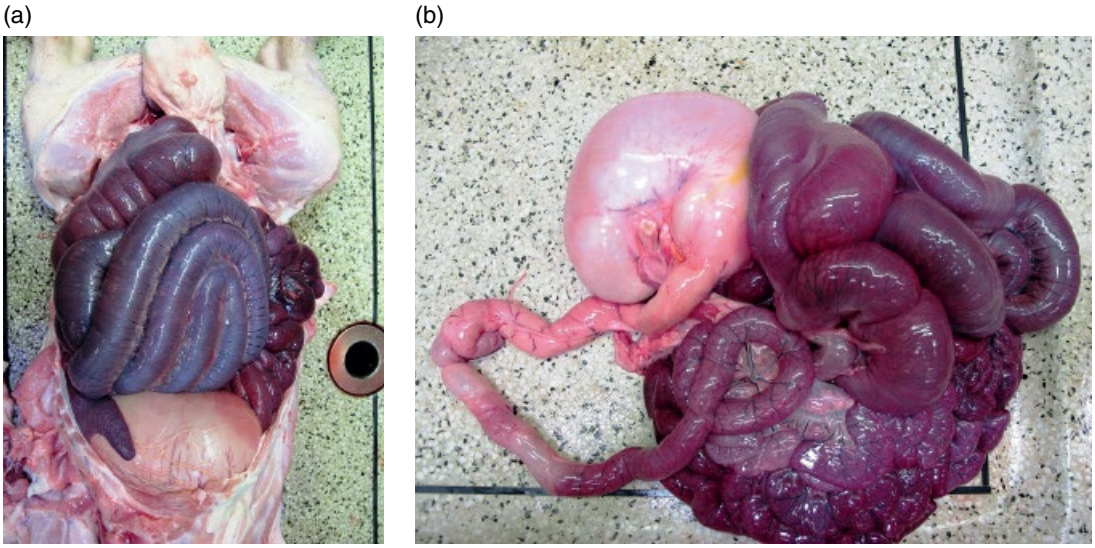


Figure 15.2 Volvulus affecting both small and large intestines. (a) Organs *in situ* in the abdominal cavity. (b) Organs removed to show unaffected stomach and duodenum, with abrupt transition to affected jejunum.

distension syndrome," or "bloody gut"), the intestines have a similar appearance to cases of intestinal torsion, but no obvious displacement of the intestines or mesenteric torsion is detected at necropsy. Typically, this occurs under circumstances similar to cases of intestinal torsion, where pigs receive a highly fermentable ration, particularly fed in liquid form. Feeding fresh whey ad libitum has been associated with increased risk of intestinal torsion and/or "hemorrhagic bowel syndrome," such that the term "whey bloat" is given to the condition. The hypothesis that such pigs can die due to excessively high intra-abdominal pressure in the absence of intestinal torsion was supported by pressure measurements in the order of 40 mmHg being recorded in pigs immediately after death (Thomson et al. 2007). In biomedical research studies involving pigs, artificially induced pressures of this magnitude were found to cause occlusion of the mesenteric veins and obstruction to venous return with fatal consequences (Gudmundsson et al. 2001). Prevention of "whey bloat" can be achieved by allowing the whey to ferment before feeding, limiting intake to no more than 20% of the ration and feeding it as part of a complete diet to avoid preferential ingestion of excessive quantities. In instances where non-whey rations are associated with the problem, efforts to reduce the rapidly fermentable properties of the diet or use "little and often" feeding strategies help to reduce the incidence of cases. The role of *Clostridium* sp. or other bacterial infections in this condition has been discounted by many authors. The potential causes and pathogenesis of "porcine intestinal distension syndrome" have been reviewed by Martineau et al. (2008). Other forms of intestinal catastrophe, such as volvulus of a short length of small intestine followed by strangulation of the affected portion, occur more rarely.

Muscular hypertrophy of the ileum

Muscular hypertrophy of the ileum can be seen as an incidental finding in finishing pigs at slaughter. Approximately 30–50 cm of the terminal ileum is thickened, pale, and firm. Internally the lumen is narrowed, the mucosa and submucosa are normal, but both internal and external muscular layers are hypertrophied, suggesting that the process is secondary to a functional obstruction. Some cases show an annular stricture in the terminal ileum or at the ileocecal orifice. Many samples of ileum submitted for examination do not extend to the junction with normal tissue, so the cause cannot be confirmed. Although the basis of this condition is unknown, it is possible that a previous focus of inflammation and ulceration has led to stricture formation. Externally, and on palpation, the lesion can be confused with porcine proliferative enteropathy (PPE)/necrotic ileitis associated with *Lawsonia intracellularis* infection. Histopathology readily differentiates the two conditions.

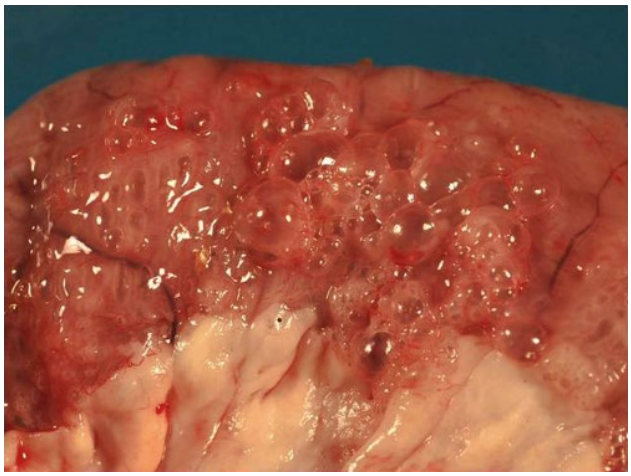


Figure 15.3 Intestinal emphysema (pneumatosis, "bubbly gut") in a 5-month-old slaughtered pig. *Source:* Courtesy of M. Hazlett.

Intestinal emphysema

Intestinal emphysema, pneumatosis intestinalis, or "bubbly gut" is another apparently incidental finding at slaughter (Lazier et al. 2008). The serosal surface shows numerous gas-filled, thin-walled cystic structures along the intestinal wall, in the mesentery, and occasionally in the mesenteric lymph nodes (Figure 15.3). Lazier et al. (2008) described a 20% incidence of cases from one herd, and although there were no clinical signs of illness in affected pigs, condemnation of the intestines caused significant financial loss for the packer. The pathogenesis of the condition involves gas accumulation in the lymphatics, but the cause is unknown.

Infectious conditions affecting the intestines

Many forms of inflammatory and degenerative changes can affect small and large intestines. Mechanisms of diarrhea include hypersecretion, malabsorption, inflammation, and increased intestinal permeability (Table 15.1). Hypersecretory diarrhea is watery, without gross lesions, and is associated with enteropathogenic *E. coli* and sometimes rotavirus. Enterocyte necrosis and loss of surface enterocytes occur with *Cystoisospora* (previously *Isospora*), some of the enteropathogenic *E. coli*, and viral infections, including rotaviruses and coronaviruses. Fluid exudation is a consequence of epithelial loss and results in watery diarrhea and dehydration. Villous atrophy is particularly associated with coronavirus and rotavirus infections. In these conditions, the intestines are fluid filled and flaccid, with no gross evidence of inflammation. Intestinal erosion and necrotizing, hemorrhagic enteritis in young pigs are often associated with *Clostridium perfringens* type C infection. *Cryptosporidium* (*C. parvum*) infection in neonatal piglets is an infrequent cause of villous

Table 15.1 Mechanisms of diarrhea.

	Primary pathophysiologic mechanism of diarrhea		
Infectious insult	**Hypersecretion**	**Malabsorption**	**Inflammation**
Escherichia coli	+++	+	
Clostridium perfringens A	+		+
C. perfringens C		+	+++
Clostridium difficile	+	+	+++
Cystoisospora		++	++
TGEV/PEDV		+++	
Rotaviruses	+	++	
Salmonella	+		+++
Lawsonia		++	++
Strongly hemolytic *Brachyspira; B. pilosicoli*		+	++

atrophy, stunting, and fusion of villi, with diarrhea due to malabsorption. Intestinal ulceration is associated with salmonellosis and *L. intracellularis* infection. Large intestinal ulcerative typhlocolitis is present with swine dysentery, *Salmonella*, and *L. intracellularis*. So-called button ulcers alert concerns of CSF, but they are also associated with *Salmonella* infections. Infrequent reports have implicated PDNS, bovine viral diarrhea virus (Terpstra and Wensvoort 1988), and other insults as well. Degeneration of intestinal crypt epithelium is associated with coccidial infection and bovine viral diarrhea virus infection. In coccidiosis, due to *Cystoisospora suis*, damage caused by coccidial development in the villus epithelium will result in villous atrophy, intestinal erosions, and fibrinonecrotic enteritis, mainly affecting the distal jejunum and ileum. The enteric lesions of pseudorabies are characterized by foci of crypt necrosis that extend to necrosis of the mucosa, submucosa, and the muscular layers of the intestinal wall (Narita et al. 1998). Hyperplasia of crypt epithelium is the major feature of *L. intracellularis* infection and leads to thickening of the mucosa in the ileum, cecum, jejunum, and/or colon. While this is generally a widespread and fairly uniform change in the affected areas, occasionally it can cause polypoid-like growths or larger protruding lesions resembling intestinal tumors or in some cases fibrinonecrotic enteritis.

Inflammatory cell infiltration occurs in response to any cause of disruption to the epithelial barrier as well as enteropathogenic infections. Mucosal microabscesses are a feature of yersiniosis; both *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* affect pigs. Granulomatous inflammation is associated with viral enteropathogens such as PCV2 and certain coronavirus infections. Hemorrhage into the intestinal tract can occur with acute clostridial enteritis in unweaned piglets. Porcine proliferative hemorrhagic enteropathy (*L. intracellularis*) results in major blood loss into the ileum and is uniquely associated with large clots of fresh blood that can be found in the absence of any obvious intestinal ulceration. Watery, port-wine-colored colonic contents and diarrhea may be seen. Gastric ulcers can give rise to mild subclinical blood loss or major hemorrhage resulting in obvious melena. In ulcerative typhlocolitis due to swine dysentery, there is hemorrhagic colonic content and increased mucus production resulting in mucohemorrhagic diarrhea containing fresh-looking blood. *T. suis* (whipworm) may also cause mucohemorrhagic diarrhea in heavy infestations. Fresh blood in feces can also occur in pigs that have suffered anorectal trauma, such as rectal prolapse cases. Anticoagulant or dicumarol poisoning can cause intestinal hemorrhage. All these hemorrhagic conditions are eminently life threatening and require prompt diagnosis and treatment where possible. Severe intestinal blood loss occurs in intestinal torsion and intestinal hemorrhage syndrome, but death occurs before fecal hemorrhage is seen.

Diseases affecting the intestinal tract are among the most important economic problems affecting pig production. The prevalence of diseases in pigs varies between countries, farming systems, and units with different health status. Within farms, the disease situation is dynamic, and the prevalence can change quite dramatically between batches for no apparent reason. Multiple enteric infections can occur concurrently, giving rise to complex clinical disease patterns and difficulties in arriving at successful control measures. The major diseases are mentioned only briefly here; detailed descriptions of all are given in respective chapters (Tables 15.2 and 15.3).

Table 15.2 Differential diagnosis of some common gastrointestinal conditions of swine.

Cause	Age	Key clinical signs
Escherichia coli (*ETEC, EPEC*)	Neonatal: 1–4 days old	Watery, yellowish diarrhea; dehydration
	Post weaning: 2–3 weeks after weaning	Diarrhea, ill-thrift, sudden deaths; eyelid edema; neurological signs
Rotavirus	1 day to 7 weeks; most frequent at 2–3 weeks of age	Watery to pasty diarrhea; dehydration; may be subclinical
Clostridium perfringens type C	1–14 days	Hemorrhagic or watery diarrhea; sudden death
Clostridium perfringens type A	3–28 days	Creamy diarrhea; usually mild but can affect growth rate
Clostridium difficile	5–21 days, rarely older	Creamy diarrhea; dehydration
Cryptosporidium spp.	3 days to weaning	Mild to moderate yellowish diarrhea
Cystoisospora suis (*Isospora*)	5–21 days (sometimes older)	Watery yellowish diarrhea to no diarrhea; retarded growth
Coronaviruses: TGE, PED, PDCoV	All ages	Watery diarrhea; rapid dehydration; vomiting; rapid increased mortality
Porcine circovirus type 2	6–12 weeks, sometimes older	Ill-thrift; depression, diarrhea; wasting; often multisystemic signs
Gastric ulceration	Any age after weaning	Asymptomatic; dark feces; melena; sudden death with anemia and pallor
Lawsonia intracellularis (PE, PHE, PIA)	5 weeks old to young adults	Broad range of severity of diarrhea; can be hemorrhagic
Strongly hemolytic *Brachyspira* spp.	Approximately 6 weeks old to adult	Mucoid diarrhea; usually mucohemorrhagic
Brachyspira pilosicoli	5 weeks to 4 months of age	Pasty, sloppy, mucoid diarrhea
Salmonella spp.	All ages, usually after weaning	Variable; watery to bile stained; mucohemorrhagic with fibrin flecks
Oesophagostomum dentatum	From weaning to adult	Mild diarrhea
Trichuris suis	From weaning to adult	Mucoid, occasionally mucohemorrhagic diarrhea
Yersinia spp.	From 6 weeks to 4 months of age	Pasty, sloppy diarrhea

ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; TGE, transmissible gastroenteritis; PED, porcine epidemic diarrhea; PDCoV, porcine deltacoronavirus; PE, proliferative enteropathy; PHE, proliferative hemorrhagic enteropathy; PIA, proliferative intestinal adenomatosis.

Enteric diseases in suckling piglets

Accurate diagnosis relies on differentiating endemic and epidemic infectious agents of neonatal diarrhea as well as noninfectious risk factors for disease expression. Although infectious agents are discussed in detail in respective chapters, it is important to realize that many are endemic herd infections, seizing opportunity to proliferate and express disease. ETEC remains a very important disease. *C. perfringens* type C causes characteristic lesions of segmental hemorrhagic necrosis, usually in the proximal two-thirds of the small intestine (Figure 15.4). This disease is somewhat restricted geographically, mostly in outdoor farming systems. Both bacterial diseases can be controlled by vaccination of breeding stock, provided piglets receive adequate and timely colostrum intake and ongoing adequate milk intake. The availability of breeding stock that is genetically resistant to *E. coli* F4 (K88) strains offers a control option through genetic selection, but the uptake of this technology in commercial pig farming has been very limited.

Other infections that are commonly reported in young unweaned piglets include rotavirus types A, B, and C and coccidiosis (*C. suis*), with cryptosporidia also being implicated in some production units. It is relatively rare for *Salmonella* infection to cause significant enteric disease in unweaned pigs though subclinical infection and shedding can occur as a result of contact with sow feces. The enteric coronavirus diseases, including transmissible gastroenteritis (TGE), PED, and porcine deltacoronavirus, can cause severe morbidity and mortality in susceptible piglets and have reportable status in many countries. *Clostridium difficile* is a recognized cause of toxin-mediated necrotizing colitis and mesocolonic edema in suckling pigs. Likewise, *C. perfringens* type C has been long associated with segmental necrohemorrhagic enteritis. Neonatal enteric disease has not been

Table 15.3 Pathology and diagnostic confirmation of some common gastrointestinal conditions of swine.

Cause	Gross lesions	Histological lesions	Common laboratory tests
Escherichia coli (*ETEC, EPEC*)	Fluid ingesta; intestinal congestion; neonates have milk in stomach	Mucosa congestion, edema; bacteria attached to epithelium	Culture; serotype; PCR virulence genes; IHC
Rotavirus	Fluid-filled and pale intestines	Moderate villous atrophy	Detect with PCR, ELISA, or PAGE; lesion with FAT; IHC
Clostridium perfringens type C	Segmental hemorrhage and necrosis	Necrosis and gram-positive rods associated with lesions	Histopath; isolation; toxin ELISA; PCR testing
Clostridium perfringens type A	Watery, creamy intestinal content; no gross lesions	Mild; loss of epithelium from villus tips; neutrophils; inflammation	Histopath and isolate high numbers; toxin ELISA; PCR testing
Clostridium difficile	Mesocolonic edema; watery to creamy diarrhea	Colitis with multifocal ulcers and suppuration	Histopath; toxin A/B via ELISA and LFDs; isolation
Cryptosporidium spp.	Fluid intestinal content	Cryptosporidia oocysts intimate with surface epithelium	Histopath; high oocysts with smear or fecal float
Cystoisospora suis (*Isospora,* coccidiosis)	Thick ileum; fibrinous enteritis; feces variable	Villous atrophy, fibrinonecrotic enteritis, intracellular coccidial forms	Histopath (lesions and coccidia); impression smear; fecal float
Coronavirus (TGEV, PEDV, PDCoV)	Thin-walled intestine; watery; rapid dehydration	Severe and diffuse villous atrophy	IHC, ISH, or FAT to visualize; PCR to detect virus
Porcine circovirus type 2	Lymph node enlargement, edema of mesocolon, pneumonia	Histiocytic lymphadenitis with lymphoid depletion; granulomatous enteritis	Histopath; IHC or ISH to visualize; qPCR is suggestive
Gastric ulceration	Ulceration or fibrosis of pars esophagea; hemorrhage in stomach; dark blood/melena in intestines	Hyperkeratosis (mild to severe) to necrosis, hemorrhage, fibrosis, granulation	Gross exam (examine pars esophagea); histopath; occult blood in feces
Lawsonia intracellularis (PHE)	Proliferation or necrosis in small or large intestine; PHE can have fresh blood clots in ileum; pallor	Crypt enterocyte hyperplasia; cryptitis; small curved rods in epithelial cells	Histopath; IHC, ISH, or FAT to visualize; detect with PCR on feces or mucosa; serology
Brachyspira hyodysenteriae; strongly hemolytic *Brachyspira* spp.	Lesions only in large intestine; mucohemorrhagic typhlocolitis; erosions with fibrinous exudates	Erosion of epithelium; goblet cell hyperplasia, inflammation, fibrin exudate	Histopath; IHC stains; ISH to visualize; detection by culture or PCR
Brachyspira pilosicoli	Mucoid colitis is mild to moderate	Mild mucoid colitis; end-on attachment of spirochetes	Histopath; IHC stains; ISH to visualize; detection by culture or PCR
Salmonella spp.	Fibrinous or hemorrhagic colitis or enteritis with focal ulcers	Ulceration, suppuration, fibrin thrombi; paratyphoid nodules in liver	Histopath; detect with culture or PCR; serotype; mix-ELISA serology
Oesophagostomum dentatum	Erosions, edema, granulomas in cecum and proximal colon	Granulomatous typhlocolitis with nematode parasites	Histopath; parasitology; fecal float
Trichuris suis	Typhlocolitis, erosions; parasites visible 3 weeks after infestation	Mucoid mixed typhlocolitis; embedded nematodes	Histopath; gross exam; parasitology; fecal float
Yersinia spp.	Mild enteritis and/or colitis	Chronic active enterocolitis; microabscesses, granulomas	Histopath; bacterial culture

ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; PHE, proliferative hemorrhagic enteropathy; TGE, transmissible gastroenteritis; PED, porcine epidemic diarrhea; PDCoV, porcine deltacoronavirus; PCR, polymerase chain reaction test; IHC, immunohistochemistry; FAT, immunofluorescence; ISH, *in situ* hybridization; PAGE, polyacrylamide gel electrophoresis; LFDs, lateral flow devices.

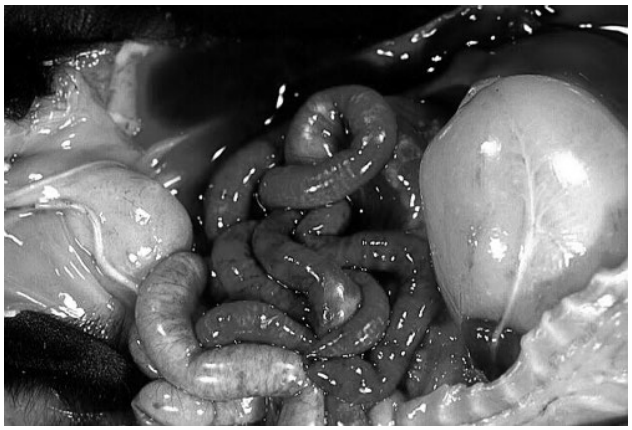


Figure 15.4 Necrosis and hemorrhage in the jejunum of a 4-day-old piglet caused by *Clostridium perfringens* type C.

reproduced with *C. perfringens* type A. The diagnosis of clostridial diseases relies in large part on demonstrating compatible lesions by histopathology. In general, the detection of endemic agents by culture or PCR (bacteria, viruses, protozoans) without typical or compatible gross and/or microscopic lesions is of dubious diagnostic significance.

Recombinant strains of enteric coronaviruses have been recently reported in Europe, which raises concerns over future potential virus transformations (Belsham et al. 2016, Boniotti et al. 2016).

Strongyloides sp. can affect pigs ranging from 10 days of age up to 3 months old. Parasites colonize the anterior half of the small intestine and cause villous atrophy and granulomatous enteritis, resulting in diarrhea and ill-thrift. *Enterococcus durans* has been associated with enteropathy in suckling piglets (Cheon and Chae 1996) by virtue of heavy enterocyte colonization, usually secondary to other pathogens or risk factors. Similarly, other organisms cited as occasional causes of diarrhea include *Bacteroides fragilis* (Collins et al. 1989) and *Chlamydia suis* (Rogers and Andersen 2000). Hemagglutinating encephalomyelitis virus is reported to cause vomiting and wasting in suckling piglets but is rarely a cause of clinical disease. The ongoing reports of porcine adenovirus, bovine viral diarrhea virus, kuboviruses, astroviruses, and a host of other viruses that have been detected by sensitive techniques such as PCR or metagenomic sequencing have not been serious or ongoing disease threats. To reiterate, detection of common endemic agents by sensitive techniques such as PCR is not sufficient to establish causation or definitive diagnosis in absence of well-defined history and clinical presentation or ideally collaborative lesions, as these organisms can be carried by normal healthy piglets and are expected to be present in the population. That stated, new and emerging diarrhea syndromes in suckling pigs can result in major economic losses until the cause and specific control measures can be devised. Investigation of serious diarrhea syndromes merits considerable effort to characterize the condition and determine the cause (Astrup et al. 2010; Gin et al. 2010).

Diseases from weaning onward

A number of the aforementioned preweaning infectious agents are also capable of causing disease in young weaners. *E. coli* continues to be a major cause of post weaning diarrhea, enterotoxemia, and mortality. Edema disease caused by Shiga toxin-producing strains of *E. coli* results in sudden deaths or neurological signs in weaner and grower pigs. Intestinal mucosal lesions are not apparent, although edema of the gastric submucosa, mesocolon, eyelids, and forehead can be prominent. Diseases such as rotavirus, coccidiosis, and cryptosporidiosis can affect piglets up to 6 weeks of age or older. Edema of the mesocolon has been reported in weaner pigs affected with PCV2 systemic disease. Diarrhea is a feature of some PCV2 disease outbreaks and can be a direct consequence of the viral enteritis associated with PCV2 infection (Kim et al. 2004; Ségales et al. 2004). The distal small intestinal and colon mucosa may be thickened, sometimes with edema of the mesocolon. Histologically, there is villous atrophy, granulomatous inflammation, and histiocytosis with multinucleated giant cells. The gut-associated lymphoid tissue shows lymphocytolysis and atrophy of the follicles, as also occurs in other lymphoid organs. PCV2 viral inclusions can be visualized microscopically, and antigen deposits can be demonstrated readily by immunohistochemistry. Coinfection with other agents such as *Salmonella* spp., *Brachyspira* spp., and/or *L. intracellularis* can be common (Järveots et al. 2016; Zlotowski et al. 2008). Grossly, PCV2-associated enteritis can resemble PPE; hence differentiation by histopathology is warranted (Jensen et al. 2006). Another equally important feature associated with PCV2-associaed enteritis is the presence of mild nonspecific colitis. Often bacterial cultures fail to show any recognized bacterial pathogen but yield a mixed growth of commensal-type organisms. Histology of the colon shows mixed bacterial infection of crypts and surface epithelium, accompanied by crypt inflammation and goblet cell hyperplasia in chronic cases. This suggests that there is an opportunistic bacterial overgrowth or dysbacteriosis in the colon of affected pigs, possibly associated with altered enteric immune processes.

Through growing and finishing periods, the most important economic-limiting diseases continue to be swine dysentery, PPE, salmonellosis, and porcine colonic spirochetosis. Prevention and control of PPE caused by *L. intracellularis* include vaccination, the timing of which has improved with modern diagnostic testing technologies. The development of serological tests allows herd

profiling for best application of oral or intramuscular vaccines. In contrast, no commercially available serological tests for swine dysentery or porcine colonic spirochetosis are available, and to date, vaccine efficacy is limited. *Brachyspira* infections are important causes of diarrhea and reduced growth rates in many countries, particularly those caused by strongly beta-hemolytic pathogenic *B. hyodysenteriae* and *Brachyspira hampsonii* that cause typical swine dysentery. Infections with these may also be mild, subclinical, or asymptomatic, depending upon the presence or absence of virulence factors and/or interactions with diet and enteric flora. The prevalence of pathogenic *Brachyspira* spp. varies between countries. Of the most recently described species, *B. suanatina* appears to be limited to Scandinavia, and *B. hampsonii* mostly limited to North America (Råsbäck et al. 2007, Chander et al. 2012). Mixed infections involving *Brachyspira* sp., *L. intracellularis*, *Salmonella* sp., and *Yersinia* sp. are common. Achieving the correct diagnosis and finding effective control measures pose significant challenges for the swine veterinarian.

The disease patterns in swine production units change dramatically when immunosuppression is a feature and the global emergence of PCV2-associated diseases in the first decade of the twenty-first century dramatically illustrated the pathogenic potential of endemic potential pathogens as well as coinfections with other agents such as *Salmonella*, *Brachyspira*, and/or *L. intracellularis* (Zlotowski et al. 2008). Histopathology is critical to confirm a pathologic role for the multitude of agents detected in combined infections (Jensen et al. 2006). Histopathology is also critical to confirm dysfunction when no specific causal agent is demonstrable, as in cases of nonspecific colitis associated with opportunistic bacterial overgrowth or dysbacteriosis.

The prevalence of parasitic infections varies considerably between units. Many units have achieved total freedom of enteric parasites, such as *A. suum*, *Oesophagostomum* sp., *Trichuris* sp. and *Hyostrongylus* sp. However, the threat of parasitism remains, particularly on small farms that may be enrolled in niche or organic markets, in outdoor production status where routine anthelmintic use is not permissible, or where recycled lagoon water is utilized. *A. suum* is a zoonotic parasite.

Trichuris suis and *Oesophagostomum* sp. can be causes of colitis that are easily overlooked until no response to antibiotic agents results in further investigation. Heavy infestations with *T. suis* can cause mucohemorrhagic typhlocolitis that grossly resembles swine dysentery. Differentiation by gross lesions is difficult until the parasites become visible, starting about 3 weeks after infestation. Diarrhea, dehydration, and chronic ill-thrift can all ensue. Recent studies have shown that immunity to *T. suis* is established by 8–9 weeks postinfection due to T2 helper cell immune responses (Andreasen et al., 2015). *O. dentatum* infection is more persistent than *T. suis*, possibly due to the immune-suppressing abilities of the parasite that delay development of the host's T2 helper cell immune reaction (Andreasen et al. 2016).

Some *Eimeria* sp. are considered to be potentially pathogenic, causing villous atrophy and enteritis, and can affect older pigs when exposed to yards and pasture contaminated with oocysts. Infrequently, coccidiosis (*C. suis* or *Eimeria* sp.) can cause acute enteritis and colitis, including hemorrhage, in naive young adult pigs that are introduced into a heavily infected environment and must be differentiated from the hemorrhagic enteropathy form of PPE.

Macracanthorhynchus hirudinaceus, the thorny-headed worm, is a zoonotic acanthocephalan that can be found in the small intestine of grazing pigs. It can cause granulomatous nodules; affected pigs suffer ill-thrift, anemia, and occasionally intestinal perforation. *Balantidium coli* are commonly present in the large intestine of pigs as commensals that can invade degenerative or necrotizing lesions from other causes. Other incidental parasites that can occur in the colon are the paramphistomes *Gastrodiscoides* and *Gastrodiscus* that require aquatic snails to complete their life cycle. They are not considered to be of any pathogenic significance in pigs, but they are zoonotic and heavy infestations can cause serious illness in humans.

Serious epidemic diseases such as CSF and African swine fever (ASF) have an important enteric component. Suspected outbreaks should be reported to proper authorities and aggressively investigated to obtain a rapid, accurate diagnosis to limit spread and control the outbreak. TGE and PED can cause severe diarrhea in all age groups of pigs in addition to the serious morbidity and mortality that occur in suckling piglets. Pseudorabies virus (PRV) can cause necrotizing enteritis in distal small intestine, but less commonly than the more typical necrotizing rhinitis and tonsillitis. Prevention and control strategies for TGE, PED, CSF, ASF, and PRV vary considerably. Vaccination is used in some countries where disease eradication is not possible or feasible.

The peritoneal cavity

The peritoneal cavity is intrinsically involved with the health and normal function of the digestive system. The area of the peritoneal lining is considerable, exceeding the area of skin. When normal, it is smooth, and its moisture is maintained by hydrostatic peritoneal fluid that moves readily across serosal surfaces.

The most common congenital abnormalities that affect the peritoneal cavity of pigs are umbilical or inguinal hernias as discussed above. Occasionally, the remnants of vestigial structures can be encountered as incidental findings at postmortem examination. Rarely,

these can be responsible for intestinal entrapment, with fatal consequences.

Abnormal contents of the peritoneal cavity include blood, excess serous fluid, inflammatory products, intestinal contents, or urine. Severe trauma and rupture of organs can lead to abnormal intra-abdominal contents. Hemoperitoneum associated with a ruptured liver is a common finding in piglets overlain by sows. Ascites can arise for a number of reasons including hypoproteinemia, anemia, uremia, hepatic diseases, heart failure, systemic illnesses, intoxications, and endotoxemia. Discussions of all these circumstances are beyond the scope of this chapter, but the basis of ascites in relation to specific conditions is discussed in the relevant chapters.

Peritonitis may be associated with systemic bacterial infections, the most common of which are *Haemophilus parasuis* (Glässer's disease), *S. suis*, *Actinobacillus suis*, and *Mycoplasma hyorhinis* infections. Pigs that recover spontaneously or following treatment may develop fibrous abdominal adhesions that impair normal gut function, cause discomfort, reduce appetite, and lower growth rates that affect the overall productivity of the unit if significant numbers are involved. Other circumstances that can give rise to peritonitis are umbilical infection in neonatal piglets and infection associated with castration wounds. Environmental or opportunist types of organisms such as *E. coli*, *Trueperella pyogenes*, *Staphylococcus*, and *Streptococcus* spp. are usually involved. Peritonitis can occur secondary to colonic serositis in pigs with over-distension of the organ secondary to rectal stricture and in pigs with enteritis or typhlocolitis associated with invasive bacterial infections. Rare forms of peritonitis in pigs include intestinal anthrax, where the mesentery is gelatinous and hemorrhagic associated with lymphatic spread of infection, and tuberculosis, where lesions are more localized and often adhered to the spleen. In pigs with *Stephanurus dentatus* infection, localized peritonitis with inflammation and edema of perirenal tissue can be caused by larval migration from the liver to the kidneys. Neoplastic lesions affecting the peritoneum can occur in aged pigs due to metastatic spread from primary sites.

Ossification of the mesentery or the peritoneal lining is seen occasionally in adult pigs. The lesion is usually confined to a focal area and is thought to arise as a long-term consequence of tissue injury or scarring. At the root of the mesentery, there can be radiating spicules of calcification and bone formation. The cause is uncertain, but we postulate that it could result from "stretch and shear" damage. Generally these lesions are limited in extent and of no consequence. However, intestinal obstruction, perforation, and mesenteric torsion involving heterotopic mesenteric bony elements have been described (Forsythe 1987; Sanford and Rehmtulla 1994). In the latter reference, a y-shaped bony lesion measuring approximately 28 cm served as a fulcrum for the mesenteric torsion.

Tumors involving the peritoneal cavity are relatively rare in pigs. Cases of multicentric lymphoma invariably involve the mesenteric lymph nodes in addition to the liver and spleen. Marked edema of the mesentery can result from obstructions to the efferent lymphatic drainage caused by tumor metastasis. Uterine, ovarian, and intestinal carcinomas have all been found to cause extensive peritoneal lesions.

Splenic torsion involving the lengthy gastrosplenic ligament in pigs is a common finding. Complete torsions are rapidly fatal as the spleen becomes engorged with blood and ultimately ruptures. Pigs with partial torsions can survive successfully, depending upon the amount of spleen affected. The resulting lesions can be seen as incidental findings in slaughtered animals or those that have died from other causes. Typically, one-third to half of the spleen has been entrapped by the ligament and mesentery, resulting in chronic adhesions and atrophy of the affected portion of the spleen.

The liver

Congenital anomalies affecting the pig liver are rare. One recognized condition is a cystic anomaly affecting bile ducts in which the liver is enlarged and shows numerous fluctuating cystic lesions containing bile. The enlarged liver causes abdominal distension, and piglets have poor viability.

Traumatic conditions affecting the liver are important. In neonatal piglets, rupture of the liver and fatal hemorrhage is a consequence of trauma, usually inflicted accidentally by the sow. Torsion of one or more liver lobes can affect pigs of any age. It usually affects the left lateral lobe and results in infarction of the lobe (Figure 15.5a and b). Death occurs due to shock or hepatic rupture and hemorrhage.

Hepatosis dietetica is a noninfectious diet-associated condition in which there is massive hepatic necrosis. Experimentally, concurrent deficiencies of sulfur-containing amino acids, tocopherols, and selenium are required for development of hepatosis dietetica. The pathogenesis is not fully understood, but it is thought to be associated with formation of free radicals and their subsequent adverse effects similar to the related condition "mulberry heart disease" in which there is acute cardiomyopathy and myocardial hemorrhage results in congestive heart failure and marked congestion of the liver. Enlarged, heavy, and turgid livers occur with various causes of congestive heart failure, including vegetative endocarditis.

Many systemic diseases cause nonspecific changes in the liver, including congestion and inflammatory cell infiltration. Hemorrhages are a feature of septicemia. Salmonellosis, especially when caused by *Salmonella* Choleraesuis, can cause multifocal white pyogranulomatous nodules, often referred to as "paratyphoid nodules."

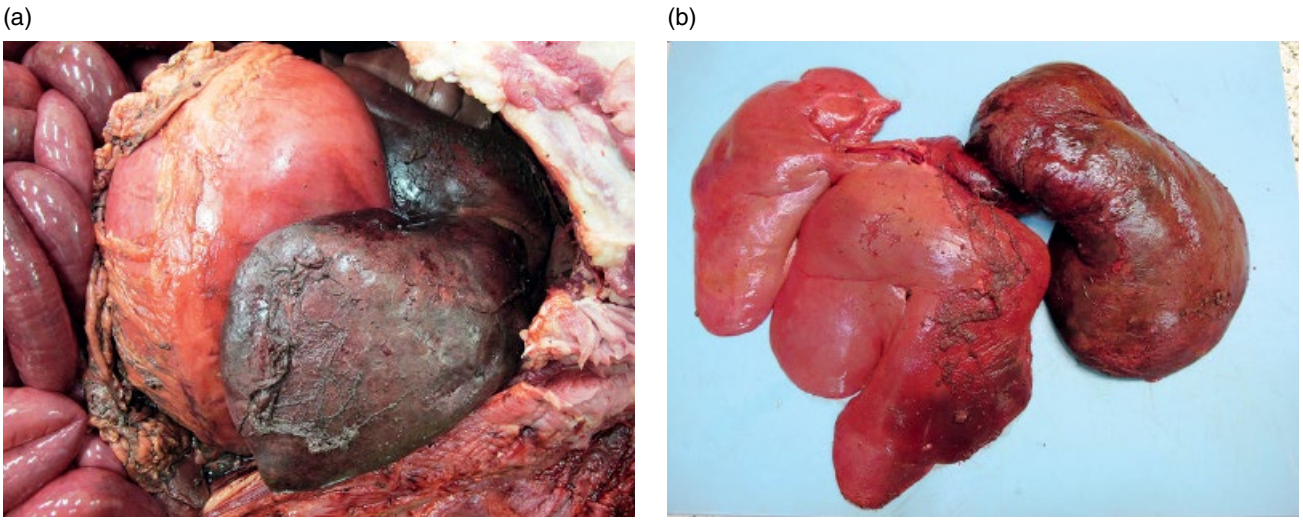


Figure 15.5 Torsion of liver lobe. (a) Liver *in situ* showing necrotic left lobe with fibrin coating. (b) Liver removed, showing appearance of affected and unaffected lobes. *Source:* Courtesy of Fiona Howie.

Parasitism is the most common condition affecting the liver. Migrating *A. suum* larvae cause mechanical damage in the form of hemorrhagic tracts that initiate intense inflammation. Reactive changes, both repair of tissue and hypersensitivity reaction to excretory and secretory products of larvae, cause eosinophil infiltration and fibrosis seen grossly as "white spots" or "milk spots," resulting in economic losses to the farmer. In heavy infestations, adult ascarids can migrate up the bile duct or pancreatic duct, causing obstruction, jaundice, and cholangitis. Other parasitic infections affecting the liver include *Cysticercus tenuicollis*, the metacestode of the tapeworm *Taenia hydatigena* (of dogs). Cysts can sometimes be found in the pig peritoneal cavity, often attached to the liver. *S. dentatus* infection results in migratory tracts and hepatitis. Portal phlebitis with thrombus formation in the portal vein are additional features following oral infection. *Fasciola hepatica* and *Fasciola gigantica* are both capable of infecting pigs, though this is rare. The lancet liver fluke *Dicrocoelium dendriticum* has resulted in nodular hepatic lesions causing liver condemnations in outdoor-reared pigs. The latter is a fascinating parasite that requires two intermediate hosts (aquatic snail and ant).

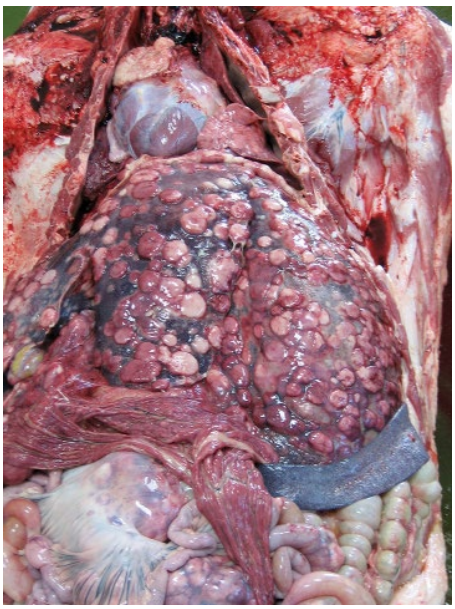


Figure 15.6 Hepatic lymphoma in a 4-month-old gilt. The mesenteric lymph nodes were also severely infiltrated.

Toxicities affecting the liver can be acute or chronic in nature. Cresol toxicity is caused by exposure to tar compounds that might have been used in construction of piggery buildings, accidental environmental spillage, road materials, or "clay pigeons" used as shooting targets. Lesions include severe hepatocellular necrosis leading to sudden death. Chronic cresol toxicity results in jaundice, ascites, and anemia on account of chronic, progressive destruction of hepatic tissue. Iron toxicity occurs occasionally in neonatal piglets, with deaths occurring within 24 hours of iron-dextran administration. Toxicity is associated with marginal or deficient vitamin E and selenium status. Iron-catalyzed lipoperoxidation occurs in the liver and muscle, resulting in hepatic necrosis and hepatic hemorrhages.

Aflatoxicosis is caused by the use of cereals contaminated with *Aspergillus* species or *Penicillium puberulum*. Lesion development is a chronic process in which there is liver hypertrophy and progressive fibrosis. The condition results in reduced growth rates and liver condemnations.

Tumors of the liver are uncommon apart from metastatic lymphoma (Figure 15.6).

The pancreas

There has been little focus on pancreatic conditions in pigs. Pancreatic hypoplasia is rare and associated with poor growth in individual weaner pigs. The pancreatic duct can be invaded by *A. suum* in piglets with heavy infestations. This can lead to obstruction of the pancreatic duct, resulting in pancreatic necrosis and acute pancreatitis. Inflammation of the pancreas can be observed in some cases of mycotoxicosis (fumonisin B2) or systemic viral infections (PCV2).

Acknowledgments

Extracts from *Diseases of Swine 9th edition* (2006), Chapter 59 entitled "Prolapses," have been included in the text of this chapter, with kind permission of the authors W.J. Smith and B.E. Straw.

References

Accioly JM, Durmic Z, McDonald DE, et al. 1998. In Proceedings of the 15th International Pig Veterinary Society Congress, Vol. 3, p. 242.
Adeola O, Cowieson AJ. 2011. *J Anim Sci* 89:3189–3218.
Amass SF, Schinckel AP, Clark LK. 1995. *Vet Rec* 137:519–520.
Andreasen A, Petersen HH, Kringel K, et al. 2015. *Vet Parasitol* 207:249–258.
Andreasen A, Skovgaard K, Klaver EJ, et al. 2016. *Parasite Immunol* 38: 53–63.
Andrews JJ 1979. *Vet Pathol* 16:432–437.
Asplund K, Hakkinen M, Bjorkroth J, et al. 1996. *J Appl Bacteriol* 81:217–222.
Astrup P, Larsen KV, Jorsal SE, et al. 2010. In Proceedings of the 21st International Pig Veterinary Society Congress, p. 751.
Aumiller T, Mosenthin R, Weiss E, et al. 2015. *Livest Sci* 172:16–32.
Ayles HL, Ball RO, Friendship RM, et al. 1996a. *Can J Anim Sci* 76:607–611.
Ayles HL, Friendship RM, Ball RO. 1996b. *J Swine Health Prod* 4:211–216.
Bach Knudsen KE, Canibe N. 2000. *J Sci Food Agric* 80:1253–1261.
Bach Knudsen KE, Jørgensen H. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 109–120.
Bach Knudsen KE, Jensen BB, Andersen JO, et al. 1991. *Br J Nutr* 65:233–248.
Bach Knudsen KE, Jensen BB, Hansen I. 1993. *J Nutr* 123:1235–1247.
Backstrom L, Crenshaw T, Shenkman D. 1981. *Am J Vet Res* 42:538–543.
Baele M, Decostere A, Vandamme P, et al. 2008. *Int J Syst Evol Microbiol* 58:1350–1358.
Bailey M, Miller BG, Telemo E, et al. 1993. *Int Arch Allergy Immunol* 101:266–271.
Baintner K. 1986. *Intestinal Absorption of Macromolecules and Immune Transmission from Mother to Young*. Boca Raton, FL: CRC Press, pp. 1–216.
Barbosa AJA, Silva JCP, Nogueira AMMF, et al. 1995. *Vet Pathol* 32:134–139.
Beal JD, Moran CA, Campbell A, et al. 2001. In Lindberg JE and Ogle B (eds). *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 351–353.
Becker HN, Van der Leek M. 1988. *Proc Congr Int Pig Vet Soc* 10:395.
Belsham GJ, Rasmussen TB, Normann P, et al. 2016. *Transbound Emerg Dis* 63:595–601.
Berends BR, Urlings HAP, Snijders JMA, et al. 1996. *Int J Food Microbiol* 30:37–53.
Berg RD. 1999. *Adv Exp Med Biol* 473:11–30.
Berg JD, Roberts PV, Matin A. 1986. *J Appl Bacteriol* 60:213–220.
Berruecos JM, Robinson OW. 1972. *J Anim Sci* 35:20–23.
Bertschinger HU, Eggenberger E, Jucker H, et al. 1978. *Vet Microbiol* 3:281–290.
Biernat M, Gacsalyi U, Rådberg K, et al. 2001. In Lindberg JE and Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 46–48.
Bikker P, Dirkzwager A, Fledderus J, et al. 2006. *J Anim Sci* 84:3337–3345.
Bindels LB, Delzenne NM, Cani B, et al. 2015. *Nat Rev Gastroentero* 12: 303–310.
Bolduan G, Jung H, Schnabel E, et al. 1988. *Pig News Inf* 9:381–385.
Boniotti M, Papetti A, Lavazza A, et al. 2016. *Emerg Infect Dis* 22(1): 83–87.
Bosi P, Casini L, Finamore A, et al. 2004. *J Anim Sci* 82:1764–1772.
Boudry G, Péron V, Le Huërou–Luron I, et al. 2004. *J Nutr* 134:2256–2262.
Boyd JS, Taylor DJ, Reid J. 1988. *Proc Congr Int Pig Vet Soc* 10:403.
Brestoff JR, Artis D. 2013. *Nat Immunol* 14: 676–684.
Brockman JB, Patterson NW, Richardson WS. 2004. *Surg Endosc* 18(3):536–539.
Brooks PH, Geary TM, Morgan DT, et al. 1996. *Pig J* 36:43–64.
Brown PJ, Bourne FJ. 1976. *Am J Vet Res* 37:9–13.
Brunsgaard G. 1998. *J Anim Sci* 76:2787–2798.
Burrin DG, Stoll B, Jiang R, et al. 2000. *Am J Clin Nutr* 71:1603–1610.

Burrin DG, Stoll B, van Goudoever JB, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 75–88.
Burrough ER, Arruda BL, Patience JF, et al. 2015. *PLoS One* 10(11):e0141337.
Burt S. 2004. *Int J Food Microbiol* 94:223–253.
Cameron BF, Wong CW, Hinch GN, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 66–68.
Canibe N, Steien SH, Øverland M, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 288–290.
Casper HH, Berg IE, Crenshaw JD, et al. 1991. *J Vet Diagn Invest*:3(2):172–173.
Cera KR, Mahan DC, Cross RF, et al. 1988. *J Anim Sci* 66:574–584.
Chae C. 2004. *Vet J* 168: 41–49.
Chagnon M, D'Allaire S, Drolet R. 1991. *Can J Vet Res* 55:180–185.
Chander Y, Primus A, Oliveira S, et al. 2012. *J Vet Diagn Invest* 4:903–910.
Cheon DS, Chae C. 1996. *J Vet Diagn Invest* 8:123–124.
Cohen IT, Nelson SD, Modley RA, et al. 1991. *J Pediatr Surg* 26:598–601.
Collins JE, Bergeland ME, Meyers LL, et al. 1989. *J Vet Diagn Invest* 1:349–351.
Creuz E, Perez JF, Peralta B, et al. 2007. *Zoonoses Public Health* 54:314–319.
Dahl J. 2008. *Pig J* 61:6–11.
Daniel M. 1975. Thèse de Doctorat Vétérinaire. École Nationale Vétérinaire d'Alfort.
Davies PR. 1993. In Proceedings of the A. D. Leman Swine Conference, University of Minnesota, pp. 129–135.
Davies PR, Grass JJ, Marsh WE, et al. 1994. *Proc Congr Int Pig Vet Soc* 13:471.
De Witte C, Ducatelle R, Smet A, et al. 2016. *Proc Congr Int Pig Vet Soc* 24:264.
Deen J. 1993. In Proceedings of the A. D. Leman Swine Conference, pp. 137–138.
Demeckova V, Moran CA, Caveney C, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 291–293.
Dionissopoulos L, deLange CFM, Dewey CE, et al. 2001. *Can J Anim Sci* 81:563–566.
Dirkzwager A, Elbers ARW, Vanderaar P, et al. 1998. *Livest Prod Sci* 56:53–60.
Douglas RGA. 1985. *Vet Rec* 117:129.
Driesen SJ, Fahy VA, Spicer EM. 1987. *Proc Pig Production (Sydney Univ)* 95:1007–1017.
Duncan SH, Flint HJ, Stewart CS. 1998. *FEMS Microbiol Lett* 164:283–288.
Durmic Z. 2000. PhD thesis, Murdoch University, Murdoch, Western Australia.
Durmic Z, Pethick DW, Mullan BP, et al. 2000. *J Appl Microbiol* 89:678–686.
Ebner S, Schoknecht P, Reeds PJ, et al. 1994. *Am J Physiol* 266:R1736–R1743.
Eisemann JH, Morrow WEM, See MT, et al. 2002. *J Am Vet Med Assoc* 220:503–506.
Elbers ARW, Hessing MJC, Tielen MJM, et al. 1995. *Vet Rec* 136:588–590.
Elbers ARW, Vos JH, Bouma A, et al. 2003. *Vet Microbiol* 96:345–356.
Embaye H, Thomlinson SR, Lawrence TLJ. 1990. *J Comp Pathol* 103:253–264.
Février C, Gotterbarm G, Jaguelin-Peyraud Y, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 192–194.
Forsythe DW. 1987. *Pig J* 18:99–100.
Foss DL, Kopta LA, Paquette JA et al. 2013. *J Swine Health Prod* 21:242–247.
Fouhse JM, Zijlstra RT, Willing BP. 2016. *Anim Front* 6:30–36.
Gabert VM, Sauer WC, Schmitz M, et al. 1995. *Can J Anim Sci* 75:615–623.
Gallois M, Oswald IP. 2008. *Arch Zootech* 11(3):15–32.
Garden S. 1985. *Proc Cong Int Pig Vet Soc* 15:100–107.
Garden S. 1988. Rectal prolapse in pigs. *Vet Rec* 123:654.
Gardner IA, Hird DW, Franti CE, et al. 1988. *Vet Rec* 123:222–225.
Gebert A, Rothkotter HJ, Pabst R. 1994. *Cell Tissue Res* 276:213–221.
Gibson GR, Roberfroid M 1995. *J Nutr* 125:1401–1412.
Gin T, Guennec J le, Morvan H, et al. 2010. In Proceedings of the 21st International Pig Veterinary Society Congress, pp. 758.
Glitsø LV, Brunsgaard G, Højsgaard S, et al. 1998. *Br J Nutr* 80:457–468.
Graham TE, Sgro V, Friars D, et al. 2000. *Am J Physiol Endocrinol Metab* 278:E83–E89.
Grosse-Beilage E, Grosse-Beilage I. 1994. *Deut Tier Wochenschr* 101(10):383–387.
Gudmundsson FF, Gislason HG, Dicko A, et al. 2001. *Surg Endosc* 15:854–860.
Guilloteau P, Zabielski R, Hammon H, et al. 2010. *Nutr Res Rev* 23:4–22.
Guise HJ, Penny RHC. 1990. *Anim Prod* 49:511–515.
Haesebrouck F, Pasmans F, Flahou B, et al. 2009. *Clin Microbiol Rev* 22:202–223.
Hampson DJ. 1986. *Res Vet Sci* 40:32–40.
Hampson DJ, Robertson ID, La T, et al. 2000. *Vet Microbiol* 73:75–84.
Hampson DJ, Pluske JR, Pethick DW. 2001. In Lindberg JE, Ogle B, eds. *Dietary Manipulation of Enteric Disease*. Wallingford, UK: CAB International, pp. 247–261.
Häni H, Scholl E. 1976. *Schweiz Arch Tierheilkd* 118:325–328.

Harms PA, Sorden SD, Halbur PG, et al. 2001. *Vet Pathol* 38:428–539.
Hazzledine M, Partridge GG. 1996. In Proceedings of the 12th Annual Carolina Swine Nutrition Conference, pp. 12–33.
Hedde RD, Lindsey TO, Parrish RC, et al. 1985. *J Anim Sci* 61:179–186.
Heinritz SN, Weiss E, Eklund M, et al. 2016. *PLoS One*. doi: 10.1371:0154329.
Hellemans A, Chiers K, Maes D, et al. 2007. *Vet Rec* 161:189–192.
Heo JM, Opapeju FO, Pluske JR, et al. 2012. *J Anim Physiol Anim Nutr* 97: 207–237.
Heo JM, Kim JC, Yoo J, et al. 2015. *Anim Sci J* 86: 286–293.
Hessing JJC, Geudeke MJ, Scheepens CJM, et al. 1992. *Tijdschr Diergeneeskd* 117:445–450.
Högberg A, Melin L, Mattsson S, et al. 2001.In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 296–299.
Högberg A, Lindberg JE, Leser T, et al. 2004. *Acta Vet Scand* 45:87–98.
Hontecillas R, Wannemeulher MJ, Zimmerman DR, et al. 2002. *J Nutr* 132:2019–2027.
Hooper PT, Scanlan WA. 1977. *Aust Vet J* 53:109–114.
Horter DC, Yoon K-J, Zimmerman JJ. 2003. *Anim Health Res Rev* 4:143–155.
Hou C, Liu H, Zhang J, et al. 2015. *PLoS One* 10:E0119505.
Isaacson R, Kim HB. 2012. *Anim Health Res Rev* 13:100–109.
Ivascu I, Christea I, Gatina L. 1976. *Proc Congr Int Pig Vet Soc* 4:Z15.
Jacobson M, Fellstrom C, Lindberg R, et al. 2004. *J Med Microbiol* 53:273–280.
Jansman ASM, 2016. *J Anim Sci* 94:12–21.
Järveots T, Saar T, Põdersoo D, et al. 2016. *Pol J Vet Sci* 19(2):291–301.
Jensen V. 1989. *Dansk Vet* 72(10):557–565.
Jensen BB, Jørgensen H. 1994. *Appl Environ Microbiol* 60:1897–1904.
Jensen BB, Mikkelsen LL. 1998. In Garnsworthy PC, Wiseman J, eds. *Recent Advances in Animal Nutrition*. Nottingham, UK: Nottingham University Press, pp. 107–126.
Jensen KH, Pedersen LJ, Nielsen EK, et al. 1996. *Physiol Behav* 59(4/5):741–748.
Jensen TK, Vigre H, Svensmark B, et al. 2006. *J Comp Pathol* 135:176–182.
Jha R, Berrocoso JFD. 2015. *Animal* 9:1441–1452.
Jha R, Berrocoso JFD. 2016. *Anim Feed Sci Technol* 212:18–26.
Junli H, Li W, Nenqi RLX, et al. 1997. *Water Res* 31:455–460.
Keeler RF, Crowe RW. 1983. *Clin Toxicol* 20:49–58.
Keeliside J. 2006. In 8th Annual Swine Technology Workshop, Red Deer, Alberta, Canada.
Kelly D. 1990. *Res Vet Sci* 48:250–356.
Kelly D, Coutts APG. 1997. In Laplace JP, Février C, Barbeau A, eds. Digestive Physiology of Pigs. Proceedings of the 7th International Symposium on INRA-SRP and EEAP, St-Malo, France, pp. 163–170.
Kelly D, Smyth JA, McCracken KJ. 1991. *Br J Nutr* 6:181–188.
Kiarie E, Romero LF, Nyachoti CM. 2013. *Nutr Res Rev* 26:71–88.
Kiers JL, Nout MRJ, Rombouts FM, et al. 2001a. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 277–279.
Kiers JL, Nout MRJ, Rombouts FM, et al. 2001b. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 261–263.
Kim J, Ha Y, Jung K, et al. 2004. *Can J Vet Res* 68:218–221.
Kirchgessner M, Gedek B, Wiehler S, et al. 1992. *J Anim Physiol Anim Nutr* 68:73–81.
Kirkwood RN, Huang SX, McFall M, et al. 2000. *J Swine Health Prod* 8:73–76.
Kjar HA. 1976. *Proc Congr Int Pig Vet Soc* 4:6.
Kleingebbink GAR, Sutton AL, Williams BA, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 269–271.
Klose V, Bruckbeck R, Henikl S, et al. 2010. *J Appl Nicrobiol* 108(4):1271–1280.
Kolden A. 1994. *Norsk Vet* 106(10):731–736.
Konstantinov SR, Smidt H, Akkermans AD, et al. 2008. *FEMS Microbiol Ecol* 66(3):599–607.
Kopinski JS, McKenzie RA. 2007. *Aust Vet* 85:356–361.
Kopinski JS, Foggarty R, McVeigh J. 2007. *Aust Vet* 85:362–367.
Krakowka S, Rings DM, Ellis JA. 2005. *Am J Vet Res* 66:945–952.
de Lange CFM, Pluske J, Gong J, et al. 2010. *Livest Sci* 134:124–134.
Lawlor PG, Flood C, Fitzpatrick E, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 54–56.
Lawrence BV, Anderson DB, Adeola O, et al. 1998. *J Anim Sci* 76:788–795.
Lazier A, Friendship R, Hazlett M, et al. 2008. In Proceedings of the Annual Meeting of the American Association of Swine Veterinarians, pp. 45–48.
Lee YK, Mazmanian SK. 2010. *Science* 330:1768–1773.
Levast B, Berri M, Wilson HL, et al. 2014. *Dev Comp Immunol* 44:235–244
Levesque CL, Yu H, Gong J, et al. 2012. *J Anim Sci* 90:448–450.
Levesque CL, Hooda S, Swanson KS, et al. 2014. *PLoS One* 9(9):e108472.
Lindecrona RH, Jensen TK, Jensen BB, et al. 2003. *Anim Sci* 76:81–87.
Lindecrona RH, Jensen TK, Moller K. 2004. *Vet Rec* 154:264–267.

Longland AC, Low AG, Quelch DB, et al. 1993. *Br J Nutr* 70:557–566.
Longpré J, Fairbrother JM, Fravalo P et al. 2016. *J Anim Sci* 94:1053–1063.
Mack DR, Michail S, Wie S, et al. 1999. *Am J Physiol* 276:G941–G950.
Magras C, Cantet F, Koffi G, et al. 1999. *J Rech Porc France* 31:395–399.
Mahanta S, Chaudhury B. 1985. *J Vet Med Mycol* 23:395–397.
Makkink CA, Negulescu GP, Guixin Q, et al. 1994. *Br J Nutr* 72:353–368.
Martineau GP, Morvan H, Decoux M. 2008. *J Rech Porc* 40:33–42.
Mathews CJ, MacLeod RJ, Zheng SX, et al. 1999. *Gastroenterology* 116:1342–1347.
McCracken BA, Gaskins HR, Ruwe-Kaiser PJ, et al. 1995. *J Nutr* 125:2838–2845.
McCracken BA, Spurlock ME, Roos MA, et al. 1999. *J Nutr* 129:613–619.
McDonald DE, Pethick DW, Pluske JR, et al. 1999. *Res Vet Sci* 67:245–250.
McDonald DE, Pethick DW, Mullan BP, et al. 2001. In Lindberg JE, Ogle B, eds. *In Digestive Physiology of pigs*. Wallingford, UK: CAB International, pp. 280–282.
McDonald P, Edwards RA, Greenhalg JED, et al. 2002. In *Animal Nutrition*, 6th ed. Harlow, Essex: Pearson Education Ltd, pp. 616–629.
McLaughlin CL, Byatt JC, Curran DF et al. 1997. *J Anim Sci* 75(4):959–67.
Melin L, Katouli M, Jensen-Waern M, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 294–296.
Melnichouk SI. 2002. *Can Vet J* 43:23–25.
Melnichouk SI, Friendship RM, Dewey CE, et al. 1999. *Swine Health Prod* 7:201–205.
Mendes EN, Queiroz DM, Rocha GA, et al. 1990. *J Med Microbiol* 33:61–66.
van der Meulen J, de Graaf GJ, Nabuurs MJA, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 329–331.
Michiels J, Missotten J, Dierick N, et al. 2008. *J Sci Food Agric* 88:2371–2381.
Mikkelsen LL, Jensen BB. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 285–288.
Moeser AJ. 2016. *Proc AASV*:7–10.
Mølbak L, Thomsen LE, Jensen TK, et al. 2007. *J Appl Microbiol* 103:1853–1867.
Moore JC. 1989. *Pig Vet J* 23:124.
Moran CA, Ward G, Beal JD, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 266–268.
Morin M, Sauvageau R, Phaneuf JB, et al. 1984. *Can Vet J* 25:440–442.
Muirhead MR. 1989. *Int Pig Lett* 9(1):3.
den Hond E, Hiele M, Peeters M, et al. 1999. *J Parenter Enteral Nutr* 23:7–11.
Naqid IA, Owen JP, Maddison BC, et al. 2015. *Anim Feed Sci Technol* 201:57–65.
Narita M, Kimura K, Tanimura N, et al. 1998. *J Comp Pathol* 118:329–336.
Nemcová R, Bomba A, Gancarciková S, et al. 1999. *Berl Münch Tieräztl Wochenschr* 112:225–228.
Niekamp SR, Sutherland MA, Dahl GE, et al. 2007. *J Anim Sci* 85:93–100.
Nielsen EK, Ingvartsen KL. 2000. *Acta Agric Scand A* 50:30–38.
Niewold TA, van Dijk AJ, Geenen PL, et al. 2007. *Vet Microbiol* 124:362–369.
Norrish JG, Rennie JC. 1968. *J Hered* 59:186–187.
Nyachoti CM, Omogbenigun FO, Rademacher M, et al. 2006. *J Anim Sci* 84:125–134.
O'Sullivan T, Friendship RM, Ball RO, et al. 1996. In Proceedings of the Annual Meeting by American Association of Swine Practitioners, pp. 151–153.
Panter KE, Keeler RF, Buck WB. 1985. *Am J Vet Res* 46:1368–1371.
Papatsiros V, Athanasiou L, Tzivara A, et al. 2012. *Scientific Reports* 1(11):516. doi:https://doi.org/10.4172/scientific reports.516.
Partanen KH, Mroz Z. 1999. *Nutr Res Rev* 12:1–30.
Peace RM, Campbell J, Polo J, et al. 2011. *J Nutr* 141:1312–1317.
Pearce SC, Mani V, Weber TE, et al. 2013.*J Anim Sci* 91:5183–5193
Penny RHC, Hill FWG. 1973. *Vet Annu* 14:55–60.
Perez JF, Reeds PJ. 1998. *J Nutr* 128:1562–1569.
Perfumo CJ, Sanguinetti HR, Giorgio N, et al. 2002. *Arch Med Vet* 34:245–252.
Petkevicius S, Bach Knudsen KE, Nanse P, et al. 1997. *J Parasitol* 114:555–568.
Pfeifer CW. 1984. Nat Hog Farmer, January 15, p. 64.
Phadtare S, Alsina J, Inouye M. 1999. *Curr Opin Microbiol* 2:175–180.
Pieper R, Janczyk P, Urubschurov V, et al. 2010. *Livest Sci* 133:98–100.
Pieper R, Bindell J, Malik G, et al. 2012. *Arch Anim Nutr* 66:163–179.
Pieper R, Villodre Tudela C, Taciak M, et al. 2016. *Anim Health Res Rev*. doi: https://doi.org/10.1017/S14662523160000141.
Pierzynowski SG, Valverde-Piedra JL, Hommel-Hansen T, et al. 2001. In Piva N, Bach Knudsen KE, Lindberg JE, eds. *Gut Environment of Pigs*. Nottingham, UK: Nottingham University Press, pp. 43–62.
Piva A, Galvano F, Biagi G, et al. 2006.In *Biology of Nutrition in Growing Animals*. London, UK: Elsevier, pp. 3–31.
Pluske JR. 2001. In Piva N, Bach Knudsen KE, Lindberg JE, eds. *Gut Environment of Pigs*. Nottingham, UK: Nottingham University Press, pp. 1–27.

Pluske JR, Siba PM, Pethick DW, et al. 1996a. *J Nutr* 126:2920–2933.
Pluske JR, Williams IH, Aherne FX. 1996b. *Anim Sci* 62:145–158.
Pluske JR, Hampson DJ, Williams IH. 1997. *Livest Prod Sci* 51:215–236.
Pluske JR, Durmic Z, Pethick DW, et al. 1998. *J Nutr* 128:1737–1744.
Pocock EF, Bayley HS, Roe CK. 1968. *J Anim Sci* 27:1296–1302.
Pocock EF, Bayley HS, Slinger SJ. 1969. *J Anim Sci* 29:591–597.
Potkins ZV, Lawrence TLJ. 1989. *Res Vet Sci* 47:68–74.
Powell RW, Dyess DL, Collins JN, et al. 1999. *J Pediatr Surg* 34:193–198.
Prange H, Uhlemann J, Schmidt A, et al. 1987. *Monatsh Veterinarmed* 42:425–428.
Prohaszka L, Baron F. 1980. *Zentralbl Veterinarmed B* 27:222–232.
Pujols J, Ségales J, Polo J, et al. 2016. *Porcine Health Manage* 2:4. doi: https://doi.org/10.1186/540813-016-0021-6.
Queiroz DMM, Rocha GA, Mendes EN, et al. 1996. *Gastroenterology* 111:19–27.
Rådberg K, Biernat M, Linderoth A, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 25–28.
Råsbäck T, Jansson, DS, Johansson KE, et al. 2007. *Environ Microbiol* 9:983–981.
Reeds PJ, Burrin DG, Stoll B, et al. 1997. *Am J Physiol* 273:E408–E415.
Reese NA, Muggenburg BA, Kowalczyk T, et al. 1966. *J Anim Sci* 25:14–20.
Regina DC, Eisemann JH, Lang JA, et al. 1999. *J Anim Sci* 77:2721–2729.
Reid CA, Hillman K. 1999. *Anim Sci* 68:503–510.
Reiners K, Hessel EF, van der Weghe HFA. 2008. *J Anim Sci* 86:3600–3607.
Rist VTS, Weiss E, Eklund M, et al. 2013. *Animal* 7: 1067–1078.
Roberfroid M, Van Loo J, Gibson GR. 1998. *J Nutr* 128:11–19.
Rogers DG, Andersen AA. 2000. *J Vet Diagn Invest* 12:233–239.
Rossi F, Ewodo C, Fockedey R, et al. 1997. In Hartemink R ed. Proceedings of the International Symposium on Non-digestible Oligosaccharides, Wageningen, The Netherlands, p. 143.
Rossi F, Cox E, Coddeeris B, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 299–301.
Roth FX, Windisch W, Kirchgessner M. 1998. *Agribiol Res* 51:167–175.
van Sambraus HH. 1979. *Deut Tier Wechr* 86:58–62.
Sanford E, Rehmtulla AJ. 1994. *J Swine Health Prod* 2:17–18.
Sanford SE, Josephson GKA, Rehmtulla AS. 1994. *Proc Congr Int Pig Vet Soc* 13:476.
Sangild PT. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 3–17.
Sangild PT, Trahair JF, Silver M, et al. 1997. *Proc Eur Assoc of Anim Prod* 88:194–197.
Sangild PT, Trahair JF, Loftager MK, et al. 1999. *Pediatr Res* 45:595–602.
Scholten RHJ, van der Peet-Schwering CMC, Verstegen MWA, et al. 1999. *Anim Feed Sci Technol* 82:1–19.
Schon V. 1985. *Dansk Vet* 68:715–772.
Searcy-Bernard R, Gardner IA, Hird DW. 1994. *JAVMA* 204:1660–1664.
Ségales J, Rosell C, Domingo M. 2004. *Vet Microbiol* 98:137–149.
Si W, Gong J, Tsao R, et al. 2006a. *J Appl Microbiol* 100:296–305.
Si W, Gong J, Chanas C, et al. 2006b. *J Appl Microbiol* 101:1282–1291.
Siba PM, Pethick DW, Hampson DJ. 1996. *Epidemiol Infect* 116:207–216.
Smith WJ. 1980. *Proc Congr Int Pig Vet Soc* 6:356.
Smith WJ, Edwards SA. 1996. *Proc Congr Int Pig Vet Soc* 14:693.
Smith VG, Kasson CW. 1991. *J Anim Sci* 69:571–577.
Smith F, Clark JE, Overman BL, et al. 2010. *Am J Physiol Gastrointest Liver Physiol* 298:352–363.
Spencer RJ, Chesson A. 1994. *J Appl Bacteriol* 77:215–220.
Stokes CR, Bailey M, Haverson K. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 59–66.
Stoll B, Henry J, Reeds PJ, et al. 1998. *J Nutr* 128:606–614.
Stoll B, Burrin DG, Henry J, et al. 1999. *Am J Physiol* 277:E168–E175.
Strachan WD, Edwards SA, Smith WJ, et al. 2002. In Proceedings of the 17th International Pig Veterinary Society Congress, Vol. 2. pp. 214.
Straw B. 1987. *Cornell Extension Bull*, Fall
Straw B, Bates R, May G. 2009. *J Swine Health Prod* 17:28–31.
Sutton AL, Mathew AG, Scheidt AB, et al. 1991. In Versegren MWA, Huisman J, den Hartog LA, eds. Proceedings of the 5th International Symposium on Digestive Physiology in Pigs. Wageningen, The Netherlands, pp. 442–427.
Szeredi L, Palkovics G, Solymosi N, et al. 2005. *Acta Vet Hung* 53:371–383.
Tako E, Glahn RP, Welch RM, et al. 2008. *Br J Nutr* 99:472–480.
Telemo E, Bailey M, Miller BG, et al. 1991. *Scand J Immunol* 34:689–696.
Terpstra C, Wensvoort G. 1988. *Res Vet Sci* 45:137–142.
Thillainayagam AV, Hunt JB, Farthing MJ. 1998. *Gastroenterology* 114:197–210.

Thomke S, Elwinger K. 1998. *Ann Zootech* 47:245–271.
Thomsen LE, Bach Knudsen KE, Hedemann MS, et al. 2006. *Vet Parasitol* 142:112–122.
Thomsen LE, Bach Knudsen KE, Jensen TK, et al. 2007. *Vet Microbiol* 119:152–163.
Thomson JR, Edwards SA, Strachan WD, et al. 2004. In *Proc 18th Congr Int Pig Vet Soc*, Vol. 2. pp. 885.
Thomson JR, Miller WG, Woolfenden NJ, et al. 2007. *Pig J* 59:152–159.
Torrallardona D, Conde R, Badiola I, Polo J. 2007. *Livest Sci* 108:303–306.
Torremorrell M, Calsamiglia M, Pijoan C. 1998. *Can J Vet Res* 62:21–26.
Tucker AL, Widowski TM. 2009. *J Anim Sci* 87:2274–2281.
Tucker, AL, Widowski TM, Friendship RM. 2010. *J Swine Health Prod* 18:68–74.
Uzal FA, Plattner BL, Hostetter JM. 2016. In *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*, 6th ed., Vol. 2. St. Louis: Elsevier, pp. 29, 57–59.
Vega-Lopez MA, Telemo E, Bailey M, et al. 1993. *Vet Immunol Immunopathol* 37:49–60.
Vega-Lopez MA, Bailey M, Telemo E, et al. 1995. *Vet Immunol Immunopathol* 44:319–327.
Verdonk JMAJ, Spreeuwenberg MAM, Bakker GCM. 2001a. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 332–334.
Verdonk JMAJ, Spreeuwenberg MAM, Bakker GCM, et al. 2001b. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 347–349.
Von Muller E, Schoon HA, Schultx LC. 1980. *Deut Tier Wochenschr* 87:196–199.
Vonderfecht HE. 1978. *Vet Med Small Anim Clin* 73:201–206.
Wang L, Lei XG. 2011. *J Anim Sci* 89(E-1):187.
Wellock IJ, Fortomaris PD, Houdjik JGM, et al. 2008. *Br J Nutr* 99:520–530.
Weström BR, Svendsen J, Ohlsson BG, et al. 1984. *Biol Neonate* 46:20–26.
Wilberts BL, Arruda PH, Kinyon JM, et al. 2014. *PLoS One* 9(12):e114741.
Wilcock BP, Olander HJ. 1977a. *Vet Pathol* 14:36–42.
Wilcock BP, Olander HJ. 1977b. *Vet Pathol* 14:43–55.
Wilson MR. 1984. *Int Pig Lett* 4(5):4.
Wilson AD, Stokes CR, Bourne FJ. 1989. *Res Vet Sci* 46:180–186.
van der Wolf PJ, van Schie FW, Elbers AR, et al. 2001. *Vet Q* 121–125.
Wondra KJ, Hancock JD, Kennedy GA, et al. 1995a. *J Anim Sci* 73:421–426.
Wondra KJ, Hancock JD, Behnke KC, 1995b. *J Anim Sci* 73:2564–2573.
Yamaguchi M, Takemoto T, Sakamoto K, et al. 1981. *Am J Vet Res* 42:960–962.
Yen JT, Nienaber JA, Hill DA, et al. 1991. *J Anim Sci* 69:2001–2012.
Yi GF, Carroll JA, Allee GL, et al. 2005. *J Anim Sci* 83:634–643.
Yin YL, Baidoo SK, Liu KYG, et al. 2001. In Lindberg JE, Ogle B, eds. *Digestive Physiology of Pigs*. Wallingford, UK: CAB International, pp. 145–147.
Zamora CS, Reddy VK, Frandle KA, et al. 1980. *Am J Vet Res* 41:885–888.
Zhao X, Zhi-Qiang Du, Vukasinovic N, et al. 2009. *Am J Vet Res* 70:1006–1012.
Zinter DE, Migaki G. 1970. *J Am Vet Med Assoc* 157:301–303.
Zlotowski P, Corrêa AMR, Barcellos DESN, et al. 2008. *Pesq Vet Bras* 28(6):313–318.
Zucker BA, Krüger M. 1998. *Berl München Tierärztl* 111(6): 208–211.

16

Immune System

Christopher Chase and Joan K. Lunney

Introduction

The continuing explosion of knowledge on the immune system has substantial implications for swine health. This contains key concepts, including immune proteins and cell subsets, genetics, microbiome–immune interactions, and vaccine responses. The immune system develops in the fetus, and immune responses begin where microorganisms and/or their products interact with epithelial cells of the mucosa and skin. This interaction is necessary for proper development including immune system maturation and regulation and maintenance of homeostasis.

The immune response starts where microorganisms breech the various barriers and engage the immune system (Figure 16.1). The first line of defense is the innate immune response with phagocytic cells and production of various cytokines, chemokines, and proteins that provide antimicrobial protection, recruit T cells through the inflammatory process, and activate the adaptive, or acquired, immune response (Figure 16.2). Natural killer (NK) cells, like many cellular components of the innate immune system, have a dual function: an innate response to attack virus-infected cells and to produce cytokines for assisting in the activation of acquired immunity (Gerner et al. 2009; Mair et al. 2014). The adaptive immune response, with its myriad of B cells, T cells, cytokines, and antibodies, provides the pathogen-specific memory for protection from subsequent infections with the same pathogen (Figure 16.2). Pattern recognition receptors (PRR), including Toll-like receptors (TLRs), monitor pathogen-associated molecular patterns and induce different signaling pathways to activate the immune system against infection.

The mucosal barrier and microbiome

The mucosal epithelium is important for secretion and absorption in the gut and clearance of particulates and pathogens for proper lung function in the upper respiratory tract (URT). In addition, the enterocytes in the gut and ciliated epithelial cells (CEC) in the URT are the first responders to microorganisms at those surfaces. The goblet cells in the mucosa secrete mucus and mucins that provide the initial barrier (Pelaseyed et al. 2014; Varum et al. 2010) with the mucosal epithelial cells (Johansson and Hansson 2014). The mucosal barrier contains antimicrobial peptides (AMPs) produced by the enterocytes and CEC (Figure 16.3). Secretory immunoglobulin A (sIgA) is produced when dimeric IgA is secreted by the plasma cells in the lamina propria (LP) and is transported to the mucosal surface of the epithelial cell. The inner mucus layer along with the AMP and sIgA form a "killing zone" that only a few pathogens or commensals have evolved strategies to penetrate (Figure 16.3). The "killing zone" along with the tight junctions that knits the enterocytes and CEC form a "barrier" against pathogens.

The microbiome is essential for immune development in the neonatal pig after which the microbiome–gut–immune–brain axis maintains the health of the pig (Kim and Isaacson 2015; Mayer et al. 2015; Sherman et al. 2015). As piglets develop, there is a "succession" of microbes that culminates in a "climax" community as the gut transitions to an anaerobic environment (Kim and Isaacson 2015). The outcome of succession is influenced by nutrition, stress, and environment. The microbial community of commensals and their metabolites influence the health of the gut mucosa and the underlying immune cells in the LP (Figure 16.1) (Fouhse et al. 2016; Kim et al. 2016). The composition of the microbiome varies by gut location, with the numbers and diversity of populations increasing dramatically from the stomach to the colon and to the ileum, a key organ for microbial–immune development. An understanding of the complexity of the gut microbial ecosystem (the microbiome), which has evolved to help protect the pig by improving barrier and immune function, is essential (Benis et al. 2015; Stokes 2017).

Diseases of Swine, Tenth Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson.

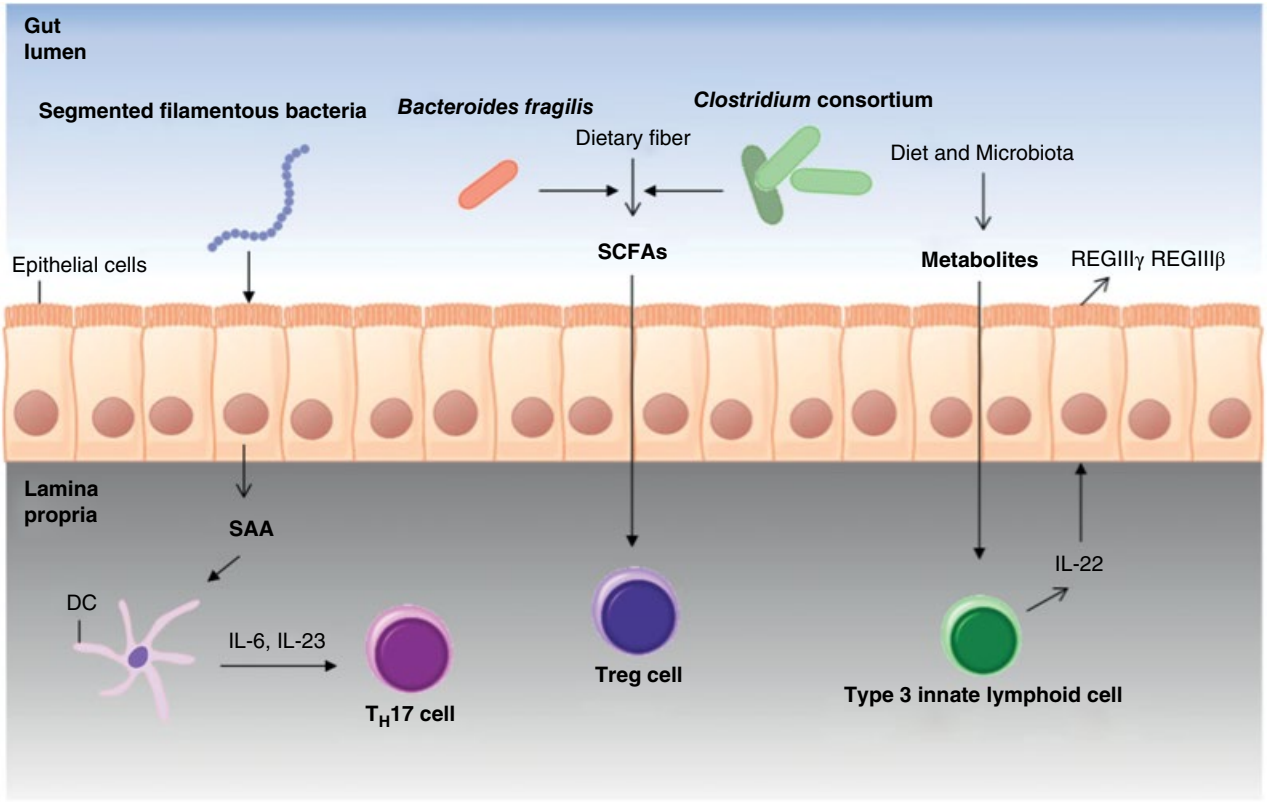


Figure 16.1 Gut microbiota and their products shape the development of epithelial cells and immunity. Segmented filamentous bacteria (related to *Clostridium*) promote the production of serum amyloid A (SAA) protein from epithelial cells, which activates dendritic cells (DCs) to produce IL-6 and IL-23, resulting in the generation of Th17 cells that are important for T-cell development. *Clostridium* consortium and *Bacteroides fragilis* produce short-chain fatty acids (SCFAs) from dietary carbohydrates that induce the differentiation of Treg cells to help minimize inflammatory response. Diet- or microbiota-derived metabolites upregulate the number of IL-22-secreting type 3 innate lymphoid cells (ILC3s) that induce the production of antimicrobial/host defense peptides (AMP/HDP-REGIIIB and REGIIIγ) from epithelial cells. *Source:* Adapted from Kim et al. (2016). Reproduced with permission of Springer Nature.

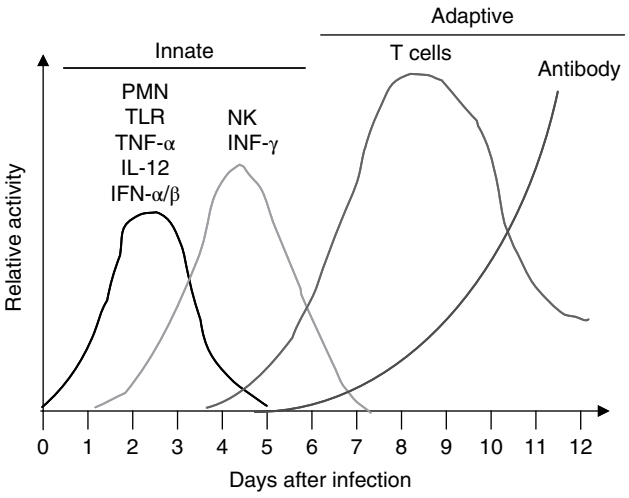


Figure 16.2 The timing of the host response to infection: mobilization of the innate and adaptive response. *Source:* Courtesy of D. Topham, PhD, Rochester, NY, from Introduction to Viral Immunology: Part I.

Piglet age and the act of weaning result in major microbial population shifts (dysbiosis) in the microbiome because of the stress of sudden removal from the sow, the mixing of pigs in a new environment, and the abrupt diet changes. Microbiome shifts can lower defenses against pathogen entry, leading to increased risk of disease because it results in depletion of the "killing zone." The mucus layer becomes thinner, and the amount of sIgA and AMP declines precipitously, weakening the barrier to allow pathogens to interact with the mucosa and cause disease. Low feed and water intake also contribute to dysbiosis and post weaning diarrhea (Fouhse et al. 2016). There is a reduction of lactic acid-producing bacteria (*Lactobacillus*), which raises intestinal pH, increasing susceptibility to pathogens because low gut pH is bactericidal to *Escherichia coli* (Fouhse et al. 2016). Applied research aims to minimize dysbiosis by increasing symbiotic microbes and decreasing opportunistic pathogens.

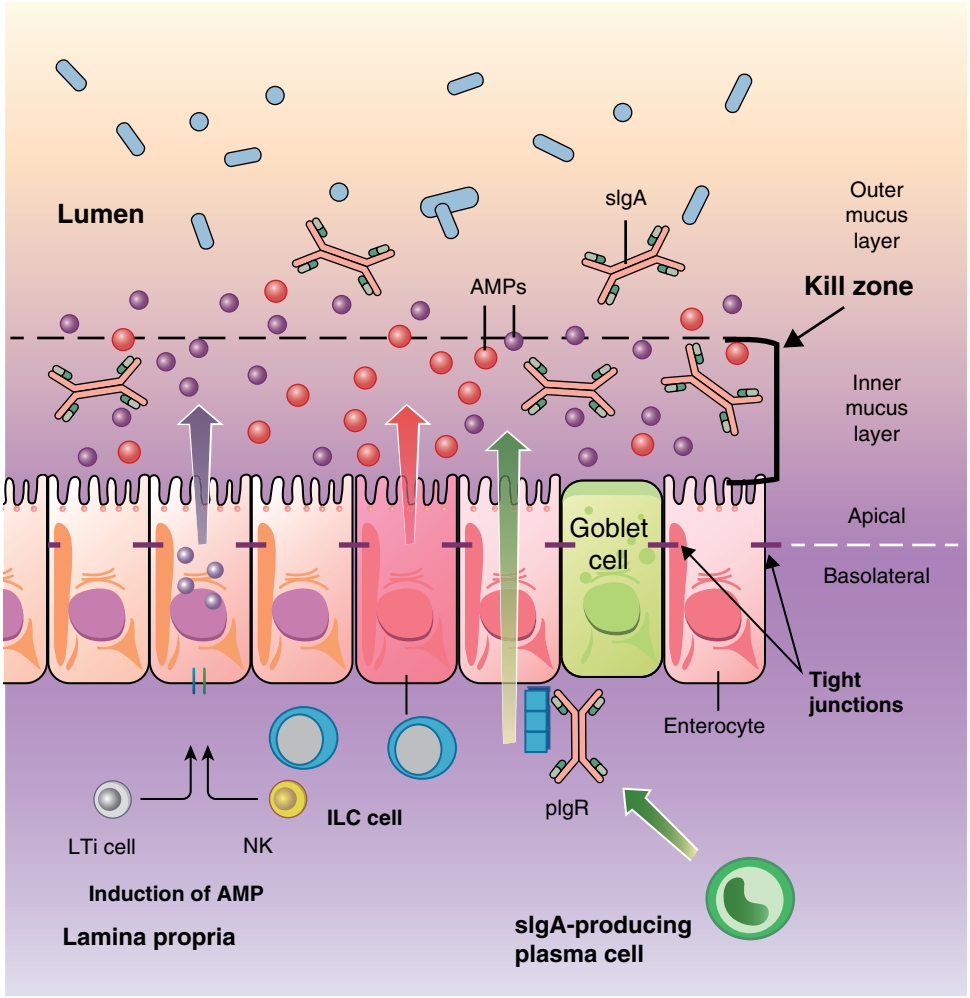


Figure 16.3 Mucosal defenses. Distinct subpopulations of intestinal epithelial cells (IECs) enterocytes are integrated into a continuous single-cell layer that is divided into apical and basolateral regions by tight junctions. Epithelial cells sense the microbiota to induce the production of antimicrobial peptides (AMPs). Goblet cells produce mucin that is organized into a dense, more highly cross-linked inner proteoglycan gel that forms an adherent inner mucus layer and a less densely cross-linked outer mucus layer. The outer layer is highly colonized by constituents of the microbiota. The inner mucus layer is largely impervious to bacterial colonization or penetration due to its high concentration of bactericidal AMPs, as well as commensal-specific secretory IgA (sIgA), which is moved from their basolateral surface, where it is bound by the receptor, to the inner mucus layer. Responding to the microbiota, innate lymphoid cells (ILC), lymphoid tissue inducer cells (LTi), and natural killer (NK) cells produce cytokines, which stimulate AMP production and maintain the epithelial barrier. A similar scheme is in respiratory mucosa. *Source:* Adapted from Maynard et al. (2012). Reproduced with permission of Springer Nature.

Unique features of the swine lymphoid system

The swine lymphoid system consists of five major organized tissues: lymph nodes, lymphoid follicles, tonsils, thymus, and spleen (Rothkotter 2009; Wilson and Obradovic 2015). The structure of porcine lymph nodes is inverted compared with other domestic species; the B-cell germinal centers are in the interior of the node rather than the cortical regions, and there is a much larger cortical and paracortical region that normally contains the T cells (Binns et al. 1986; Pabst and Binns 1986). The lymphoid follicle organization of the gut is also unique. Rather than the discrete lymphoid follicles (Peyer's patches [PP]) present in the ileum in many species, the pig has discrete follicles in the jejunum and a continuous PP in the ileum (Liebler-Tenorio and Pabst 2006). The lymphoid follicles and germinal centers within the PP were significantly smaller in formula-fed compared with sow-fed piglets, suggesting less immune stimulation (Yeruva et al. 2016).

The tonsils are the first contact point to the immune system for many pathogens. The largest tonsils are those of the soft palate, but the pig also has pharyngeal and lingual tonsils. The tonsils contain many lymphoid elements in crypts and interfacing with the surface, often

described as lymphoepithelium. The tonsil is a very important sample for the detection of infectious disease agents (Horter et al. 2003) as well as a source of their continued shedding (Stenfeldt et al. 2014). The thymus is the primary lymphoid organ for the development of T cells in the prenatal and neonatal pig. The spleen, like other mammals, has two main functions: red blood cell management in the red pulp and induction of immune responses against bacterial sepsis in the white pulp.

Innate defense mechanisms

Innate defense mechanisms are present in normal animals and do not require previous exposure to antigen and have no "memory." Innate immunity enables the pig to respond almost immediately to an infectious agent (Figure 16.2). The innate immune system controls infection until the adaptive immune system (with memory) can be activated. Components of the innate system help activate the adaptive immune system to produce antibody- and cell-mediated immune responses.

Physical, chemical, and microbial barriers

Physical, chemical, and microbial barriers to infection at body surfaces are a very important part of resistance to disease (Figure 16.4). These factors include the epithelial cells, bactericidal fatty acids, normal flora, and the mucus layer, which are modulated by the flow of mucus, low pH, bile, and numerous enzymes.

Cells

An important function of the cells of the innate immune system is phagocytosis to remove pathogens. Phagocytic cells engulf, kill, and digest invading bacteria and also play important roles in controlling viral infections and fungal infections and killing cancer cells. The two main types of phagocytic cells are the granular leukocytes, including neutrophils, basophils, mast cells, and eosinophils, and the mononuclear phagocytes, which include the circulating blood monocytes and tissue macrophages (Mair et al. 2014) (Figure 16.5). Phagocytic cells are capable of all the reactions described below for neutrophils. Macrophages are also important in processing antigens as they are critical antigen-presenting cells (APCs) that interact with lymphocytes to stimulate cell-mediated and humoral immune responses.

Granulocytes

Neutrophils (polymorphonuclear neutrophils, PMNs) produced in the bone marrow are released into the bloodstream with a half-life of approximately 8 hours. In healthy pigs PMNs are eliminated via the intestinal tract and lungs. PMNs tend to marginate in the capillaries by loosely associating with endothelial cells and are activated very early in the inflammatory response. The principal function of PMNs is the phagocytosis and destruction of invading microorganisms.

The PMNs are attracted to the vicinity of the invading microorganism by chemotaxis. Chemotactic factors are produced directly by microorganisms, by the cleavage of complement (C′) components, by endothelial activation with inflammatory mediators and chemokines, or by factors released by lymphocytes at the site of infection or inflammation. These factors diffuse in a gradient from the inflammatory site, cause increased expression of adhesion molecules in capillary endothelial cell membranes, and result in an increase in PMN numbers in the capillaries. PMNs enter the tissues, migrate along the gradient toward the site of infection, and ingest those microorganisms susceptible to phagocytic activity. Most pathogens must be opsonized by the attachment of specific antibody and/or C′ to their surface before they can be ingested by PMNs.

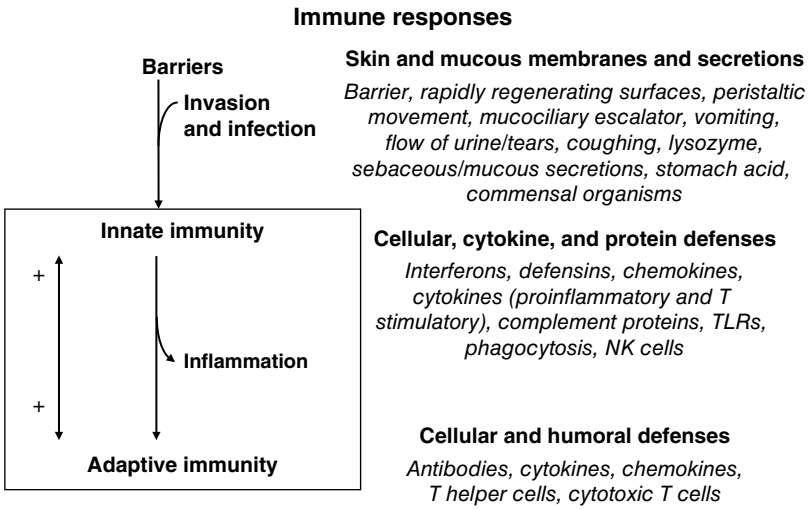


Figure 16.4 Immune responses: the barrier, innate, and adaptive immune components. *Source:* Courtesy of D. Topham, PhD, Rochester, NY, from Introduction to Viral Immunology: Part I.

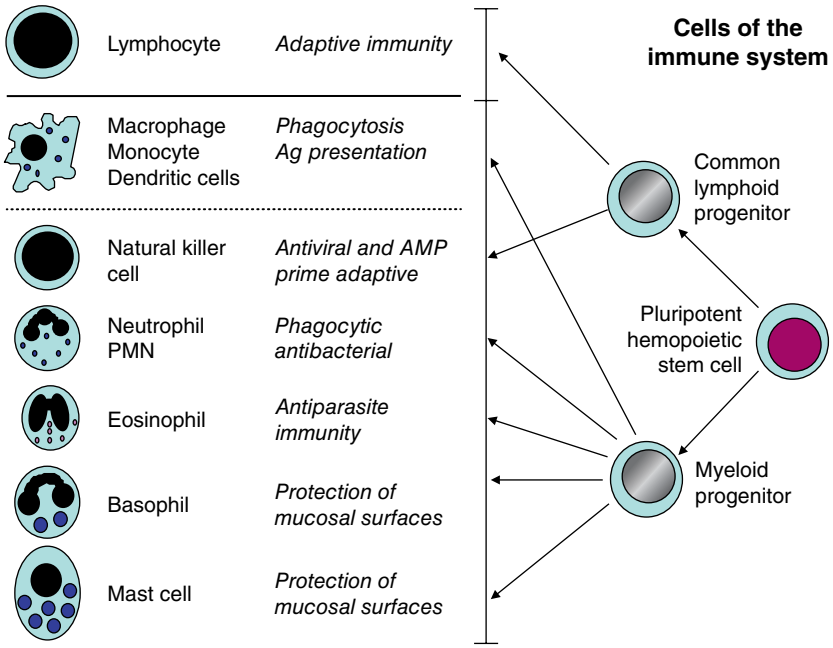


Figure 16.5 Mononuclear blood cells of the immune system. The innate and acquired immune cell lines have overlap with the macrophages and natural killer cells having important innate and acquired responses. AMP, antimicrobial peptides; Ag, antigen. *Source:* Courtesy of D. Topham.

The PMN cytoplasm contains membrane-bound lysosomes with numerous hydrolytic enzymes with bactericidal activity and antibacterial peptides (Sang and Blecha 2009). PMNs die after a short time at sites of inflammation, with release of hydrolytic enzymes to contribute to the inflammatory response and tissue destruction. PMN granule proteins induce adhesion, attract inflammatory monocytes to the site of inflammation, and create extracellular defenses by the formation of neutrophil extracellular traps (NETs) (de Buhr et al. 2017). PMNs use a potent oxidative metabolism system to kill bacteria; when stimulated by an opsonized particle, a burst of oxidative metabolism results in the production of reactive oxygen intermediates (ROI) that are the most potent of bactericidal mechanisms of PMNs, potentially fungicidal and virucidal. PMNs control certain viral infections via a mechanism referred to as antibody-dependent cell-mediated cytotoxicity (ADCC) in which antibodies form a bridge between the PMN and the virus-infected target cell. Porcine PMNs are very active at ADCC, even in the fetus and newborn (Yang and Schultz 1986).

The eosinophil is capable of the same phagocytic and metabolic functions as the PMNs but focuses the host's defense against the tissue phase of certain parasitic infections (Figure 16.5). Eosinophils are more capable of exocytosis by efficiently attaching to and killing migrating parasites that are too large to be ingested. Eosinophils are also important in helping to control certain types of allergic responses and maintaining an anti-inflammatory environment.

Basophils and mast cells have been associated primarily with allergic reactions because of their binding of IgE. These cells have an important regulatory role by release of inflammatory mediators necessary for the activation of the acquired immune response (Abraham and St. John 2010; Galli and Tsai 2010). In pigs, mast cells also have a major effect on intestinal barrier integrity particularly in early weaned pigs (<21 days) (Moeser et al. 2007; Smith et al. 2010). Early weaned pigs have higher numbers of mast cells in the intestinal LP, and treatment of early weaned pigs with a mast-cell degranulation blocker inhibits intestinal mucosal dysfunction.

Mononuclear phagocytes: macrophages and monocytes

The mononuclear phagocytic system consists of circulating monocytes, tissue macrophages, migrating macrophages (histiocytes), and dendritic cells (DCs) (Figure 16.5). Monocytes are produced in the bone marrow, released to the bloodstream, and circulate before migrating into the tissues to become macrophages and DCs (Ezquerra et al. 2009). Fixed macrophages line the endothelium of capillaries, particularly in the lungs, as well as in the sinuses of the spleen, bone marrow, and lymph nodes. Tissue macrophages trap and remove foreign antigens from the bloodstream and lymph as well as serve as APC for T-cell stimulation. Migrating macrophages are derived from blood monocytes and are found throughout the tissues of the body. In certain locations, they differentiate into DCs and act as APCs. Specialized APCs include the glial cells in the nervous system, Langerhans cells in the skin, and Kupffer cells in the liver. Immature DCs located in various tissues will, upon activation, migrate to the peripheral lymphoid organs where they mature and become the focus for the activation and differentiation of T lymphocytes

(Auray et al. 2016; Bautista et al. 2002; Summerfield and McCullough 2009). Macrophages are capable of all the bactericidal activities described for PMNs and act as the second line of innate cell defense. They are slower to arrive at sites of inflammation and are not as aggressive as neutrophils following infection. However, unlike neutrophils, macrophages sustain activity against pathogens, allowing them to kill bacteria that are resistant to killing by neutrophils. This is especially true if activated by cytokines secreted by T lymphocytes. Macrophages and DCs as APCs provide an essential step in the initiation of a cell-mediated acquired immune response and facilitate an efficient antibody response by B lymphocytes.

Alveolar macrophages are specialized to phagocytize inhaled particles or pathogens, including low numbers of bacteria that they may encounter (Chitko-McKown et al. 1991) and then leave the alveolus by one of two pathways, either through the airways and then up the mucociliary escalator or out of the alveolus between alveolar epithelial cells entering the lymphatic drainage. The latter route leads alveolar macrophages to local lymph nodes where they present captured antigens to lymphocytes to initiate an immune response. Alveolar macrophages are a major target of porcine reproductive and respiratory syndrome virus (PRRSV) infection, thus preventing effective antiviral responses and leading to secondary bacterial infections (Gómez-Laguna et al. 2010; Jung et al. 2010; Loving et al. 2015).

Pulmonary intravascular macrophages adhere to endothelial cells in the blood vessels of the lungs (Chitko-McKown and Blecha 1992). They are prominent in pigs, primarily involved in defense against septicemia rather than protection from respiratory disease. Pulmonary intravascular macrophages that are actively clearing bacteria (especially gram-negative bacteria) or free endotoxin from the bloodstream may release cytokines and arachidonic acid metabolites that contribute significantly to pulmonary inflammation.

Natural killer cells

NK cells are lymphoid cells of the innate immune system and can kill a variety of nucleated cells without previous antigenic stimulation. They are activated quickly (within 1–2 days) following infection. Like macrophages, NK cells are an important component of the innate defense mechanisms and participate in activating the adaptive immune response (Shekhar and Yang 2015) (Figures 16.2; 16.5). NK cells can be detected with monoclonal antibodies (mAbs) (Mair et al. 2016) and differ markedly from NK cells found in other species in that their activity is mediated by small granular lymphocytes that have the cluster of differentiation 2 (CD2) T-cell marker (Gerner et al. 2009; Kim and Ichimura 1986). The killing activity of swine NK cells is enhanced in the presence of a variety of cytokines: interferon-γ (IFN-γ), interleukin (IL)-2, human IFN-α, human IL-1α, and interferon (IFN) inducer (poly I:C) (Gerner et al. 2009). Stimulated NK cells produce IFN-γ that activates components of the cell-mediated immune system, such as cytotoxic T lymphocytes (CTLs), macrophages, and NK cells, and also induces T helper (Th) cell differentiation to the Th1 pathway important for cell-mediated immunity and memory (Pintaric et al. 2008). In swine influenza infection, IFN-γ production by NK cells was not increased, but tumor necrosis factor-α (TNF-α) was increased in the lung, which may enhance the proinflammatory response and exacerbate disease (Forberg et al. 2014). Overall, NK cells are an important part of the innate defense mechanisms and participate in a cell-mediated immune response by enhanced activity through cytokine activation.

Innate immune factors

Defensins

AMPs or host defense peptides (HDPs) are a family of molecules that form a chemical barrier to limit infection at epithelial surfaces and attack invading bacteria (Figure 16.3). The HDPs are relatively small cationic peptides found predominantly at mucosal surfaces and in phagocytic cells that vary in structure and antimicrobial functional activity. AMPs/HDPs are induced by the microbiota in the lumen of the gut and respiratory tracts (Maynard et al. 2012; Yeoman and White 2014) and by NK and innate lymphoid cells (ILC) known as lymphoid tissue inducer (LTi) cells. It is likely that HDP antimicrobial and immunoregulatory functions are separate, thus providing opportunities for new designs for antimicrobial drug alternatives (Xiao et al. 2015).

Complement

The complement (C′) system is an enzymatic cascade system that, via at least 20 serum proteins, greatly amplifies its multiple innate functions during the cascade. The C′ system is divided into the classical pathway, the alternative pathway, and the mannan-binding pathway and involves the membrane attack complex and regulatory proteins. The C′ system mediates the inflammatory response, controls bacterial infections, and plays a prominent role in allergic and hypersensitivity reactions. The trigger for the classical C′ pathway is antigen–antibody complexes (IgG and IgM). The alternative C′ pathway is activated by antigen–antibody complexes (IgA and IgE), certain bacterial products such as endotoxin, and proteases released by tissue damage. The mannan-binding pathway recognizes molecules on the surface of bacteria that differ from those present on the host cells. All three pathways end in the splitting of the third component of C′ and start the formation of the membrane attack complex. Activation of any of these C′ pathways causes

vasodilation and increased vascular permeability, resulting in serum components (including antibody and C′) entering the tissues to help control infection. C′ components produced during activation are chemotactic and attract phagocytic cells to the site of infection, as well as coat or opsonize infectious agents to increase their uptake by phagocytic cells. The C′ system is important for mediating inflammation and controlling bacterial infections, but it is also capable of causing serious and even life-threatening damage if unregulated; therefore it is closely regulated to control and stop the C′ reaction once it has started.

Toll-like receptors

TLRs are a family of cell surface molecules (Table 16.1) that bind to various microbial molecules such as lipopolysaccharide, peptidoglycans, cytosine guanine dinucleotide (CpG)-rich unmethylated oligonucleotides, and double-stranded RNA and are a key component of innate immunity (Uenishi and Shinkai 2009). They are the primary method for early detection and response to microbial invasion. Binding of microbial components to TLRs initiates an inflammatory response that helps to activate other aspects of innate immunity and to initiate the acquired immune response.

Bacteria-derived vaccine adjuvants can enhance immune response to vaccines through binding to TLRs, 10 of which are affirmed in the swine genome (Dawson et al. 2013) and are similar to humans. Major efforts are under way to identify functional single nucleotide polymorphisms (SNPs) that may identify pigs with improved innate responses against pathogens associated with SNPs in TLR genes (Clop et al. 2016; Shinkai et al. 2012). Toka and Golde (2013) demonstrated that TLR7 and TLR8 agonists indirectly or directly activate porcine NK cells but that optimum levels of activation require cytokine secretion by accessory cells activated by these compounds. Such activated NK cells were cytotoxic against foot-and-mouth disease virus (FMDV)-infected cells *in vitro*.

Table 16.1 Ligand and pig pathogen responses associated with TLRs and NLRs in pigs.

Gene	Ligand	Effect on immune response	References
TLR1	Lipopeptides	Response to gram-positive bacteria	Uenishi and Shinkai (2009)
TLR2	Peptidoglycan, lipoteichoic acid, heat shock protein	Increased TLR2 in gnotobiotic pig intestine with *Escherichia coli* and *Lactobacillus*	Willing and Van Kessel (2007)
TLR2, TLR6		Recognition of *Mycoplasma hyopneumoniae* cell bodies in porcine alveolar macrophages	Muneta et al. (2003)
TLR3	Double-stranded RNA	Response to viruses. Activate TLR3 with increase IFN-α to decrease PRRSV in macrophages	Miller et al. (2009)
TLR4	Lipopolysaccharide (LPS) (endotoxin), heat shock protein	Response to gram-negative bacteria (LPS and *Salmonella* spp.) and damaged host tissue	Burkey et al. (2009)
TLR5, TLR9	Flagellin, DNA containing high unmethylated CpG	Upregulation after feeding of *Salmonella* spp.	Burkey et al. (2007)
TLR6	Lipopeptides	Response to gram-positive bacteria	Uenishi and Shinkai (2009)
TLR7	Single-stranded RNA	Response to viruses. Transcriptional activation of TLR7-induced genes in swine macrophages	Fernandez-Sainz et al. (2010)
TLR7, TLR8	Single-stranded RNA	Response to viruses. NK cells activated by TLR agonists were cytotoxic against FMDV-infected cells	Toka et al. (2009)
TLR9	DNA containing high unmethylated CpG	Response to bacteria and viruses. PCV2 via CpG–TLR9 signaling of cytokine inducers and inhibitors	Hasslung et al. (2003), Vincent et al. (2007), Wikström et al. (2007), Shimosato (2003)
NOD1	Immunobiotic. Peptidoglycan of lactic acid bacteria	Induction of proinflammatory cytokines	Tohno et al. (2008)
NOD2	Immunobiotic. Muramyl dipeptide (MDP) response	Induction of proinflammatory cytokines	Jozaki et al. (2009), Tohno et al. (2008)

Source: Adapted from Table 6.2 (Lunney et al. 2010a). Reproduced with permission of CABI.

Cytokines: Type I IFNs, tumor necrosis factor-α (TNF-α), IL-6, and IL-8

Cytokines are small protein or glycoprotein molecules secreted by cells and serve as intercellular signaling molecules; all cells of the immune system are capable of secreting and being influenced by cytokines. Cytokine secretion is usually transient, occurs in response to specific stimuli, and typically acts locally in low concentrations. A cytokine will only act on a cell that has specific receptors for it; regulation of cytokine receptor expression is an important mechanism for controlling the response to cytokines. Much is known of porcine cytokines because of the economic and biomedical research importance of pigs (Bailey 2009; Charley et al. 2006; Dawson et al. 2005; Murtaugh et al. 2009). One group of cytokines, important in mediating innate immunity, includes the type I IFNs (IFN-α/β) and the proinflammatory cytokines (IL-1β, IL-6, and TNF-α). Porcine type I IFNs are composed of at least 39 functional genes with diverse expression profiles and antiviral activities (Sang et al. 2010). Type I IFNs can be detected within a few hours of infection, increase cell resistance to virus infection, increase NK cell activity, and increase the major histocompatibility complex (MHC) or swine leukocyte antigen (SLA) molecule expression on cell surfaces, thus increasing antigen presentation to T cells (Lunney et al. 2009). Other cytokines and IFN-γ are discussed below in adaptive immunity.

The proinflammatory cytokines (IL-1, IL-6, and TNF-α) require no previous pathogen exposure and are produced by either macrophages in response to bacterial infection or TNF-α by NK cells (Figure 16.6) in response to viral, protozoal, or fungal infections and tissue damage. The proinflammatory cytokines stimulate the liver to produce acute-phase proteins, stimulate the release of amino acids from muscle tissue, and may induce cachexia or wasting in chronic infections. In addition, they induce fever, loss of appetite, and fatigue if present in high enough concentrations. In low levels, these cytokines promote leukocyte adhesion to endothelial cells, diapedesis of leukocytes into the tissues, and migration of macrophages and DCs to the secondary lymph nodes, resulting in the activation of the adaptive immune response. Their presence in small amounts is required for effective stimulation of adaptive immune responses; however, in large quantities, they can induce hypovolemic shock and death.

Adaptive immunity

Lymphocyte populations

B and T lymphocytes and their products are the components of the adaptive immune response system, an antigen-driven system that requires 2–3 weeks after first exposure to antigen to reach optimal functional capacity (Figure 16.2). With second exposure to antigen, the immune response reaches optimal activity much more rapidly due to the anamnestic, or memory, response. B and T lymphocytes also enhance resistance to disease by activating the innate defense mechanisms (phagocytic cells, NK cells, and C′), thus increasing their efficiency (Figures 16.4 and 16.6).

Pigs have high blood lymphocyte counts compared with most other mammals (approximately 10^7/mL), and lymphocyte subpopulations in the blood of young pigs

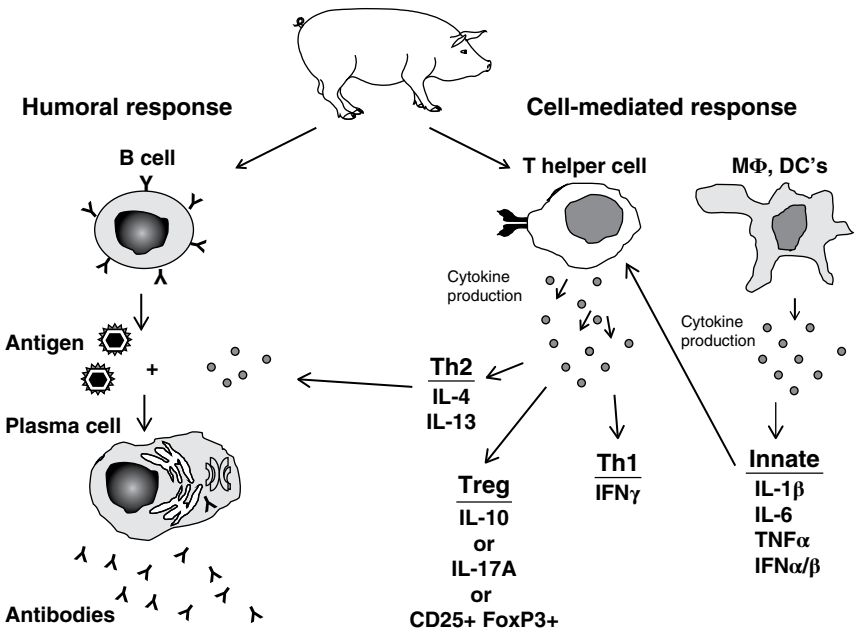


Figure 16.6 Cell-mediated (T-cell) and humoral (B-cell) activation of the adaptive immune response of the pig. T-cell subsets express certain polarizing cytokines, the interleukins (ILs) or interferons (IFNs), as the immune response matures.

differ markedly from other species (Sinkora and Butler 2016). Porcine T cells are divided into subpopulations, which includes the most abundant T cells expressing T-cell receptors (TCR) with αβTCR chains (αβT cells) as well as a prominent fraction of T cells with γδTCR chains (γδT cells) (Duncan et al. 1989; Hirt et al. 1990; Saalmüller and Bryant 1994). Pigs and ruminants have a much higher population of γδT cells in the blood than other mammals. γδT cells do not recirculate between the blood and lymphatic tissues and do not have NK cell activity. Like αβT cells, these γδT cells can express CD8α and SLA class II, potential activation markers of T cells, suggesting that they express cytolytic activity or are involved in antigen presentation (Gerner et al. 2009). As in other species, αβT cells are SLA class-I-restricted CD8+ CTL, CD4+ Th, or regulatory T (Treg) cells. In pigs, Th cells express other activation-related markers, including CD8α, SLA class II, and CD45RC. Certain pig Treg cells have a phenotype similar to humans and mice and can suppress proliferation of other T cells and produce IL-10 (Gerner et al. 2009, 2015).

Swine αβT lymphocytes have three unusual properties compared with other species (Lunney and Pescovitz 1987). First, approximately 25% of swine peripheral blood T cells express both the CD4 and CD8 antigens on their surface rather than just a single antigen like most other species; the functional significance of having both CD4 and CD8 on the same cells is not known (Pescovitz et al. 1994; Zuckermann and Husmann 1996). Second, the normal ratio of CD4+ to CD8+ T cells is approximately 0.6 in pigs, which is reversed compared with other species. Third, resting CD8+ cells in swine preferentially express class II MHC antigens. The significance of these differences between swine T lymphocytes and those of other species is not understood.

γδT cells are located predominantly along mucosal surfaces, especially as intraepithelial lymphocytes in the intestine. They are thought to be important in protecting mucosal surfaces from infection and perhaps in oral tolerance (Thielke et al. 2003). γδT cells proliferate in the intestine and actively recirculate through the intestinal lymphatics to the bloodstream and then to the gut. The role of the thymus and intestinal epithelium in the development of γδT cells is not known. A subset of circulating porcine γδT cells can act as APCs and are capable of producing IFN-γ, proliferating in response to recall antigens *in vitro* and being cytotoxic (Lee et al. 2004; Takamatsu et al. 2002).

Lymphocyte circulation

The inverted lymph node structure provides for recirculation of lymphocytes from blood to lymphoid tissues, important for antigen presentation and facilitating cellular interactions needed for the induction of the immune response (Figure 16.7). Lymphocytes produced in the bone marrow mature in the thymus (αβT cells) or the secondary lymphoid tissues (B cells) and circulate in the blood for approximately 30 minutes before entering the tissue. Lymphocytes enter lymph nodes via afferent lymphatics from tissues or directly from blood by adhering to high endothelial venule (HEV) cells of the lymph node and then migrating into the node. In contrast to other species where lymphocytes exit the lymph node and return to circulatory system via the thoracic duct, lymphocytes in swine directly reenter the blood rather than go to the next draining lymph node through the efferent lymphatics (Binns et al. 1986; Roth and Thacker 2006).

Lymphocyte subpopulations in swine show a distinct preference for circulation to either gut-associated lymphoid tissues (GALTs) or surface nodes (Figure 16.7). For instance, mesenteric lymph node cells (both T and B lymphocytes) preferentially home to the gut (Salmon 1986). In swine, approximately equal numbers of lymphocytes in the mammary gland come from GALT or from peripheral lymph nodes. The dual origin of mammary lymphocytes suggests that the local mammary immune response may not depend solely on oral immunization (Salmon 1987).

Cell-mediated immunity

Antigen presentation

APCs (macrophages and DCs) phagocytize and process antigens and then present these antigenic fragments bound to SLA molecules as they contact T lymphocytes (Figure 16.6). CD4 Th cells can only efficiently recognize foreign antigens that are on a cell surface bound to SLA class II molecules. CD8+ CTL are important for killing cells infected with intracellular pathogens and cancer cells. CTL recognize the foreign antigens processed intracellularly and transported to a cell surface bound to SLA class I molecules. T cells do not respond to free soluble antigen or to whole bacteria or viruses; thus SLA class I and class II molecules play a key role in antigen presentation and have significant influence on the nature of the immune response. The SLA genes are highly polymorphic, differing genetically between individuals (Chardon et al. 1999; Lunney et al. 2009). The set of SLA molecules that a pig inherits can influence their immune response to pathogens and vaccinations and their ability to resist some infectious diseases.

In addition to antigen and class II MHC molecule contact, the Th cell requires the presence of cytokines released by the APCs and other T cells and contact with costimulatory molecules on the surface of the APC for complete activation. IL-1 released by macrophages is a key mediator of the host response to infection through its ability to induce fever and neutrophilia. IL-1 acts on

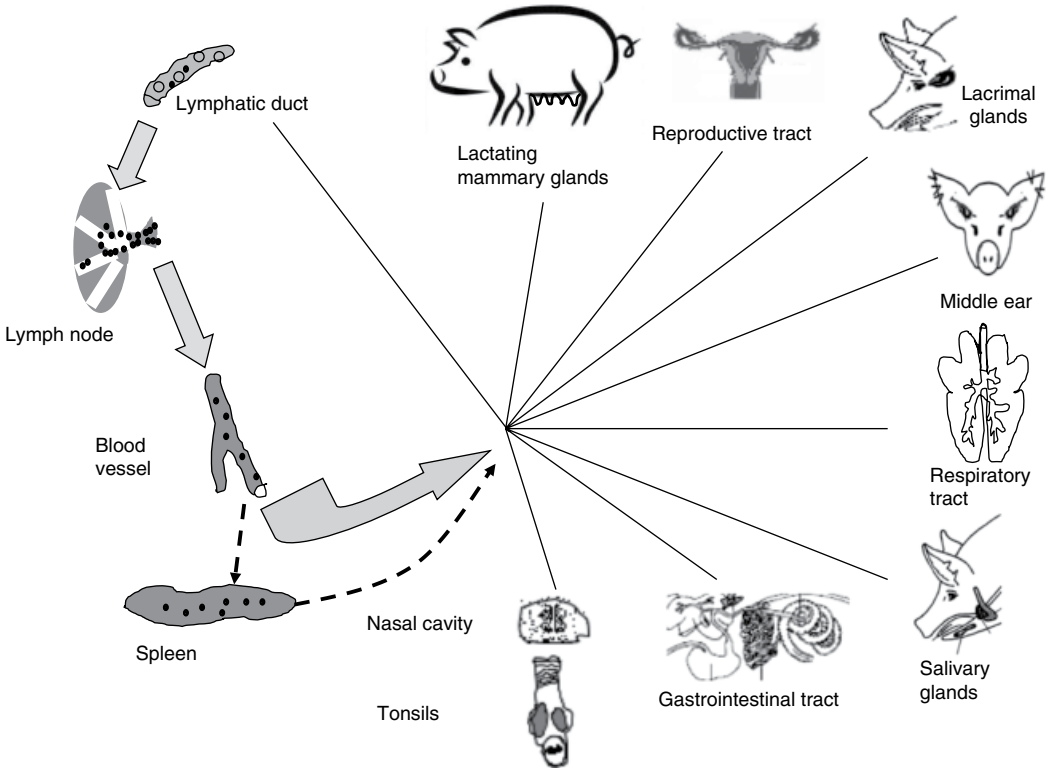


Figure 16.7 Lymphocyte circulation and common mucosal immune system of the pig. As illustrated on the left side of the figure, the pig has unique lymphocyte circulation with lymphocytes entering the lymph nodes by afferent lymphatics but exiting via blood vessels rather than efferent lymphatics. The common mucosal system involves the circulation of B and T cells between lymphoid tissues on mucosal surfaces.

Th cells to cause secretion of IL-2 that induces T cells to undergo mitosis and clonal expansion. B cells are also capable of processing antigen and presenting it to Th cells on MHC II molecules. During secondary immune responses, B cells are thought to act as APCs.

Th cells are critical in initiating the B-cell response resulting in antibody production (Figure 16.6). B cells contact antigen through immunoglobulins (Igs) bound to their surface, which act as the B-cell receptors (BCR). B-cell antigens do not need to be presented on MHC class II molecules by macrophages, although optimal B-cell response to antigen requires Th cell release of cytokines and contact with costimulatory molecules on the Th cell surface. This Th cell help is needed for B-cell mitosis and clonal expansion and for switching the class of antibody produced from IgM to IgG, IgA, or IgE.

Clonal selection and expansion

Clonal selection is basic to understanding the immune response. Each mature T or B lymphocyte in the body has receptors on its surface that it uses to recognize an antigen. All the antigen receptors (TCR and BCR) on each single lymphocyte recognize exactly the same antigen. All the lymphocytes that recognize exactly the same antigen make up a "clone," and they are from the same ancestor cell. Lymphocytes are in a resting stage as they circulate through blood, migrate through the lymph nodes, and reenter the bloodstream. In the lymph nodes or secondary lymphatic tissues, T lymphocytes come into contact with antigen on APC (DCs or macrophages) following their activation in the periphery. B lymphocytes can directly bind antigen that enters the lymph node. Each lymphocyte's TCR or BCR responds to only one specific antigen that it recognizes through its antigen receptor. Swine T-cell specificity is defined by the TCR variable regions as they interact with foreign antigen peptides presented by SLA molecules on the APC (Gerner et al. 2009; Piriou-Guzylack and Salmon 2008).

The vast majority of lymphocytes that contact an antigen in the lymph node cannot respond to it. Thus, in an animal never exposed to a particular infectious agent, there are relatively few lymphocytes in each clone that can recognize a particular antigen. The first step in producing an effective primary immune response is

expansion of the clone of lymphocytes that recognize the antigen. Within a few days, there will be enough lymphocytes in the clone to mount an effective humoral and/or cell-mediated immune response. Animals exposed previously to the antigen will have an expanded clone of lymphocytes, so fewer cycles of cell division are required to produce enough lymphocytes to mount an immune response. The cells present in the expanded clone are called memory cells. If the previous exposure has been relatively recent, there will still be circulating antibody and effector T lymphocytes that can act immediately to control the infection.

Th1, Th2, and Treg cells

Cytokines secreted by macrophages, DCs, and other T cells play crucial roles in the initiation and maintenance of immune responses against both viral and bacterial pathogens in pigs (Dawson et al. 2005; Thanawongnuwech and Thacker 2003; Thanawongnuwech et al. 2000, 2001; Zuckermann et al. 1998). Similar to other species, CD4+ T cells differentiate into Th cells with characteristic cytokine secretion profiles including the production of IFN-γ by Th1 cells, activating macrophages and stimulating T- and B-cell proliferation. Release of IL-10 is associated with the induction of one subset of Treg cells and is important in suppressing macrophage function and maintaining homeostasis of the respiratory tract. The timing and balance between cytokine expression following PRRSV infection clearly affect the efficiency of the antiviral response and likely viral persistence (Charerntantanakul et al. 2006; Loving et al. 2015; Lunney et al. 2010b, 2016; Suradhat and Thanawongnuwech 2003).

A group of cytokines regulates lymphocyte activation, growth, and differentiation. IL-2 stimulates T and B cells that have recognized antigen to proliferate and activates NK lymphocytes to have increased cytotoxic activity. IL-4 is important for effective IgE–mast-cell–eosinophil inflammatory reactions required to control some parasites and may result in allergic signs to nonparasitic antigens. In other species, IL-12 activates NK lymphocytes and induces CD4+ cells to differentiate; in swine, the lack of upregulation of the IL-12 receptor means that such activation is less prominent (Solano-Aguilar et al. 2002). IFN-γ causes cells to be resistant to virus infection (similar to IFN-α/β) and is a potent activator of macrophages, neutrophils, and NK cells. TNF-α often acts synergistically with IFN-γ to activate phagocytic cells. TNF-α can also activate endothelial cells, resulting in diapedesis of leukocytes into sites of inflammation.

Another group of cytokines, the colony-stimulating factors (CSFs), stimulates hematopoiesis through the expansion and differentiation of bone marrow progenitor cells. They include IL-3, which stimulates the production of all types of leukocytes; granulocyte–macrophage CSF (GM-CSF), which stimulates the production of granulocytes and macrophages; and granulocyte CSF (G-CSF), which stimulates the production of granulocytes only. The CSFs also enhance the antimicrobial activities of mature neutrophils and macrophages.

Acquired immune defense mechanisms

T lymphocytes are the predominant population of cells that secrete cytokines; some are cytolytic to abnormal cells through secretion of perforins and granzymes. Antibodies produced by B cells are very specific for their respective antigens, whereas cytokines are not. The cytokines produced during an immune response play an important role in orchestrating host defense against pathogens, partially through their direct activities and partially by enhancing the activity of both the innate immune system (i.e. C′, phagocytic cells, and NK cells) and the adaptive immune response by Th cells as described earlier.

CTLs are an important part of the cell-mediated immune response to virus infection and tumors. CTLs are CD8+ and recognize antigen associated with MHC class I molecules on a cell surface. MHC class I molecules present peptide antigens derived from proteins synthesized within the cell, such as viral proteins. CTLs directly attack host cells that have foreign antigen (e.g. viral antigen) presented on MHC class I molecules on their surface; they do not attack free bacteria or viruses. CTL activity specific for hog cholera virus, African swine fever virus, and pseudorabies virus (PRV) has been demonstrated in pigs that have recovered from infection (Martins et al. 1993; Pauly et al. 1995; Zuckermann et al. 1990), but there are limited CTL responses to PRRSV infection (Costers et al. 2009). CTLs kill target cells by making direct contact, releasing granzymes onto the cell surface, and inducing apoptosis (programmed cell death) in the target cells. Production of cytokines, including IL-12 and IFN-γ, by Th1 cells is required for the activation of CTL and elimination of cells infected with intracellular pathogens, especially viruses.

Humoral immunity

Immunoglobulins (Igs)

Swine B-cell development is a function of Ig gene rearrangement and modification (Butler et al. 2009a,c, 2017; Schroeder and Cavancini 2010). After birth, B-cell development begins in the bone marrow and is independent of antigen stimulation, and its fate becomes increasingly dependent on its response to antigen. Immature B cells express IgM but leave the bone marrow and become mature B cells that begin to express both IgM and IgD (Figure 16.8). The secreted pre-immune antibodies of

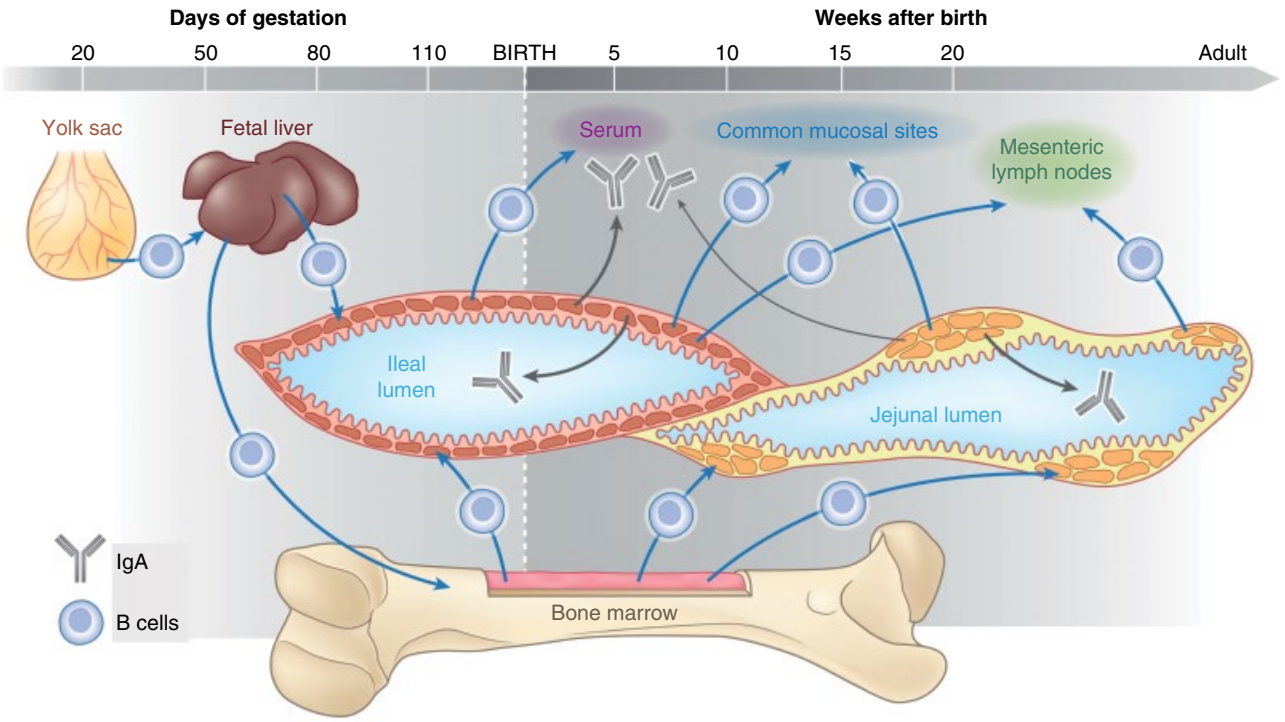


Figure 16.8 B-cell development and antibody production during the pig's lifetime. B-cell lymphogenesis begins in the yolk sac and fetal liver and continues in the bone marrow. The ileal lumen is lined with continuous Peyer's patches (IPP) as the fetal mucosal immune system develop; it is the source of T-independent natural immunoglobulin A (IgA) responses in newborn piglets. Bacterial colonization of the newborn promotes migration of IgA+ B cells to other mucosal sites and secretion of natural IgA antibodies. Several weeks later the segmented jejunal Peyer's patches (JPP) develop with associated T-dependent mucosal IgA responses. *Source:* Butler et al. (2017). Reproduced with permission of Annual Reviews.

the fetus include mainly IgM, IgA, and IgG3; this last isotype may provide a type of first responder mucosal immunity (Butler et al. 2017). Pigs are unique in that their light-chain loci rearrange before the heavy-chain loci, resulting in the Ig light chain selecting the heavy-chain repertoire (Sinkora et al. 2017).

B cells recirculate through the blood, the secondary lymphoid organs, and the bone marrow. An encounter with compatible antigen can cause the B cell to become a memory B cell or a plasma cell. B cells from clones that have never been stimulated by antigen have monomeric IgM (their BCR) on their surface; all of the IgM molecules on one B cell are specific for the same antigen. When stimulated by antigen and cytokines produced by CD4+ Th cells, a B cell begins to undergo mitosis, resulting in many more B cells with IgM recognizing the same antigen; some differentiate into plasma cells that secrete IgM. As the antigen-specific IgM level begins to increase in the blood, activated Th cells produce the cytokines that signal B cells to undergo class switch from IgM to IgD, IgG, IgA, or IgE production (Figure 16.9) (Crawley et al. 2003; Sinkora and Butler 2016). These B cells then rearrange their genetic material to produce antibody molecules with the same antigenic specificity (i.e. the same light-chain structure and variable portion of the heavy chain) but of a different antibody class (i.e. the constant heavy portion of the antibody molecule is changed). Changing the antibody class or isotype gives the antibody molecules different properties. The class of Ig that the Th cells induce the B cells to produce depends on the nature of the antigen and the location in the body where the antigen was trapped. Th cells located in lymph nodes and the spleen tend to induce B cells to switch to IgG production; those located in PP or under other mucosal surfaces tend to induce B cells to switch to IgA and/or IgE production, depending on the nature of the antigen and the genetic predisposition of the individual (Figure 16.8).

The molecular cascade involved in swine Ig expression has been explored in depth by Butler and his colleagues (Butler 2009a–c; Butler and Wertz 2012; Butler et al. 2017; Lunney et al. 2010a). Regions of each Ig heavy-chain gene are encoded by genes (e.g. IGHV, IGHD, IGHJ, IGHG1) that must be joined to form the

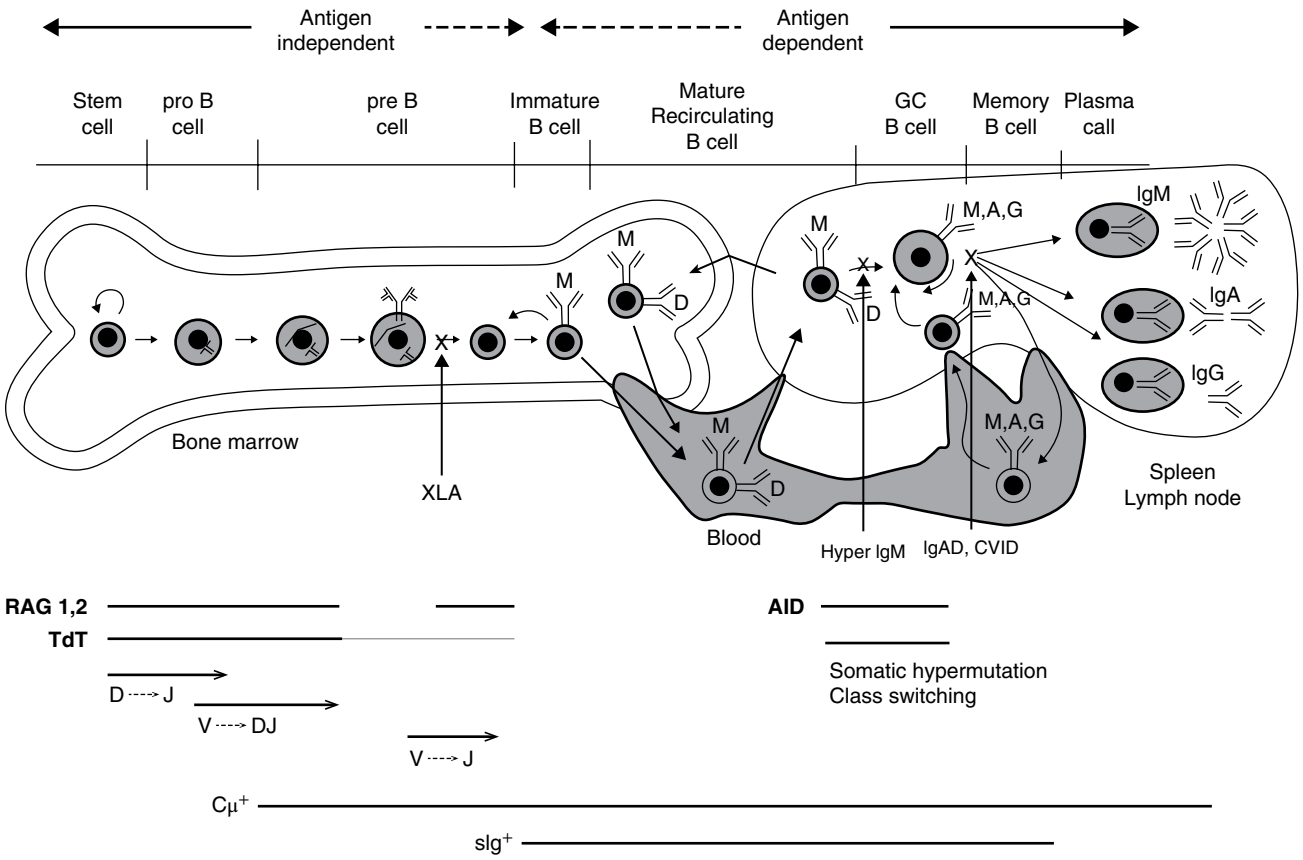


Figure 16.9 B-cell development and antibody production. *Source:* Adapted from Schroeder and Cavancini (2010). Reproduced with permission of Elsevier.

mature IgG antibody molecule (Figure 16.9). There are six expressed IgG subclass genes in swine. As in other mammals, major differences among swine subclasses are located in the hinge region. B-cell lymphogenesis is initiated when lymphocyte stem cells begin to rearrange their Ig genes. For IgG, this starts with IGHD to IGHJ (D-J) rearrangements followed by rearrangement to an IGHV gene. Recombination–activating gene (RAG)-mediated somatic hypermutation of Ig gene segments involves the excision of the intervening sequences between exons. Because swine have only two functional IGDH segments and one functional IGJH segment, the process of B-cell lymphogenesis is much easier to follow than in mice or humans (Butler and Wertz 2012).

Antibody molecules have a variety of activities in host defense, although they alone cannot kill infectious agents. Antibody molecules can coat infectious agents to prevent them from attaching to or penetrating host cells, agglutinate infectious agents to reduce their infectivity, and directly bind to and neutralize toxins. A very important function of antibody is that it marks infectious agents for destruction by C′, phagocytic cells, and/or cytotoxic cells.

Classes of immunoglobulins

Characteristics of the various classes of porcine Igs were thoroughly reviewed in a previous edition of this book (Porter 1986) and in more recent reviews (Butler et al. 2009b,c, 2017; Crawley et al. 2003). IgG is the predominant Ig class in the serum of the pig and other species, accounting for more than 80% of the Ig in serum and colostrum. As in other mammals, IgA is the major mucosal Ig for swine, including in mature milk (Klobasa and Butler 1987; Porter 1969). The IgA and IgM repertoires were more diverse in ileal than in jejunal piglet PP, reflecting a more diversified microflora in the ileal PP (Levast et al. 2010). IgM accounts for approximately 5–10% of the total Ig in serum and colostrum; it is a large pentameric protein held together by disulfide bonds. Zhao et al. (2002) affirmed the presence of IgD genes in artiodactyls, suggesting that IgD may have some as yet unknown biological properties, distinct from those of IgM.

The porcine immune system produces far more IgA than any other class of antibody; however, most of the IgA is found on mucosal surfaces rather than in the serum (Figure 16.8). IgA is present in swine serum as monomers and dimers (two monomers bound together

with a J chain) (Porter and Allen 1972). IgA at mucosal surfaces is mostly dimeric IgA with a J chain and associated secretory component (see section "MUCOSAL IMMUNITY").

Porcine IgE has the same physicochemical properties as other species, including the characteristic of losing biological activity when serum is heated to 56°C (Roe et al. 1993). A polyclonal antiserum for porcine IgE inhibited a passive cutaneous anaphylaxis reaction, identified a sparse population of plasma cells in the LP of the gut and mesenteric lymph nodes of parasitized pigs, and reacted with human IgE in Western blotting (Schmied et al. 2013).

Polyclonal and monoclonal antibodies (mAbs)

Antibodies produced by an animal in response to an infection or vaccination are polyclonal and recognize multiple antigens. Infectious agents are complex antigens with many different antigenic specificities (epitopes) on their surface; therefore, they stimulate many clones of B and T lymphocytes to respond. This results in a heterogeneous mixture of polyclonal antibodies that recognizes a wide variety of surface molecules on the microorganism.

The broad spectrum of antibodies produced and present in serum is most helpful to the animal in overcoming infection. This is a disadvantage, however, for using serum for developing diagnostic reagents for certain diagnostic tests. Moreover, the polyclonal antibodies produced in response to one infectious agent may cross-react with another infectious agent and thus interfere with the specificity of the assay.

mAbs are produced in research laboratories to overcome the disadvantages of polyclonal antisera for diagnostic and (less commonly) for therapeutic purposes. Mouse mAbs are the result of hybridoma production and expansion of one clone from a single B lymphocyte; therefore mAbs are identical and specific for a single antigenic determinant. Hence the problem of cross-reactivity between microorganisms in diagnostic tests is reduced.

mAb can be produced against a protective antigen on a microorganism and then used in therapy or for prevention of disease. mAbs can also be produced against immune cells (CD antigens) and proteins (ILs, IFNs, etc.), providing a means to track the complexity of swine immune responses and helping to delineate protective from pathogenic responses to vaccination and infection (Haverson et al. 2001; Lunney et al. 1994). Since mAb can be produced in very high concentrations and purity, a much lower volume of mAb compared with a polyclonal antibody solution can be used to passively immunize or treat animals. This reduces the risk of serious reaction to the passively administered antibody and its extraneous protein.

Mucosal immunity

Mucosal immunity: Role of the mucosal epithelium

The mucosal immune system provides the first immune defense barrier for over 90% of potential pathogens. The gut mucosal immune system contains more than 10^{12} lymphocytes and has a greater concentration of antibodies than any other tissue in the body (Burkey et al. 2009). It protects against harmful pathogens yet must also induce immune system tolerance to dietary antigens and normal microbial flora (Levast et al. 2014). The mucosal immune system is very rudimentary in the newborn pig and gradually develops in four stages over the first 6 weeks of life (Table 16.2).

In addition to actual immune cells, the mucosal immune system includes epithelial cells that help with antigen recognition and immune modulation. The epithelial cells are coated with mucus–glycocalyx layer that helps in barrier functions but at the same time allows the epithelial cells of the mucosa to be continually in contact with commensal and pathogenic organisms (Figure 16.2). Epithelial cells express TLR on their inner cell membranes, not on their surfaces, and will only be upregulated when the cell is infected (Philpott et al. 2001). Epithelial cells express chemokines such as CCL25 that is chemotactic and binds the chemokine receptor CCR9 on mucosal system T cells (Cheroutre

Table 16.2 Stages in the development of the mucosal immune response in the neonatal pig.

Stage	Pig age	Immune status
1	Newborn pig	Rudimentary Peyer's patches
		Small numbers of mucosal APCs and T cells
2	1–14 days	Nonspecific expansion or Peyer's patches and B cells
		Appearance of some conventional activated helper T cells
		Influx of MHC II+ cells in lamina propria
3	2–4 weeks	Appearance of mature helper T cells in lamina propria and IgM+ B cells in intestinal crypt areas
4	4–6 weeks	Expansion of B-cell repertoire to IgA+ B cells in intestinal crypt area
		Appearance of memory cytotoxic T lymphocytes in the epithelium (intraepithelial T cells) and in lamina propria

Adapted from Bailey et al. (2005b) with permission from Elsevier and Bailey and Haverson (2006).

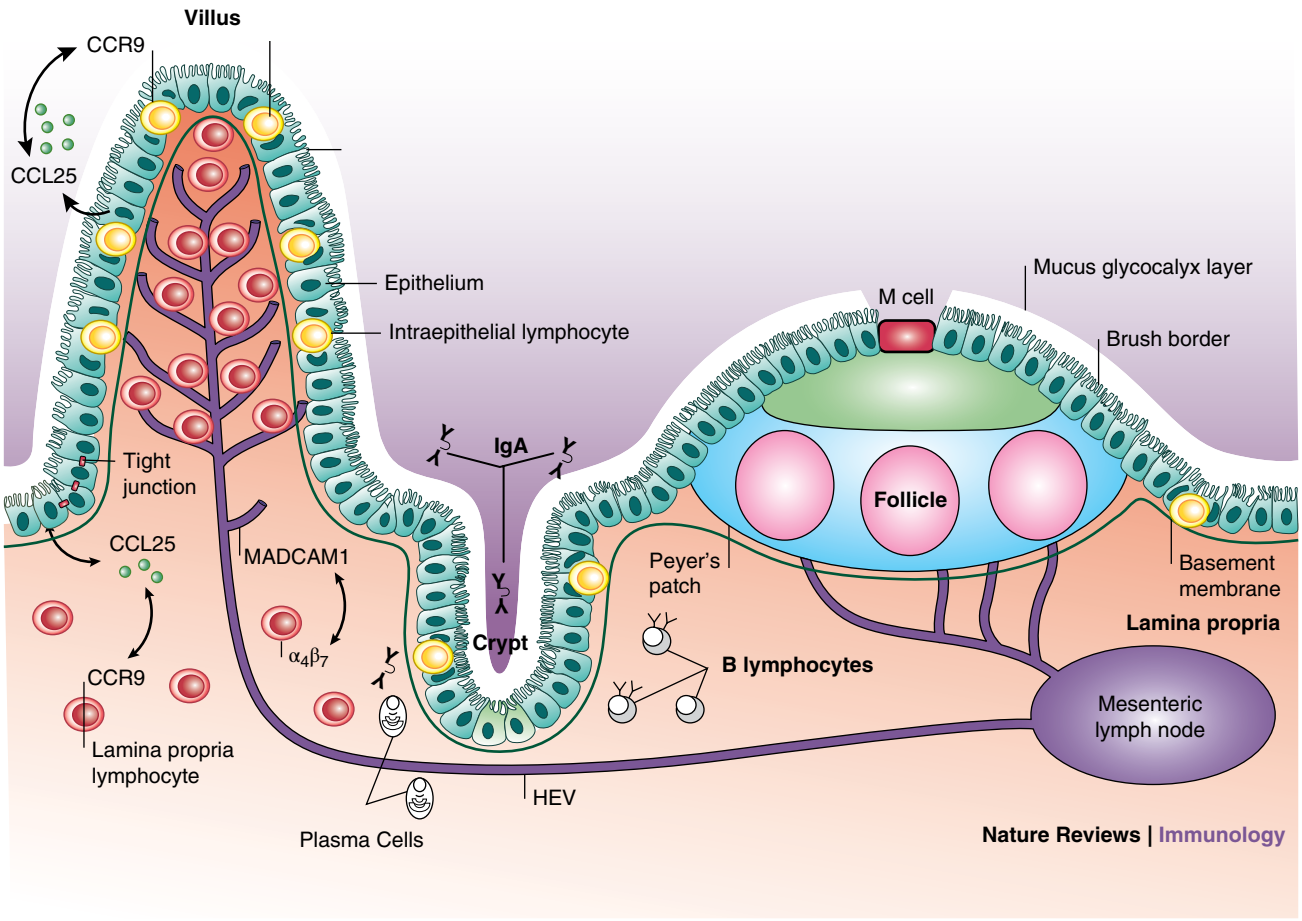


Figure 16.10 Mucosal immune system of the gut epithelium. The lamina propria (LP) contains scattered T cells and lies beneath the epithelium that contains intraepithelial lymphocytes (IEL). B cells are scattered in the LP but are more frequent in the crypt regions along with plasma cells that produce IgA that is transported and secreted into the lumen. M cells facilitate antigen uptake and delivery to the organized lymphoid tissues. T cells activated in the Peyer's patch and mesenteric lymph node express the $\alpha_4\beta_7$ integrin, which interacts with cell adhesion molecule MADCAM1, on the high endothelial venules (HEVs), assisting in homing these T cells to the mucosal LP. The chemokine CCL25 produced by epithelial cells recruits lymphocytes expressing CCR9 receptors to the LP. *Source:* Adapted from Cheroutre and Madakamutil (2004). Reproduced with permission of Springer Nature.

and Madakamutil 2004) (Figure 16.10). The production of CCL25 by epithelial cells recruits these lymphocytes to the LP and into mucosal epithelium.

Another important component of the mucosal immune response is neutrophils, which migrate into the intestinal lumen in large numbers in response to antigen–antibody complexes. The recruitment of neutrophils into the intestinal lumen is dependent on the presence of circulating IgG antibody (Bellamy and Nielsen 1974), colostral antibody (Sellwood et al. 1986), or locally induced IgA class antibody (Bhogal et al. 1987). The migration of neutrophils into the lumen of the gut and their subsequent destruction resulted in increased concentrations of lactoferrin, lysozyme, and cationic proteins. These substances may also contribute to immunity to bacterial infections in the gut.

Intraepithelial lymphocytes and LP immune cells

Intraepithelial T lymphocytes are important mediators of immunity at mucosal surfaces (Figure 16.10) (Burkey et al. 2009; Dunkley et al. 1995). This is especially true for respiratory and enteric infections. T lymphocytes also play a role in immunity in the intestinal tract. Pigs have high numbers of intraepithelial lymphocytes that are predominantly γδ T cells and CTLs (Salmon 1987; Thielke et al. 2003). These intraepithelial cells appear at 4–6 weeks of age. CTLs in contact with intestinal epithelial cells are likely to be important in destroying virus-infected epithelial cells. The γδ T cells proliferate in the intestine and recirculate through the lymphatic and blood vessels back to the intestine. They can produce IFN-γ, be cytotoxic, and act as APCs through MHC II molecules (Lee et al. 2004; Takamatsu et al. 2002).

The LP is the thin layer of connective tissue that lies beneath the mucosal epithelium of the respiratory, gastrointestinal, and urogenital tracts. Besides the smooth muscle cells, blood vessels (including HEV), and lymphatics, the LP contains many immune cells including macrophages, DCs, neutrophils, mast cells, and lymphocytes (Figure 16.10). DCs are the first cells to appear in the LP, increasing in the first few weeks. Mast cells also appear in the LP within the first few weeks, and their numbers are much higher in early weaned pigs (<21 days) than in pigs weaned at 28 days (Moeser et al. 2007; Smith et al. 2010). LP lymphocytes appear within the first week of life, while mature Th cells appear at 2–3 weeks of age (Table 16.2). LP T lymphocytes require more signals for activation, produce higher levels of cytokines, and are memory cells; CTLs appear later, at 4–6 weeks of age, and are also memory cells. B cells first appear near the crypt areas in the LP at 2–4 weeks of age (Table 16.2) and IgA+ B cells do not appear until 4–6 weeks of age(Figure 16.8; Table 16.2).

Homing is an important function of LP lymphocytes (Bailey 2009; Cheroutre and Madakamutil 2004). After T and B cells recognize antigen and mature in mucosal lymphoid follicles like PP, they express adhesive molecules like α4β7 integrin. The lymphocytes then travel to mesenteric lymph nodes, enter the blood circulation, and home back to the LP where the lymphocyte adhesion molecule binds to homing receptors like mucosal addressin cell adhesion molecule 1 (MADCAM1) on the HEV. The lymphocytes then emigrate out of the venules into the LP (Figure 16.10).

Secretory IgA

The predominant Ig secreted by the mucosal immune system is IgA. Dimeric IgA is secreted by plasma cells in the LP, binds to the polyimmunoglobulin receptor on the basal membrane of mucosal epithelial cells, and is transported to the mucosal surface of the epithelial cell (Figures 16.3 and 16.10). The cleavage product is called the secretory component and remains bound to the dimeric IgA. The secretory component is important for protecting the IgA molecule from proteolytic enzymes and also serves to anchor the IgA into the mucus layer so that it forms a protective coating on the mucosal surface. sIgA plays an important role in immunity at mucosal surfaces by agglutinating infectious agents, preventing attachment of infectious agents to epithelial cells, and neutralizing toxins.

Organized and diffuse mucosal lymphocytes

Organized mucosal-associated lymphoid tissues (MALT) are widely distributed in mucosal surfaces throughout the body (Liebler-Tenorio and Pabst 2006). MALT is the initial induction site for mucosal immunity for antigens sampled from mucosal surfaces (Figure 16.10). These mucosal aggregates or follicles of B cells, T cells, and APCs are covered by the epithelium that contains specialized epithelial cells called dome or M cells that are found in the gut and bronchus-associated lymphoid tissues (BALT). Dome cells pinocytose antigen and transport it across the epithelial layer. The antigen may then be processed by APCs and presented to T and B lymphocytes; indeed, intestinal APCs play a central role in the induction and maintenance of mucosal immunity (Inman et al. 2012). These follicles are organized like lymph nodes with T-cell areas and B-cell germinal centers. The lymphocytes that emigrate from these organized areas into the surrounding LP are referred to as diffuse lymphocytes (Bailey and Haverson 2006). The hallmark of this system is that local stimulation will result not only in memory T and B cells in the nearby mucosal tissue but also in other mucosal tissues.

Common mucosal system

Lymphocytes can be divided into two populations, those that circulate between the bloodstream and the systemic lymphoid tissues and those that circulate between the bloodstream and lymphoid tissues associated with mucosal surfaces. In the mucosal lymphoid tissues, mature T cells and B cells that have been stimulated by antigen and induced to switch to produce IgA will leave the submucosal lymphoid tissue and reenter the bloodstream (Wilson and Obradovic 2015). These lymphocytes will exit the bloodstream through HEV as described above and locate in the LP. B cells will differentiate into plasma cells that will secrete dimeric IgA. Many of these cells will return to the same mucosal surface from which they originated (Bailey 2009), but others will be found at different mucosal surfaces throughout the body. This homing of lymphocytes to other MALT sites throughout the body is referred to as the "common immune system" (Figure 16.7). Therefore, oral immunization can result in the migration of IgA precursor cells to the bronchi and subsequent secretion of IgA onto the bronchial mucosa. There is a special affinity for lymphocytes sensitized in the gut of the sow to migrate to the mammary gland to become plasma cells and secrete IgA into the milk.

Environmental influences, nutraceuticals, and development of mucosal immunity

Mucosal immunity, particularly the GALT, is greatly influenced by environmental factors on the surface of the mucosa (Bailey et al. 2005a,b; Bailey and Haverson 2006; Inman et al. 2010). The GALT of the neonatal pig is poorly developed and undergoes a rapid period of development and expansion (Table 16.2), which

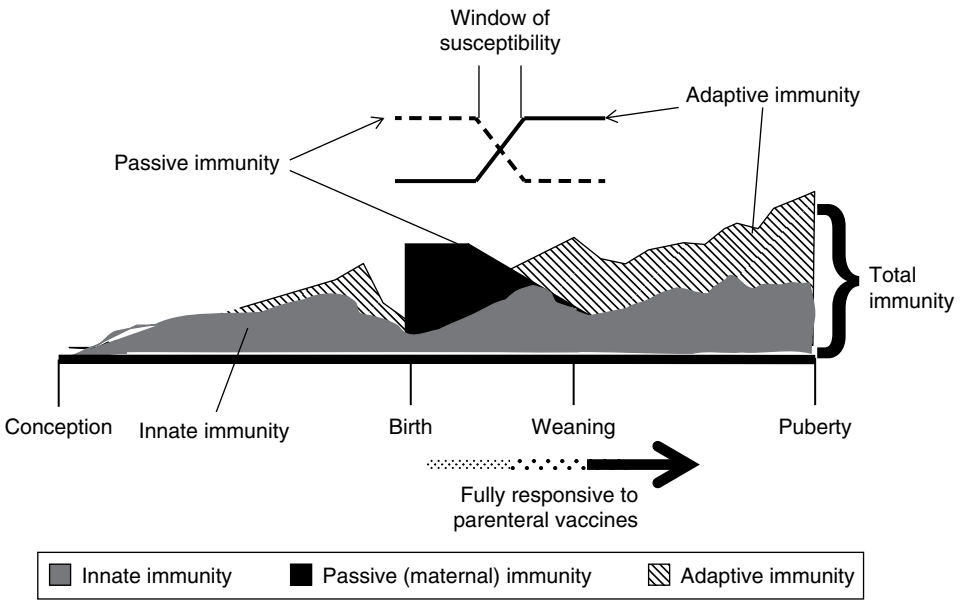


Figure 16.11 Development of the immune response in the pig: from conception to puberty. The pig's passive maternal immunity is only transferred after birth due to its unique placenta. *Source:* Adapted from Chase et al. (2008). Reproduced with permission of Elsevier.

remains incomplete when commercial pigs are weaned at 14–24 days (Lalles et al. 2007). The developing GALT makes one of two different responses to antigens, either an active protective response against pathogens or an active tolerance to commensal organisms and dietary antigens (Levast et al. 2014). The two critical control points for environmental influences in the GALT are immediately after birth and at weaning (Bailey et al. 2005a). Colostrum is important for gut development and growth and for non-antigen-specific immune development (Bailey and Haverson 2006). Colostrum is also important for providing pathogen-specific Ig. It contains high levels of IL-4 and transforming growth factor-β (TGF-β) that has anti-inflammatory effects and accelerates the switch of IgA antibody to common food proteins along with preventing expression of active immune responses and promoting the development of tolerance against nutrient antigens (Bailey et al. 2005a; Nguyen et al. 2007). The presence of commensal microbial flora (microbiome) is essential for the development of GALT in the neonatal pig (Bailey and Haverson 2006). Studies in rearing conditions using either high hygiene (pigs reared in an isolator) or low hygiene (pigs nursed on the sow) demonstrated that a substantial number of the low-hygiene pigs had a more complex microbial flora similar to older pigs raised in low hygiene, and they had more DCs (Inman et al. 2010). These pigs also had higher levels of IL-4, a cytokine associated with an anti-inflammatory effect and the production of IgA.

The second major environmental control point for GALT is weaning. At weaning, the pig is capable of active immune responses (Figure 16.11) (Bailey et al. 2005b). The weaning period is characterized by diet change and sometimes accompanied by low feed intake, poor growth and development, diarrhea, and increased risk for disease from enteric pathogens. Unfortunately, the maternal milk factors that modulate the immune response (TGF-β) and provide specific immunity in the newborn (Ig) are no longer available at weaning, and the balance between tolerance and active immunity at weaning is disturbed. The magnitude and severity of this "weaning" GALT crisis is dependent on how much the immune system was expanded during the pre-weaning period (Bailey et al. 2005a). Unfortunately, the weaning age imparted by a swine production system does not match when the immune system is "ready" for weaning. Managing the immune system for optimal disease prevention at early weaning ages will continue to be problematic.

The effect of nutraceuticals, probiotics, and other dietary supplements on swine mucosal immunity and health has generated much interest. Prebiotics (oligosaccharides and fiber), fiber metabolites (butyric acid and other short-chain fatty acids [SFCA]), organic acids (i.e. formic acid, citric acid), and botanicals (i.e. vanilla, oregano, pepper oil) may enhance the tight junctions in mucosal barrier and have an anti-inflammatory effect on the mucosa (Grilli et al. 2015, 2016; Heinritz et al. 2016; Melo et al. 2016; Zou et al. 2016). Probiotics (i.e. yeast, *Lactobacillus, Bifidobacteria* and their metabolites) increase sIgA and decrease local inflammatory and TLR responses to improve mucosal immunity (Deng et al. 2013; Vlasova et al. 2016).

Immunity in the fetus and neonate

All components of the native and acquired immune systems of the pig develop *in utero* and are functional at birth; however, they are generally less efficient than in the adult (Hammerberg et al. 1989). Since the normal newborn piglet has not yet been exposed to antigens, the humoral and cell-mediated immune responses to infectious agents have not yet been developed. After exposure to infectious agents, it will take 7–10 days for a primary antibody- or cell-mediated immune response to develop.

During this time, resistance to infection depends on the actions of the innate defense mechanisms and the antibody or immune components passively transferred from the sow to the piglet through colostrum and milk (Figure 16.11). There is virtually no transfer of antibody across the placenta prenatally due to the epitheliochorial placentation of the sow; the tissue layers between maternal and fetal circulation effectively prevents all antibody transfer prior to parturition. The sow concentrates antibody in the colostrum during the last weeks and days of gestation that, when ingested, will be transferred intact across the gut epithelial cells into the circulation of the newborn piglet. The passive transfer of antibody from sow to piglet in the colostrum and milk is very important for neonatal survival. The presence of high concentrations of Th2 cytokines like IL-4 or immunoregulatory cytokines such as TGF-β in colostrum is thought to play a critical role in allowing acquisition of the normal commensal microflora in the intestine (Chattha et al. 2013; Nguyen et al. 2007).

Innate defense mechanisms

The newborn piglet has low levels of hemolytic C′ activity, with relative concentrations related to birth weight; heavier pigs have significantly higher C′ concentrations in the serum (Rice and L'Ecuyer 1963). Piglets allowed to suckle colostrum have higher titers of hemolytic C′ than colostrum-deprived piglets during the first 3 weeks of life. The level of natural IFN-α production by porcine peripheral blood mononuclear cells (PBMC) is low at birth and gradually increases until adult age, with a significant increase around puberty (Nowacki et al. 1993).

Phagocytic cells are present in newborn animals but generally have reduced phagocytic activity as compared with adult animals (Osburn et al. 1982). Alveolar macrophages from 1-day-old pigs had reduced oxidative killing mechanisms compared with adult pigs, but by 7 days of age, these aspects of alveolar macrophage function had reached adult levels (Zeidler and Kim 1985). Neonatal pigs have low numbers of pulmonary intravascular macrophages that can increase up to 14-fold by 30 days of age (Winkler and Cheville 1987). Since phagocytes depend on C′ and/or antibodies to opsonize many infectious agents, the overall efficiency of phagocytosis in young pigs may be reduced due to inadequate levels of C′ and antibodies. Neutrophils from fetal pigs have ADCC activity that is comparable with that of adult pigs. Neutrophils from neonatal pigs rapidly emigrate into the lumen of the gut in response to the presence of *E. coli* and colostral antibody (Sellwood et al. 1986; Yang and Schultz 1986).

Adaptive immune mechanisms

The percentage of CD2+, CD4+, and CD8+ blood T lymphocytes increases with age over the first several weeks of life in specific-pathogen-free pigs (Bianchi et al. 1992; Joling et al. 1994). The PBMC blastogenic responsiveness to mitogens is low at birth and increased by 4 weeks of age (Becker and Misfeldt 1993). The mucosal lymphoid system is also less developed at birth and matures over the next few weeks of life (Jericho 1970; Ramos et al. 1992). NK cell activity is absent in peripheral blood of fetal pigs and low in pigs of less than 2 weeks of age (Yang and Schultz 1986).

Passive transfer in the neonate

Pigs are born with almost no serum antibody. Antibody is absorbed from colostrum that is three- to fourfold higher in IgG3 and IgA than serum (Butler and Wertz 2012; Roth and Thacker 2006). Colostrum has approximately the same concentration of IgM as serum. After initial suckling, colostrum ceases and is replaced with milk that has fivefold lower IgG content. From 3 days of age until the end of lactation, IgA is the predominant Ig found in sow milk. The majority of milk Ig is synthesized in the mammary gland, whereas colostral Ig is mostly derived from serum, although this varies by Ig class.

All three major classes of Ig (IgG, IgA, and IgM) are absorbed from the colostrum into the circulation of newborn pigs (Curtis and Bourne 1971; Porter 1969). IgA is absorbed less efficiently than the other classes of antibody (Hill and Porter 1974; Porter 1973) because porcine colostrum is dimeric IgA lacking secretory component. Neonatal colostrum-deprived piglets express secretory component in the gut that tends to localize in the mucus of the crypt areas. Because of the affinity of the dimeric IgA and IgM for secretory component, it has been suggested that IgA and IgM are bound in association with secretory component and held in the mucus of the crypt areas and are, therefore, less efficiently absorbed from the colostrums (Butler et al. 1981). The IgA present in sow's milk throughout the suckling period may also bind to the secretory component in the crypt areas and provide relatively continuous protection against intestinal pathogens.

Intestinal absorption of Ig from the colostrum normally ceases by 24–36 hours after birth. If pigs suckle

normally, the efficiency of absorption decreases with a half-life of about 3 hours (Speer et al. 1959). Lecce and Morgan (1962) found that the period of time that the intestine could absorb antibodies was extended up to 5 days in starved pigs that were maintained by parenteral administration of nutrients. Therefore, piglets that have not had an opportunity to eat during the first 24–36 hours may still benefit from colostrum ingestion. Neonatal pigs absorb colostral lymphocytes from their intestinal tract into the bloodstream (Tuboly et al. 1988; Williams 1993). By 24 hours, cells derived from colostrum were found in the liver, lungs, lymph nodes, spleen, and gastrointestinal tissue. Pigs that had absorbed the colostral lymphocytes had higher lymphocyte blastogenic responses to mitogens than control pigs. It is not clear if the passively transferred lymphocytes also transfer clinically significant cell-mediated or antigen-specific immunity from the sow to the piglet.

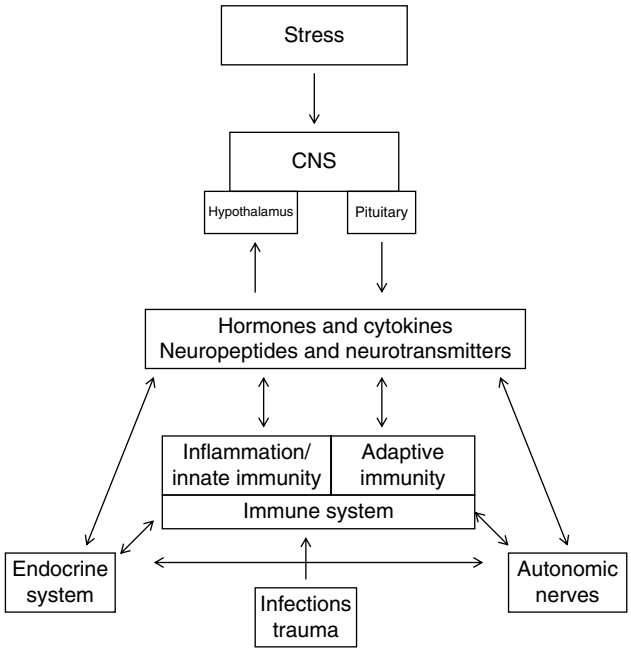


Figure 16.12 Stress effects on immunity. Network of bidirectional communication among central nervous system (CNS) and peripheral nervous system, endocrine system, adipose tissue, and immune systems. *Source:* Adapted from The Lancet Oncology, Reiche et al. (2004). Reproduced with permission of Elsevier.

Stress, immunosuppression, nutrition, and immunity

Physical and psychological stress: Interactions of the central nervous, endocrine, and immune systems

There is ample evidence that both physical and psychological distress can suppress immune function in animals, which may lead to an increased incidence of infectious disease. Excess heat or cold, crowding, mixing, weaning, limit feeding, shipping, noise, and restraint are stressors that are often associated with intensive animal production and influence immune function in various species (Blecha et al. 1985; Kelley 1985; Miró et al. 2016; Westly and Kelley 1984; Yen and Pond 1987). Also, social status, genetics, age, and the duration of stress (chronic vs. acute) are important in the pig's response to stress (Salak-Johnson and McGlone 2007).

The immune system and the central nervous system (CNS) are a bidirectionally linked "two-way street," each influencing the other (Borghetti et al. 2009; Miró et al. 2016) (Figure 16.12). In particular, there is a critical balance that exists between hormones such as growth hormone (GH), glucocorticoids (GC), prolactin (PRL), catecholamines, and insulin with the proinflammatory mediators (IL-1, IL-6, and TNF-α) of the immune system.

The immune system and CNS interaction is influenced through two pathways of the nervous system: the neuroendocrine (hypothalamic–pituitary axis) and the autonomic nervous systems (hypothalamic–sympathetic) (Borghetti et al. 2009; Miró et al. 2016; Salak-Johnson and McGlone 2007). Stress on the CNS affects both inflammatory/innate and adaptive responses through the neuroendocrine activation of the hypothalamic–pituitary–endocrine responses. Activation of the hypothalamus results from either stressors or the acute-phase response following infection when proinflammatory cytokines, IL-1, TNF-α, and IL-6, are released (Figure 16.12). This hypothalamus activation then stimulates hormones from the pituitary gland. The hormones released by the pituitary include thyroid-stimulating hormone (TSH), PRL, and GH, which are linked to insulin-like growth factor 1 (IGF-1) activity, and adrenocorticotropin (ACTH), which activates a number of different endocrine systems including GC from the adrenal gland.

The study of these multisystem endocrine interactions initially focused on the secretion and influence of GC, which suppress several aspects of immune function directly and indirectly by the production of anti-inflammatory cytokines like IL-10. However, pigs are more resistant to the immunosuppressive effects of GC compared with some other species (Flaming et al. 1994), and other mechanisms exist whereby the neuroendocrine system can alter immune function. Neurotransmitters (catecholamines, acetylcholine, neuropeptides, vasoactive intestinal peptide, and substance P) affect the immune system. Catecholamines inhibit IL-12 and enhance IL-10 production (Salak-Johnson and McGlone 2007). Somatotropic hormones like GH and IGF-1 have a positive effect on the immune response including increased proliferation of immune cells in the bone marrow, production of the proinflammatory cytokines, increased cytotoxic activity of CTL and NK cells, and

T-cell development in the thymus, to name a few (Borghetti et al. 2009). TSH, GH, ACTH, PRL, and neuropeptides influence the thymus and the development of T cells.

Recently, the importance of adipokines, the cytokines produced by adipose tissue, has been recognized (Borghetti et al. 2009). Leptin, the most well-studied adipokine, has many positive effects on the immune system including survival of T cells in the thymus, increased killing activity in monocytes and neutrophils, and maturation of DCs. Adipose tissue also produces proinflammatory cytokines. Interestingly, runt pigs have higher adipokine gene expression prior to weaning including higher proinflammatory cytokines (Ramsay et al. 2010).

The immune system is capable of altering the activity of the neuroendocrine system (Kelley 1988; Miró et al. 2016). Immune activation at very early age (10–16 days of age) resulted in much lighter pigs at 28 days of age (Fangman et al. 1998; Schinckel et al. 1995). Pigs receiving high levels of antigens had decreased weight gain, feed efficiency through 107 days of age, and took 5.6 more days to reach 230 lb and 3.6 days to reach market weight of 264 lb (Schinckel et al. 1995). This likely involves an effect of immune cytokines on IGF-1 production. Activated Th, CTL, and thymic cells, like thymic epithelial cells, can produce hormones like GH, PRL, ACTH, and TSH. A thymic hormone, thymulin, can have a direct effect on the pituitary to release endocrine hormones (Borghetti et al. 2009).

One of the most important areas in immune development affected by stress is Th1/Th2 balance. In most cases, a balanced Th1/Th2 response means that both a cell-mediated response (Th1) and a humoral response (Th2) will be produced. Skewing it in one direction or the other may be inappropriate for certain pathogens. Typically, extracellular organisms require a Th2 response, while intracellular organisms require a Th1 response, for eliminating the pathogen. Mixing, cold stress, heat stress, crowding, restraint, and weaning age all affect the Th1/Th2 balance (Salak-Johnson and McGlone 2007).

Weaning is a stressful event for the young pig. Research indicates that weaning before 3 weeks results in long-term negative effects on the immune system and mucosal development (Davis et al. 2006; Hameister et al. 2010; Moeser et al. 2007; Niekamp et al. 2007; Smith et al. 2010). Weaning at 2, 3, or 4 but not 5 weeks of age decreased the *in vivo* and *in vitro* response of porcine lymphocytes to undergo clonal expansion to initiate an immune response (Blecha et al. 1983). These same parameters were suppressed in artificially reared neonatal piglets compared with their sow-reared littermates (Blecha et al. 1986; Hennessy et al. 1987). Early weaning of pigs at 3 weeks of age suppressed the ability of mesenteric lymph node cells to produce IL-2 (Bailey et al. 1992). Immunizations 24 hours after weaning at 5 weeks of age decreased the antibody response, while vaccinating 2 weeks prior to weaning did not decrease the antibody response (Blecha and Kelley 1981). Early weaning results in prolonged increase in corticotropin-releasing factor (CRF) as well as increases in mast cells, which results in intestinal mucosal dysfunction (Moeser et al. 2007; Smith et al. 2010). However, successful vaccination strategies at the time of weaning are anecdotally but frequently reported in the field. Recent work in 10-week-old grower pigs indicated that chronic social stress from mixing pigs and increasing density resulted in decreased growth, impairment of the epithelial barrier, and immunosuppression (Li et al. 2017).

An area of increased interest is the effect of stress on the pregnant sow and its subsequent effects on the immune system of the developing piglet (Bate and Hacker 1985; Bellinger et al. 2008; Tuchscherer et al. 2002). Cold stress 2 days prior to parturition resulted in increased serum IgG in the piglet but decreased the ability of the piglet to produce IgG. Heat stress in the last 2 weeks of gestation resulted in decreased serum IgG through 21 days of age (Machado-Neto et al. 1987). Restraint stress (5 minutes a day for 35 days prior to farrowing) resulted in decreased IgG serum levels in the 1- and 3-day-old pigs; the thymus was smaller in 1- and 35-day-old pigs. In an assessment, a variety of T- and B-cell proliferation responses, along with NK killing activity, were lower at 35 days of age in pigs from the stressed sows (Tuchscherer et al. 2002). Another report indicated that tethering (continuous restraint) of sows suppressed antibody synthesis, which reduced the amount of antibodies that were transmitted through the colostrum into the blood of the piglets (Kelley 1985).

Social stress (mixing gilts together twice a week for 4 weeks) resulted in significant decrease in the total numbers of white blood cells, lymphocytes, and granulocytes from 26 days of age to study termination at 60 days of age (Couret et al. 2009). It also decreased the CD4+/CD8+ T-cell ratio on day 4. Endotoxin induced TNF-α production on day 60 and increased the mitogen-induced lymphocyte proliferation on D4 and D60. Another social stress study in sows with a similar design demonstrated that there was an increase in pituitary and hypothalamus neurotransmitters of the piglets both at 28 days of age (weaning) and at 60 days of age following a relocation stressor (Otten et al. 2010). Although this study did not directly show an effect on the immune system, changes to neurotransmitters can influence the immune system both directly and indirectly (Figure 16.12).

Immune dysfunction and infectious agents

Many infectious agents are capable of inducing immune dysfunction, particularly of the innate response, making the animal more susceptible to secondary infections and/or enhancing immunopathology. For example, infection with *Mycoplasma hyopneumoniae, Actinobacillus*

pleuropneumoniae, virulent or vaccine strains of hog cholera virus, PRRSV, or PRV increases the severity of *Pasteurella multocida*-associated pneumonia (Fuentes and Pijoan 1986, 1987; Pijoan and Ochoa 1978; Smith et al. 1973). The functions of neutrophils, another innate cell, are compromised following bacterial infection with *M. hyopneumoniae, Salmonella typhimurium*, or *Salmonella choleraesuis* (Roof et al. 1992a,b). Excessive NET formation also results in collateral damage (Bardoel et al. 2014; Döhrmann et al. 2016; Porto and Stein 2016). Porcine epidemic diarrhea virus (PEDV) inhibits IFN (Ding et al. 2014), while PRRSV effect on IFN varies with PRRSV strain (Overend et al. 2017). A number of swine pathogens impair or kill tissue macrophages (often alveolar macrophages) and monocytes including *A. pleuropneumoniae* (Dom et al. 1992; Tarigan et al. 1994), PRV (Chinsakchai and Molitor 1992; Iglesias et al. 1989a,b, 1992), influenza A virus(IAV) (Kim et al. 2009), PRRSV (Bautista et al. 1993; Charley 1983), and PCV2 (Chang et al. 2006). PRV and PRRSV infection also inhibit IFN-α (Brukman and Enquist 2006; Lunney et al. 2016). CD163 genetically modified pigs or alveolar macrophages are resistant to PRRSV infections (Burkard et al. 2017; Whitworth et al. 2016). IAV increases production of proinflammatory cytokines in macrophages, resulting in a "cytokine storm" and causing severe immunopathology (Janke 2014; Tisoncik et al. 2012). IAV also reduces NK cells in the lung (Forberg et al. 2014). Porcine parvovirus replicates in alveolar macrophages, as well as lymphocytes and impaired macrophage phagocytosis and lymphocyte proliferation (Harding and Molitor 1988).

PRRSV and PCV2 both modulate the immune response at many levels as discussed elsewhere in this book. However, it is important to recognize that much of the impact of PRRSV and PCV2 on the swine industry is due to their ability to modulate or alter the ability of the immune system to control other pathogens (Loving et al. 2015; Lunney et al. 2016). This applies not only to PRRSV infections but also to modified live vaccines (MLVs), thus reducing vaccine efficacy (Wang et al. 2016). *M. hyopneumoniae* coinfection increases PCV2 Th1 and Treg responses that were correlated with increases in PCV2 antigen and lung lesions (Zhang et al. 2011).

Nutritional influences on immunity

Immune system functions require energy, protein, vitamins, and trace minerals. Both malnutrition and overfeeding may result in impairment of immune function and increased susceptibility to disease. Swine in modern production typically have a completely controlled diet. Key vitamins and minerals for optimal immune function include vitamins A, C, E, and B complex and copper (Cu), zinc (Zn), magnesium (Mg), manganese (Mn), iron (Fe), and selenium (Se). The balance of these constituents is especially important since an excess or deficiency in one component may influence the availability or requirement for another (Wintergerst et al. 2007).

Zinc metabolism is greatly influenced by the acute-phase reaction (APR) following infection (Borghetti et al. 2009). Zn is an essential cofactor for the thymic hormone thymulin and T-cell development. High levels of GC and IL-1 and IL-6 result in hypozincemia due to redistribution of Zn among various tissues, particularly the liver, and the consistent loss of Zn in urine and feces. Zn deficiency associated with stress and high levels of GC causes a decrease in the resistance to infection and a continuous imbalance of Th1/Th2 favoring Th2 bias. Zn deficiency also decreased recruitment of naive T cells, NK cell activity, and the precursors of CTL and hypoplasia of lymphoid organs, including lymph nodes, thymus, spleen, and PP (Borghetti et al. 2009).

There are very little research data to predict the optimal diet for immune function for swine. The dietary requirements for optimal immune function may differ from the requirements to avoid deficiencies as judged by traditional methods. In addition, stress or the demands of rapid growth may change dietary requirements for optimal immune function.

Vaccines and immunity

General principles regarding vaccine efficacy and vaccine failure are found here. Information regarding protective immunity and vaccination for specific diseases are found in respective chapters of this book.

Developing a vaccination program

A swine vaccination program should first start with assessment of the disease risks in a particular herd. The common "blanket vaccination" programs suggested for many pathogens are not appropriate for all herds. A careful review of endemic disease agents and risks of external introduction of agents is warranted before recommending a vaccination program.

Secondly, the effect of maternal immunity and the age of the pig should be considered. The relationship is generally linear for efficacy of vaccination for active immunity. The younger the pig, the poorer the response, and the older the pig, the better the immune response. However, for protection derived from passive maternal immunity, the younger pig generally has better protection than the older pig because of the high initial levels of maternal antibody derived via colostral transfer that wane as pigs get older (Figure 16.11).

Thirdly, certain management practices such as minimizing exposure of piglets to pathogens via high hygiene, using all-in/all-out systems, and executing an effective biosecurity program can provide enhanced protection

and give an extended window before vaccination is necessary. However, in those swine management systems where continuous flow and lower biosecurity is in place, a more aggressive vaccination program may be warranted.

Herd immunity

Herd immunity is often used to describe the threshold proportion of immune individuals that should lead to a decline in incidence of infection attributable to immunity acquired via infection and/or vaccination. An implication is that the risk of infection to susceptible individuals in the herd is reduced because of the presence, proximity, and number of immune individuals present in the population. With regard to swine vaccination, one of the key factors to consider is that the level of protection conferred by most vaccines does not fully protect the herd against infection (Rose and Andraud 2017). In this case, there may be a "critical threshold," that is, the fraction of the population that should be immunized (according to the level of immunization conferred by the vaccine) to mitigate a disease outbreak. The partial protection of a herd from vaccines results from a variety of factors: the vaccine can reduce the susceptibility of pigs to the infection; it can decrease pathogen disease severity once the pig is infected, or it can accelerate the elimination of the pathogen by reducing pathogen replication or duration of shedding. Vaccine efficacy varies by pathogen and formulation. For example, PCV2 herd immunity following vaccination is generally high. In contrast, PRRSV vaccines only provide a limited protection from an epidemiological point of view and would be generally called a "leaky vaccine" (Rose and Andraud 2017).

Interval between vaccinations

Vaccination incites expansion in the populations of responding T- and B-cell clones (Figure 16.13). However, to have a complete and mature immune response, this clonal expansion must not only stop, but an active process of cell death (apoptosis) must also occur. This period allows for "culling" by apoptosis of those T or B cells that may be poor responders or err toward autoimmunity (Wagner 2007). The whole process from vaccination to achieving mature immune response homeostasis takes at least 3 weeks, at which time the response can be boosted to get a true anamnestic secondary response. In practice, swine vaccine primary and booster doses are often administered at 2-week intervals. In young pigs, occasionally this is done to provide an opportunity to assure that the pigs develop a primary response in the face of maternal immunity. The adjuvants that are used with most commercial vaccines usually provide superior immune development over older-generation adjuvants

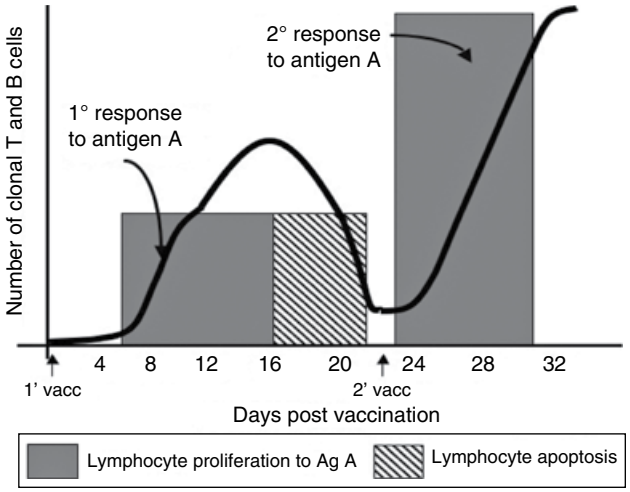


Figure 16.13 Importance of vaccine timing and the booster response. The primary response to vaccine antigen (Ag A) and the anamnestic response to secondary challenge. *Source:* Courtesy of D. Topham.

like alum (Awate et al. 2013; Wilson-Welder et al. 2009). Therefore, in most instances, if primary vaccination occurs after 3 weeks of age, booster vaccination beyond 3 weeks and even longer will be efficacious. The dogma that revaccination must occur within 2 weeks of the primary vaccination is simply not true; the anamnestic response will be better if the interval between vaccinations is longer.

Route of vaccination

Vaccination protection of the animal from infection at mucosal surfaces such as the intestinal tract, respiratory tract, mammary glands, and reproductive tract is especially difficult for the immune system. The antibodies responsible for humoral immunity and the lymphocytes responsible for cell-mediated immunity are predominantly in the bloodstream and tissues; they are typically not found on the mucosal surfaces. Therefore, while lymphocytes assist in preventing systemic invasion through the mucosal surface, they are often not very effective at controlling infection on the mucosal surface. Even in the lungs and the mammary gland where IgG and lymphocytes are found in relative abundance, they are not able to function as effectively as in the tissues. Protection on mucosal surfaces is due in large part to sIgA, CTL, and γδ T cells as discussed earlier.

The route of vaccine administration can be important when attempting to induce mucosal immunity (Wales and Davies 2017). To induce sIgA production at mucosal surfaces, it is best for the vaccine to enter the body via a mucosal surface (Zhu et al. 2017). This can be accomplished by feeding the vaccine to the animal, aerosolizing the vaccine so the animal will inhale it, or

by intramammary exposure. If a sow is exposed to an infectious agent in her intestinal tract, the sow may respond by producing sIgA not only in the intestinal tract but also in the mammary gland (Figure 16.7). The sow passes the IgA against the infectious agent to the piglet via milk when it suckles, thus protecting the piglet from infectious agents present in the sow's intestine. This protection will only last as long as the piglet continues to suckle. Enteric infections by many organisms are not controlled by the presence of IgG and IgM in the bloodstream or by a systemic cell-mediated immune response. If an MLV is given by injection, but goes to a mucosal surface to replicate, it may also induce a sIgA response. In addition, killed vaccines with newer adjuvant formulations have proven to stimulate mucosal IgA and CTL cell response for the respiratory pathogens *M. hyopneumoniae* and IAV (Kitikoon et al. 2006; Marchioro et al. 2013).

Adjuvants and nanoparticles

Adjuvants provide several opportunities to improve vaccine performance (Awate et al. 2013; Wilson-Welder et al. 2009). They are used with inactivated vaccines to enhance innate immune response and antigen presentation. They can be immunostimulants that can direct the immune response to either Th1 or Th2 responses. Adjuvanted parenteral vaccines are often used to overcome preexisting immunity (maternal or active immunity) (Awate et al. 2013; Morein et al. 2002). There are more adjuvants available for veterinary use than human use, with the most commonly used adjuvants in swine being alums, oil in water, and Carbopol (Table 16.3).

Adjuvants are sometimes used to enhance the immature immune system, to stimulate a Th1 response for balance of the skewed Th2 response in young pigs, or for immunosuppressive pathogens like PRRSV (Awate et al. 2013; Charerntantanakul 2009). Two experimental vaccine systems have demonstrated the ability to break the Th2 bias in very young pigs. Small DNA sequences called oligodeoxynucleotides (ODN) containing one or more unmethylated CpG motif (CpG ODN) are potent stimulators of Th1 immune responses when used as vaccine adjuvants. One-day-old piglets vaccinated with attenuated PRV with an adjuvant system containing CpG ODN induced significant cellular proliferation and IFN-γ production in response to vaccine antigen within the first week after vaccination (Linghua et al. 2007). This vaccine also induced significant antibody titers. An even better Th1 immune response was obtained by adding a plasmid expressing the proinflammatory Th1-inducing porcine IL-6 to CpG ODN adjuvanted PRV vaccine (Linghua et al. 2006).

Nanoparticles (NP) are biodegradable polymers that contain encapsulated antigens (Dhakal et al. 2017). The NP protects the antigen from degradation under physiological conditions, which is critical when vaccine is delivered to mucosal sites as they are protected from proteolytic degradation and are readily taken up by immune cells at the mucosal sites of the respiratory tract. NP intranasal PRRSV and IAV vaccines protect pigs from clinical disease (Binjawadagi et al. 2014; Dhakal et al. 2017). NP have the benefit of slow release of the antigen for a long period of time (4–8 weeks) when administered by parenteral route that helps in prolonged immune activation (Dhakal et al. 2017).

Use of autogenous vaccines and "planned exposure"

Autogenous vaccines are composed of agents or antigens that are derived organisms present in the individual herd in which they are to be used. The use of autogenous vaccines for a variety of bacterial and viral agents is a common practice in swine production (Chase 2004; Geldhof et al. 2013). The greatest value of autogenous

Table 16.3 Adjuvants currently used in licensed vaccines.

Species	Humans	Livestock
Location	United States, United Kingdom, and European Union	Worldwide
Adjuvants/vehicles	Aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate (alum)	Aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate (alum)
	Calcium phosphate	Saponin (QS-21)
	MF59 (squalene), AS03 (oil-in-water emulsion)	Oil emulsions,[a] paraffin, mineral oil, lanolin, squalene, ISA-70, Montanide (IMS)
	AS04 (liposome formulation), virosomes	Glycerine, Carbopol (polymer)

Adapted from Wilson-Welder et al. (2009), with permission from the American Association of Pharmaceutical Sciences. Updated based on Awate et al. (2013).

[a] Many livestock adjuvant–vaccine formulations are proprietary, and their compositions have not been disclosed.

vaccine is when diagnostic or molecular techniques are able to detect immunologically applicable differences in the field strains from those strains in commercial vaccines. The science-based application of autogenous vaccines is important for prevention of certain diseases; any cost savings from using autogenous vaccines should not be the major factor in selecting an autogenous vaccine over a commercial vaccine.

Infectious agents do not uniformly infect all animals simultaneously. The intention of a planned or controlled exposure procedure is to expose all animals in a population to a live infectious agent derived from the herd while minimizing the impact of any associated disease. From the immunology perspective, this practice is simply letting an infection (rather than vaccine) generate an immune response. An example is the management practice of using serum containing live PRRSV to infect certain populations of pigs, such as gilts prior to their introduction into the breeding herd. This is done to achieve uniform exposure and immunity against PRRSV. This practice is not without risks. Careful consideration of benefits and risks is warranted before executing controlled exposure programs.

Vaccination failure

There are many reasons why animals may develop disease even though they have been vaccinated (Roth 1999). The major reasons for these failures include (1) vaccine administered in the face of maternal immunity, (2) vaccine administered after natural infection has occurred, (3) improper handling of vaccines and/or administration equipment, (4) improper timing of vaccination and/or age when administered, (5) poor cross-protection against heterologous pathogens, and (6) immune dysfunction or compromise at the time of vaccination.

One of the major challenges in developing an active immune response in young pigs has been interference from maternal immunity (Hodgins et al. 2004; Ma and Richt 2010; Opriessnig et al. 2008; Rose and Andraud 2017). The timing of many vaccines administrated by the parenteral route involves estimating when the level of maternal antibody is low enough for an active immune response to progress sufficiently to provide vaccine immunity (Hodgins et al. 2004; Opriessnig et al. 2008). Maternal antibody half-life in pigs ranges from 11.3 to 20 days compared with bovine half-life of 16–28 days (Fulton et al. 2004). Specific examples for half-life for swine maternal antibodies include 16.2 days for PRRSV (Yoon et al. 1995), 14 days for AIV (Fleck and Behrens 2002), 19 days for PCV2 (Opriessnig et al. 2004), 11.3 days for PRV (Mueller et al. 2005), 11 days for classical swine fever virus (Mueller et al. 2005), 20 days for parvovirus (Paul et al. 1982), and 15.8 days for *M. hyopneumoniae* (Morris et al. 1994).

The optimal window for vaccination can range from a few weeks of age to 3 months. As illustrated in Figure 16.11, this can vary by animal and depends on the level of maternal antibody and the particular vaccine antigen. Maternal interference can present a major obstacle for achieving an adequate vaccine response. Antibody levels often decay to a level still high enough to block responses to vaccine, but not high enough to resist a field infection, which creates a window of opportunity for infecting organisms.

The pig requires several days after vaccination before an effective immune response will develop. If infection occurs prior to or near the time of vaccination, the vaccine may not have time to induce immunity (Rose and Andraud 2017); hence the animal may develop clinical disease. Disease that occurs shortly after vaccination may easily be misinterpreted as vaccine failure or even falsely attributed to the vaccine itself. Some MLVs consisting of attenuated organisms may be capable of producing disease in immunosuppressed animals. Improperly handled and administered vaccines may fail to induce the expected immune response in normal healthy animals.

Modified live bacterial and viral vaccines are only effective if the agent in the vaccine is viable and able to replicate in the vaccinated animal. Observing proper storage conditions and proper methods of administration are very important for maintaining vaccine viability. Failure to store the vaccine at refrigerator temperatures or exposure to light may inactivate the vaccine. Even when stored under appropriate conditions, many vaccines lose viability over time. Therefore, vaccines that are past their expiration date should not be used. The use of chemical disinfectants on syringes and needles can inactivate MLVs if there is any residual disinfectant.

The use of an improper diluent or the mixing of vaccines in a single syringe may also inactivate MLVs. Diluents for lyophilized vaccines are formulated specifically for each vaccine. A diluent that is appropriate for one vaccine may inactivate a different vaccine. Some vaccines and diluents contain preservatives that may inactivate other MLVs. For these reasons, and many others, different vaccines should not be mixed and given as a single dose.

The timing of the vaccination is important. Vaccination of young animals may be ineffective due to age of the animal and/or because of the presence of maternal antibody. However, if the vaccine is administered after all maternal antibodies are gone, there may be a period of vulnerability to infection before they develop their own immune response. Although considered impractical to vaccinate young pigs frequently because of economic and logistical constraints, frequent vaccination may be justified in cases of unusually high disease incidence or pressure.

Immune dysfunction or decreased immunocompetence can be due to a variety of factors including stress, malnutrition, and concurrent infections; immaturity or senescence of the immune system may also lead to vaccination failure. If the immune dysfunction occurs at the time of vaccination, the vaccine may fail to induce an adequate immune response. If the immune dysfunction occurs some time after vaccination, then disease may occur due to reduced immunity in spite of an adequate response to the original vaccine. Therapy with immunosuppressive drugs, for example, GC, may also cause this to occur.

Acknowledgments

The authors have adapted their chapter from that written for the 9th (Roth and Thacker 2006) and 10th editions.

References

Abraham SN, St. John AL. 2010. *Nat Rev Immunol* 10:440–452.
Auray G, Keller I, Python S, et al. 2016. *J Immunol* 197:4791–4806.
Awate S, Babiuk LA, Mutwiri G. 2013. Mechanisms of action of adjuvants. *Front Immunol* 4:1–10.
Bailey M. 2009. *Dev Comp Immunol* 33:375–383.
Bailey M, Haverson K. 2006. *Vet Res* 37:443–453.
Bailey M, Clarke CJ, Wilson AD, et al. 1992. *Vet Immunol Immunopathol* 34:197–207.
Bailey M, Haverson K, Inman C, et al. 2005a. *Proc Nutr Soc* 64:451–457.
Bailey M, Haverson K, Inman C, et al. 2005b. *Vet Immunol Immunopathol* 108:189–198.
Bardoel BW, Kenny EF, Sollberger G, et al. 2014. *Microbe* 15:526–553.
Bate LA, Hacker RR. 1985. *Can J Anim Sci* 65:87–93.
Bautista EM, Goyal SM, Yoon IJ, et al. 1993. *J Vet Diagn Invest* 5:163–165.
Bautista EM, Gregg D, Golde WT. 2002. *Vet Immunol Immunopathol* 88:131–148.
Becker BA, Misfeldt ML. 1993. *J Anim Sci* 71:2073–2078.
Bellamy JE, Nielsen NO. 1974. *Infect Immun* 9:615–619.
Bellinger DL, Lubahn C, Lorton D. 2008. *J Immunotoxicol* 5:419–444.
Benis N, Schokker D, Suarez-Diez M, et al. 2015. *BMC Genomics* 16:556.
Bhogal BS, Nagy LK, Walker PD. 1987. *Vet Immunol Immunopathol* 14:23–44.
Bianchi ATJ, Zwart RJ, Jeurissen SHM, et al. 1992. *Vet Immunol Immunopathol* 33:201–221.
Binjawadagi B, Dwivedi V, Manickam C, et al. 2014. *Int J Nanomedicine* 9:679–694.
Binns RM, Pabst R, Licence ST. 1986. *Swine Biomed Res* 3:1837–1853.
Blecha F, Kelley KW. 1981. *J Anim Sci* 53(2):439–447.
Blecha F, Pollmann DS, Nichols DA. 1983. *J Anim Sci* 56(2):396–400.
Blecha F, Pollmann S, Nichols DA. 1985. *Am J Vet Res* 46:1934–1937.
Blecha F, Pollmann DS, Kluber IEF. 1986. *Can J Vet Res* 50:522–525.
Borghetti P, Saleri R, Mocchegiani E, et al. 2009. *Vet Immunol Immunopathol* 130:141–162.
Brukman A, Enquist LW. 2006. *J Virol* 80:6345–6356.
de Buhr N, Reuner F, Neumann A, et al. 2017. *Cell Microbiol* 19:e12649.
Burkard C, Lillico SG, Reid E, et al. 2017. *PLoS Pathog* 13:e1006206.
Burkey TE, Skjolaas KA, Dritz SS, et al. 2007. *Vet Immunol Immunopathol* 115:309–319. PMID: 17178162.
Burkey TE, Skjolaas KA, Minton JE. 2009. *J Anim Sci* 87:1493–1501.
Butler JE, Wertz N. 2012. *Front Immunol* 3:153.
Butler JE, Klobasa F, Werhahn E. 1981. *Vet Immunol Immunopathol* 2:53–65.
Butler JE, Lager KM, Splichal I, et al. 2009a. *Vet Immunol Immunopathol* 128:147–170.
Butler JE, Wertz N, Deschacht N. et al. 2009b. *Immunogenetics* 61:209–230.
Butler JE, Zhao Y, Sinkora M, et al. 2009c. *Dev Comp Immunol* 33:321–333.
Butler JE, Wertz N, Sinkora M. 2017. *Annu Rev Anim Biosci* 5:255–279.
Chang H-W, Jeng C-R, Lin T L, et al. 2006. *Vet Immunol Immunopathol* 110:207–219.
Chardon P, Renard C, Vaiman M. 1999. *Immunol Rev* 167:179–192.
Charerntantanakul W. 2009. *Vet Immunol Immunopathol* 129:1–13.
Charerntantanakul W, Platt R, Roth JA. 2006. *Viral Immunol* 19:646–661.
Charley B. 1983. *Ann Virol* 134:51–59.
Charley B, Riffault S, Van Reeth K. 2006. *Ann N Y Acad Sci* 1081:130–136.
Chase CCL. 2004. *Dev Biol* 117:69–71.
Chase CCL, Hurley DJ, Reber AJ. 2008. *Vet Clin Food Anim* 24:87–104.
Chattha KS, Vlasova AN, Kandasamy S. 2013. *Vaccine* 31:1916–1923.
Cheroutre H, Madakamutil L. 2004. *Nat Rev Immunol* 4: 290–300.
Chinsakchai S, Molitor TW. 1992. *Vet Immunol Immunopathol* 30:247–260.

Chitko-McKown CG, Blecha F. 1992. *Ann Rech Vet* 23:201–214.
Chitko-McKown CG, Chapes SK, Brown RE, et al. 1991. *J Leukoc Biol* 50:364–372.
Clop A, Huisman A, van As P, et al. 2016. *Genet Sel Evol* 48:28.
Costers S, Lefebvre DJ, Goddeeris B, et al. 2009. *Vet Res* 40:46.
Couret D, Jamin A, Kuntz-Simon G, et al. 2009. *Vet Immunol Immunopathol* 131:17–24.
Crawley A, Raymond C, Wilkie BN. 2003. *Vet Immunol Immunopathol* 91:141–154.
Curtis J, Bourne FJ. 1971. *Biochim Biophys Acta* 236:319–332.
Davis ME, Sears SC, Apple JK, et al. 2006. *J Anim Sci* 84:743–756.
Dawson HD, Beshah E, Nishi S, et al. 2005. *Infect Immun* 73:1116–1128.
Dawson H, Loveland JE, Pascal G, et al. 2013. *BMC Genomics* 14:332.
Deng J, Li Y, Zhang J, Yang Q. 2013. *Res Vet Sci* 94:62–68.
Dhakal S, Hiremath J, Bondra K, et al. 2017. *J Control Release* 247:194–205.
Ding Z, Fang L, Jing H, et al. 2014. *J Virol* 88:8936–8945.
Döhrmann S, Cole JN, Nizet V. 2016. Conquering neutrophils. *PLoS Pathog* 12:e1005682.
Dom P, Haesebrouck F, De Baetselier P. 1992. *Am J Vet Res* 53:1113–1118.
Duncan IA, Binns RM, Duffus WPH. 1989. *Immunology* 68:392–395.
Dunkley M, Pabst R, Cripps A. 1995. *Immunol Today* 16:231–236.
Ezquerra A, Revilla C, Alvarez B, et al. 2009. *Dev Comp Immunol* 33:284–298.
Fangman TJ, Ostlund EN, Tubbs RC, et al. 1998. *Vet Rec* 143:327–330.
Fernandez-Sainz I, Gladue DP, Holinka LG, et al. 2010. *J Virol* 84(3):1536–1549.
Flaming KP, Goff BL, Frank DE, et al. 1994. *Comp Hematol Int* 4:218–225.
Fleck R, Behrens A. 2002. In 2002 Proceedings of the AASV. pp. 109–110.
Forberg H, Hauge AG, Valheim M. 2014. *PLoS One* 9:e100619.
Fouhse JM, Zijlstra RT, Willing BP. 2016. *Anim Front* 6:30–36.
Fuentes M, Pijoan C. 1986. *Vet Immunol Immunopathol* 13:165–172.
Fuentes MC, Pijoan C. 1987. *Am J Vet Res* 48(10):1446–1448.
Fulton RW, Briggs RE, Payton ME, et al. 2004. *Vaccine* 22:643–649.
Galli J, Tsai M. 2010. *Eur J Immunol* 40(7):1843–1851.
Geldhof MF, Van Breedam W, De Jong E, et al. 2013. *Vet Microbiol* 167:260–271.
Gerner W, Kaser T, Saalmuller A. 2009. *Dev Comp Immunol* 33:310–320.
Gerner W, Talker SC, Koinig HC, et al. 2015. *Mol Immunol* 66(1):3–13.
Gómez-Laguna J, Salguero FJ, Barranco I, et al. 2010. *J Comp Pathol* 142:51–60.
Grilli E, Tugnoli B, Passey JL, et al. 2015. *BMC Vet Res* 11:96.
Grilli E, Tugnoli B, Foerster, CJ. 2016. *J Anim Sci* 94:433–436.
Hameister T, Puppe B, Tuchscherer M, et al. 2010. *Berl Munch Tierarztl Wochenschr* 123:11–19.
Hammerberg C, Schurig GG, Ochs DL. 1989. *Am J Vet Res* 50(6):868–874.
Harding MJ, Molitor TW. 1988. *Arch Virol* 101:105–117.
Hasslung FC, Berg M, Allan GM, et al. 2003. *J Gen Virol* 84:2937–2945.
Haverson K, Saalmuller A, Alvarez B, et al. 2001. *Vet Immunol Immunopathol* 80:5–23.
Heinritz SN, Weiss E, Eklund M, et al. 2016. Intestinal. *PLoS One* 11:e0154329.
Hennessy KJ, Blecha F, Pollmann DS, et al. 1987. *Am J Vet Res* 48:477–480.
Hill IR, Porter P. 1974. *Immunology* 26:1239–1250.
Hirt W, Saalmuller A, Reddehase MJ. 1990. *Eur J Immunol* 20:265–269.
Hodgins DC, Shewen PE, Dewey CE. 2004. *J Swine Health Prod* 12:10–16.
Horter DC, Yoon KJ, Zimmerman JJ. 2003. *Anim Health Res Rev* 4:143–155.
Iglesias G, Pijoan C, Molitor T. 1989a. *Arch Virol* 104:107–115.
Iglesias G, Pijoan C, Molitor T. 1989b. *J Leukoc Biol* 45:410–415.
Iglesias G, Pijoan C, Molitor T. 1992. *Comp Immunol Microbiol Infect Dis* 15:249–259.
Inman CF, Haverson K, Konstantinov SR, et al. 2010. *Clin Exp Immunol* 160:431–439.
Inman CF, Laycock GM, Mitchard L, et al. 2012. *PLoS One* 7(3):e33707.
Janke BH. 2014. *Vet Pathol* 51:410–426.
Jericho KWF. 1970. *Res Vet Sci* 2:548–552.
Johansson ME, Hansson GC. 2014. *Cell Host Microbe* 15:251–252.
Joling P, Bianchi ATJ, Kappe AL, et al. 1994. *Vet Immunol Immunopathol* 40:105–117.
Jozaki K, Shinkai H, Tanaka-Matsuda M, et al. 2009. *Mol Immunol* 47:247–252.
Jung K, Gurnani A, Renukaradhya GJ, et al. 2010. *Vet Immunol Immunopathol* 136:335–339.
Kelley KW. 1985. In Moberg GP, ed. *Animal Stress*. Bethesda, MD: American Physiological Society, pp. 193–223.
Kelley KW. 1988. *J Anim Sci* 66:2095–2108.
Kim YB, Ichimura O. 1986. *Swine Biomed Res* 3:1811–1819.
Kim HB, Isaacson RE. 2015. *Vet Microbiol* 177:242–251.
Kim B, Ahn KK, Lee YH, et al. 2009. *J Vet Med Sci* 71(5):611.
Kim D, Yoo S-A, Kim W-U. 2016. *Arch Pharm Res* 39:1565–1576.
Kitikoon P, Nilubol D, Erickson BJ, et al. 2006. *Vet Immunol Immunopathol* 112:117–128.
Klobasa F, Butler JE. 1987. *Am J Vet Res* 48:176–182.
Lalles J-P, Bosi P, Smidt H, et al. 2007. *Proc Nutr Soc* 66:260–268.
Lecce JG, Morgan DO. 1962. *J Nutr* 78:263–268.
Lee J, Choi K, Olin MR, et al. 2004. *Infect Immun* 72:1504–1511.
Levast B, De Monte M, Melo S, et al. 2010. *Dev Comp Immunol* 34:102–106.

Levast B, Berri M, Wilson HL, et al. 2014. *Dev Comp Immunol* 44:235–244.
Li Y, Song Z, Kerr KA, et al. 2017. *PLoS One* 12:e0171617.
Liebler-Tenorio E, Pabst R. 2006. *Vet Res* 37:257–280.
Linghua Z, Yong G, Xingshan T, et al. 2006. *Dev Comp Immunol* 30:589–596.
Linghua Z, Xingshan T, Fengzhen Z. 2007. *Vaccine* 25:1735–1742.
Loving CL, Osorio FA, Murtaugh MP, et al. 2015. *Vet Immunol Immunopathol* 167(1–2):1–14.
Lunney JK, Pescovitz MD. 1987. *Vet Immunol Immunopathol* 17:135–144.
Lunney JK, Walker K, Goldman T, et al. 1994. *Vet Immunol Immunopathol* 43:193–206.
Lunney JK, Ho C-S, Wysocki M, et al. 2009. *Dev Comp Immunol* 33:362–374.
Lunney JK, Eguchi-Ogawa T, Uenishi H, et al. 2010a. Chapter 6: Immunogenetics. In Ruvinsky A, Rothschild M, eds. *The Genetics of the Pig*, 2nd ed. Wallingford, UK: CAB International, pp. 101–133.
Lunney JK, Fritz ER, Reecy JM, et al. 2010b. *Viral Immunol* 23:127–134.
Lunney JK, Fang Y, Ladinig A, et al. 2016. *Annu Rev Anim Biosci* 4:129–154.
Ma W, Richt JA. 2010. *Anim Health Res Rev* 11:81–96.
Machado-Neto R, Graves CN, Curtis SE. 1987. *J Anim Sci* 65:445–455.
Mair KH, Sedlak C, Käser T, et al. 2014. *Dev Comp Immunol* 45(2):321–343.
Mair KH, Stadler M, Talker SC, et al. 2016. *Front Immunol* 7:263.
Marchioro SB, Maes D, Flahou B, et al. 2013. *Vaccine* 31:1305–1311.
Martins CLV, Lawman MJP, Scholl T, et al. 1993. *Arch Virol* 129:211–225.
Mayer EA, Tillisch K, Gupta A. 2015. *J Clin Invest* 125:926–938.
Maynard CL, Elson CO, Hatton RD, et al. 2012. *Nature* 489:231–241.
Melo ADB, Silveira H, Bortoluzzi C, et al. 2016. *Genet Mol Res* 15.
Miller LC, Lager KM, Kehrli ME Jr. 2009. *Clin Vaccine Immunol* 16:360–365.
Miró S, Tecles F, Ramón M, et al. 2016. *Vet Res* 12(1):171.
Moeser AJ, Ryan KA, Nighot PK, et al. 2007. *Am J Physiol Gastrointest Liver Physiol* 293:G413–G421.
Morein B, Abusugra I, Blomqvist G. 2002. *Vet Immunol Immunopathol* 87:207–213.
Morris CR, Gardner IA, Hietala SK, et al. 1994. *Prev Vet Med* 21:29–41.
Mueller T, Teuffert J, Staubach C, et al. 2005. *J Vet Med B Infect Dis Vet Public Health* 52:432–436.
Muneta Y, Uenishi H, Kikuma R, et al. 2003. *J Interferon Cytokine Res* 23:583–590. PMID: 14585198
Murtaugh MP, Johnson CR, Xiao Z, et al. 2009. *Dev Comp Immunol* 33:344–352.
Nguyen TV, Yuan L, Azevedo MSP, et al. 2007. *Vet Immunol Immunopathol* 117:236–248.
Niekamp SR, Sutherland MA, Dahl GE, et al. 2007. *J Anim Sci* 85:93–100.
Nowacki W, Cederblad B, Renard C, et al. 1993. *Vet Immunol Immunopathol* 37:113–122.
Opriessnig T, Yu S, Thacker EL, et al. 2004. *J Swine Health Prod* 12:186–191.
Opriessnig T, Patterson AR, Elsener J, et al. 2008. *Clin Vaccine Immunol* 15:397–401.
Osburn BI, MacLachlan NJ, Terrell TG. 1982. *J Am Vet Med Assoc* 181(10):1049–1052.
Otten W, Kanitz E, Couret D, et al. 2010. *Domest Anim Endocrinol* 38:146–156.
Overend CC, Cui J, Grubman MJ, et al. 2017. *Vet Res Commun* 41:15–22.
Pabst R, Binns RM. 1986. *Swine Biomed Res* 3:1865–1871.
Paul PS, Mengeling WL, Pirtle EC. 1982. *AJVR* 43: 1376–1379.
Pauly T, Elbers K, Konig M, et al. 1995. *J Gen Virol* 76:3039–3049.
Pelaseyed T, Bergström JH, Gustafsson JK, et al. 2014. *Immunol Rev* 260:8–20.
Pescovitz MD, Sakopoulos AG, Gaddy JA, et al. 1994. *Vet Immunol Immunopathol* 42:53–62.
Philpott DJ, Girardin SE, Sansonetti PJ. 2001. *Curr Opin Immunol* 13:410–416.
Pijoan C, Ochoa G. 1978. *J Comp Pathol* 88:167–170.
Pintaric M, Gerner W, Saalmüller A. 2008. *Vet Immunol Immunopathol* 121:68–82.
Piriou-Guzylack L, Salmon H. 2008. *Vet Res* 39:54.
Porter P. 1969. *Biochim Biophys Acta* 181:381–392.
Porter P. 1973. *Immunology* 24:163–176.
Porter P. 1986. Immune system. In Leman AD, Straw RD, Glock WL, et al., eds. *Diseases of Swine*. Ames, IA: ISU Press, pp. 44–57.
Porter P, Allen WD. 1972. *J Am Vet Med Assoc* 160:511.
Porto BN, Stein RT. 2016. *Front Immunol* 7:311.
Ramos JA, Ramis AJ, Marco A, et al. 1992. *Am J Vet Res* 53:1418–1426.
Ramsay TG, Stoll MJ, Caperna TJ. 2010. *Comp Biochem Physiol* 155:97–105.
Reiche EMV, Nunes SOV, Morimoto HK. 2004. *Lancet Oncol* 5:617–625.
Rice CE, L'Ecuyer C. 1963. *Can J Comp Med Vet Sci* 27:157–161.
Roe JM, Patel D, Morgan KL. 1993. *Vet Immunol Immunopathol* 37:83–97.
Roof MB, Kramer TT, Kunesh JP, et al. 1992a. *Am J Vet Res* 53:1333–1336.
Roof MB, Roth JA, Kramer TT. 1992b. *Compend Contin Educ Pract Vet* 14:411–424.
Rose N, Andraud M. 2017. *Porcine Health Manag* 3:8.
Roth JA. 1999. *Adv Vet Med* 41:681–700.
Roth JA, Thacker E. 2006. Chapter 2: Immune system. In Diseases of Swine, 9th ed., pp. 15–35.
Rothkotter H-J. 2009. *Dev Comp Immunol* 33:267–272.
Saalmüller A, Bryant J. 1994. *Vet Immunol Immunopathol* 43:45–52.
Salak-Johnson JL, McGlone JJ. 2007. *J Anim Sci* 85:E81–E88.
Salmon H. 1986. *Swine Biomed Res* 3:1855–1864.
Salmon H. 1987. *Vet Immunol Immunopathol* 17:367–388.

Sang Y, Blecha F. 2009. *Dev Comp Immunol* 33:334–343.
Sang Y, Rowland RR, Hesse RA, et al. 2010. *Physiol Genomics* 42:248–258.
Schinckel AP, Clark LK, Stevenson G, et al. 1995. *J Swine Health Prod* 3:238–234.
Schmied J, Rupa P, Garvie S, et al. 2013. *Vet Immunol Immunopathol* 154(1–2):17–24.
Schroeder HV, Cavancini L. 2010. *J Allergy Clin Immunol* 125:S41–S42.
Sellwood R, Hall G, Anger H. 1986. *Res Vet Sci* 40: 128–135.
Shekhar S, Yang X. 2015. *Vet Immunol Immunopathol* 168(1–2):30–34.
Sherman MP, Zaghouani H, Niklas V. 2015. *Pediatr Res* 77:127–135.
Shimosato T, Kitazawa H, Katoh S, et al. *Biochim Biophys Acta* 2003;1627:56–61.
Shinkai H, Arakawa A, Tanaka-Matsuda M, et al. 2012. *Comp Immunol Microbiol Infect Dis* 35:523–532.
Sinkora M, Butler JE. 2016. *Dev Comp Immunol* 58:1–17.
Sinkora M, Sinkorova J, Stepanova K. 2017. *J Immunol* 198(4):1543–1552.
Smith IM, Hodges RT, Betts AO, et al. 1973. *J Comp Pathol* 83:307–321.
Smith F, Clark JE, Overman BL, et al. 2010. *Am J Physiol Gastrointest Liver Physiol* 298:G352–G363.
Solano-Aguilar GI, Zarlenga D, Beshah E, et al. 2002. *Vet Immunol Immunopathol* 89:133–148.
Speer VC, Brown H, Quinn L, et al. 1959. *J Immunol* 83:632.
Stenfeldt C, Pacheco JM, Rodriguez LL, et al. 2014. *PLoS One* 9:e106859.
Stokes CR. 2017. *J Anim Sci Biotechnol* 26(8):12.
Summerfield A, McCullough KC. 2009. *Dev Comp Immunol* 33:299–309.
Suradhat S, Thanawongnuwech R. 2003. *J Gen Virol* 84:2755–2760.
Takamatsu HH, Denyer MS, Wileman TE. 2002. *Vet Immunol Immunopathol* 87:223–224.
Tarigan S, Slocombe RF, Browning GF, et al. 1994. *Am J Vet Res* 55:1548–1557.
Thanawongnuwech R, Thacker EL. 2003. *Viral Immunol* 16:357–367.
Thanawongnuwech R, Thacker B, Thacker E. 2000. In: Proceedings, the 16th International Pig Veterinary Society Congress, Melbourne, Australia, p. 173.
Thanawongnuwech R, Young TF, Thacker BJ, et al. 2001. *Vet Immunol Immunopathol* 79:115–127.
Thielke KH, Hoffmann-Moujahid A, Weisser C, et al. 2003. *Eur J Immunol* 33:1649–1656.
Tisoncik JR, Korth MJ, Simmons CP. 2012. *Microbiol Mol Biol Rev* 76:16–32.
Tohno M Shimazu T, Aso H, et al. 2008. *Mol Immunol* 45:1807–1817.
Toka FN, Golde WT. 2013. *Immunol Lett* 152:135–143.
Toka FN, Nfon CK, Dawson H, et al. 2009. *Clin Vaccine Immunol* 16(6):866–878.
Tuboly S, Bernath S, Glavits R, et al. 1988. *Vet Immunol Immunopathol* 20:75–85.
Tuchscherer M, Kanitz E, Otten W, et al. 2002. *Vet Immunol Immunopathol* 86:195–203.
Uenishi H, Shinkai H. 2009. *Dev Comp Immunol* 33:353–361.
Varum FJO, Veiga F, Sousa JOS, et al. 2010. *Eur J Pharm Sci* 40:335–341.
Vincent IE, Balmelli C, Meehan B, et al. 2007. *Immunology* 120:47–56.
Vlasova AN, Kandasamy S, Chattha KS, et al. 2016. *Vet Immunol Immunopathol* 172:72–84.
Wagner DH. *Clin Immunol* 2007;123:1–6.
Wales AD, Davies RH. 2017. A review. *Zoonoses Public Health* 64:1–13.
Wang X, Mu G, Dang R, et al. 2016. *Vet Microbiol* 197: 68–71.
Westly HJ, Kelley KW. 1984. *Proc Soc Exp Biol Med* 177:156–164.
Whitworth KM, Rowland RRR, Ewen CL, et al. 2016. *Nat Biotechnol* 34:20–22.
Wikström FH, Meehan BM, Berg M, et al. 2007. *J Virol* 81:4919–4927.
Williams PP. 1993. *Can J Vet Res* 57:1–8.
Willing BP, Van Kessel AG. 2007. *J Anim Sci* 85: 3256–3266. PMID: 17785595
Wilson HL, Obradovic MR. 2015. *Mol Immunol* 66(1): 22–34.
Wilson-Welder JH, Torres MP, Kipper MJ, et al. 2009. *J Pharm Sci* 98:1278–1316.
Winkler GC, Cheville NF. 1987. *Microvasc Res* 33:224–232.
Wintergerst EV, Maggini S, Hornig DH. 2007. *Ann Nutr Metab* 51:301–323.
Xiao H, Shao F, Wu M, et al. 2015. *J Anim Sci Biotechnol* 6(1):19.
Yang WC, Schultz RD. 1986. *Dev Comp Immunol* 10: 405–418.
Yen JT, Pond WG. 1987. *J Anim Sci* 64:1672–1681.
Yeoman CJ, White BA. 2014. *Annu Rev Anim Biosci* 2:469–486.
Yeruva L, Spencer NE, Saraf MK, et al. 2016. *BMC Gastroenterol* 16:40.
Yoon KJ, Zimmerman JJ, Swenson SL, et al. 1995. *J Vet Diagn Invest* 7:305–312.
Zeidler RB, Kim HD. 1985. *J Leukoc Biol* 37:29–43.
Zhang H, Lunney JK, Baker RB, et al. 2011. *Vet Immunol Immunopathol* 140:152–158.
Zhao Y, Kacskovics I, Pan Q, et al. 2002. *J Immunol* 169:4408–4416.
Zhu L, Zhao X, Yin Q, et al. 2017. *Sci Rep* 7:46408.
Zou Y, Xiang Q, Wang J, et al. 2016. *Biomed Res Int* 2016: 11, Article ID 5436738.
Zuckermann FA, Husmann RJ. 1996. *Immunology* 87:500–512.
Zuckermann FA, Zsak L, Mettenleiter TC, et al. 1990. *J Virol* 64:802–812.
Zuckermann FA, Husmann RJ, Schwartz R, et al. 1998. *Vet Immunol Immunopathol* 63:57–67.

17

Integumentary System

Skin, Hoof, and Claw

Jerry Torrison and Ranald Cameron

Structure and function of skin

The skin acts as a barrier to provide protection of the interior environment from exterior environment. The skin and its cellular or adnexal adaptations provide both specialized structures (hoof, claw, secretory glands, specialized cells) and functions (e.g. temperature regulation, electrolyte balance, vitamin D production, immunoregulation, antimicrobial actions, secretions, excretions, and sensory perceptions). The skin is the site of microbial flora, with the ecology influenced by pH, salinity, moisture, and albumin and fatty acid levels. There is an ongoing competitive balance between normal nonpathogenic resident flora and (potential) pathogens. The skin is the largest body organ in the pig. Skin represents between 10 and 12% of body weight at birth and around 7% in adult animals, although in some breeds such as the Meishan, skin can be 10–12% of adult body weight.

The histologic structure is similar to that of other domestic animals and, compared with other species, has many more similarities to human skin. There are two primary layers of the skin, the epidermis and dermis. The epidermis is relatively thick, the major cells being the keratinocytes in the stratum basale, the polyhedral cells in the stratum spinosum, the flattened cells in the stratum granulosum, and the cornified cells in the stratum corneum; the stratum lucidum is absent except in the snout. The thickness of the epidermis varies considerably in different areas of the body (Meyer et al. 1978), with the skin of the cranial and dorsal regions generally thicker and hairier than the skin of the ventral and caudal regions. Maximum thickness is between the toes and on lips, snout, and the shield uniquely found on the scapular and costal region in older boars. The thinnest layers are in the axilla, eyelids, and ventral areas of the thorax and abdomen (Marcarian and Calhoun 1966).

The dermis consists of two ill-defined layers overlying a prominent layer of adipose tissue (hypodermis). The two layers of the dermis, stratum papillare and stratum reticulare, are composed of connective tissue containing blood vessels, nerves, lymphatics, and associated epidermal appendages. Cells found in the dermis include fibroblasts, melanocytes, and mast cells. The origins of hair follicles and sweat glands are in the hypodermis.

The holocrine sebaceous glands of the pig are branched alveolar and open into the neck of the main hair follicle. The coiled, tubular apocrine sweat glands are present in all areas except the snout, but there are relatively few (about 25/cm^2) compared with other species. The short, stout hair follicles possess *arrectores pilorum* muscles attached to the outer root sheath. The bristles occur either singly or in groups of two or three. The hair coat consists of 60–70% bristles and 30–40% fine downy hair. Specialized forms of hair are the tactile hairs in the region of the snout (Marcarian and Calhoun 1966; Mowafy and Cassens 1975). Specialized seromucoid glands are in the carpal glands located behind the carpus and in the mental (mandibular) organ located in the intermandibular space.

Examination and diagnosis of skin

Skin diseases either only involve skin or can be cutaneous manifestations of internal disease. Examples of diseases restricted to the skin are ear necrosis, pityriasis rosea, and swinepox, whereas skin lesions symptomatic of a more general pathophysiological condition are erysipelas, classical swine fever (CSF), and dermatitis/nephropathy syndrome. It is essential to gather an accurate history and perform a thorough clinical examination of the population and affected individuals to get an accurate individual or herd diagnosis. Characterization of the lesions (i.e. specific types, distribution, and progression) is essential in formulating the differential diagnosis. Collection and preservation of appropriate samples for laboratory testing often is warranted to confirm the primary diagnosis.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

History

On-farm examinations with due consideration of available verbal and recorded history provides valuable insight. The type of husbandry or housing system influences disease expression: extensive or free-range systems may predispose to environmental hazards, whereas intensive indoor housing may predispose to conditions such as pressure sores in sows, ear necrosis in pigs, or certain bacterial disease. Evaluation of environmental conditions regarding hygiene, variation in ambient temperatures or relative humidity, and high stocking density provides clues to risk factors for outbreaks of bacterial infections. A seasonal pattern may be evident for some skin lesions such as insect bites. Signs and sources of trauma, including self-inflicted trauma due to pruritus associated with sarcoptic mange or lice infestation, are useful clues. Recent movement of pigs, commingling of ages or groups, and introduction of new stock are risk factors because mixing of pigs results in fighting, which provides sites for bacterial infection, as well as may introduce or expose pigs to new or different infectious agents.

Evaluation of nutrition and diet may be helpful, as deficiencies of the B vitamins, zinc, or essential fatty acids can result in dry, scaly, dandruff-like dermatitis or parakeratosis. Predilection to particular breed, genotype, or lineage may indicate a congenital or hereditary condition such as pityriasis rosea, dermatosis vegetans, or epitheliogenesis imperfecta.

Some diseases are more frequent in certain ages or age groups. For example, exudative epidermitis rarely affects pigs older than 6 weeks of age, pityriasis rosea is most common in pigs between 2 and 10 weeks of age, and teat necrosis usually occurs within 24 hours after birth, but nutritional deficiencies are unlikely to occur before weaning.

The onset, duration, and location of lesions that define the disease progression or clinical evolution are important to note. Poxviruses and some vesicular lesions follow the progression of macules to vesicles that rupture and form pustules and dark circumscribed crusts before healing. Exudative epidermitis often commences as macules and pustules around the eyes before spreading over the entire body of piglets.

Clinical examination

A thorough clinical examination at the herd, pen, and individual animal level (see Chapter 1) is necessary to determine overall character and impact of the disease on individuals and the population. Proper examination of the skin lesions requires proper restraint with careful examination and a thorough description of character and variation of skin lesions present. Although the skin lesions may be visible and obvious, other clinical signs such as anorexia, depression, loss of body condition, reluctance to move, or diarrhea may indicate that the skin lesions are the result of an internal disease. It is important to determine whether the skin disease is a symptom of an internal disease (e.g. erysipelas, salmonellosis, CSF) or is a primary skin insult (e.g. vesicular diseases, poxvirus, trauma, bacterial or mycotic infections). Many septicemias or toxemias can cause erythema or cyanosis characterized by red to purple discoloration, especially on the extremities, which are easy to see in the white breeds. The number of animals affected over time may suggest a contagious nature, and the presence of mortalities may suggest an infectious disease. Diseases such as exudative epidermitis or erysipelas tend to spread more rapidly, whereas congenital and hereditary conditions such as pityriasis rosea usually occur at a constant rate within a herd. A critical appraisal of the response to therapy can aid in differentiating viral, bacterial, and fungal infections. Pityriasis rosea does not respond to any treatment, whereas parakeratosis responds dramatically to the addition of zinc and essential fatty acids to the diet, and erysipelas responds quite predictably to injected antimicrobials.

Primary lesions

Skin lesions are either primary, the direct result of the disease insult type, or secondary as result of evolutionary changes to the skin dictated by factors such as the cause of the disease, secondary infection, self-trauma, and chronicity. It is important to differentiate between primary lesions and secondary lesions because often the first animals presented with most dramatic lesions may only have secondary lesions present. Examination of the entire body of several animals may be necessary to locate primary lesions of acute cases. Familiarity with terms to describe skin is useful, particularly when visual images are not available.

Macules are defined as circumscribed flat discolorations less than 1 cm in diameter, and papules – more solid, raised areas of skin of varying color – are seen in the early stages of exudative epidermitis, erysipelas, and swinepox. Plaques are elevated superficial lesions more than 0.5 cm in diameter, scattered over the whole body surface of young growing pigs, and have been associated with erysipelas and pityriasis rosea.

Vesicles are well-demarcated, dome-shaped lesions (<1 cm) usually containing serum or inflammatory exudates. They are pale or translucent and are characteristic of a number of the viral skin diseases in swine, such as swinepox, foot-and-mouth disease, and a potpourri of swine vesicular diseases, including recently emerging *Senecavirus A* (SVA) (Segales et al. 2016) and likely infrequent porcine parvovirus infection (Kresse et al. 1985).

Pustules are elevated lesions filled with inflammatory cells (leukocytes) and can be follicular or epidermal.

They are white, yellow, or red (hemorrhagic) and often surrounded by erythema. Pustules in swine are commonly associated with streptococcal infections, exudative epidermitis, and later stages of SVA or swinepox.

Wheals are circumscribed, raised, round or oval areas of the skin due to edema in the dermis. They may be blanched or slightly erythematous. Fly and mosquito bites frequently cause wheals. Urticaria, seen as multiple pink to purple raised areas of the skin, commonly occurs in cases of erysipelas, beta-hemolytic streptococcal infections, food allergy, or insect bites.

Secondary lesions

Blue to black skin discoloration with necrosis can indicate gangrenous dermatitis, cellulitis, or myositis. Necrosis of the ears and tail of piglets is also often black. Skin pallor is often an indication of anemia, with iron deficiency or blood loss anemia still all too common. Skin icterus (jaundice) is a yellowish discoloration due to increased red cell breakdown or liver compromise occurring with hepatic disease or diseases causing extravascular hemolysis.

Hemorrhage is often blotchy red to purple to black, depending on location and extent from extravasation of blood from dermal blood vessels as seen with contusions, bruises, or thrombocytopenic purpura.

Scales are flakes of keratinized epidermis indicating chronic inflammation or irritation. They may be due to nutritional (e.g. parakeratosis) or hormonal dysfunction or caused by ectoparasites, such as *Sarcoptes scabiei*, or by bacterial skin diseases. They occur on the thinner skin of piglets with exudative epidermitis, on the inner side of the margin of the ringlike lesions of pityriasis rosea, and on the outer periphery of ringworm lesions. They also may be mixed with sebum and sweat, giving a greasy or oily appearance known as seborrhea.

Crusts are a very common secondary skin lesion of swine and are due to a combination of serum, sebum, blood, and cutaneous debris adhering above the normal skin surface. They are seen following bacterial infections and viral vesicular diseases, especially swinepox, and as a result of pruritus associated with sarcoptic mange or lice infestation.

Hyperkeratosis, an increased thickening of the stratum corneum, develops with nutritionally related metabolic disorders such as vitamin A, zinc, and fatty acid deficiencies or with local callus formation due to trauma associated with pressure and friction. Erythema and intense pruritus with hyperkeratosis and acanthosis have been associated with in-feed tiamulin (macrolide) therapy (Laperle et al. 1988).

Erosions involve the epidermis only and are localized loss of partial thickness of epidermis that occurs with trauma, irritants, sunburn, or mild exudative epidermis. Ulcers are localized full thickness loss of epidermis from loss of vitality of epidermis, with necrosis and sloughing, and are seen with trauma or pressure (decubital ulcers) or certain deep bacterial infections (*Staphylococcus* spp., *Streptococcus* spp., *Fusobacterium necrophorum*, and the spirochete *Treponema pedis*).

Edema of the skin can indicate a systemic disease, for example, hypoproteinemia, vasculitis, and increased vascular permeability such as in malignant edema caused by *Clostridium septicum* and in edema disease associated with *Escherichia coli*.

Pruritus results in scratching that occurs with a variety of conditions, most notably sarcoptic mange or lice infestation. The presence of sarcoptic mange in ears can manifest as shaking of the head and can result in aural hematomas. Aural hematoma is the acute distension of the ear with blood, usually the result of trauma from fighting or head shaking. Ear hematomas are more common in breeds with longer ears. Intervention beyond segregation usually is not warranted since draining or lancing the hematoma usually results in continued hemorrhage or infection.

Alopecia and excoriation may also be an indicator of intense scratching seen in chronic sarcoptic mange. Severe scratching will result in alopecia, commonly seen over the shoulders and hindquarters in pigs with sarcoptic mange or lice and in pigs irritated by insects such as flies and mosquitoes.

Thorough lesion characterization should allow the clinician to formulate a differential diagnosis. Laboratory tests may be warranted to confirm a diagnosis (skin scraping, culture, or biopsy) in order to decrease uncertainty or increase confidence of an accurate diagnosis. Obtaining an accurate diagnosis aids in development of effective interventions, treatments, and prevention strategies and likely will save time and money in the longer run (Table 17.1).

Diagnostic tests

Frequently used tests to confirm a diagnosis of skin disease in swine include skin biopsy for histopathological examination, direct smears or scrapes for PCR or isolation for detection and identification of bacteria, viruses, and fungi, and euthanasia and necropsy to submit tissues sufficient to address all differentials. Antimicrobial sensitivity testing is sometimes appropriate and requires isolation of the organism; PCR detection does not provide that information unless resistance genes are targeted or sequencing is performed.

Skin biopsy and direct examination

Skin biopsy is appropriate for most lesions, including suspected neoplasm, persistent ulceration, and lesions not responding to treatment. Fully developed primary lesions or early vesicles and pustules are best for biopsy, whereas biopsy of chronic or secondary, lesions from

Table 17.1 Differential diagnosis of skin lesions by location.

Location	Lesions and signs	Diseases
Head and neck	Macules, pustules, greasy exudate (seborrhea), crusts; in suckling pigs and weaners	Exudative epidermitis (*S. hyicus*)
	Pustules, crusts, and abscesses	Streptococcosis
	Plaques, pustules, crusts, alopecia with pruritus; ear pinnae common	Sarcoptic mange
	Pustules, erosions, necrosis, crusts below the eye, cheek, and lips in suckling piglets	Facial necrosis
	Edema: palpebral, forehead; in weaners and young growers	Edema disease (*E. coli*)
	Edema of the head and throat; injection site necrosis	Malignant edema (*Clostridium* sp.)
	Vesicles, pustules, erosions on the snout, lips, mouth, and tongue	Vesicular diseases; *Senecavirus A*; other viruses; idiopathic
	Multifocal vesicles, erosions, "spots" of black crusts	Swinepox; poxviruses
Ears	Black necrosis, ulcers on the tips and posterior edge of the pinna in piglets	Ear necrosis, salmonellosis, erysipelas; idiopathic
	Deep ulcers at the base of the pinna in growers; often bilateral	Ulcerative spirochetosis; trauma
	Erythema, red to purple blotchy discoloration	Systemic bacteria and viruses; sunburn; photosensitization
	Plaques, brown or gray crusts on the inner ear, ear shaking, pruritus	Sarcoptic mange
	Macules, pustules, and black crusts	Exudative epidermitis, streptococcosis
	Circular macules, patches, small scales, pink to red discoloration	Ringworm (microsporosis)
Dorsum and shoulder	Hyperkeratosis, dry scales along spine, some alopecia	Parakeratosis (Zn); fatty acids; vitamin A, C, or E; mange
	Complete absence of epithelium (large red shiny areas) in newborn piglets	Epitheliogenesis imperfecta
	Pressure necrosis or deep ulcer or crust over spine of scapula, last ribs, and lumbar area in sows	Pressure necrosis from trauma; recumbent sows in poor body condition; confinement
	Large deep discrete ulcer, necrosis, and crust over spine of scapula in sows often in poor body condition	Pressure sore from poor condition, due to confinement on solid or mesh floors; low energy intake
Ventral abdomen	Erythema, pustules, dark brown crusts, exudate	Exudative epidermitis, streptococcosis, sarcoptic mange, candidiasis, biotin deficiency,
	Erythema, round to diamond-shaped red plaques, fever, anorexia, arthritis	Erysipelas, *Actinobacillus suis*
	Papules; ringlike lesions; collarettes, flakes and scales (3- to 14-week-old pigs)	Pityriasis rosea
	Circular pink to red macules, scales, or crusts around periphery	Ringworm (microsporosis, trichophytosis)
	Papules, thick crusts, fissures, exudate	Zinc deficiency (parakeratosis), dermatosis vegetans, exudative epidermitis
	Vesicles, pustules, black scabs, round raised areas with depressed centers	Swinepox; poxviruses
	Necrosis (red or black scabs) of teats, especially pectoral teats in piglets	Trauma and bacterial infection due to rough floors

Table 17.1 (Continued)

Location	Lesions and signs	Diseases
Lateral abdomen and flank	Erythema, round to diamond-shaped red plaques, fever, anorexia, arthritis	Erysipelas, *A. suis*, flank biting – vice
	Pustules, scales, thick wrinkled skin, alopecia	Sarcoptic mange
	Erythema, erosion, or ulcer on flank; poor hair coat	Niacin, pantothenic acid, riboflavin, fatty acid, or vitamin A deficiency
	Circular pink to red macules varying in size, scales or crusts around periphery	Ringworm (microsporosis, trichophytosis)
Hindquarters	Erythema of the scrotum, vulva, and perineum	Septicemia, sunburn
	Tail necrosis, ulceration, abscesses (growers)	Tail biting; tail necrosis (piglets)
	Erythema, expanding and coalescing purple spots, perineal area, and flanks	Porcine dermatitis and nephropathy syndrome (PDNS)
	Small round raised wheals, urticarial reaction	Insect bites: flies, mosquitoes, fleas
Legs (limbs)	Erythema, red to purple discoloration	Septicemia
	Plaques seen as ringlike lesions, collarettes	Pityriasis rosea
	Papules, thick crusts, fissures, papillomas	Parakeratosis (Zn def), exudative epidermitis
	Complete absence of epithelium in newborn piglets	Epitheliogenesis imperfecta
	Thick fibrotic areas over joints (hocks, elbows)	Callosities, bursitis, adventitious bursa
	Necrosis of carpus and hocks in suckling piglets	Trauma due to rough farrowing crate floors
Distal limb, coronary band, feet	Vesicles, pustules, erosions around the coronary band and accessory digits, with lameness	Vesicular diseases; *Senecavirus A*; other viruses; idiopathic
	Thick, dry crusts, deep fissures	Parakeratosis, mange, exudative epidermitis, dermatosis vegetans
	Abscesses, discharges, swelling of the coronary band	Bush foot, ascending infections of hoof
	Hoof lesions	Biotin, mineral and vitamin deficiency, trauma
	Thickening, ridges and furrows parallel to coronary band	Dermatosis vegetans

previously medicated animals may be of little value. Proper restraint and local anesthetic aid in collection. Immerse the portion of the biopsy for histopathology immediately in a fixative such as 10% neutral phosphate-buffered formalin.

The technique usually involves removal of 6–9 mm of skin using a biopsy punch or with surgical excision of a wedge biopsy, which may be more suitable for larger lesions, vesicles, and pustules and where the skin is very thick. It is important to include the transition zone representing affected and normal skin in submitted biopsies; multiple samples may be appropriate.

Skin biopsies that are chilled and not fixed can be used for isolation or PCR for detection and characterization of bacteria, fungal agents, or viruses. For microbial isolation, the skin should be cleaned with water or saline only and not with alcohol. Samples should be stored and transported at 4 °C in appropriate transport medium.

Direct examination of smears from a biopsy or skin scraping is common for identification of bacteria, parasites, or fungi. For bacteria, smear samples of pus or exudate from pustules, macules, or ulcers on glass slides, air-dried, and stained with methylene blue, Gram stain, or Diff-Quik for light microscopy. Skin scrapings or direct touch impressions can be used for suspected fungal diseases, which are best collected after removing fat from the skin with alcohol. Scrapings are warmed in a 20% solution of sodium hydroxide, and spores appear as round highly refractile bodies in chains or mosaics in hair follicles, in epithelial scales, and on the surface of hair fibers. Skin scraping is useful for identification of mange (see section on "Parasitic Diseases").

Culture and PCR

PCR is often preferred to confirm the presence of specific pathogens because of the high sensitivity and specificity. However, interpretation of PCR results should be in context of clinical signs, lesion type, and particular agent since endemic agents or potential pathogens are readily detected irrespective of whether they have a role in disease present.

Cultures of open sores (erosions, ulcers, and sinuses) generate confusion because lesions often contain mixed environmental flora that can be difficult to interpret.

Best results are obtained by aspirating samples from intact pustules, vesicles, or abscesses with a needle and syringe.

Clearly communicate history, clinical signs, sample identification/location, and testing expectations with the reference laboratory as approaches to testing often depend greatly on the differential diagnosis.

Bacterial diseases of skin

See the respective chapters for details of various bacterial diseases. A few comments specific to skin diseases caused by bacteria are discussed here.

Exudative epidermitis (greasy pig disease, impetigo contagiosa, seborrhea contagiosa)

Exudative epidermitis occurs with those strains of *Staphylococcus hyicus* that produce a heat-labile exfoliative toxin (Andresen et al. 1993). Lesions can occur at any age, but severe disease in individuals or outbreaks generally occur in pigs less than 8 weeks of age, with suckling piglets the most commonly and severely affected age group. The morbidity varies considerably, up to 80% in some groups. The disease can occur sporadically in older swine as well. Disease may be acute, subacute, or chronic. Lesions most frequently occur around the eyes, nose, lips, and gums and behind the ears as macules increase in size to form damp and oily greasy exudates of sebum, sweat, and serum (Figure 17.1). Erythema is marked, and in some pigs the lesions become generalized, affecting nearly the entire body. Occasionally there are lesions in the kidney involving the renal pelvis and tubules (Blood and Jubb 1957). Ulcerative glossitis and stomatitis were associated with *S. hyicus* by Andrews (1979), and nervous signs (sepsis) were observed in an outbreak of exudative epidermitis by Blood and Jubb (1957), supporting occasional septicemia that can occur with this organism. The differential diagnosis includes sarcoptic mange, parakeratosis associated with zinc and other nutritional deficiencies, swinepox, pityriasis rosea, pustular dermatitis, and ringworm.

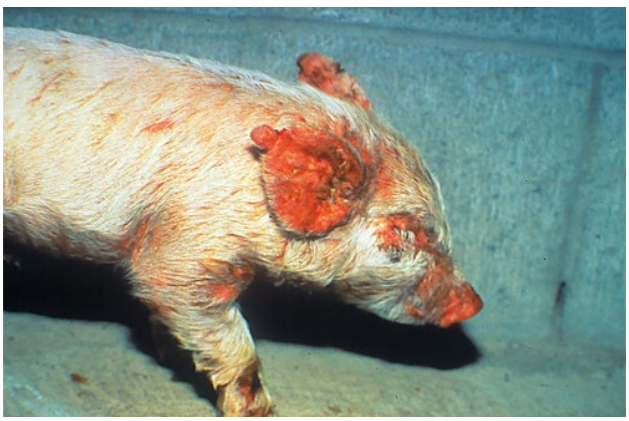


Figure 17.1 Exudative epidermitis.

Pustular dermatitis (contagious pyoderma)

Pustular dermatitis is a result of streptococcal infections that cause skin necrosis and pustular dermatitis. Transmission of pyogenic streptococci is directly from sows to newborn piglets. Disease results when organisms colonize breaches in integument, such as skin abrasions from fighting and environmental hazards or from traumatic tissue damage associated with tail docking, ear notching, castration, or clipping needle teeth. Infected wounds on any region of the body can result in cellulitis, necrosis, abscess formation, ulceration, and occasionally sepsis. Differential diagnosis includes ulcerative dermatitis, exudative epidermitis, sarcoptic mange, swinepox, and erysipelas.

Ear necrosis

Ear necrosis is a syndrome seen in pigs, usually from one to 12 weeks of age, characterized by bilateral or unilateral necrosis of any part of the ear (Figure 17.2). In young pigs, the tips of ears are commonly affected, but it can also affect the posterior edge of the pinna or base of the ear. In growers, necrosis at the base of the ears or tips of the ears can occur, with outbreaks that can have up to 80% of a pen affected and high morbidity within the group. A major consequence of ear necrosis is septic embolic that may cause infection and abscesses in lung and joints.

The etiology and pathogenesis of ear necrosis is obscure, is likely multifactorial, and may vary from outbreak to outbreak. Some lesions are the result of a mixed

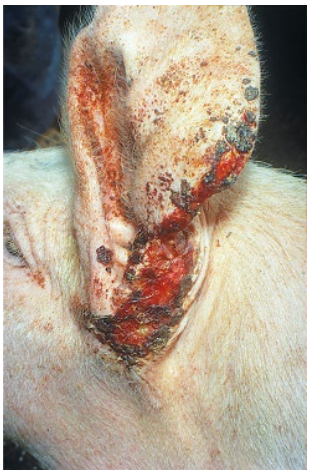


Figure 17.2 Ear necrosis (spirochetosis).

infection following damage to the skin, for example, toxin-induced epidermitis from *S. hyicus* followed by the more invasive streptococci and spirochetes (see "Ulcerative Dermatitis" below), which results in necrosis and ulceration (Fraser et al. 1991; Park et al. 2013). Biting following mixing of pigs is a common predisposing factor. Ear biting can be a vice similar to flank and tail biting, leading to infections. Risk factors such as high stocking rate, high ambient temperature, or high humidity were associated with outbreaks of ear necrosis (Busch et al. 2002); the same study reported no association with porcine reproductive and respiratory syndrome virus (PRRSV) or PCV2-associated disease or with concurrent tail lesions. Self-inflicted trauma resulting from the irritation of *S. scabiei* infection in the ears can initiate the early lesions. Systemic toxemia (e.g. ergot) and frostbite are unusual causes of ear tip necrosis.

Frequently, however, infectious agents are not consistently found in outbreaks of bilateral symmetrical ear necrosis, suggesting the possibility of a vascular- or immune-mediated ischemia (Figure 17.3). Arruda et al. (2015) investigated an ear necrosis outbreak using histopathology and metagenomics techniques. Aseptically collected punch biopsies were obtained for histopathology, molecular diagnostics, and metagenomics from acutely affected, chronically affected, and unaffected ears of pigs. Histologic examination of the epidermis and dermis of acutely affected pigs revealed deep dermal inflammation with minimal changes to the epidermis and no lesions in age- and site-matched internal controls, suggesting a primary lesion of vascular damage rather than epidermal insult. Metagenomics analysis found increased *Actinobacillus* and *Haemophilus* genera in acutely affected pigs compared with unaffected controls. However, the roles for bacterial sepsis and immune complex mechanisms remain speculative.

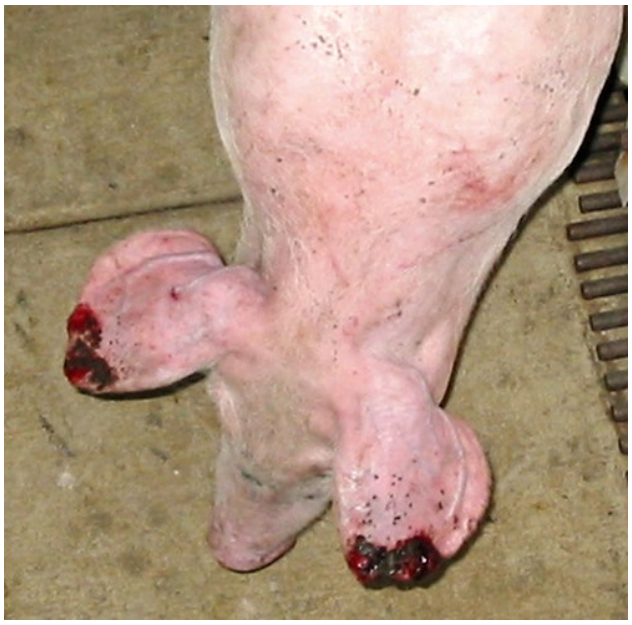


Figure 17.3 Ear tip necrosis (idiopathic).

Ulcerative dermatitis

Ulcerative dermatitis can occur as ulcerative, necrotic, or tumor-like lesions found on the body surface. Common sites include the buccal cavity of pigs and sites of ear necrosis, facial necrosis, infected bursa, or ulcers and calluses over joints and bony prominences.

The etiology involves initial trauma of the skin, followed infection, often involving several organisms. In the more proliferative or granuloma-like lesions from ears, feet, legs, or shoulder ulcers, microscopy may demonstrate spiral-shaped organisms. *T. pedis* is implicated as having a contributing role for shoulder, flank, ear (Figure 17.2), and tail ulcers (Clegg et al. 2016; Karlsson et al. 2014; Park et al. 2013; Pringle and Fellstrom 2010). The lesions usually contain *S. hyicus* and beta-hemolytic streptococci as well, with *Trueperella pyogenes* a common secondary invader (Cameron 1984). Skin trauma or damage that results in infections can be associated with bite wounds, especially around the face and head and on the flank and tail. Infection following castration, pressure sores, and ulceration of swollen bursas and calluses can lead to spirochetosis. Gum damage following teeth clipping can result in lesions in the buccal cavity. A differential diagnosis would include foreign body abscesses, neoplasms, other infectious granulomas, and pressure necrosis.

Facial necrosis (facial pyemia)

Facial skin necrosis is a common condition in suckling pigs less than 1 week of age, characterized by bilateral necrotic ulcers usually covered by hard brown crusts that extend from the side of the face to the lower jaw area. The condition is the result of infection of wounds inflicted by piglets by "needle teeth" on each other during feeding. Although clipping of needle teeth has been widely practiced, perhaps a more important risk factor is mammary gland availability and milk flow for each pig. Facial necrosis is more common in disadvantaged weaker piglets in large litters or in litters where sows have agalactia or hypogalactia.

Facial necrosis occurs during the first few days of life. Initially lesions are striated lacerations caused by bites from other piglets, which then are infected with organisms such as *F. necrophorum*, *Streptococcus* spp., and *T. pedis*, resulting in shallow ulcerations covered with hard brown crusts. The encrustation may extend over a large area involving the lips and eyelids, making it difficult for the piglet to open its mouth or eyes. These animals have

difficulty in feeding and may starve. Facial necrosis can predispose to outbreaks of exudative epidermitis.

The condition is easily diagnosed by the nature and distribution of the lesions on the face of young piglets. Careful removal of the crusts and application of a mild disinfectant or antibiotic cream will help remove the infection as well as soften the lesions. Prevention is assuring healthy underline and milking capability of dams through proper genetic selection and feeding and by fostering piglets to minimize teat competition. The clipping of the canine and lateral incisor teeth just above the level of the gum surface during the first 24 hours of life with instruments thoroughly cleaned and disinfected between litters has been a common practice but is not considered necessary.

Specific bacterial diseases

Erysipelas, the disease caused by *Erysipelothrix rhusiopathiae*, occurs as septicemia, acute or chronic arthritis, vegetative endocarditis, skin lesions, or abortion. In acute septicemia, the skin of the extremities is erythematous or cyanotic, particularly in younger pigs. The color varies from pink to purple, typical of many systemic infections and not necessarily diagnostic of erysipelas. Skin lesions more specific for erysipelas but not consistently present in outbreaks first appear as small pink or red raised areas (papules) or larger plaques ranging from 3 to 6 cm in diameter (Figure 17.4). Many of these lesions will develop the characteristic diamond or rhomboid shape and are raised, firm, and easily palpated, with the outer area pink in color and the center becoming blue to purple (necrosis) as the disease progresses. These discrete lesions are result of arteritis characterized microscopically by small arterioles having acute suppurative infiltration and cellular thrombi (Jubb et al. 1985). In the chronic stage, skin lesions become more necrotic and appear dark, dry, and firm; the superficial skin may easily peel away from the underlying tissues, and, occasionally, sloughing of the ears, tail, or a foot can occur.

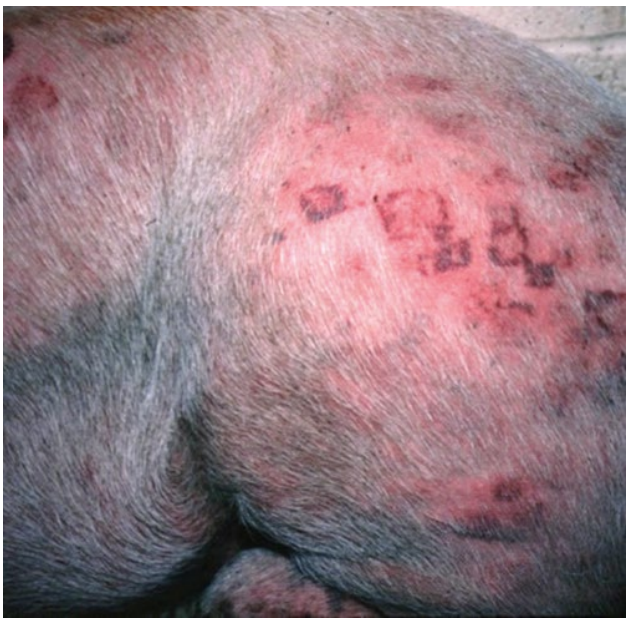


Figure 17.4 Erysipelas ("diamond skin disease").

Figure 17.5 Cyanosis – acute bacterial septicemia. (erysipelas).

Actinobacillus suis causes septicemia, pneumonia, and rapid deaths and occasionally will cause skin lesions virtually identical to those of erysipelas. Salmonellosis, most notably *Salmonella choleraesuis*, also causes septicemia and skin lesions (necrosis) or changes in skin color (cyanosis). Pigs from weaning to 4 months of age are most frequently affected, but all age groups can be affected. Skin necrosis is uncommon but can involve the ears, tail, and feet in young pigs.

Erythema, cyanosis (Figure 17.5), or other patterns of skin discolorations occur with gram-negative sepsis or systemic diseases, including those with bacterial, viral, metabolic, toxic, or parasitic involvement, which emphasizes the need for thorough clinical assessment and laboratory testing to confirm diagnosis.

Viral diseases

Swinepox (contagious impetigo, louse-borne dermatitis) is a typical poxvirus infection mainly affecting young pigs. There is little to no systemic illness, and lesions are usually confined to the ventrolateral abdomen and thorax. The lesions follow the typical pox evolution of erythematous macules becoming papules and then vesicles progressing to pustules, which rupture and form crusts. Lesions occur mainly on the side of the body, ventral abdominal wall, and inner thighs. Occasionally lesions occur on the back, face, and udder. Piglets infected *in utero* may be born with generalized pox lesions affecting any portion of the skin as well as oral cavity and esophagus (Figure 17.6). Poxviruses from other species are suspected to induce lesions in

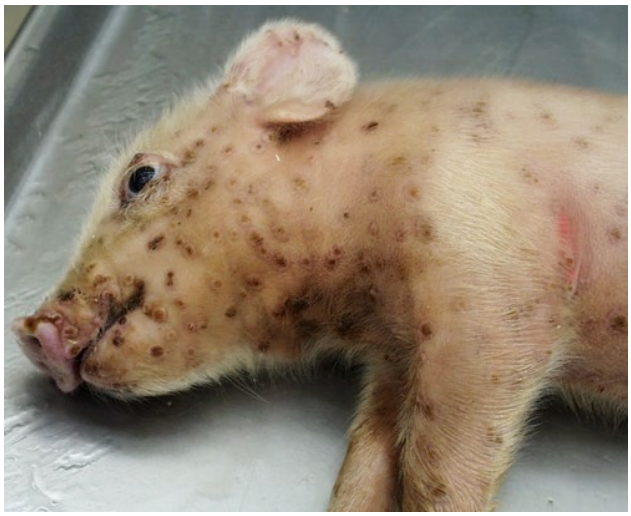


Figure 17.6 Swinepox (congenital swinepox).

some cases but are rarely characterized since disease is generally very sporadic and self-limiting.

CSF (hog cholera) can cause diffuse erythema followed by purplish discoloration of the skin over the abdomen, snout, ears, and thighs in acute cases. Necrosis of the edges of the ears, tail, and vulva may develop, with purple blotching of the ears often present. Generalized hypotrichosis is reported in the chronic form of the disease. Congenital alopecia has been reported in piglets infected *in utero* (Carbrey et al. 1966).

The vesicular diseases include foot-and-mouth disease, swine vesicular disease, vesicular stomatitis, and vesicular exanthema, with other members of the family *Picornaviridae* such as SVA all capable of causing vesicular skin lesions in swine; all produce very similar lesions with similar distribution. Aggressive diagnostic investigation is warranted when vesicles are present, particularly when lesions are present on snout or coronary bands, as many of these diseases are reportable or under regulatory authority.

African swine fever causes general signs of fever, depression, anorexia, and incoordination similar to hog cholera, both of which can have skin changes that include cyanotic blotching and purple discoloration of the limbs, snout, abdomen, and ears. Hemorrhages may also occur on the skin of the ears and flanks. Another syndrome, porcine dermatitis and nephropathy syndrome (PDNS), is thought to be associated with viral infection(s) as discussed as follows.

Porcine dermatitis and nephropathy syndrome

PDNS is widely reported in pigs (Cameron 1995; Hélie et al. 1995; Smith et al. 1993; White and Higgins 1993) and is characterized by multifocal skin lesions, weight loss, edema of the limbs, vasculitis, and glomerulonephritis. See Chapter 22 for additional discussion of the renal component.

The causes and mechanisms by which PDNS occurs are not completely understood; however, histopathological and immunological findings suggest that the pathogenesis involves an immune complex disorder (antibody–antigen complex deposition) associated with infectious agent(s). Thibault et al. (1998) suggested that PRRSV infection might play a role in the pathogenesis of the disease because of detection of PRRSV antigens by immunochemistry in macrophages located around vessels of skin and kidney tissue examined in acute and chronic cases. However, Wellenberg et al. (2004) considered that PCV2 was more likely to be a more common agent in the development of PDNS. They reported excessively high PCV2 antibody levels in a case–control field study of PDNS and hypothesized that PCV2 plays an important clinical and immunopathological role in the development of PDNS. They suggested that the excessive high levels of PCV2 antibodies trigger the development of immune complex deposition in kidneys that initiate an inflammatory process in the vascular or glomerular capillary walls. They also found an epidemiological association with herds that experienced post weaning multisystemic wasting syndrome (PMWS), also caused by PCV2. Porcine circoviruses (PCV2, PCV3) have a strong association with occurrence of PDNS (Opriessnig and Langohr 2013; Palinsky et al. 2016).

Seen mainly in growing swine from 20 to 65 kg, the most obvious clinical signs are skin lesions and a rapid loss in body weight with concurrent depression. The skin lesions range from large areas of erythema, macules, and hemorrhagic papules to dark brown to black thick crusts of necrosis on the ears, face, lower limbs, hindquarters (Figure 17.7), scrotum in boars (Figure 17.8), and vulva of

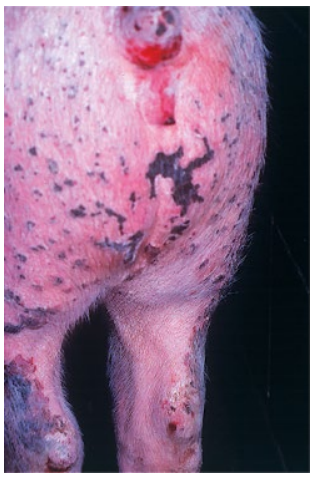


Figure 17.7 Porcine dermatitis and nephropathy syndrome (PDNS) in a grower pig.

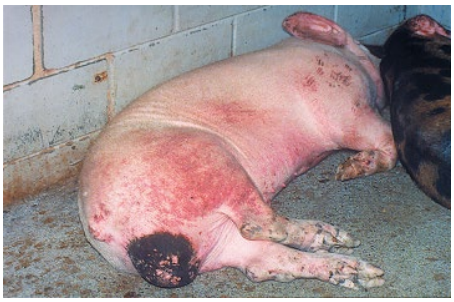


Figure 17.8 Porcine dermatitis and nephropathy syndrome (PDNS) in a mature boar.

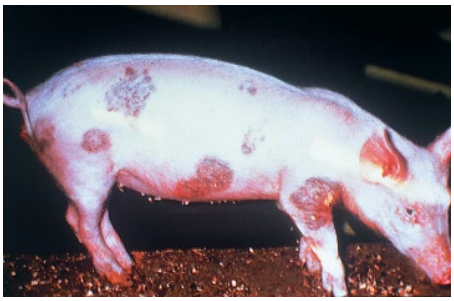


Figure 17.9 Ringworm (*Microsporum nanum*).

sows. Other clinical signs include subcutaneous edema along the ventral abdominal wall extending to limbs, sometimes with swelling of the joints.

The condition could be confused with erysipelas or other causes of skin necrosis, but another serious concern is that the clinical signs and lesions closely resemble those of CSF and African swine fever. Necropsy reveals enlarged, pale, spotted kidneys with petechiae, excess fluid in the body cavities, subcutaneous edema, and perhaps excessive synovial fluid in the joints. The frequency of gastric ulceration and gastric hemorrhage may be increased. The histopathology of the kidney lesions is consistent with a diffuse necrotizing and proliferating glomerulonephritis, including formation of hyaline/granular casts and distended tubules. Microscopy of skin reveals necrotizing vasculitis of arterioles in the dermis and subcutis. Small-vessel vasculitis also occurs in other organs, including lymph nodes, spleen, stomach, liver, bladder, brain, and joints (Higgins 1993). Control is difficult because the actual causative agent(s) and disease mechanism(s) are poorly understood. Anecdotally, refining the PCV2 vaccination program (age of administration as well as the frequency or number of doses of vaccine given to grower pigs, gilts in development, and/or the sow herd) has usually mitigated occurrence of PDNS.

Fungal diseases (ringworm)

Fungal diseases of swine tend to be superficial mycoses involving the keratinized epithelial cells and hair only. Fungi reported in swine include *Microsporum nanum, Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton tonsurans, Trichophyton verrucosum*, and *Candida albicans*.

Ringworm occurs in both extensively and intensively reared swine. All age groups can be affected, and the incidence appears to be higher where sanitation is poor and stocking densities are high with moderate temperatures and high humidity. Bedding may be an important source of infection. Fungal spores can remain viable for many years in a dry and cool environment. Mycelial growth is optimized when the environmental conditions are warm and humid with a slightly alkaline skin pH. Ringworm fungi are strictly aerobic.

Ringworm lesions associated with *Microsporum* spp. (most commonly *M. nanum*) can be found on almost any part of the body. Lesions begin as circumscribed spots, which tend to enlarge in a circle, some to enormous size covering the complete side of the pig. The skin is reddish to light brown in color, roughened but not raised. Dry crusts form around the periphery, the hair is usually not lost, and no pruritus develops (Figure 17.9). Experimental infection with *M. nanum* (Connole and Baynes 1966) produced lesions that first appeared as pustules or moist brown areas of desquamated tissue 2 cm in diameter. As the lesions extended, fresh pustules were often seen near the periphery. Scales, crusts, and deposits of black material similar to the natural infection appeared. Lesions developed in 2–3 weeks and resolved by 9 weeks. Chronic infections are often seen behind the ears of adult swine and appear as thick brown crusts that spread over the ear and neck.

Trichophytosis is most commonly associated with *T. mentagrophytes*, but *T. rubrum, T. tonsurans*, and *T. verrucosum* also cause ringworm in swine. Lesions are found on the thorax, flank, and neck, behind the ears, and on the legs. The size and shape of lesions vary; some measure up to 12.5 cm across and are roughly circular. Typical lesions are red or covered by a thin brownish dry crust. The disease tends to be self-limiting and lasts about 10 weeks (McPherson 1956). Arora et al. (1979) described lesions caused by *T. rubrum* as rough and reddish and appearing on several sites; 10% of piglets and 4% of sows in a herd were affected.

Cutaneous lesions caused by the yeast *C. albicans* can occur when host resistance is compromised. Disease with 40% morbidity was reported in grower pigs fed garbage and kept in unsanitary conditions. The lesions on the most severely affected animals consisted of circular areas approximately 2 cm in diameter coated with moist gray exudate. Lesions were found on all limbs and the lateral and ventral surfaces of the abdomen. The skin was thickened, wrinkled, devoid of hair, and hung loose in folds (Reynolds et al. 1968).

Diagnosis of ringworm is by biopsy, by microscopic examination of scrapings, or by fungal culture. Treatment of fungal infections, if indicated, consists of removal of the crusts and local application of antifungal products. Control is by maintaining good sanitation, nutrition, and strategic disinfection of premises.

Parasitic diseases

Sarcoptic mange (scabies) is the most commonly encountered parasitic skin disease of swine, is of major economic importance globally, and is caused by the mange mite *S. scabiei var. suis*. The first skin lesions appear about 3 weeks after contact with mites as small encrustations around the ears, eyes, and snout that develop into plaques about 5 mm in diameter. The lesions in the ear may regress and disappear in 12–18 weeks. Early pruritus is due to the local irritation from the mites establishing themselves in the skin. As the initial lesions regress, focal erythematous papules associated with hypersensitivity occur on the rump, flank, and abdomen. Mites are not usually found in these lesions but often remain present and detectable in ear scrapes. The hypersensitivity causes further pruritus, which results in excessive rubbing and the liberation of tissue fluids, giving the animal a greasy or shiny appearance. This is followed by coagulation and drying of the serum, sebum, and sweat to form crusts. In more chronic cases, excessive keratinization and proliferation of the connective tissue occur, with the result that the skin becomes thickened and wrinkled. A common sign seen in grower pigs is shaking of the ears and, in some, the development of large hematomas on the inner surface of the ear. Chronic cases, usually adults, will develop thick gray-colored, loosely attached scales lining the inner surface of the ears, around the neck, and down the lower limbs, especially over the hock joints. Considerable loss of hair is associated with chronic mange (Figure 17.10).

Demodectic mange (follicular mange) is of little economic importance in swine. Clinical signs occur when pigs are in a poor or debilitated condition. The disease is caused by *Demodex phylloides*, which lives in the hair follicles or sebaceous glands of the skin. The mites can invade the soft skin of the snout and around the eyes but can spread over the entire body. Infection is not uncommon on the abdomen between the legs. Lesions start as small red spots, which become scaly with a nodular appearance. The nodules contain white caseous material and many mites. Treatment is usually not successful, and severely affected animals should be culled.

Figure 17.10 Sarcoptic mange.

Haematopinus suis, a louse that affects pigs only, causes severe irritation, resulting in continual scratching and rubbing against objects. Adult lice are easily visible around the neck, base of the ears, and inner ears and inside the legs and flank, and the white eggs can also be seen on the bristles, especially in colored pigs. The blood feeding causes considerable irritation, scratching, and rubbing against objects, resulting in lacerations and hemorrhage. Lice tend to congregate around the areas where skin damage has taken place. The continual irritation results in loss of body weight and reduces weight gains. Lice may spread swinepox virus, erysipelas, and likely other disease agents.

Fleas (*Ctenocephalides canis, Ctenocephalides felis, Pulex irritans*, and *Echidnophaga gallinacea*), mosquitoes (*Aedes* spp.), and flies (*Musca domestica, Stomoxys calcitrans, Culicoides* sp.) commonly affect swine. Clinical signs can include varying degrees of rubbing, scratching resulting in alopecia, excoriations, and bleeding from the skin and circumscribed, raised, rounded (wheal) lesions or edema associated with urticarial reactions.

Environmental diseases

Sunburn

Sunburn caused by the direct effect of ultraviolet rays on skin occurs in white pigs managed under open-range conditions without adequate protection from sunlight. Young pigs and pigs not previously exposed to sunlight are often seriously affected. Erythema occurs within a few hours of exposure and develops most commonly on the back and behind the ears. Edema can develop, and affected areas become warm and painful to the touch. Severely affected pigs walk very carefully, may have sudden bouts of muscular twitching, may jump into the air, or may suddenly drop to sternal recumbency, presumably in response to pain. The skin becomes dry, scales develop, and the skin peels. In young pigs the tail and ears become necrotic and slough. A simple and effective treatment is to cover the skin with bland oil, for example, vegetable oil or light mineral oil. Animals should be removed from direct sunlight, and adequate shade provided for prevention.

Photosensitization

Photosensitization is a condition seen in extensive, free-range-managed swine exposed to photodynamic agents and sunlight. Photosensitivity (hypersensitivity to light) results from the ingestion of photodynamic agents such as hypericin found in St. John's wort (*Hypericum perforatum*) and fagopyrin found in buckwheat (*Fagopyrium esculentum* and *Polygonum fagopyrum*). Other plants, including rape (*Brassica* sp.), lucerne (*Medicago sativa*), and *Trifolium* sp., cause photosensitization of unknown etiology. Other substances reported to cause photosensitization in swine are phenothiazine, tetracyclines, sulfonamides (Amstutz 1975), and possibly aphids (McClymont and Wynne 1955).

Lesions are seen in white-skinned breeds and on areas most directly exposed to the sunlight. The severity of the condition depends on the concentration of the photodynamic agent and the length of exposure to light (Jubb and Kennedy 1970).

Erythema and edema develop and serum may exude from the skin and become dry and matted in the hair. Pain causes swine to walk carefully; severely affected animals may suddenly drop into sternal recumbency and immediately rise again or stagger sideways (Hungerford 1990), similar to acute sunburn. The ears become thickened, and congestion of the conjunctiva may occur with matting together of the eyelids (Amstutz 1975). Skin becomes dry, hard, and fissured and extremely pruritic. Areas of the skin may become necrotic and peel off in strips. Ears and tail may slough.

Sunburn, erysipelas, and sarcoptic mange have to be included in a differential diagnosis. The typical lesions confined to unpigmented or white areas of skin exposed to sunlight and a history of ingestion of a photodynamic agent or plants known to cause photosensitization will suggest a diagnosis.

Affected animals should be placed in darkened housing. Treatment is palliative, and control is by preventing access to photodynamic agents, grazing only at night, or keeping pigs indoors.

Frostbite

Frostbite can develop in pigs exposed to severely cold temperatures. Ear tips and other extremities are common sites for frostbite in all pigs, and the scrotum can be affected in boars. Boars affected by frostbite may also become infertile. Lesions develop due to the freezing of tissues, with skin turning white and then red initially and, in severe cases, black and necrotic thereafter. Frostbite arises in extensively raised swine housed without adequate shelter and dry bedding or during transport in extremely cold conditions.

Skin necrosis and trauma in piglets

Skin necrosis in piglets most frequently affects the knees, fetlocks, hocks, elbows, teats, coronets, and soles of the feet. Necrosis of the hocks, vulva, and tail is common in piglets with splayleg. Necrosis of the knees (carpus) is very common in weaker and smaller piglets in large litters or with sow agalactia or hypogalactia. In piglets, necrosis starts as small abraded areas often developing 12–24 hours after birth and reaching maximum severity in 7 days (Penny et al. 1971). Lesions are due to trauma from hard abrasive floors, especially rough concrete in farrowing crates. The alkaline pH of new concrete floors and slats may also affect older pigs. Necrosis of the soles of the feet can be caused by the abrasive surface of rusting wire mesh or metal floors.

Teat necrosis is most noticeable at 3 days of age (Stevens 1984) with the lesions consisting of blackish-brown scales or crusts that easily peel off, leaving a new, fresh wound. It usually affects the pectoral (first four) teats, resulting in blind, nonfunctional teats. The highest incidence was found in pigs on heated concrete floors, with decreasing incidence on expanded-metal floors, rubber mats, and plastic-coated wire mesh. A genetic basis for teat necrosis associated with sire lines was demonstrated, but nongenetic causes were more common than genetic causes (Stevens 1984). Females are most commonly affected. Besides environmental abrasion, estrogenic stimulation has been postulated as a risk factor. Necrosis of the tail starts at the base, usually encircling the whole tail, which becomes black and may slough. Cause is not known but may be related to risk factors already stated.

Control in piglets should be aimed at avoiding rough, wet contact surfaces and providing bedding or rubber mats in the creep area of farrowing crates.

Skin necrosis and trauma in older swine

In sows, skin necrosis is common on the shoulder, over the hip region, and on the side of the jaw and is generally due to a combination of pressure from lying for long periods on hard floors (both concrete and mesh) and poor body condition associated with rapid weight loss during lactation, limit feeding, or old age. The condition can occur in young sows after their first litter.

Skin necrosis of sows is best prevented by maintaining good body condition through appropriate feeding before and during lactation, using plastic-coated floors in farrowing crates, and encouraging sows to stand and exercise frequently.

Calluses

Calluses are epidermal hypertrophy with fibrosis of the skin over joints and bony prominences. They are seen mainly over the fetlocks, elbows, hocks, and tuber ischii.

A callus may become very large, hard, or fluctuant and may contain fluid that is termed an adventitious bursa. The bursa may become infected and result in subcutaneous abscesses. Pigs with leg weakness, foot lesions, or muscular weakness or that spend a lot of time lying down due to illness frequently develop callosities or bursitis.

Parakeratosis and nutritional diseases of skin

Swine parakeratosis is a nutrition-related metabolic disorder of growing pigs characterized by a generalized nonpruritic crusting dermatosis (Figure 17.11). The cause of this condition is considered to be complex, involving deficiencies of zinc and essential fatty acids or high levels of calcium, phytates, and other chelating agents that affect zinc absorption. Gastrointestinal diseases may also predispose to a more severe zinc deficiency and parakeratosis.

Early lesions (macules and papules) develop on the ventral surface of the abdomen, medial thighs, and distal parts of the legs (Figure 17.11). The lesions rapidly become covered with scales and then hard dry keratinized crusts that get progressively thicker and form deep fissures. The surface of the skin may be dry and rough, but moist brownish sebum, dirt, and debris accumulate in the fissures. In severe cases animals will have reduced growth rates and reduced appetite, diarrhea, and in some cases vomiting. Testicular development may be affected. Mortalities are rare.

The condition has to be differentiated from chronic sarcoptic mange, exudative epidermitis, and deficiencies of the B vitamins and iodine. A history of feeding a diet likely to be deficient in zinc and essential fatty acids, or including factors that may interfere with zinc absorption together with the characteristic lesions and their distribution, will suggest parakeratosis.

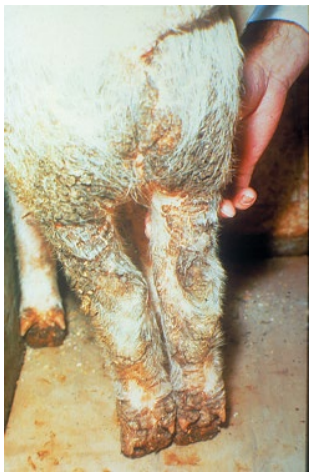


Figure 17.11 Parakeratosis.

Skin biopsy from animals early in disease course for histopathology to demonstrate typical and severe parakeratotic hyperkeratosis is valuable for confirming diagnosis. Serum alkaline phosphatase and zinc levels may be decreased in serum and liver. Response to supplementary zinc and essential fatty acids will support a diagnosis.

A host of nutrients can affect skin and hair health. Dietary levels or ratios of fat-soluble and B vitamins, microminerals, fatty acids, and amino acids all have impact on skin integrity and healthy appearance (see Chapter 67).

Congenital and hereditary diseases of skin

Pityriasis rosea

Pityriasis rosea in swine is the name used to describe a dermatitis with quite characteristic gross lesions. This condition is seen only in young swine and mainly on the ventral abdomen and inner thighs. The disease is self-limiting and has little clinical or pathologic similarity to pityriasis rosea in humans. Although the actual cause is unknown, the condition appears to be at least partially heritable; individuals that have had the condition themselves are more likely to produce affected progeny, and incidence may be higher in Landrace breed. Attempts to transmit the disease or demonstrate an infectious agent have failed. Pityriasis rosea has been reported in piglets derived by hysterectomy and reared in isolation.

The disease affects young pigs 3–14 weeks of age. Entire litters or only a few piglets in a litter may be affected. It begins as small erythematous papules on the skin of the abdomen and inner thighs. The papules are raised with a central crater and rapidly expand to produce a ring, or collarette, with a raised bright red periphery behind, which are scales. As the ring expands, the central area returns to normal. The rings coalesce as they expand to produce mosaic patterns. The hair is usually not lost and pruritus is not present (Figure 17.12). The condition usually lasts about 4 weeks, regressing slowly and leaving normal skin as lesions heal. Lesions may become infected with bacteria and resemble an exudative dermatitis. The condition has to be differentiated from ringworm, dermatosis vegetans, and swinepox.

Skin biopsy will show psoriasiform epidermal hyperplasia and superficial perivascular dermatitis. There is mild to moderate mucinous degeneration of the superficial dermis, and the predominant inflammatory cells are eosinophils and neutrophils. Parakeratotic hyperkeratosis is usually prominent, with an eosinophilic

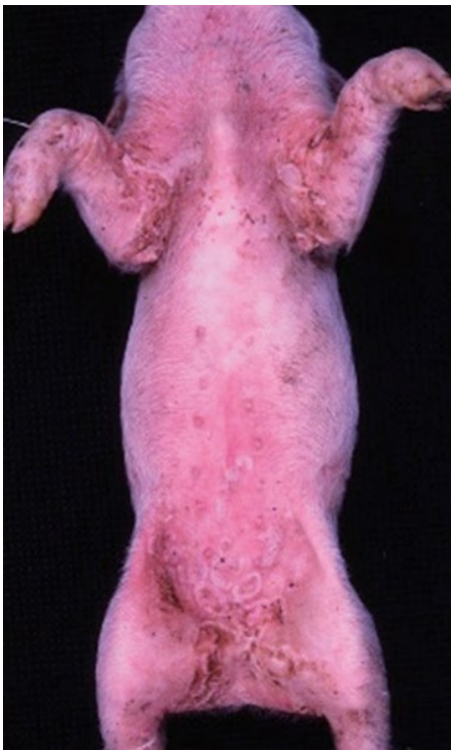


Figure 17.12 Pityriasis rosea.

inflammatory component not present in nutritional parakeratosis.

Treatment does not appear to affect the course of the disease. Good hygiene will reduce the chance of secondary infections, whereas overstocking with high humidity and high temperatures appears to increase the incidence. It may be best to cull breeding stock known to produce progeny that develop the condition from the breeding herd.

Dermatosis vegetans

Dermatosis vegetans is a hereditary and often congenital disease of swine due to a semilethal autosomal recessive factor believed to have originated in the Danish Landrace breed (Don et al. 1967). The condition occurs as an erythematous maculopapular dermatitis with lesions on the coronary band and hoof together with giant cell pneumonia. The main features of the condition are skin lesions, abnormalities of the feet, poor growth, and respiratory dysfunction. The skin lesions may be present at birth or, more commonly, develop within 2–3 weeks of birth. They first develop on the abdomen and inside the thighs as small (0.5–2.0 cm in diameter) raised pink swellings that enlarge rapidly. The lesions spread over the flanks and back of the pigs and become covered with yellowish-brown, brittle, papillomatous crusts that are easily removed, leaving a pink granular surface. The lesions become very thick with a hard, horny surface that develops cracks and fissures, giving a characteristic papillomatous appearance. Swine may die after 5–8 weeks, but some will survive and the skin lesions regress.

Lesions of the hoof are usually present at birth consisting of marked swelling and erythema over the coronary band of both the main and the accessory digits and covered with a yellowish-brown greasy exudate. The walls of the hooves are thickened with ridges and furrows parallel to the coronary band. The horn becomes discolored and blackened.

The condition has to be differentiated from pityriasis rosea, chronic exudative epidermitis, and vitamin deficiencies. The clinical appearance of the skin and hoof lesions seen in young pigs 2–3 weeks of age is characteristic. The very thick papillomatous crusts of the skin lesions are typical and, together with respiratory distress, will suggest a diagnosis of dermatosis vegetans. Skin biopsy reveals intraepidermal pustular dermatitis and microabscesses containing eosinophils and neutrophils, with parakeratotic hyperkeratosis. Older lesions show hyperplastic superficial perivascular dermatitis with multinucleated giant cells in the dermis (Percy and Hulland 1967, 1968).

There is no treatment for the condition, and prevention is aimed at detection and removal of breeding stock known to have produced affected progeny.

Epitheliogenesis imperfecta (aplasia cutis)

Epitheliogenesis imperfecta is an inherited congenital condition of both white and colored swine, caused by a simple autosomal recessive trait thought to result in a primary failure of embryonic ectodermal differentiation. The lesions appear as clearly demarcated discontinuities of the squamous epithelium of varying sizes and shapes but usually on the back, loins, or limbs. The condition may be seen in individual piglets or with a familial incidence in litters. The defect may also affect the dorsal and anterior ventral surface of the tongue with concurrent hydroureter and hydronephrosis (Jubb and Kennedy 1970). Lesions develop as large ulcers and frequently become infected; they may fail to heal or cause septicemia, which may lead to death (Figure 17.13).

Neoplastic diseases of skin

Tumors are reported relatively rarely in swine; however, a variety of neoplastic conditions have been reported, including lymphangioma, rhabdomyoma, papilloma, sweat gland adenoma, fibroma, and hemangioma.

Melanomas, the result of proliferation of melanoblasts, have been reported most commonly in the Duroc breed and are often found at birth. The tumors are frequently

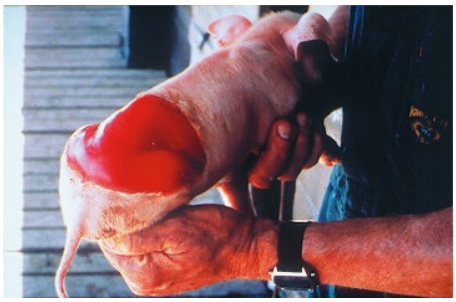


Figure 17.13 Epitheliogenesis imperfecta.

seen on the flank or mammary region but can occur at any location. The tumors are usually 1–4 cm in diameter, raised with an irregular black shiny surface. Metastases have been seen in lymph nodes, kidney, liver, lung, heart, brain, and skeletal muscle. Rhabdomyosarcomas have been reported as solitary or multiple tumors on piglets less than 1 week old in at least 25 piglets in a short period of time in one herd in the Netherlands, indicating a common, possibly genetic cause (Vos et al. 1993).

Swine papillomas occur as small fibrous polyps or wartlike lesions and have been observed on sows and growing pigs, especially around the neck, along the back, and on the ears. These lesions frequently bleed and can easily be removed under local anesthetic.

The feet, hoof, and claw

Introduction

Lesions of feet (hooves and claws) are frequently associated with lameness, which can adversely affect growth rates and reproductive performance due to early culling in the breeding herd as well as having a significant impact on welfare (Allerson et al. 2008; Anil et al. 2005; Bradley et al. 2008; Penny et al. 1963, 1965). Reports suggest that the prevalence and severity of lesions of the feet and claws have increased over the last half century with production trends toward increased population density in confined housing with solid or slotted floors, particularly those of concrete. More recently, increased foot lesions and lameness are reported in production systems transitioning from individually housed sows back to group housing systems (Johnston and Li, 2013). Foot lesions have been reported in suckling piglets (Zoric et al. 2009), post weaned pigs (Gillman et al. 2009), and finisher pigs (Mouttotou et al. 1997, 1999) as well as sows and boars (Anil et al. 2007). Factors influencing the prevalence or severity of lesions include foot, claw, and skeletal conformation, housing and floor surfaces, nutrition, population density and commingling, and infectious agents.

Structure and function of the hoof and claw

The pig is tetradactylous, having two large weight-bearing digits (third and fourth) and two non-weight-bearing accessory digits (second and fifth digits or "dewclaws"). The first digit is absent in the pig, the third digit is medial, and the fourth is lateral on each foot. The term "foot" usually refers to the entire region from the toe to the top of the dewclaws. Each digit includes the claw or hoof, which is the keratinized horny tissue of the hard wall, hard sole, and soft heel. Each claw covers the third phalanx and the distal part of the second phalanx. The accessory digits are caudal to the larger digits and have a full complement of phalanges. The volar surface of the claws consists of the large and prominent heel (which houses the digital cushion), the sole, and the wall. The sole in the pig covers a relatively small area, and a nonpigmented soft wall (white line [WL]) forms the junction between the wall of the claw and the sole. The lateral digit is usually larger than the medial digit on both the front and hind feet. The disparity in size is usually more prominent on the hind feet.

A cross section of the claw consists of bone of the phalanx, subcutis, corium or dermis, vascular basement membrane, and epidermis. The corium located just below the epidermis forms the supportive connective tissue layer for the epidermis, containing blood vessels and nerves. From this layer nutrients and hormones are provided to the stratum basale or germinal layer for the production of the epidermal cells. All distal layers of the epidermis are derived from these cells by a process of proliferation and differentiation. The corium and its basement membrane are critical structures for keratin or soft/hard horn formation. Keratins are produced by complex processes of differentiation of the epidermal cells. Formation and biochemical binding of keratin proteins and synthesis and exocytosis of intracellular cementing substance are the hallmarks of keratinization (Tomlinson et al. 2004).

The corium is covered by the densely arranged cells of the stratum basale, which are pushed into the next layer and enter the process of differentiation to make up the stratum spinosum. Toward the end of differentiation, basophilic dense keratohyalin granules accumulate in the cells, forming the stratum granulosum. It is this layer that forms the border of cornification in which the cells die (cornify) and turn into horn cells, which is known as the stratum corneum. The process is dependent on an appropriate supply of nutrients, including vitamins, minerals, and trace elements. These nutrients are essential for the integrity of hoof horn. Decreasing nutrient supply to keratinizing epidermal cells can lead to inferior horn quality and increase its susceptibility to chemical, physical, or microbial damage from the environment, which may result in clinical lesions of the claws and lameness.

Classification, pathology, and scoring of foot lesions

Foot and claw lesions can involve the heel, sole, WL, wall, and accessory digits. Virtually anything that predisposes to breach of integrity of parts of the foot and claw will increase the risk of pododermatitis, arthritis, osteomyelitis, or cellulitis. Anil et al. (2007) generally characterized claw lesions as erosion, cracks, and overgrowths.

Heel lesions start as dark discolored areas or bruises under the volar surface, followed by erosions usually seen as a rough "ragged" lesion with underlying hemorrhage. Ulceration of the heel may follow. Continuous trauma can result in hyperkeratinization, excessive granulation, and necrosis (Penny et al. 1963), often extending to the interdigital cleft. Bradley et al. (2008) described heel erosion occurring in a circular pattern resembling tissue degeneration or irritation with cracks resembling a cut or tissue separation crossing the heel diagonally from the WL to the rear of the heel.

Sole lesions also occur as bruising with dark brown to red areas of hemorrhage under the surface, most frequently along the junction of the heel and sole. Hyperkeratinization of the sole can be a sequel to erosion and ulceration.

WL lesions of the claw, either axial or abaxial, start as hard tissue wear (erosion) and separation between the hard and soft tissue of the hoof wall and sole or heel or both (Bradley et al. 2008; Penny et al. 1965). The lesions can cause extensive separation with impaction with dirt, feces, and foreign material often resulting in deformity of the claw and separation of the wall. WL lesions provide a site for infection to enter the hoof and corium.

Cracks of the wall can be vertical, starting at the WL (false sand cracks) or at the coronary band (true sand cracks). Horizontal cracks can also occur. False sand cracks can vary in severity from a fine crack to a deep fissure with necrotic edges (Penny et al. 1963).

Coronary band abscesses (bush foot) are most likely an infection of the laminae with an abscess-like lesion along the coronary band. The area above the coronary band becomes very swollen, and a granulating sinus develops, producing a purulent discharge. Infection may also involve deeper areas of the hoof and joints of the foot, causing severe lameness. Accessory digit (dewclaw) lesions, more common in adult sows and boars, can involve loss of the horn with hemorrhage and infection of the underlying tissue or lengthening of the digit (overgrowth).

Da Silva et al. (2010) found a number of claw lesions to be highly correlated with inflammation of the corium in lame sows. Although the pathology and etiology of inflammation of the corium is not the same for each lesion type, there is potential for claw lesions to contribute to additional inflammation of the corium. Due to the large number of nerves associated with the corium, inflammation is likely to result in pain and lameness. Indeed, the histologic changes in sow claws are consistent with laminitis in other species (Newman et al. 2015; Varagka et al. 2016a).

Deen and Winders (2008) have suggested using a consistent scoring system of 0–3 (i.e. none, mild, moderate, severe) for the types of pathology. First, score the heel overgrowth and erosion (HOE), which is a common lesion ranging from slight overgrowth and/or erosion of the soft heel tissue to severe overgrowth and cracks. Next, score heel–sole crack (HSC) lesions at the junction between the heel and the sole with severe lesions involving all or most of the junction. A third score is for WL lesion involving separation along the WL in the juncture of the claw wall. Fourth, score the cracked wall horizontal (CWH), cracks horizontally along the claw wall with severe lesions involving multiple or deep cracks. A fifth score is for the cracked wall vertical (CWV), vertical cracks in the claw wall and severe lesions involving multiple or deep cracks. Sixth, score toe (T) lesions involving excess lengthening of one or more claws where severe lesions significantly affect gait. Finally, score dewclaw (DC) involving moderate or severe lengthening of the claw (score 1 or 2) or torn or missing claw (3).

Prevalence of foot and claw lesions

Foot lesions are common, reported in all age groups of swine. The prevalence in suckling piglets of sole bruising ranged from 49.4 to 100% and sole erosion from 15.5 to 49.1% in the studies of Mouttotou and Green (1997) and KilBride et al. (2009), respectively. Zoric et al. (2004) found 87% of suckling piglets with sole bruising at 3–10 days of age and 39% still affected at 17 days. Prevalence in weaner pigs has been reported as 50.2% with at least one digit affected (Mouttotou et al. 1998) and an overall prevalence of 39.6% reported by Gillman et al. (2009).

In two studies the prevalence of lesions in finisher pigs were reported as 65.1 and 93.8% of pigs with at least one lesion by Penny et al. (1963) and Mouttotou et al. (1997), respectively. Lesions in weaners and finishers were similar and included heel, sole, and toe bruising and erosions, heel flaps, WL lesions, wall separation, and false sand cracks.

The early reports (Hogg 1952; Osborne 1950; Penny et al. 1963) noted a high prevalence of foot lesions in sows. A more recent study found that more than 96% of culled sows from loose housing and 80% from confined housing had at least one lesion (Gjein and Larssen 1995a). Anil et al. (2007) found that out of 184 gestating sows, 88.6% had at least one wall lesion and 86.4% had one heel lesion. Lesions between the junction of the heel and sole were seen in 66.3% of sows, and 60.9% had WL

lesions. Lesions were more severe on the forelimbs, and a higher proportion of sows had lesions on the lateral claws than the medial claws.

Surveys are prone to bias. The reported prevalence from surveys and studies is heavily dependent on scoring system used and the study design and definitions applied. Ultimately, a high rate of lesions is likely the result of the cumulative effects of various flooring types and management practices experienced over the lifetime of the pigs surveyed.

Association between foot lesions and lameness

Lameness in sows is an important welfare issue that has many risk factors, including group housing. Pluym et al. (2017) performed a longitudinal study in 15 randomly selected herds to investigate the incidence of sow lameness and risk factors present within the first days of group housing. They found a mean lameness incidence of 13.1% and that increased floor area decreased risk of lameness, while floor characteristics and degree of sow aggression did not influence development of lameness.

The presence of a lesion does not equate to the presence of lameness or pain, since not all lesions result in perceptible lameness in pigs. Studies of lesions in sows (Hogg 1952; Osborne 1950; Penny et al. 1963) reported an association between the severity of claw lesions and presence of lameness, especially in sows and boars. Lameness is more likely when lesions are visibly infected or when lesions (heel erosions, WL cracks, infected vertical cracks, abscesses of the coronary band) are classified as severe.

Anil et al. (2007, 2008) found a significant association between lameness and WL lesions in sows and later reported that lesions of the heel as well as of the WL were associated with lameness. In a study of the prevalence of lameness and claw lesions in two commercial herds where the prevalence was relatively low (<4% had serious lameness), the most common lesions related to lameness were heel erosions and overgrowth of dewclaws. WL and sidewall lesions were not a feature of lameness in these herds (Sonderman et al. 2009).

Early recognition and assessment of lameness in sows is important for early intervention and treatment. However Anil et al. (2008) showed that clinical assessment in correctly grading lameness scores was difficult for mild cases and non-lame sows. Accurate assessment of lameness requires experience and specific training. Heinonen et al. (2013) reported within herd incidence of all lameness causes of 8.8–16.8%, to which the hoof and claw contribute only a portion. Lameness due to other causes may predispose animals to skin, hoof, and claw lesions by restricted motion or postural effects.

Foot lesions in sows have also been correlated with performance and behavior in sows, with reduced numbers of pigs weaned per litter for sows with outer hoof wall cracks and lighter weaning weights for sows with elongated toes (Fitzgerald et al., 2012).

Factors affecting prevalence of foot and claw lesions

Research into the prevalence and severity of foot and claw lesions has identified a number of associated factors including claw conformation, environment (in particular housing type and floor type), nutrition, and infectious agents.

Claw conformation

The lateral claw is generally larger than the medial claw, particularly in hind limbs, and lesions tend to be more prevalent and/or more severe on the lateral claw, particularly of the hind limbs (Penny et al. 1963). They surmised that the larger lateral claw somewhat protected the smaller medial claw, such that the medial claws take less weight and hence suffers less trauma, while the lateral claw is subject to more trauma due to its size and position. Subsequent reports are in general agreement with these earlier findings (Anil et al. 2007, 2009; Bradley et al. 2008; Gjein and Larssen 1995b; Van Amstel and Doherty 2010).

Anil et al. (2007) found lesions more severe on forelimbs than on hind limbs and that the proportion of sows with lesions on the lateral claws was greater than the proportion of sows with lesions on the medial claw. In discussion and with reference to other studies, Anil et al. (2007) considered that weight distribution of sows may be important in determining the development of lesions on different claws and different limbs (Kroneman et al. 1993). Hoof lesions may be more common on lateral claws because of a greater weight-bearing surface than medial claws (Tubbs 1988). More than 75% of the weight of the pig is born by the lateral digit, and 80% of the injuries affect these digits (Webb 1984). Kroneman et al. (1993) suggested that the strength of different parts of the foot varies, whereby junctions between hard and soft areas may be more susceptible to injury. Maximum weight is born by the heel bulb, followed by the junction between the heel bulb and abaxial hoof wall of the lateral digit. The tip of the toe is the greatest weight-bearing region on the medial digit.

Amstel et al. (2009) found that the front feet have better weight-bearing stability than the rear feet with the rear medial claw least stable. It was suggested that this may result in more weight being transferred to the more stable rear lateral claw, which may explain the higher reported incidence of lesions observed in that claw.

The disparity in claw size in piglets was suspected in part to be hereditary (Penny et al. 1963) and was considered to have low heritability by others. Jörgensen and Andersen (2000) found values of 0.13 and 0.19 in Landrace and Large White pigs, respectively. Tarres et al. (2006) examined sow longevity and feet and leg scores in Large White sows in Swiss herds and concluded that culling on extreme feet and leg scores from 1999 to 2003 has contributed to the improvement of sow longevity in these herds.

Type of flooring

The association between housing and type of flooring and the prevalence of foot/claw lesions has been the subject of many reports in suckling piglets, weaners, finishers, and sows. Foot lesions in suckling piglets are more likely to occur where farrowing crate floors and creep areas are solid, especially solid concrete (Mouttotou et al. 1999; Zoric et al. 2004, 2009). By reducing the roughness and abrasiveness of farrowing crate floors or by covering with sufficient straw, the prevalence and severity of lesions in piglets can be reduced (Zoric et al. 2009). Sole bruising increases in prevalence in piglets on slatted floors compared with solid concrete floors even with bedding. Slatted floors increase the risk of sole erosions (KilBride et al. 2009).

Gillman et al. (2009) reported the prevalence of lesions in weaners up to 14 weeks old and held on solid, totally or partially slatted floors, or outside on a soil base (all with bedding). They found that toe erosion was positively associated with deep bedding and that deep bedding on a soil base was negatively associated with the prevalence of heel and sole erosion. Heel flaps and heel–sole bruising were also associated with slatted floors.

Claw lesions, especially wall cracks and WL cracks, were more common in finisher pigs kept on solid concrete floors (Osborne 1950). Finishers kept on straw-bedded floors had fewer sole–heel erosions and heel flaps but more WL lesions, false sand cracks, wall separation, and toe erosion compared with pigs on bare concrete. Lesions increased when floors were partially or fully slatted (Mouttotou et al. 1999). Smith and Morgan (1998) found that finisher pigs kept on totally or partially slatted concrete had claw lesions on both floor types. The prevalence of lesions rose as floors became more abrasive. The prevalence on good quality totally slatted floors (low to moderate values of abrasion, slipperiness, slat edge, slat gap, and nature of slat edge) was lower than on floors with unsuitable slat quality. The authors recommended that the edge of slats should be rounded and smooth, a slat width of 13–14 cm, a void width of 1.8–2.4 cm, and a total linear edge of 10.0–17.7 m/pig. The effect of floor type on claw health in group-housed sows was recently reviewed (Pluym et al. 2013; Vermeer 2014).

The prevalence of claw lesions in pregnant sows reportedly is higher in loose housing systems compared with sows confined in stalls, especially where the floors are partially or fully slatted. However deep litter based on straw reduces the incidence and severity of lesions, while poor floor hygiene and high stocking density increase the severity of claw lesions (Gjein and Larssen 1995b; Holmgren et al. 2000).

Roughness of floors contributes to a higher incidence of claw lesions and increased lameness. The pH of concrete from 7.4 to 8.3 did not appear to influence prevalence; however, dirty wet conditions may soften the volar aspect of the feet, making them more susceptible to trauma (Penny et al. 1965). All types of claw lesions tend to be higher in sows kept in loose housing with fully slatted floors compared with sows confined to fully slatted stalls (Anil et al. 2007). The use of electronic feeders, which may contribute to aggression on entry to the feeder, may further increase prevalence of claw and foot lesions. Increase body weight has been associated with increased wall lesions, and higher backfat was associated with more heel lesions but less overgrowth of heels (Anil et al. 2007).

Nutrition and hoof horn integrity

In a review of keratins and hoof horn development in the bovine, Tomlinson et al. (2004) stated that keratinization of hoof epidermis is controlled and modulated by a variety of bioactive molecules and hormones dependent on an appropriate supply of nutrients including vitamins, minerals, and trace elements. The quality and functional integrity of hoof horn are dependent on nutrient flow to the epidermal cells. Hormones are important in horn growth with insulin, epidermal growth factor, prolactin, and glucocorticoids, all with impact on nutrient flow.

Nutrients required for horn growth include amino acids (cysteine, histidine, methionine), minerals (calcium, zinc, copper, selenium, manganese), and vitamins (A, D, E, and biotin). Except for biotin, very little peer-reviewed literature related to nutritional requirements for hoof growth in the pig is available; hence present knowledge is often an extrapolation from research in cattle and horses. Subsequent studies (Misir and Blair 1986; Webb et al. 1984; Simmins and Brooks 1988) also reported the benefit of biotin-supplemented rations in relation to hoof lesions, hoof wall hardening, and foot pad resilience.

Amino acids play key roles in establishing the structural integrity of the keratinocyte. Calcium, zinc, and copper are all important in the keratinization and cornification process. Zinc is important for tissue healing and, as with copper, strength and elasticity of the hoof tissue. Zinc supplementation in dairy cows has shown a reduction in cases of foot rot, heel cracks, interdigital dermatitis, and laminitis (Moore et al. 1989). Also in cattle, copper

deficiency can result in heel cracks, foot rot, and sole abscesses. Selenium is an antioxidant and may contribute to the protection and maintenance of physiological function of the lipid-rich intracellular cementing substance (Tomlinson et al. 2004). Excessive selenium and selenium toxicity will result in inferior hoof horn, resulting in lameness and deformed claws. The form or salt of a supplemented mineral may have some physiologic impact. Varagka et al. (2016b) reported that partial substitution of the inorganic zinc, copper, and manganese in sows' diet with their chelated complexes provided a comparative advantage against a conventional inorganic mineral source diet. Manganese is essential for skeletal and tendon development, joint, and cartilage strength. Vitamins A, D, and E all play an integral role in developing the structure and quality of keratinized horn tissue. Vitamin D is also important in calcium metabolism.

The role of biotin for hoof and claw integrity was extensively researched in the pig (Hill 1992). Tomlinson et al. (2004) considered biotin the vitamin of greatest importance to the keratinization process and in the integrity of keratinized tissues (skin, hair, claws, and footpads). Biotin is essential for the formation of the complex lipid molecules in the intracellular cementing substance (Mülling et al. 1999). Hill (1992) cites a number of early experiments involving individual biotin deficiencies in pigs resulting in alopecia, dermatosis, cracked hooves, and lameness. Penny et al. (1980) reported using biotin supplementation in a herd of 116 sows and gilts with severe lameness problems and found no improvement in animals already affected, but gilts entering the herd as replacements and receiving biotin supplement showed significantly fewer lesions of heel and WL. The authors suggested that biotin was an essential vitamin for young stock entering the breeding herd as the hoof horn would be better able to withstand the trauma of the environment. Kornegay (1986) reviewed evidence that biotin increases the hardness of the hoof wall and was of the opinion that numerous environmental and nutritional factors may influence the occurrence of biotin deficiency in swine and alter biotin required in the diet. Watkins et al. (1991) considered that the discrepancy in the efficiency of biotin supplementation seen in the literature most likely reflects the wide range of environmental, nutritional, and management practices used in the swine industry. Availability of biotin for the pig may vary significantly depending on the type of grain and storage conditions. Presence of mold can reduce availability of biotin in corn (Hamilton and Veum 1984). Biotin in grains has been reported to be poorly absorbed in the pig, and diets based on wheat or barley may not contain adequate levels of bioavailable biotin (Misir and Blair 1986). Other factors such as age, lactation, antibiotic medication, enteric disease, and access to feces (coprophagic pigs) may also influence biotin levels in the pig.

Overgrown hooves

The length and the vertical angle of the wall of the hoof are a balance between growth and wear of the hard and soft tissues of the hoof as well as foot and leg conformation. Floor type (abrasiveness) and level of physical activity influenced the rate of wear; hence overgrowth of hoof can occur in pigs housed on soft nonabrasive surfaces. Seedstock genetically selected to avoid poor foot and leg conformation is common.

References

Allerson M, Deen J, Ward TL. 2008. If sows were cows. In Proceedings of the American Association of Swine Veterinarians Annual Meeting, pp. 177–180.

Amstel SV, Ward T, Winders M, et al. 2009. Claw size in cull sows, quantification of a potential factor in lameness and culling. In Proceedings of the American Association of Swine Veterinarians Annual Meeting, pp. 375–476.

Amstutz JE, 1975. Heat stroke, sunburn, and photosensitization. In *Diseases of Swine*, 4th ed. Ames, IA: Iowa State University Press, p. 1014.

Andresen LO, Wegener HC, Bille-Hansen V. 1993. *Microb Pathog* 15:217–225.

Andrews JJ. 1979. *Vet Pathol* 16:432–437.

Anil SS, Anil L, Deen J. 2005. *J Am Vet Med Assoc* 226:956–961.

Anil SS, Anil L, Deen J, et al. 2007. *J Swine Health Prod* 15:78–83.

Anil SS, Anil L, Deen J. 2008. Association between claw lesions and sow lameness. In Proceedings of the International Pig Veterinary Society Congress, p. 282.

Arora BM, Das SC, Patgiri GP. 1979. *Indian Vet J* 56:791–793.

Arruda BL, Burrough E, Schwartz K, et al. 2015. Lesion characterization and exploration of infectious etiologies in growing pigs with ear tip necrosis. In Proceedings of AAVLD, San Diego.

Blood DC, Jubb KV. 1957. *Aust Vet J* 33:126–127.

Bradley CL, Maxwell CV, Johnson ZB, et al. 2008. The effect of parity and body weight on different claw measurements in the University of Arkansas sow herd over an 18 month period of time. In Proceedings of the International Pig Veterinary Society Congress, p. 283.

Busch ME, Nielsen EO, Hassing AG, et al. 2002. Risk factors for ear necrosis in growing-finishing pigs. In Proceedings of IPVS. Ames, Iowa.

Cameron RDA. 1984. Skin Diseases of the Pig. *Univ Sydney Post-Grad Found Vet Sci Proc Vet Rev* 23:9.

Cameron RDA. 1995. *Pork J* 16:28.

Carbrey EA, Stewart WC, Young SH, et al. 1966. *J Am Vet Med Assoc* 149:23–30.

Clegg SR, Sullivan LE, Bell J, et al. 2016. *Res Vet Sci* 104(February):64–70.
Connole MD, Baynes ID. 1966. *Aust Vet J* 42:19–24.
Da Silva A, Deen J, Ossent P, et al. 2010. In Proceedings of American Association of Swine Veterinarians Annual Meeting, p. 425.
Deen J, Winders M. 2008. Development of a claw lesion scoring guide for swine. In Proceedings of the International Pig Veterinary Society Congress, p. 579.
Don JT, Loosmore RM, Saunders CN. 1967. *Vet Rec* 80:292–297.
Fitzgerald RF, Stalder KJ, Karriker LA, et al. 2012. *Livest Sci* 145(1–3):230–38.
Fraser CM, Bergeron JA, Mays A, et al. 1991. *The Merck Veterinary Manual*, 7th ed. Merck & Co. Inc.: Rahway, NJ, p. 308.
Gillman CE, KilBride AL, Ossent P, et al. 2009. *Prev Vet Med* 91:146–152.
Gjein H, Larssen RB. 1995a. *Acta Vet Scand* 36:433–442.
Gjein H, Larssen RB. 1995b. *Acta Vet Scand* 36:443–450.
Hamilton CR, Veum TL. 1984. *J Anim Sci* 59:151–157.
Heinonen M, Peltoniemi O, Valros A. 2013. *Livest Sci* 156(1–3):2–9.
Hélie P, Drolet R, Germain MC, et al. 1995. *Can Vet J* 36:150–154.
Higgins RJ. 1993. Glomerulonephropathy syndrome. *Pig Vet J* 31:160–163.
Hill MA. 1992. Skeletal system and feed. In *Diseases of Swine*, 7th ed. Ames, IA: Blackwell Publishing, pp. 163–195.
Hogg AH. 1952. *Vet Rec* 64:39–42.
Holmgren N, Eliasson-Selling L, Lundecheim N. 2000. Claw and leg injuries in group housed dry sows. In Proceedings of the International Pig Veterinary Society Congress, p. 352.
Hungerford TG. 1990. *Diseases of Livestock*, 9th ed. Sydney: McGraw-Hill, p. 678.
Johnston LJ, Li YZ. 2013. *J Anim Sci* 91(12):5937–5945.
Jörgensen B, Andersen S. 2000. *Anim Sci* 71:427–434.
Jubb KVF, Kennedy PC. 1970. *Pathology of Domestic Animals*, Vol. 2. New York: Academic Press, p. 591.
Jubb KVF, Kennedy PC, Palmer N. 1985. *Pathology of Domestic Animals*, Vol. 1, 3rd ed. New York: Academic Press, p. 110.
Karlsson F, Klitgaard K, Jensen TK. 2014. *Vet Microbiol* 171(1–2):122–131.
KilBride AL, Gillman CE, Ossent P, et al. 2009. *BMC Vet Res* 5:31.
Kornegay ET. 1986. *Livest Prod Sci* 14:65–89.
Kresse JI, Taylor WD, Stewart WW, et al. 1985. *Vet Microbiol* 10:525–531.
Kroneman A, Vellenga L, Can de Wilt FJ, et al. 1993. *Vet Q* 15:26–29.
Laperle A, Morin M, Sauvageau R. 1988. Acute dermatitis in feeder pigs administered tiamulin. *Proc Congr Int Pig Vet Soc* 10:250.
Marcarian NQ, Calhoun ML. 1966. *Am J Vet Res* 27:765–772.
McClymont GL, Wynne KN. 1955. *Aust Vet J* 31:112.
McPherson EA. 1956. *Vet Rec* 68:710–711.
Meyer W, Schwartz R, Neurand K. 1978. *Curr Probl Dermatol* 7:39–52.
Misir R, Blair R. 1986. *Res Vet Sci* 40:212–218.
Moore CL, Walker PM, Winter JR, et al. 1989. Zinc methionine supplementation for dairy cows. *Trans Illinois State Acad Sci* 82:99–108.
Mouttotou N, Green LE. 1997. *Vet Rec* 145:160–165.
Mouttotou N, Green LE, Hatchell FM, et al. 1997. *Vet Rec* 141:115–120.
Mouttotou N, Hatchell FM, Green LE. 1998. The prevalence and distribution of foot lesions in weaners and their association with floor characteristics. In Proceedings of the International Pig Veterinary Society Congress, p. 201.
Mouttotou N, Hatchell FM, Green LE. 1999. *Vet Rec* 144:629–632.
Mowafy M, Cassens RG. 1975. *J Anim Sci* 41:1281–1290.
Mülling C, Bragulla H, Reese S, et al. 1999. *Anat Histol Embryol* 28:103–108.
Newman SJ, Rohrbach BW, Wilson ME, et al. 2015. *J Swine Health Prod* 23(2):91–96.
Opriessnig T, Langohr I. 2013. *Vet Pathol* 50(1):23–38.
Osborne HG. 1950. *Aust Vet J* 26:316–317.
Palinsky R, Pineyro P, Shang P, et al. 2016. *J Virol* 91(1).
Park J, Friendship RM, Poljak Z, et al. 2013. *Can Vet J* 54: 491–495.
Penny RHC, Osborne AD, Wright AI. 1963. *Vet Rec* 75:1225–1235.
Penny RHC, Osborne AD, Wright AI. 1965. *Vet Rec* 77:1101–1108.
Penny RHC, Edwards MJ, Mulley R. 1971. *Aust Vet J* 47:529–537.
Penny RHC, Cameron RDA, Johnson S, et al. 1980. *Vet Rec* 107:350–351.
Percy DH, Hulland TJ. 1967. *Can Vet J* 8:3–9.
Percy DH, Hulland TJ. 1968. *Pathol Vet* 5:419–428.
Pluym L, van Nuffel A, Maes D. 2013. *Livest Sci* 56(1–3):36–43.
Pluym LM, Maes D, Van Weyenberg S, et al. 2017. *Vet J* 220:28–33.
Pringle M, Fellstrom C. 2010. *Vet Microb* 142:461–463.
Reynolds IM, Miner PW, Smith RE. 1968. *J Am Vet Med Assoc* 152:182–186.
Segales J, Barcellos D, Alfieri A, et al. 2016. *Vet Pathol* 54:11–21.
Simmins PH, Brooks PH. 1988. Supplementary biotin for sows:effect on claw integrity. *Vet Rec* 122(18):431–5.
Smith WJ, Morgan M. 1998. Claw lesions their relationship with the floor surface. In Proceedings of the International Pig Veterinary Society Congress, pp. 206–207.
Smith WJ, Thomson JR, Done S. 1993. *Vet Rec* 132:42.
Sonderman J, Anil SS, Deen J, et al. 2009. Lameness prevalence and claw lesions in two commercial sow herds. In Proceedings of American Association of Swine Veterinarians Annual Meeting, pp. 285–287.
Stevens RWC. 1984. *Pig News Info* 5:19–22.

Tarres J, Bidanel J, Hofer A, et al. 2006. *J Anim Sci* 84:2914–2924.
Thibault S, Drolet R, Germain MC, et al. 1998. *Vet Pathol* 35:108–116.
Tomlinson DJ, Mülling CH, Fakler TM. 2004. *J Dairy Sci* 87:797–809.
Tubbs RC. 1988. *J Vet Med* 83:610–616.
Van Amstel S, Doherty T. 2010. *J Swine Health Prod* 18(5):239–243.
Varagka N, Lisgara M, Skampardonis V, et al. 2016a. *J Swine Health Prod* 24(2):72–80.
Varagka N, Lisgara M, Skampardonis V, et al. 2016b. *Porcine Health Manage* 2:26.
Vermeer H. 2014. *CAB Rev Perspect Agric Vet Sci Nutr Nat Resour* 9(15):1–7.
Vos JH, Borst GHA, de las Mulas JM, et al. 1993. *Vet Pathol* 30:271–279.
Watkins KL, Southern LL, Miller JE. 1991. *J Anim Sci* 69:201–206.
Webb NG. 1984. *J Agric Eng Res* 30:71–80.
Webb NG, Penny RHC, Johnston AM. 1984. *Vet Rec* 114:185–189.
Wellenberg GJ, Stockhofe-Zurwieden N, de Jong MF, et al. 2004. *Vet Microbiol* 99:203–214.
White M, Higgins RJ. 1993. *Vet Rec* 132:199.
Zoric M, Sjolund M, Persson M, et al. 2004. *J Vet Med B Infect Dis Vet Public Health* 51:278–284.
Zoric M, Nilsson E, Lundeheim N, et al. 2009. *Acta Vet Scand* 51:23.

18

Mammary System

Chantal Farmer, Dominiek Maes, and Olli Peltoniemi

Mammary gland anatomy

The microscopic and macroscopic anatomy and morphology of the porcine mammary gland was described by Barone (1978), Schummer et al. (1981), Calhoun and Stinson (1987), and Balzani et al. (2016b). The 12–18 mammary glands of swine are located in 2 parallel rows along the ventral body wall (Labroue et al. 2001), and the Meishan breed can have up to 22 mammary glands. Data from France indicates that in 2010, 65% of purebred sows had 16 or more functional teats compared with 18% in 2002. As suggested by Muirhead (1991), boars and gilts retained for breeding should have 14 well-placed normal teats to provide proper teat presentation and accessibility to the piglets since poor teat placement is a major reason for failure of sows to rear all their piglets. In fact, the number of teats has become one limiting factor for productivity with the current hyperprolific gilt lines (Rozeboom 2015). The cranial and posterior teats are generally more distant from each other compared with the middle teats, which in turn are located further away from the midline than the cranial and posterior teats (Balzani et al. 2016b).

The glands (two rows of two thoracic, four abdominal, and one inguinal) each normally have one teat (nipple) with two separate teat canals. When the teat orifice is not visible (inverted teat), it has a 50% chance of remaining blind. Functional supernumerary smaller teats can also be found. Paired vestigial nonfunctional accessory teats, not connected to glandular tissue, may also occur (Molenat and Thibeault 1977; Labroue et al. 2001).

In the nulliparous sow, the mammary gland consists of cell buds distributed among fat and connective tissue, whereas in the lactating gland, the connective tissue is largely displaced by glandular parenchyma. Mammary glands of the lactating sow are composed of tubuloalveolar tissue with the secretory units arranged in lobules. The lobules are lined by epithelial cells (lactocytes), which synthesize milk and are connected by a nonsecreting duct system to an ostium found on the teat. There are usually two complete gland systems within each mammary gland of the pig. The glandular tissue of one system usually interdigitates with the other, but the components of the two systems are independent, emerging as two orifices within each nipple (Figure 18.1). There is no muscular sphincter around the teat orifice; therefore intramammary treatment by way of the teat opening is not feasible. Diagnostic imaging by real-time ultrasound scanning allows visualization of the different tissues of the mammary gland, such as skin, subcutaneous fat layer, parenchyma, and underlying tissues (Björkman et al. 2017).

The arterial, venous, and lymphatic circulation is provided on each side of the ventral midline by a network that extends longitudinally from the axillary to the inguinal regions (Barone 1978; Schummer et al. 1981; Lignereux et al. 1996). Moreover, in swine there is a venous anastomosis between the right and left mammary gland of each pair of glands. Nerve supply to the cranial mammary glands differs from that to the inguinal glands. Cranial mammary glands receive their innervation from the last eight or nine thoracic nerves, while inguinal mammary glands receive their innervation mainly from the pudendal nerve (Gandhi and Getty 1969a,b; Ghoshal 1975). A more complete anatomical and histological description of the porcine mammary gland can be found in the seventh edition of *Diseases of Swine* (Smith et al. 1992).

Mammogenesis

Mammary growth is a major determinant of potential sow milk yield. It starts in fetal stage but occurs mainly postnatally in three periods, namely, from approximately 90 days until puberty, in the last third of gestation, and during lactation.

The mammary glands of newborn piglets have a poorly developed duct system and are largely composed of

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

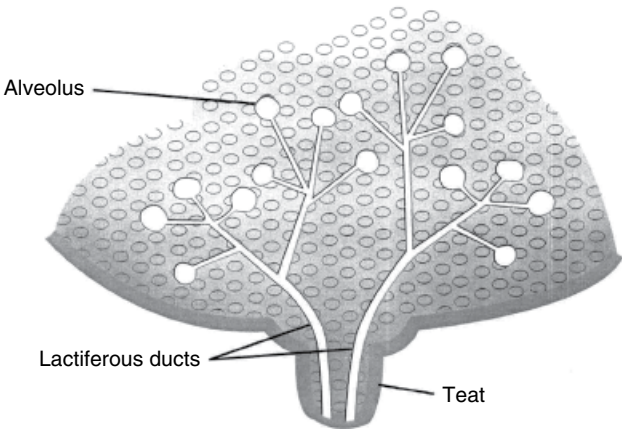


Figure 18.1 Structure of the sows' udder. *Source:* Adapted from Delouis (1986).

subcutaneous stromal tissue (Hughes and Varley 1980). Accumulation of mammary tissue and mammary DNA (which is indicative of cell number) is slow until 90 days of age, at which time the rate of accretion of mammary tissue and DNA increases four- to sixfold (Sorensen et al. 2002). By the time the gilt is mated, the mammary gland is still very small, but it consists of an extensive duct system with various bud-like outgrowths (Turner 1952).

In pregnant gilts, almost all accumulation of mammary tissue and DNA takes place in the last third of gestation (Hacker and Hill 1972; Kensinger et al. 1982; Sorensen et al. 2002; Ji et al. 2006). Wet weight of the total mammary gland increases approximately 10-fold between days 45 and 112 of gestation (Ji et al. 2006). The mammary glands undergo major histological changes as the adipose and stromal tissues are replaced by lobuloalveolar tissue to become the milk secretory apparatus (Hacker and Hill 1972; Kensinger et al. 1982). The composition of mammary tissue shifts from a high lipid content to a high protein content between early and late gestation (Ji et al. 2006); this shift is reported to be around day 90 based on DNA accretion (Sorensen et al. 2002) or day 75 based on protein accretion (Ji et al. 2006). This breakpoint is undoubtedly related to the increases in circulating concentrations of mammogenic hormones such as estrogen, relaxin, and prolactin (Ji et al. 2006). Mammary growth in pregnancy is affected by anatomical location, the largest glands generally being the middle (third, fourth, and fifth pairs) and the smallest ones being the posterior (sixth, seventh, and eighth pairs) glands (Ji et al. 2006).

Mammary development continues during lactation. Average weight of suckled glands increases linearly by 57% between days 5 and 21 of lactation, and the weight of mammary DNA per gland increases by 100% during that same period (Kim et al. 1999c). The period of maximal mammary mass coincides with the estimated peak of lactation (Hurley 2001), which occurs between weeks 2 and 4. Mammary growth in lactation is also related to the position of the gland. It is greater for the five more anterior teat pairs than for more posterior teat pairs because of their greater chance of being selected by piglets (Kim et al. 2000). Parity can affect mammary development in lactation; primiparous sows show greater development than second- or third-parity sows (Beyer et al. 1994). In first-parity sows, the increase in mammary volume during lactation is due to both cellular hyperplasia and hypertrophy (Kim et al. 1999a), whereas in multiparous sows it appears to be mainly due to hypertrophy (Manjarin et al. 2011).

Factors affecting mammary gland development

Estrogens are important for mammogenesis in growing gilts, as evidenced by the increased rate of mammary development occurring at puberty (Farmer et al. 2004; Sorensen et al. 2006). Prolactin also plays a key role for prepubertal mammary development via an increase in the number of milk secretory cells (Farmer and Palin 2005).

During the last third of pregnancy, the drastic increase in metabolic activity of mammary glands is associated with an increase in estrogens of fetal origin and decreasing progesterone concentrations (Kensinger et al. 1986). An inhibition of prolactin in that period drastically decreases mammary development in gilts (Farmer et al. 2000), and this specifically from days 90 to 109 of gestation (Farmer and Petitclerc 2003). Increasing prolactin concentrations from days 90 to 110 of gestation using the dopamine antagonist domperidone stimulates mammary epithelial cell differentiation (VanKlompenberg et al. 2013). Relaxin is another hormone that promotes mammogenesis in gilts during the last third of pregnancy. It increases growth of parenchyma and decreases the mammary fat pad while having no effect on the cellular composition of mammary parenchyma (Hurley et al. 1991).

Nutrition in the two periods of rapid mammary development (i.e. from 90 days of age until puberty and during the last third of gestation) impacts the extent of development. Gilts should be fed ad libitum from 3 months of age to puberty in order to maximize their mammary growth (Farmer et al. 2004; Sorensen et al. 2006). Decreasing energy intake of gilts between 9 and 25 weeks of age also reduces weight of parenchymal tissue and decreases mammary DNA at the end of gestation (Lyvers-Peffer and Rozeboom 2001). On the other hand, feeding a lower protein diet (14.4 vs. 18.7% crude protein) from 90 days until puberty does not hinder mammary development of gilts (Farmer et al. 2004). Providing the phytoestrogen genistein in the diet of gilts from 90 days until puberty stimulates mammary hyperplasia, most likely through its estrogenic properties (Farmer

et al. 2010b). If body conditions of gilts differ at mating and these differences are maintained during gestation, composition of parenchymal tissue will be affected (Farmer et al. 2016a). Yorkshire X Landrace gilts that are thinner (12–15 mm backfat) will have greater concentrations of protein, DNA, and RNA in their mammary parenchyma compared with fatter animals (17–19 mm backfat) at the end of gestation.

Increasing dietary protein or energy between days 75 and 105 of pregnancy does not benefit mammary development; in fact, increasing dietary energy has a negative effect (Weldon et al. 1991). Howard et al. (1994) also observed no beneficial effect of increased energy intake in gestation on mammary development. Furthermore, in the study of Kusina et al. (1995), even though protein intake in gestation favorably affected milk yield, this was not due to an improvement in mammary development. In contrast, manipulating body condition of gilts by changing their protein and energy intakes during pregnancy showed that overly fat gilts (36 mm backfat) have drastically less mammary DNA (i.e. less epithelial cells) at the end of gestation and subsequently produce less milk (7.0 vs. 9.0 L/day) than leaner gilts (24 mm backfat) (Head and Williams 1991). Decaluwé et al. (2013) indicated that changes in backfat thickness during late gestation were associated with colostrum yield in sows. They also showed (Decaluwé et al. 2014b) that sows entering the farrowing house in a moderate body condition (averaging 19.7 mm backfat) had the greatest colostrum yield and the most beneficial colostrum composition. In a recent study, feeding level of pregnant gilts was adjusted to create three body conditions at the end of gestation. Mammary development of gilts that were too thin (12–15 mm backfat) was lesser compared with that of gilts with 17–26 mm of backfat (Farmer et al. 2016b). When 2-week-old piglets were fostered onto gilts that differed in terms of number of milk secretory cells at the onset of lactation, due to overt changes in body condition, milk output was greater in gilts with 27 mm compared with 36 mm of backfat. This clearly shows that the number of secretory cells at the beginning of lactation can have a very strong effect on the amount of milk produced (Pluske et al. 1995a).

Sow nutrition during lactation has a great impact on mammary growth. Kim et al. (1999b) demonstrated quadratic effects of both total energy and protein intakes on wet weight, protein, and DNA amounts in suckled glands. There are also recent indications that feeding during pregnancy and lactation can affect postnatal mammary development of the offspring. Supplementing the diet of late-pregnant and lactating sows with 10% flaxseed tended to increase parenchymal weight and increased parenchymal protein in the mammary tissue of their female offspring at puberty (Farmer and Palin 2008). Research is needed to establish the impact of such early life events on mammogenesis and lactation performance in swine. More information on mammogenesis in swine is provided in Farmer and Hurley (2015).

Mammary gland involution

Suckled mammary glands undergo dramatic changes during the initial 7 days after weaning, with significant changes occurring as early as 2 days post weaning (Ford et al. 2003). Mammary gland involution involves losses of over two-thirds of the parenchymal mass and cells present on the day of weaning (Ford et al. 2003). Individual mammary glands that are not regularly suckled during lactation undergo involution during lactation and become nonfunctional, with no further reduction in parenchymal tissue during the first 7 days after weaning (Ford et al. 2003). Mammary gland involution is reversible within 24 hours of a teat not being used. Yet, even if it is reversible, a gland unsuckled for less than 24 hours will have a lower milk yield for the remainder of lactation (Kim et al. 2001). Involution is irreversible after 3 days of a teat not being suckled (Theil et al. 2005). Mammary gland involution in early lactation could be affected by dietary nutrient levels. Kim et al. (2001) reported a much lower regression rate of unsuckled mammary glands in sows fed a high energy and high protein diet compared with sows fed a low energy and low protein diet.

Mammary glands that are suckled during lactation are larger than the nonsuckled glands at the end of involution, suggesting that more mammary tissue is available for redevelopment in the next lactation (Ford et al. 2003). Farmer et al. (2012) demonstrated that an unused teat in first parity will produce less milk in second parity. At 56 days of age, piglets that suckled a previously unused teat weighed 1.1 kg less than piglets that suckled a previously used teat. It appears that use of a teat for 2 days in early lactation during the first parity is enough to avoid any negative effect on milk yield in second parity (Farmer et al. 2017).

The abrupt cessation of lactation when milk production is at a maximum (3–4 weeks) brings about drastic changes in metabolic activity and endocrine status. There is a decrease in milk lactose and a transient decrease in milk glucose followed by an increase in plasma lactose and glucose (Atwood and Hartmann 1995). This is likely due to alterations in the tight junctions between mammary epithelial cells. In the week following weaning, the mammary alveoli regress (Hacker 1970), and the secretory glandular mass is replaced by adipose tissue in which a new alveolar system will develop in the following pregnancy (Delouis 1986). The absence of stimulation of the mammary glands stops the regular secretion of prolactin (Benjaminsen 1981), while the concentrations of gonadotropic hormones start to increase, allowing ovarian cycles to resume (Stevenson et al. 1981).

Weaning age affects the process of mammary involution because tight junctions between mammary epithelial cells become leaky as lactation advances from 22 to 44 days (Farmer et al. 2007). It was suggested that completion of the functional changes occurring in mammary glands during involution may be required for the gland to redevelop fully in the subsequent lactation (Hurley 1989), but this was never demonstrated.

Physiology of milk production

Lactogenesis

Lactogenesis is the ability of the mammary gland to synthesize milk components such as lactose, casein, and lipids and is often described as a two-phase process. Lactogenesis phase I refers to preparation of the mammary tissue for the synthesis of milk components, and lactogenesis phase II describes the start of milk synthesis and secretion around parturition (Hartmann et al. 1995).

Milk components appear in the alveoli between days 90 and 105 of gestation, indicating the beginning of phase I (Kensinger et al. 1982); however, little secretion can be obtained until parturition. At the end of gestation and during the colostral phase, the junctions between epithelial cells surrounding the alveoli are not tight. This allows serum transudate to leak from the bloodstream into the mammary secretions and milk components from the mammary gland alveoli to leak back to the bloodstream (Figure 18.2). Although serum transudation may contribute to the volume of colostrum obtained by the piglets, its contribution is most likely very short in duration.

During lactation, plasma lactose concentrations are low (<100 mmol/L) (Hartmann et al. 1984), and most of the immunoglobulins present in the mammary secretions are synthesized locally (Bourne and Curtis 1973). Plasma concentrations of milk whey proteins (α-lactalbumin and β-lactoglobulin) are also low during the lactation phase.

The onset of milk component synthesis is closely related to the decline in serum progesterone concentrations at farrowing (Robertson and King 1974; Hartmann et al. 1984). However, exogenous progesterone administration during late pregnancy delays the onset of parturition without inhibiting lactogenesis (Whitely et al. 1990; Foisnet et al. 2010b). Nevertheless, progesterone withdrawal (i.e. lysis of the corpora lutea via prostaglandins) is a major hormonal signal that primes the mammary glands, and withdrawal of colostrum from the glands then triggers the initiation of copious milk secretion (Hartmann et al. 1995).

Relaxin is involved in the onset of parturition since its concentrations always increase before those of progesterone begin to decline at the end of pregnancy. The pre-parturient rise in prolactin followed by the decline in both progesterone and relaxin appears to form important components of the lactogenic hormonal complex (Whitely et al. 1990). Prolactin is a key hormone for the onset of lactation (Tucker 1985). In the pregnant sow, suppression of the prepartum peak of prolactin inhibits subsequent milk production (Whitacre and Threlfall 1981; Taverne et al. 1982; Farmer et al. 1998a).

Colostrum production

Colostrum is essential for piglet survival (Declerck et al. 2016a) as it provides the energy necessary for thermoregulation and body growth (Herpin et al. 2005; Le Dividich et al. 2005), passive immunity needed for

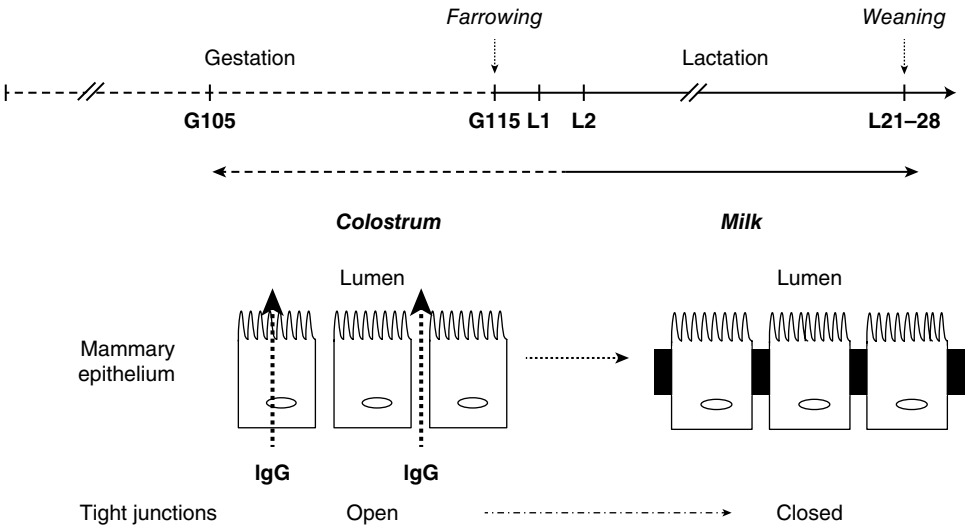


Figure 18.2 Status of mammary epithelial tight junctions in lactogenesis and galactopoiesis. *Source:* Adapted from Foisnet (2010).

Table 18.1 Contents of lipid, protein, lactose, and dry matter in colostrum, transient milk, and mature milk.

	Colostrum					
	Early	Mid	Late	Transient milk		Mature milk
Time postpartum	0 h	12 h	24 h	36 h	3 d	17 d
Chemical composition (g/100 g)						
Lipid	5.1	5.3	6.9	9.1	9.8	8.2
Protein	17.7	12.2	8.6	7.3	6.1	4.7
Lactose	3.5	4.0	4.4	4.6	4.8	5.1
Dry matter	27.3	22.4	20.6	21.4	21.2	18.9

Source: Adapted from Theil et al. (2014a).

protection against pathogens (Rooke and Bland 2002), and growth factors that stimulate intestinal growth and maturation (Xu 2003).

Colostrum composition

Colostrum is the first secretion of the mammary glands, and it contains more proteins (mainly immunoglobulins), less lipid, and less lactose than milk (Table 18.1; Theil et al. 2014a). During the colostral phase, lactose is present in high concentrations (>200 mmol/L) in the plasma of sows (Hartmann et al. 1984), and immunoglobulins (mainly IgG) found in mammary secretion originate from the plasma (Salmon et al. 2009). The transition between colostrum and milk occurs between 24 and 36 hours after the onset of parturition and is characterized by a drop in immunoglobulin concentrations with concomitant rises in fat and lactose (Table 18.1; Theil et al. 2014a).

Colostrum composition is affected by various sow and litter factors. A study using 10 commercial herds showed that colostral fat differs depending on breed, parity, and number of live-born piglets, whereas colostral protein and lactose are not affected (Declerck et al. 2015). Attempts were made at altering colostrum composition via sow management (see review by Farmer et al. 2006), and nutrition is undoubtedly the most successful tool since dietary fat affects both colostral fat content and composition.

Colostrum yield and quality

It is quite difficult to quantify colostrum yield because there is no direct measure. The most commonly used method is to obtain birth weight and individual weight gains of piglets for a specific period in early lactation and to fit these into a prediction equation (see details in Devillers et al. 2004; Theil et al. 2014b). However, this is labor intensive and requires being present at farrowing. It was established from a number of studies that sow colostrum yield is very variable and ranges between 2 and 5 kg over the first 24 hours postpartum for a litter of 8–12 piglets (see review by Farmer et al. 2006), with an average of approximately 3.3–3.6 kg (Devillers et al. 2007; Foisnet et al. 2010b; Quesnel 2011; Decaluwé et al. 2013, 2014b). Quesnel et al. (2012) indicated that piglets need to ingest a minimum of 200 g of colostrum during the first 24 hours after birth to have significantly lower preweaning mortality, acquire adequate passive immunity, and show a slight weight gain. A consumption of 250 g was recommended for piglets to achieve good health and pre- and post weaning growth. Findings using a more recent model suggest that this value is underestimated by approximately 30%, so that a minimum consumption of 325 g would be more adequate (Theil et al. 2014b).

The IgG content of colostrum is usually measured with methods that are time consuming and expensive for everyday use on the farm. Recent data suggest that a Brix refractometer could be a practical and valid method to assess colostrum IgG content in swine. It may be used during the early parturition to measure IgG levels when peak values are expected (Hasan et al. 2016). However, there are great variations between herds, indicating a significant impact of herd management (Hasan et al. 2016), and even within herds due to sow-to-sow variation (Farmer and Quesnel 2009).

Factors affecting colostrum yield

Colostrum yield over the first 24 hours postpartum is not correlated with litter size and litter birth weight and is only moderately related with litter vitality at birth (Le Dividich et al. 2005; Devillers et al. 2007; Quesnel 2011; Decaluwé et al. 2014a; Declerck et al. 2015). On the other hand, it is positively correlated with mean piglet birth weight and negatively correlated with within-litter variation in birth weight (Devillers et al. 2007; Quesnel 2011). Results suggest that colostrum yield may be influenced by general vitality of the litter at birth, i.e. the ability to reach and extract colostrum from the udder (Quesnel et al. 2015; Declerck et al. 2015). Yet, the capacity of the sow to produce colostrum also limits intake by piglets (Devillers et al. 2004).

Various nutritional, hormonal, and environmental factors affect colostrogenesis in swine (Farmer and Quesnel 2009), and it is beyond the scope of the present chapter to cover all these. One important factor is the endocrine status of the sow. Delays in progesterone decrease and prolactin increase relative to farrowing are associated with reduced colostrum yield (Foisnet et al. 2010a). Furthermore, the relative concentrations of prolactin and progesterone prepartum influence colostrum yield (Loisel et al. 2014).

An injection of azaperone at the time of expulsion of the placenta was shown to facilitate nursing behavior, increase colostrum intake by piglets (Biermann et al. 2010), and, consequently, improve piglet conditions (Miquet and

Viana 2010; Tseng et al. 2010). Parity affects colostrum yield with older sows (parity 4 and more), producing less colostrum than younger sows (Devillers et al. 2007; Decaluwé et al. 2013; Quesnel et al. 2015). Backfat gain during the last week of pregnancy appears to be positively correlated with colostrum yield, whereas backfat gain between days 85 and 109 of gestation seems to be negatively correlated with colostrum yield (Decaluwé et al. 2013).

Milk ejection

During parturition and the first hours afterward, colostrum is continuously available. At parturition, distension of the cervix for the passage of the piglets, triggering Ferguson's reflex, and movements of the sow lead to oxytocin release from the pituitary so that colostrum is available (Fraser 1984; Castren et al. 1989). After parturition, nursing can be divided in five phases: initiation, pre-ejection teat massage, milk ejection, post ejection massage, and termination of nursing. In early lactation the sow is usually initiating the nursings by vocalization (grunting). This is followed by piglets assembling at the udder for pre-ejection teat massage, which takes up to 3 minutes. Actual milk letdown then takes place with milk ejection lasting no longer than 20 seconds. This is followed by post ejection teat massage lasting several minutes (Spinka and Illmann 2015).

Following parturition, nursings are progressively developed until cyclic letdowns occur around 10–12 hours after the start of parturition (de Passillé and Rushen, 1989b; Devillers et al. 2006, 2007). Colostrum ejections are as frequent as every 10–20 minutes, and the period of high intramammary pressure permitting colostrum withdrawal may be sustained for a minute or more. Once 50–100 mL of colostrum is removed, the intramammary pressure is reduced to the point that further withdrawal is difficult (Fraser 1984). After the colostral phase, and for the rest of lactation, milk ejections are cyclical and actively synchronized by sow gruntings (Lewis and Hurnik 1985; Castren et al. 1993; Chaloupková et al. 2007). The removal of milk from the alveoli and ductal system of the mammary glands requires a neuroendocrine milk ejection reflex. This is elicited by piglets massaging the udder (Fraser 1980) and stimulating release of oxytocin, leading to milk ejection (Hartmann and Holmes 1989). Oxytocin stimulates the contraction of myoepithelial cells surrounding the alveolar lumen to force milk through the ductal system to the teats (Ellendorf et al. 1982). Udder stimulation is needed to trigger oxytocin release (Algers et al. 1990), but growth rate of piglets is not affected by the peak amplitude in intramammary pressure (Kent et al. 2003) that is linked to oxytocin levels. The increase in circulating oxytocin concentrations can occur up to 30 seconds before milk ejection (Ellendorf et al. 1982), which lasts only 10–20 seconds (Fraser 1980).

Not all nursing attempts are successful during lactation. Failed nursing attempts may affect some or all piglets in the litter. Among the latter, one must distinguish between sucklings terminated by the piglets and those terminated by the sow (Illmann and Madlafousek 1995). Unsuccessful sucklings affecting the whole litter become more frequent after the colostral phase (Castren et al. 1993; Fraser 1977). These are characterized by an absence in rise of intramammary pressure associated with no increase in plasma oxytocin (Ellendorf et al. 1982). During the first 10 days of lactation, the proportion of unsuccessful sucklings is between 20 and 40% (Fraser 1977; Jensen et al. 1991). Although frequent and requiring energy expenditure from the piglets, ejection failures may still play a role in maintaining lactation (Algers 1993) because plasma concentrations of lactogenic hormones tend to rise after an unsuccessful nursing (Rushen et al. 1993). Rushen et al. (1995) demonstrated that external stress, such as placing sows in a novel environment, increases the chance that the subsequent nursing attempt will fail. This was not due to increased concentrations of cortisol or adrenocorticotropic hormone (ACTH), but was more likely caused by an opioid-mediated inhibition of oxytocin. Another useful indicator of nursing success is when nursings are terminated by the sow instead of the piglets. Recent data suggests that at 4–6 weeks of lactation, sows terminate nursings approximately every hour, whereas piglets terminate nursings only once in 4–5 hours (Thomsson et al. 2016).

Measuring milk production

Measuring milk production in the sow is difficult because of the small and numerous teats and because milk is not available continually after the colostral phase. Milk ejection must therefore be stimulated with exogenous oxytocin. There are three ways to quantify sow milk yield, namely, using the weigh–suckle–weigh technique, the deuterium oxide (DO) dilution technique, or a prediction based on piglets or litter weight gains. The weigh–suckle–weigh implies that piglets are weighed before and after suckling seven to eight times over a 24 hour period and values are extrapolated to obtain daily milk production (Salmon-Legagneur 1965; Mahan et al. 1971). A major drawback is that one must estimate the losses due to urination and defecation between the weighings; hence milk yield may be underestimated. Milking machines are reported to give more repeatable estimates of milk production and were used to compare milk production between teats (Fraser et al. 1985) and breeds (Grun et al. 1993a). However, they are not available commercially. The DO dilution technique quantifies the water intake from colostrum by piglets. It is most accurate (Theil et al. 2007), yet its cost is exuberant and it is very labor intensive. The third method is most often

used. It estimates milk yield based on piglet growth (Noblet and Etienne 1989). However, it is essential to standardize litter size and ensure that piglets do not have access to solid feed. The amount of milk required per gram of piglet weight gain was first reported to be approximately 4.5 g (Lewis et al. 1978; Noblet and Etienne 1989). It was later shown to be affected by stage of lactation, being 3.8 g on day 3, 4.6 on day 10, and 4.9 on day 17 (Theil et al. 2002).

Pattern of milk production

Sow milk production is usually described in four phases: the colostral, ascending, plateau, and descending phases. Typical sow milk production curves are presented in Figure 18.3 (Toner et al. 1996) and are similar to those presented by others (Noblet and Etienne 1986; Shoenherr et al. 1989; Grun et al. 1993b; Hansen et al. 2012). As mentioned previously, mammary secretions are available continuously during the early colostral phase to then be episodic in release. During the ascending phase, nursing frequency doubles, going from 17 to 35 per day (Jensen et al. 1991), and the amount of milk obtained at each nursing increases from 29 to 53 g between the first and third week of lactation (Campbell and Dunkin 1982). The end of the ascending phase ranges from 10 to 28 days postpartum, its timing likely related to differences in breed, nutrition, and parity of the sows (Elsley 1971; Harkins et al. 1989; Toner et al. 1996; King et al. 1997).

During the plateau phase, piglet growth is limited by an insufficient milk supply (Reale 1987; Wolter et al. 2002), which is magnified by longer lactation and larger litters. Indeed, milk production is proportional to the number of suckled mammary glands (Auldist et al. 1998). In a 21-day lactation, the growth limitation of suckling piglets can be more than 2 kg per pig (Harrell et al. 1993). Most sows in commercial swine units do not reach the descending phase because weaning is done before 28 days of lactation.

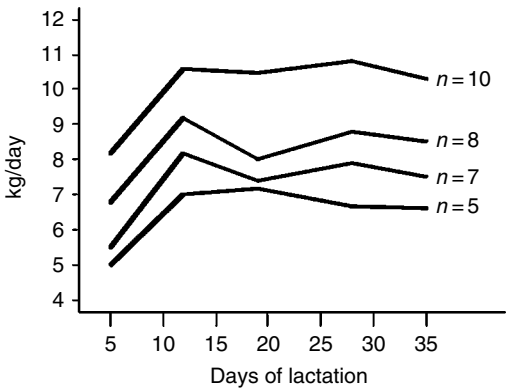


Figure 18.3 Pattern of milk production (kg/day) of first litter sows nursing litters of different sizes. *Source:* Toner et al. (1996).

Control of milk production

Milking frequency

Cyclical nursings start approximately 10 hours after the onset of farrowing (Lewis and Hurnik 1985) and develop gradually (Algers and Uvnäs-Moberg 2007). Nursing frequency has a major role in determining milk output. It reaches a maximum around days 8–10 of lactation and decreases thereafter (Puppe and Tuchscherer 2000). Nursing intervals of 36–40 minutes were reported in early lactation (days 5–6) and of 39–48 minutes in later lactation (days 18–20) (Farmer et al. 2001; Fisette et al. 2004). Nursing frequency appears similar in day and night on day 10 of lactation, whereas it decreases during the night after day 17 (van den Brand et al. 2004).

Suckling and milk removal are the major stimuli for mammary growth (Hurley 2001; Farmer 2013) and milk output (Sauber et al. 1994; Auldist et al. 2000) during lactation. The stimulus of piglets suckling or massaging the mammary glands leads to increases in circulating prolactin, a galactopoietic hormone (Spinka et al. 1999; Farmer 2001). Refilling of the mammary glands is almost complete within 35 minutes after suckling (Spinka et al. 1997), which emphasizes the importance of shorter nursing intervals to maximize total milk yield. Milk stasis in alveolar spaces triggers the end of lactation and involution of alveoli (Boyd et al. 1995). It causes an increase in intramammary pressure, which reduces blood flow to the tissue (Hurley 2001).

Sows within a farrowing room will synchronize their nursings (Wechsler and Brodmann 1996) due to the auditory stimulus from the other animals. Recorded sow suckling grunts can stimulate nursings (Stone et al. 1974), but effects of playing recordings at 35–42 minute intervals on piglet growth were only minor in a commercial farrowing house setting (Cronin et al. 2001; Fisette et al. 2004). Sow nutrition (feeding level or energy source) does not affect nursing frequency (van den Brand et al. 2004), but sow breed has an effect. The interval between nursings is shorter for Meishan-derived sows than for European white sows (Farmer et al. 2001; Fisette et al. 2004).

Hormonal control

The activation by piglets of neural receptors within the mammary glands stimulates oxytocin from the posterior pituitary as well as the release of prolactin, growth hormone (GH), ACTH, and thyroid-stimulating hormone from the anterior pituitary. Hormones from the anterior pituitary maintain the synthesis of milk from the mammary epithelial cells (Delouis 1986).

The role of GH for milk production is both direct, as a regulator of nutrient partitioning for milk component synthesis, and indirect, via IGF-1 that acts upon the mammary epithelial cells (Flint and Gardner 1994). In lactating sows, a reduction in circulating concentrations

of GH and IGF-1 by immunization against GH-releasing factor significantly decreased milk yield, yet growth rate of piglets was unaltered (Armstrong et al. 1994), suggesting that GH may have a facilitative rather than an essential role. Mammary transgenic overexpression of IGF-1 in sows also did not affect their milk yield (Ruan et al. 2005).

When the secretion of prolactin was systematically inhibited during various weeks of lactation, weight gain of piglets was suppressed (Farmer et al. 1998a); hence prolactin is considered essential for the maintenance of lactation in swine. Indeed, Plaut et al. (1989) showed that the binding of prolactin to its mammary receptor is a major effector of milk production in sows.

Thyroid hormones are required for various metabolic functions and for protein synthesis by the mammary gland (Tucker 1985). Yet, their role for lactation in swine has not been demonstrated. When thyrotropin-releasing factor was injected to lactating sows, prolactin concentrations were increased, but lactation performance was not altered (Dubreuil et al. 1990).

Water availability

Water intake increases from approximately 1 L/hour at the end of gestation to 2.6 L/hour 12 hours before the end of parturition (Klopfenstein 2003). It can be very low (less than 10 L/day) in some sows during the first 24 hours following parturition. After this period of transition, it increases gradually to reach 20–35 L/day during lactation (Figure 18.4a). The increased water intake just prior

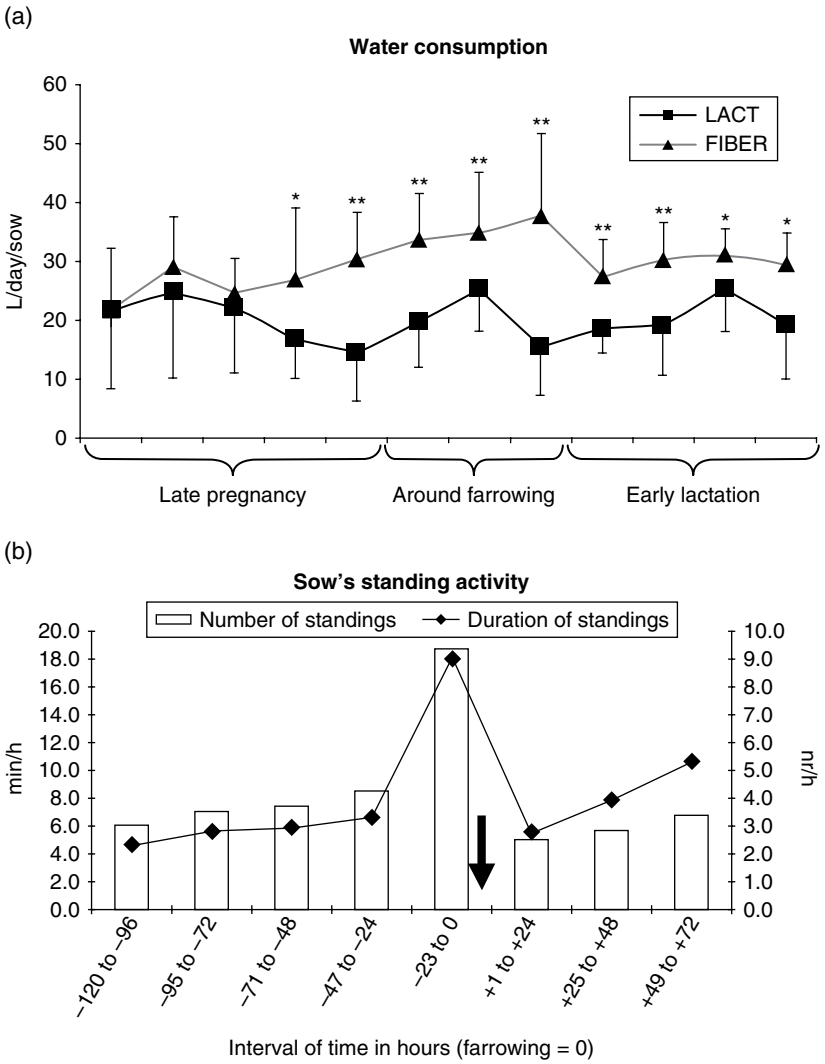


Figure 18.4 (a) Individual average daily water intake of sows fed a lactation diet low (3.8%) in crude fiber (LACT) and high (7%) crude fiber (FIBER) diet before farrowing. *Source:* Adapted from Oliviero et al. (2009). Reproduced with permission of Elsevier. The asterisk symbols indicate the power of statistical results (*0.05 and **0.01). (b) Average duration and number of times standing up per hour in the different intervals of time before and after farrowing (black arrow). *Source:* Adapted from Oliviero et al. (2008b). Reproduced with permission of Elsevier.

to farrowing is partly due to greater water needs. During the hours preceding parturition, there is a rapid increase in water content of the reproductive system to allow the process of parturition (Dobson 1988). Water intake can be increased via greater dietary fiber during the transition period (Figure 18.4a, Oliviero et al. 2009).

Composition of sow milk

Sow milk contains more than 100 chemical components (reviewed by Xu 2003). The major components are water, lactose, proteins (casein, alpha-lactalbumin, beta-globulins, serum albumins, immunoglobulins), lipids, lactocytes, leukocytes, bivalent ions (calcium, phosphorus, and magnesium) and electrolytes (sodium, potassium, and chloride). The relative concentrations of these components vary according to the stage of lactation (Table 18.2). During the first 24 hours after parturition, lacteal secretions contain more immunoglobulins, microminerals, vitamins, hormones, and growth factors and lower concentrations of lactose than in later lactation (Table 18.2). Wu et al. (2010) reported that concentrations of immunoglobulins and lactoferrin are greater in milk from anterior glands than from posterior glands. Diet may have an impact on the concentrations of some milk components such as fat, fat-soluble vitamins, minerals, and fatty acids (Hurley 2015).

Somatic cells of normal and mastitic milk

The somatic cell count (SCC) of mammary secretions from healthy sows is 1–4 million cells/mL (Evans et al. 1982; Schollenberger et al. 1986; Hurley and Grieve 1988; Magnusson et al. 1991; Drendel and Wendt 1993; Klopfenstein 2003), compared with less than 100,000 cells/mL in cows (Smith et al. 2001). Milk somatic cells are primarily leukocytes and epithelial cells shed from the lining of the mammary glands (Harmon 1994). The relative concentrations of the various cell types vary with stage of lactation. During the colostral phase most cells are leukocytes (>98%), whereas during the rest of lactation (days 7, 14, and 28), they are predominantly epithelial cells (Evans et al. 1982; Schollenberger et al. 1986; Magnusson et al. 1991).

The SCC found in milk of infected mammary glands is similar to that observed during the colostral phase or during mammary involution (Drendel and Wendt 1993). Somatic cells found in milk of infected glands are mainly leukocytes (>75%). During sow lactation, a cellular content over 12 million cells/mL with an increased proportion of leukocytes is suggestive of mammary gland alteration. Persson et al. (1983) used SCC in mammary secretions to differentiate subclinical from clinical cases of mastitis; however, the use of SCC as a diagnostic tool has no practical clinical application due to lack of sensitivity, specificity, and low predictive value.

Table 18.2 Variation of sow milk composition (mean ± SD) between the first days (days 1–2) and the plateau phase (days 10–15) of lactation.

	Day	Day		
Milk components	**1–2**	**10–15**	**Difference**	**source**
Lactose (mmol/L)	160 ± 10	190 ± 10	+30	Konar et al. (1971)
Sodium (mmol/L)	25 ± 5	18 ± 5	–7	Konar et al. (1971)
Potassium (mmol/L)	75 ± 5	50 ± 5	–25	Konar et al. (1971)
Chloride (mmol/L)	25 ± 5	18 ± 5	–7	Konar et al. (1971)
Calcium (mmol/L)	12 ± 3	50 ± 3	+38	Perrin (1955)
Phosphate(mmol/L)	12 ± 1	14 ± 1	+2	Perrin (1955)
Magnesium (mmol/L)	4 ± 1	4 ± 1	0	Perrin (1955)
Total protein (g/L)	64 ± 6	51 ± 5	–13	Klobasa et al. (1987)
Lipids (g/L)	65 ± 15	65 ± 15	0	Klobasa et al. (1987)
Somatic cells (10^3/mL)	1060 ± 790	2012 ± 990	+952	Schollenberger et al. (1986)
Leukocytes (10^3/mL)	748 ± 509	886 ± 519	+138	Schollenberger et al. (1986)
Lactocytes (10^3/mL)	152 ± 103	503 ± 315	+351	Schollenberger et al. (1986)
Anucleate cells (10^6/mL)	147 ± 160	727 ± 63	+580	Schollenberger et al. (1986)
% of leukocytes	70	44	–26	Schollenberger et al. (1986)

Source: Adapted from Klopfenstein (2003).

Manipulation of milk production

Feeding strategies

It is generally accepted that sows should maintain their body condition throughout the reproductive cycle (Einarsson and Rojkittikhun 1993). This is difficult due to increasing litter sizes and milk production (Foxcroft et al. 2007) and because low parity sows are expected to gain weight and grow over the first two parities. Top priorities for nutrient use by sows are fetal growth, colostrum production, mammary growth, and milk production (Theil et al. 2014a). Toward the end of gestation, important targets for sow nutrition can be set, such as maximizing energy intake of newborn piglets (Theil et al. 2014a) and minimizing piglet weight variation (Campos et al. 2012). Sows that are catabolic one week prior to farrowing seem unable to produce colostrum to their full potential (Decaluwé et al. 2013). Colostrum yield and composition appear optimal when sows enter the farrowing unit with a moderate body condition and are provided a high amount of feed peripartally (Decaluwé et al. 2014b). Maximizing energy intake of piglets through colostrum is a challenge because an increasing proportion of piglets (current estimation of 30%) in hyperprolific sows may not ingest enough colostrum (Foisnet et al. 2010a; Decaluwé et al. 2013; Declerck et al. 2015). Common recommendations are either a stepwise feed increment protocol in early lactation to avoid drops of feed intake during any part of lactation (Koketsu et al. 1996a,b; Soede and Kemp 2015) or ad libitum feeding with a step-down response if a drop in feed intake is observed. Milk production requires substrates derived from the diet and the sow's body reserves, the relative importance of which changes as lactation progresses. Body reserves might be sufficient in early lactation to compensate for inadequate nutrient intake, but not in late lactation (Pettigrew 1995). Accordingly, severe restriction of feed intake during lactation has no impact on litter growth in the first week postpartum, whereas it markedly decreases litter growth in later lactation (Mullan and Williams 1989; Pettigrew 1995). The magnitude of reduction depends on the dam's body reserves at farrowing; gilts with lower body reserves are affected most (Mullan and Williams 1989). Feed restriction during lactation may also lead to subsequent problems in the weaning-to-estrus interval, ovulation rate, and embryo survival.

The intake of protein/amino acids by gestating and lactating sows is critical for lactation performance. Lysine is the first limiting amino acid for lactating sows, with 26 g of dietary lysine needed per kg of litter growth/day (Sohn and Maxwell 1999). To achieve a zero protein (nitrogen) balance, Dourmad et al. (1998) demonstrated that 45–55 g/day of crude lysine is required for normal and high-yielding sows, respectively. Exceeding recommended protein values in the diet may decrease feed intake; however, in young sows, when demand for protein is at its highest, a further increment may be warranted (Smits et al. 2012; Soede and Kemp 2015). Valine and isoleucine, but not leucine, appear to increase milk production as indicated by increased litter weight gain (Kerr 1997, as cited by Sohn and Maxwell 1999). These amino acids can be metabolized to succinyl-CoA and potentially serve as a source of energy for the mammary gland (Sohn and Maxwell 1999).

Using studies designed to determine the relation between milk production and dietary energy, Williams (1995) noted that each suckling piglet grows an extra 1 g/day for each MJ of metabolizable energy consumed by the sow. Matzat et al. (1990) showed a linear relationship between milk output and energy intake of sows, whereas Pluske et al. (1995b) demonstrated that piglet growth did not respond beyond 75 MJ of metabolizable energy in gilts. Such a ceiling for lactational performance was also observed in first-parity sows offered increasing amounts of protein (King et al. 1993).

Various feeding managements were used to maximize lactational feed intake of sows and allow sufficient lean tissue gain during pregnancy in order for milk production not to be compromised. Increasing sow feed consumption by 8% with wet feeding had no impact on average daily gains (ADG) of piglets over an 18-day lactation period (Genest and D'Allaire 1995). Similarly, feeding a bulky diet in gestation increased lactational feed intake of sows by 8% without improving litter weight (Farmer et al. 1996). Increasing daily feeding frequency from 2 to 3 (Genest and D'Allaire 1995) or 2 to 4 (Farmer et al. 1996) during lactation also had no effect on feed intake. However, the use of self-feeders, whereby sows may visit the feeder up to 13 times a day, increased lactation feed intake by 10% (Hoofs and Elst-Ter Wahle 1993; van der Peet-Schwering et al. 2004; Peltoniemi et al. 2009). The addition of fat to the sow's diet did not lead to a less negative energy balance in the sows but resulted in fatter piglets when sows were fed at a high feeding level (van den Brand et al. 2000). Lactation diets with more fat may offer additional benefits under hot conditions by increasing energy intake without compromising sow comfort (Soede and Kemp 2015).

Exogenous hormones

An early study showed that exogenous GH in lactation increased milk yield by 15–22% (Harkins et al. 1989), but those results could not be reproduced (Michelchen and Ender 1991; Smith et al. 1991). A greater feed efficiency in sows was consistent in all studies.

Sows with spontaneous lactation failure have abnormally low concentrations of prolactin (Whitacre and Threlfall 1981), and a single injection of porcine prolactin to gilts (but not sows) on day 1 of lactation led to an 8% increase in litter weight gain (Dusza et al. 1991).

Prolactin injected from day 107 of gestation through lactation had no effect on milk yield (Crenshaw et al. 1989; King et al. 1996), perhaps because injections were started before there were piglets to remove milk from the mammary glands, leading to premature involution of the secretory units (Boyd et al. 1995). Prolactin injected to sows daily from days 2 to 23 of lactation did not improve piglet growth rate (Farmer et al. 1999), likely because most prolactin receptors were saturated in control sows, thereby preventing any beneficial effects of increased prolactin concentrations. Prolactin is under negative dopaminergic control, and the dopamine antagonist domperidone was used to increase secretion of prolactin in late-pregnant sows. Twice-daily feeding of domperidone from 90 to 110 days of gestation accelerated mammary cell differentiation and increased sow milk yield by more than 20% (VanKlompenberg et al. 2013), offering an avenue that merits further investigation.

Sow behavior

Sow behavior can be used to monitor their status (Oliviero et al. 2008a) and to identify animals at risk for postpartum dysgalactia syndrome (PDS). Although eating activity of sows decreases in the 24 hours preceding parturition, healthy animals seldom go completely off feed (Peltoniemi et al. 2009; Quesnel et al. 2009), whereas sows contracting PDS often go completely off feed. Loss of appetite is part of a general response to systemic inflammatory mediators (cytokines and interleukins) that may be stimulated by endotoxins (lipopolysaccharides). Products of inflammation induce fever and promote immune functions for the body to recover (Johnson 2002; Weary et al. 2009). Water intake substantially increases prior to parturition, and a decrease in water intake may be taken as a sign of greater risk of PDS. Piglets that spend more time nuzzling the udder of the sow is an indication of hunger, malnourishment, and perhaps PDS, leading to a greater risk of being crushed by the dam (Weary et al. 1996).

Under natural conditions, sows isolate themselves from the rest of the group for building a nest during the last 24 hours of pregnancy. After parturition, they stay close to the nest and piglets for the first day, leave the nest temporarily in search for food during the first week, and eventually abandon the nest with the piglets after 10 days (Jensen 1986; Stangel and Jensen 1991; Jensen et al. 1993). In confinement, the number and duration of standing incidences increase considerably (Figure 18.4b) the day before parturition. In the first 24 hours after farrowing, sows are inactive; their activity increases daily thereafter. After day 3, sows gradually spend less time in lateral recumbency (Oliviero et al. 2009).

Nursing behavior changes over lactation and is likely related to the milk supply. Piglets suckle for the first time within 20–40 minutes of birth (Tuchscherer et al. 2000; Vasdal et al. 2011; Balzani et al. 2016a). In the early colostral phase, mammary secretions are continuously available, but availability becomes cyclical later on (de Passillé and Rushen 1989a). Within 48–72 hours postpartum, piglets establish a teat order whereby each piglet consistently sucks from one or two specific teats (Fraser 1976; de Passillé et al. 1988).

In the first days after parturition, milk production exceeds the needs of piglets, and most (>85%) of the sucklings are initiated by the sow and are terminated by the piglets. When milk supply becomes limiting (weeks 3–4), most sucklings are initiated by the piglets and terminated by the sow (Jensen 1988; Jensen et al. 1991). Sows terminate suckling by leaving the piglets or by limiting teat access by lying on their belly (de Passillé and Rushen 1989b). Both the duration and intensity of teat stimulation by individual piglets influence milk production during the first 3 days of lactation (Algers and Jensen 1991), and nursing frequency also affects piglet weight gain (Spinka et al. 1997; Auldist et al. 2000; Valros et al. 2002).

Piglet growth

The main energy source for piglets over the first 12 hours postpartum is colostrum, followed by glycogen reserves until day 2 when milk becomes their sole energy source (Theil et al. 2014a). The efficacy of conversion of milk into live weight gain ranges from 3.5 to 4.2 (Noblet and Etienne 1986; Beyer et al. 1994; Pluske et al. 1998; Le Dividich et al. 2007) and is lower in restricted-fed piglets due to a greater proportion of intake devoted to maintenance (Le Dividich et al. 2007). Energy and nitrogen in sow milk have an apparent digestibility of approximately 98% (Le Dividich et al. 2007) and digestibilities of colostral lactose and fat approximate 100% (Le Dividich et al. 1994b).

Piglets that are bottle-fed with colostrum in the first 24 hours after birth have a voluntary intake exceeding 450 g/kg of birth weight, being twice the average consumption of sow-reared piglets (210–370 g/kg of birth weight), which suggests the sow is limiting colostrum intake by the piglets. However, 60–88% of sows produce enough colostrum to ensure survival of their litters since an intake of 160 g of colostrum per kg of birth weight is required for piglet survival.

Colostrum intake has lifelong effects on piglet growth. A study using multivariable analyses, correcting for the effect of birth weight, demonstrated that colostrum intake affects weaning weight as well as weight at the onset and end of the fattening period, with effects more pronounced in lightweight piglets (Declerck et al. 2016a). From birth to weaning, piglets from larger litters tend to

be lighter than piglets from smaller litters (Dyck and Swierstra 1987; Van der Lende and de Jager 1991; Le Dividich et al. 2004; Declerck et al. 2016a). This is mainly due to differences in piglet birth weight and/or lower milk supply per piglet in large litters later in lactation. Hyperprolificacy affects growth rate because the number of live piglets exceeds the number of mammary glands and low birth weight piglets are often less vigorous for nursing.

Growth rate, usually measured as ADG, is directly related to individual piglet weight at birth (Tyler et al. 1990; Castren et al. 1991; Le Dividich et al. 2004). When piglet weights are corrected for birth weight, the effect of litter size is absent on day 3, is small on day 7, and becomes greater as lactation proceeds (Klopfenstein 2003). Klopfenstein (2003) also showed that milk production becomes a limiting factor for piglet growth when the sow has reached her maximal milk production capacity (days 10–15).

Heterogeneity or variation of piglet birth weights within a litter is an increasing concern. As litter size increases from less than 10 to more than 15, mean litter birth weight decreases by 0.5 kg, and the coefficient of variation (SD/mean) increases by 10% to reach 24%. For a given sow, there is no repetition of the coefficient of variation in subsequent parities (Quesnel et al. 2008). Homogeneity is greater in young sows (parities 1 and 2) than in older sows (Table 18.3) in both normoprolific (Bolet and Etienne 1982) and hyperprolific sows (Quesnel et al. 2008) regardless of litter size, thereby indicating a parity effect that is important because the majority of published scientific data used young sows. The loss of body condition during the previous lactation and body condition in early gestation both have an impact on heterogeneity of piglet birth weights (Hultén et al. 2002; Wientjes 2013). Heterogeneity increases as sows are heavier at mating or farrowing.

The correlation between mammary gland wet weight and ADG of piglets is high, from 0.59 to 0.68 (Nielsen and Sorensen 1998; Kim et al. 2000), and significant correlations also exist between ADG of suckling piglets and mammary cell number and secretory activity per cell (Farmer et al. 2010a). This suggests a strong relationship between pig growth and mammary size. The milk production differences between mammary glands are believed to be a major source of variation in piglet weight gain (Fraser and Jones 1975). Mammary glands have different shapes depending on their anatomical location, with the anterior and middle glands only being able to expand laterally and medially, hence lacking space to grow, whereas posterior glands may be better able to expand elliptically in a longitudinal manner. Newborn piglets show preferences for certain teats and will choose the upper row of the anterior or posterior part of the udder more frequently than the middle section of the udder or the lower row (Balzani et al. 2016b). Scheel et al. (1977) showed that pigs with greater birth weight had a preference for anterior glands, but Hemsworth and Winfield (1976) and Kim et al. (2000) showed no significant relationship between birth weight of pigs and the preference for mammary glands. Overall, pigs nursing the well-developed anterior and middle mammary glands have a greater ADG than pigs nursing the remaining glands.

Piglet mortality

Despite improved production efficiency in modern swine production, piglet losses at birth and during lactation increase as litter size increases (Boulot et al. 2008). Neonatal mortality averages over 15%, and the vast majority of these deaths occur during parturition or the first 3 postnatal days (English and Morrison 1984; Dyck

Table 18.3 Effect of parity (P) on piglets' characteristics at birth (French observations on 1596 litters from a single herd).

	P1	P2	P3–4	P5–6	P7+
# Litters	432	349	470	261	86
Litter size : total born	14.0	12.3	14.5	15.3	15.1
Litter size : live born	13.2	11.7	13.5	14.4	13.3
Litter size : stillborn	0.8	0.6	0.9	1.3	1.8
Mean birth weight (kg)	1.45	1.64	1.57	1.47	1.44
Within-litter SD (kg)	0.28	0.31	0.33	0.34	0.35
Coeffi cient of variation (%)	20	20	22	24	25
Losses in small piglets	11	11	13	15	16

Source: Adapted from Quesnel et al. (2008).

and Swierstra 1987; de Passillé and Rushen 1989b; Le Cozler et al. 2004; Oliviero et al. 2008b; Edwards and Baxter 2015). Leading causes of death in the neonatal piglet include stillborn, crushing by the sow and low viability/starvation (Vaillancourt et al. 1990; Koketsu et al. 2006; Oliviero et al. 2009). The effect of litter size on mortality is quadratic, with piglet losses increasing in larger litters (Fahmy and Bernard 1971; Dyck and Swierstra 1987; Guthrie et al. 1987; Boulot et al. 2008), and is probably related to an insufficient number of functional mammary glands (Chertkov 1986; Bilkei et al. 1994). Lower birth weight piglets also have poor thermoregulation since their skin surface/kg body weight is greater than for heavier piglets. They may also be less mature at birth therefore they may be more susceptible to disease or have a slower start due to compromised colostrum and milk intake (Edwards and Baxter 2015). Greater colostrum intake is associated with lower mortality rates of suckling and weaned piglets (Declerck et al. 2016a). Energy deficit is one of the main predisposing risk factors for piglet mortality, and it was recently shown that energy supplementation could be a way of increasing survival of lightweight piglets (Declerck et al. 2016b).

Immune protection of the piglet

Newborn piglets rely on ingestion of colostrum for passive transfer of immunity (Bourne 1976), and up to a third of piglets in litters from hyperprolific sows may not ingest adequate amounts of colostrum. Management strategies are therefore needed to maximize colostrum intake, especially for small piglets and piglets born late in the birth order. Split suckling, where early-born and heavier piglets are separated from the udder to allow the small and late-born piglets to suckle, is one such management tool. Another practice is cross-fostering, where litters are standardized within the first 24 hours after parturition (Edwards and Baxter 2015). The absorption of immunoglobulins from colostrum stimulates the closure of the intestine for the passage of these large proteins (Klobasa et al. 1991) within 48 hours after birth. Le Dividich et al. (2004) showed that colostrum IgG content decreases by 31% within 3 hours after birth of the first piglet. Passive immunity in 48-hour-old piglets was 50% greater in the first than in the last-born piglet, and the difference remained significant at weaning. Piglets artificially reared and receiving 6 hourly feedings of 25 mL of sow colostrum followed by hourly feedings of cow's milk had plasmatic immunoglobulin concentrations similar to those of naturally fed piglets at 24 hours of age (Klobasa et al. 1991). Increasing the number of hourly feedings of colostrum from 6 to 12, 18, or 24 did not increase the concentrations of plasma immunoglobulins in piglets. Fasting newborn piglets for periods of up to 24 hours after birth before giving them access to their first colostrum intake did not decrease concentrations of serum immunoglobulins 12 and 18 hours after feeding (Klobasa et al. 1990). Therefore, closure of the gut system for the passage of immunoglobulins is dependent on the quantity of colostrum ingested rather than on time since birth. Six feedings of colostrum should be sufficient to give adequate immune protection to the piglets.

Studies based on radioactive labeling of immunoglobulins in colostrum showed that almost 100% of IgG, 40% of IgA, and 85% of IgM are derived from sow serum (Bourne and Curtis 1973). IgG is preferentially transferred from the blood into mammary secretions during colostrogenesis, resulting in a marked decrease in sow serum IgG levels at this time. This seems to take place both through transudation and by specific receptor-mediated transport (Schnulle and Hurley 2003; Devillers et al. 2004). The uptake of colostral immunoglobulins in neonatal piglets may not be mediated by a receptor, although FcRn is present in gut epithelial cells. Indeed, IgG, IgM, and IgA (not secretory IgA) undergo selective transcytosis into enterocytes (Devillers et al. 2006).

The quantity of IgG ingested by each piglet is affected by birth order due to the rapid changes in colostrum composition taking place between onset of farrowing and birth of the last piglet (Klobasa et al. 2004; Le Dividich et al. 2004). Piglets dying before weaning had lower plasma immunoglobulin concentrations after parturition (Hendrix et al. 1978; Tyler et al. 1990); yet this association disappears when birth weight is used as a covariate (Tyler et al. 1990). Moreover, the probability of dying is not increased among last-born piglets, even though they obtain less immunoglobulins than firstborn piglets (Le Dividich et al. 2004). These results, although surprising, suggest that most mortalities are the consequence of inadequate ongoing milk intake rather than infectious diseases.

Mastitis and postpartum dysgalactia syndrome (PDS)

Mastitis may be limited to one or a few mammary glands (uniglandular mastitis) or may involve all mammary glands (i.e. multiglandular effects, hard udder syndrome). Acute mastitis is usually accompanied by systemic and local signs, whereas the hard udder syndrome does not have systemic signs in the sow. Both conditions occur mainly within the first 3 days of parturition and rapidly lead to piglet starvation. It can be difficult to differentiate between acute multiglandular mastitis and the hard udder syndrome. Post weaning or dry-sow mastitis usually affects one or a few glands, but drying off mastitis is uncommon in sows (compared with cows). Chronic mastitis is characterized by the formation of abscesses

and granulomas in the mammary tissue, usually seen at the time of weaning or shortly after (Hultén et al. 2003). Common environmental bacteria can enter the mammary glands through teat wounds inflicted by piglets during suckling, by entry from traumatic wounds of sows that are group-housed post weaning, or by trauma associated with the particular anatomy of inguinal mammary glands of old sows.

PDS is a general term to describe a pathological condition in the postpartum sow characterized by insufficient colostrum and milk production, leading to growth retardation, starvation, and/or mortality of the piglets.

Mastitis

Acute or chronic uniglandular mastitis is common in lactating or weaned sows and is most often seen in older sows and in inguinal glands. The microorganisms involved are the same as in acute multiglandular mastitis. Important risk factors are traumatic lesions to teats and glands or inaccessibility of teats to piglets. Piglets suckling inguinal mammary glands of old sows are often unable to reach the teat during milk ejection, and a nonsuckled mammary gland is a candidate for infection. Usually, piglets have selected a specific gland by 24–72 hours after birth; hence a piglet suckling an affected gland will show growth retardation, while littermates retain a normal growth. Milk secretion may be restricted by acquired problems of mammary conformation (as in old sows), traumatic lesions, or other teat abnormalities such as blind teats. Since teat lesions and blind teats may develop anytime, integrity of the mammary glands should be checked before each farrowing.

Traumatic teat lesions can be the consequence of injuries induced by piglets, other sows, or trauma from the environment. Cranial teats appear to be more at risk of trauma than other teats (Hultén et al. 2003). Unfortunately, primary lesions often go unnoticed; hence the gland is lost for the ongoing lactation(s). In that case, the number of nursing piglets should be limited to functional glands, or the sow should be culled. Gerjets et al. (2011) found the following to be risk factors for coliform mastitis (CM) in a case–control study: a greater number of piglets born alive and stillborn piglets, gilts compared with older sows, and birth interventions.

Mastitis is a pathological entity. Infected glands have inflammation, edema, and skin congestion with fever (>40.3–40.5 °C) and anorexia in the sow (Van Gelder and Bilkei 2005). Mastitis can affect individual, multiple, or all mammary glands. Gram-negative coliform (*Escherichia*, *Enterobacter*, *Citrobacter*, and *Klebsiella)* bacteria are most frequently isolated from mastitis-affected sows (Klopfenstein et al. 2006), with gram-positive bacteria (*Streptococci*, *Staphylococci*, *Aerococcus* spp.; Menrath et al. 2010) being less frequent. Pyogenic organisms (*Trueperella*, *Streptococci*, *Staphylococci*) can be isolated from chronic infections. Because coliforms predominate, the word "coliform mastitis" was commonly reported (Gerjets and Kemper 2009); however, most studies on CM were conducted between 1970 and 1990. Data from Kemper and Gerjets (2009) showed that the prevalence of different types of common bacteria in mammary glands in PDS-positive and PDS-negative sows do not differ.

Postpartum dysgalactia syndrome (PDS)

PDS in sows is characterized by insufficient colostrum and milk production during the first days after farrowing, the consequences of which depend on its severity. Acute cases with clinical signs in sows lead to high piglet mortality, whereas subclinical PDS leads "only" to poor growth of neonatal piglets or "problem litters" (Foisnet et al. 2010a). Clinical variation confounds diagnosis as well as estimates of prevalence of PDS. Estimates of within- or across-herd prevalence depend on the criteria used for assessment of occurrence and severity, and the variations in criteria, assessments, and reporting prevent a precise definition of PDS. The multifactorial nature of PDS makes implementation of preventive and therapeutic measures very challenging for pig veterinarians.

Pathophysiology of PDS

The pathophysiology of PDS has not yet been fully elucidated, and a single pathway is unlikely to exist (Figure 18.5). There are at least three different pathways in connection with lactogenesis and galactopoiesis (Martineau et al. 2013). One is mediated by endotoxins and acts via the innate immune system. Another one is mediated by stress, and the last one is linked with the "body building syndrome," the latter further subdivided into the "fat sow syndrome" (FSS) and the "over-muscled sow syndrome" (OMSS). As illustrated in Figure 18.5, it is necessary to consider the impact on lactogenesis and galactopoiesis (step 2) in order to understand the links between the suggested mechanisms of action of PDS (step 3) and homeorrhesis and behavior (step 1).

Clinical signs and symptoms

Martineau et al. (1992) summarized a list of early and late signs of PDS that may be present in sows, piglets, and herds. Scalable measures in sows include local signs (structurally absent nipples or glands, udder edema, mastitis, agalactia, vaginal discharge) and/or general signs (absence of any milk secretion, fever, prostration, anorexia). In piglets, early signs include mortality, inanition, diarrhea, or poor growth among the litter, whereas late signs consist mainly of variation in growth within the litter and lower weaning weights. At the herd level, measures include the consequences of PDS on

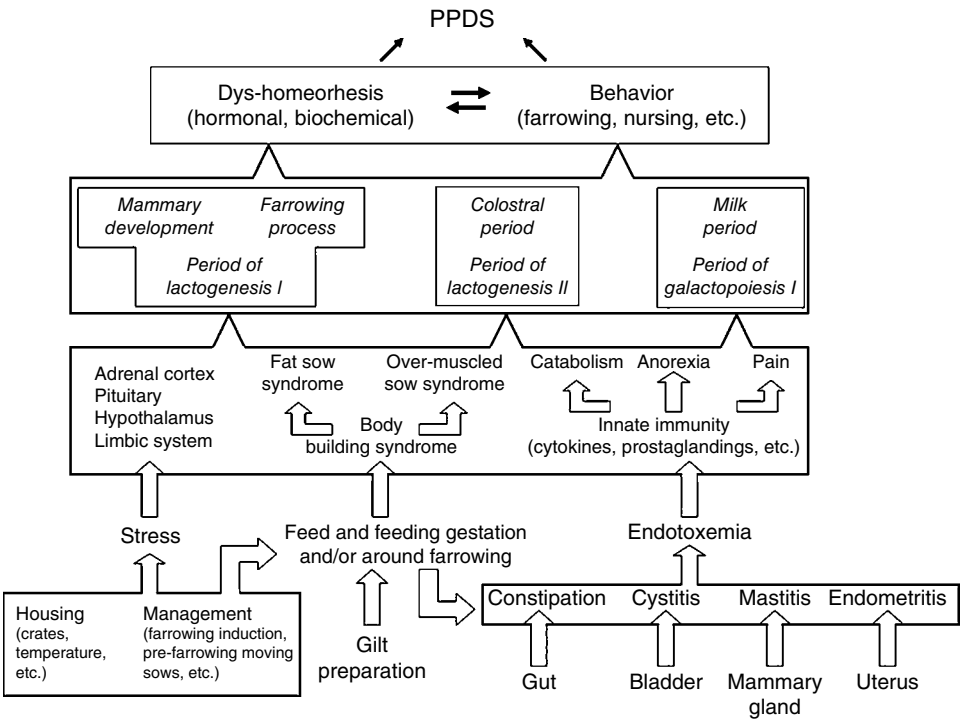


Figure 18.5 Pathophysiology of PDS. Homeorhesis is defined as "the orchestrated or coordinated changes in the metabolism of body tissues necessary to support a physiological state" (Bauman and Curie 1980). Of particular importance, the three main characteristics of homeorhetic control are (1) its chronic nature, that is, hours or days versus seconds or minutes required for homeostatic regulation; (2) its simultaneous influence on multiple tissues and systems with apparently unrelated functions; and (3) its mediation through altered responses to homeostatic signals.

productivity (number of piglets weaned/sow/year) as well as all other consequences associated with low or variable weaning weights.

The number of affected sows may vary, as does range and intensity of clinical signs. Growth retardation is the major sign of insufficient milk, identified with certainty only after passing of time. Close observation of the behavior of piglets is one of the best methods for early detection of problem litters. These piglets fight more and for longer periods, lose weight, and remain close to the sow between sucklings (Algers and de Passillé 1991). Detecting early signs in sows is difficult because most do not show differentiable signs (Klopfenstein 2003) even though milk production of sows nursing such litters is less. As reported by Foisnet et al. (2010a), the clinical presentation of sows with poor colostrum production is apparently normal.

Criteria for assessing mastitis reported in the literature can be confusing. Although the normal rectal temperature of healthy gestating sows is between 38.3 and 38.5 °C (Elmore et al. 1979; Klopfenstein et al. 1997; Messias de Bragança et al. 1997; Klopfenstein 2003), healthy lactating sows vary considerably in rectal temperature, with observed values as low as 38.4 °C and as high as 40.5 °C (Messias de Bragança et al. 1997). Elmore et al. (1979) reported a normal increase of 1–1.5 °C in rectal temperature starting approximately 24 hours before birth of the first piglet and remaining elevated until weaning (Figure 18.6a); however, there are conflicting reports (Hendrix et al. 1978; Bories et al. 2010), and Decaluwé et al. (2013) observed an increase in rectal temperature from 1 day before farrowing (38.1 °C) to day 1 of lactation (38.9 °C). This wide variation in rectal temperature is most likely the consequence of heat stress induced by the increased internal heat production and high environmental temperatures in the farrowing rooms. Some, but not all, historical reports used postpartum rectal temperatures greater than 39.3 or 39.5 °C to categorize sows as being affected by PDS (Hermansson et al. 1978b; Goransson 1989; Persson et al. 1989; Madec and Leon 1992; Hoy 2004). A Canadian study showed that rectal temperatures of sows nursing "problem litters" and characterized as PDS were similar to those of sows nursing "normal litters" both in late gestation and early lactation (Klopfenstein 2003). The greater rectal temperature observed in lactating sows must be considered as physiological hyperthermia and should not be confused with fever. Body temperature should not be misinterpreted or used as a single tool in clinical evaluation of PDS (Pepys and Hirschfield 2001; Marnell et al. 2005; Meisner 2005). The belief that higher rectal

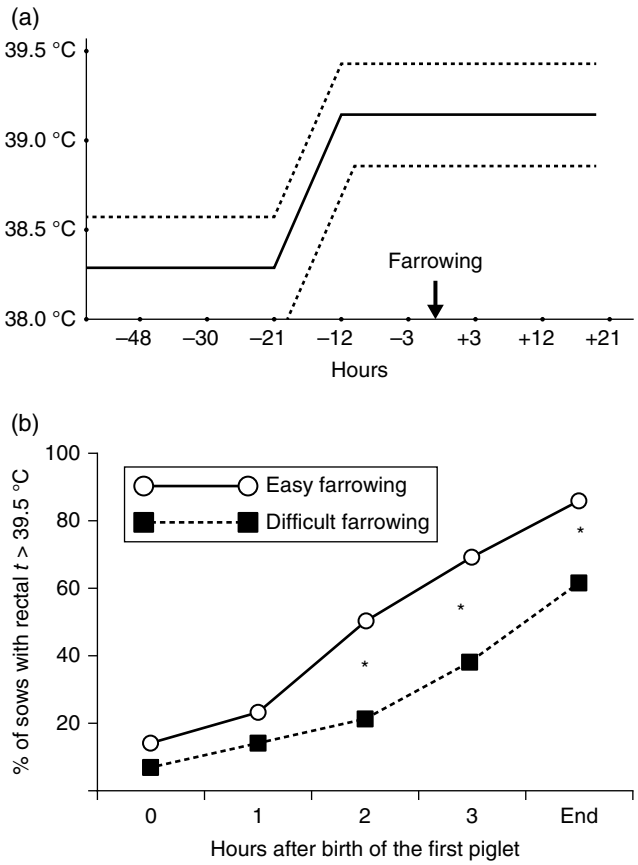


Figure 18.6 Body temperature. (a) Body temperature of preparturient sows detected by radio telemetry. The area between the dotted lines shows the range of preparturient body temperatures. *Source:* Adapted from Elmore et al. (1979). (b) Rectal temperature before and after farrowing measured in 14 sows with "difficult farrowing." Using a 39.5 °C threshold, a significantly greater proportion of sows in the "difficult farrowing" group were above this threshold after birth of the second piglet. The asterisk symbol indicates the power of the statistical results (*0.05). *Source:* Adapted from Bories et al. (2010) and Sialelli et al. (2010).

temperature identifies sows with mastitis or PDS is so generally accepted that many researchers use this criterion without estimating piglet growth and preweaning mortalities; hence many such published results on the PDS syndrome are not included in this chapter.

PDS Prevalence

The prevalence of PDS either at the animal or herd level depends upon the criteria used to assess the occurrence and the severity of the syndrome. Symptoms and criteria used to define PDS vary largely depending on the study (reviewed by Papadopoulos 2008), with diagnostic criteria including agalactia, anorexia, constipation, vaginal discharge, inflammation of mammary gland, rectal temperature higher than 39.8 °C (Jorsal 1986), or milk SCC greater than 10 million/mL even if rectal temperature is normal (<39.5 °C) and no clinical mastitis is present (Persson et al. 1996).

Generalizations regarding PDS are difficult because of the variation in criteria used for classification of hypogalactia, agalactia, mastitis, fever, appetite, piglet condition, or other clinical signs in sows or piglets (Bäckström et al. 1984; Hirsch et al. 2003; van Gelder and Bilkei 2005). As a result of this variation, prevalence data are difficult to compare and likely overestimate actual values. Bäckström et al. (1984) reported PDS in 6.9% of 16,405 farrowings recorded over 1 year in 31 swine herds in Illinois with in-herd prevalence ranging from 1.1 to 37.2%. Threlfall and Martin (1973) studied 27,656 farrowings in the state of Missouri and found that 13% of the sows were affected by PDS. The range of incidence of PDS in Swedish herds varied from 5.5% in small herds to 10.3% in large herds (Bäckström et al. 1984). More recently, in Denmark, Larsen and Thorup (2006) used a definition of PDS as one or more of the following – inappetence, abnormal mammary glands (reddening, swelling), or temperature >39.4 °C – and found an occurrence of 32.5% on the first day post farrowing, 31.5% on the second day, and 10.1% on the third day. In a survey including 110 pig herds, 34% reported to have experienced PDS problems during the year preceding the study (Papadopoulos et al. 2010).

PDS Risk factors and prevention

Risk factors for PDS are typical of multifactorial diseases, where many factors are sufficient to increase the probability of occurrence of PDS but by themselves are not necessary for causation. One of the keys in reducing the incidence of problem litters in a herd is the identification and correction of risk factors. Because different pathophysiological pathways may lead to PDS (Figure 18.5, originating from stress, feeding and endotoxemia), there are many potential risk factors identified for each of these pathways. Recent studies (Maes et al. 2010; Papadopoulos et al. 2010) suggest that modern pig herds should utilize control measures including optimization of management and feeding practices.

Animal factors and body type

The role of genetics in the occurrence of PDS is limited; Awad et al. (1990) reported a genetic predisposition for CM, and Preissler et al. (2012) showed a heritability of 0.10 for PDS, suggesting the possibility of genetic predispositions.

Sow constipation is a risk factor for PDS. One closely linked factor is endotoxemia. As sows approach farrowing, water absorption in the intestine increases due to the fluid needed for the onset of milk production (Mroz et al. 1995), which, in turn, alters feces consistency. Daily fecal scores (Oliviero et al. 2009) allowed diagnosis of constipation at both the sow and the herd level, and constipation was observed in some sows nursing problem

litters (Hermansson et al. 1978b). Feeding high fiber diets in late gestation has been widely used to decrease the incidence of early-lactation constipation related to PDS (Wallace et al. 1974). Offering feed that is low in volume and fiber can worsen constipation and increase the risk of absorbing bacterial endotoxins (Smith 1985a, b). Studies with constipated sows showed direct effects on the occurrence of PDS (Hermansson et al. 1978a; Persson et al. 1996). Constipation can result in a solid mass of feces as a physical obstacle for birthing (Cowart 2007) and perhaps be a source of intestinal pain for the sow, thus jeopardizing animal welfare. This pain induces opioid production that suppresses oxytocin release and might compromise the farrowing process (Oliviero et al. 2010b). Insufficient water intake just before farrowing can enhance postpartum constipation. Low postpartum water intake and low activity level of sows were proposed as risk factors for early lactation problems (Fraser and Phillips 1989).

Two "body building syndromes," namely, FSS and OMSS, are recognized as risk factors for PDS. Visual body condition scores only approximate true body condition (Charette et al. 1996; Maes et al. 2004). Sows that are fat at farrowing have a greater risk of peripartal hypophagia, prolonged farrowing, and PDS (Goransson 1989; Oliviero et al. 2009) and have a lower colostrum yield than nonfat sows (Decaluwé et al. 2014b), but high backfat thickness does not necessarily lead to severe PDS problems (Cools et al. 2014). Control of the FSS is not easy because small errors in the amount of feed distributed over the whole gestation period can lead to overweight or underweight sows at the time of parturition (Martineau and Klopfenstein 1996). Sows maintained in pens without individual feeding have more variable body weights, the most aggressive sows often being overweight, while submissive animals are underweight (Martineau 1990; Marchant 1997).

Control of the OMSS is also challenging as it is a new emerging syndrome (Solignac et al. 2010) and there is no consensus on the best feeding strategy for the modern hyperprolific sow. This OMSS is the direct consequence of genetic improvement and hyperprolificacy (Solignac 2008; Solignac et al. 2010). Modern and hyperprolific sows are leaner, thereby increasing the risk of an early catabolism phase before farrowing. An important contributor to this catabolism is the increased accretion rate in terms of weight of individual fetuses during the last 10 days of gestation, i.e. 50 versus 4–5 g/day during early gestation (Ji et al. 2005), with faster protein deposition (Whittemore 1998). Mammary gland growth also increases during the last third of gestation (Sorensen et al. 2002). Therefore, around and often before farrowing, the sow has already switched to a catabolic state, which continues during lactation, using her body reserves to produce large quantities of milk (van den Brand and Kemp 2006).

Rising levels of blood nonesterified fatty acids (NEFAs) are a clear indicator of a catabolic state associated with severe loss of body weight and low feed intake (Messias de Bragança and Prunier 1999). Le Cozler et al. (1998) and Oliviero et al. (2009) found that circulating concentrations of NEFAs increased rapidly a few days before farrowing, reaching a peak on the day of parturition. A catabolic state can also be quantified by circulating urea and creatinine concentrations; creatinine is a more efficient indicator of muscle catabolism than urea since it is a direct product of creatine metabolism in the muscles (Mitchell and Scholz 2001). It was found that increasing the dietary energy in late pregnancy can negatively affect feed intake during early lactation due to a reduction in glucose tolerance and to insulin resistance (Boren and Carlson 2006). Sows with high backfat levels have greater leptin concentrations, which is also a risk for reduced feed intake (Cools et al. 2013).

Housing and environment

Oliviero et al. (2010a) showed that the farrowing process lasts longer in crates than in pens, implying a potential indirect effect of housing conditions on PDS via farrowing duration. The incidence of PDS was also greater in sows housed in crates 60 cm wide compared with 67 cm wide (Cariolet, 1991). Despite numerous reports over the last 30 years, there is no clear conclusion regarding impact of housing, environment, or movement of sows prior to parturition (Klopfenstein et al. 1995; Papadopoulos et al. 2010), but a short period of adaptation of the sow to the new farrowing–lactation environment appears to be warranted. The use of slatted floors in the farrowing pens was associated with a decreased risk of chronic mastitis in sows (Hultén et al. 2004). Fully slatted floors may be hygienic, but they do not appear to support social behavior (Munsterhjelm et al. 2008) and may sometimes reduce air quality.

The effects of heat stress on lactating sows include decreased feed intake and milk production (Quiniou and Noblet 1999). High ambient temperatures have a direct effect on milk production, independent of the reduction in feed intake (Messias de Bragança and Prunier 1999). Newborn piglets require a localized warm environment, but the temperature requirement of the sow is less since her zone of thermoneutrality is much lower. It is generally recommended that room temperature be maintained warm (20–22 °C) for the 2–3 days after farrowing to favor piglet survival. However, after this crucial period, room temperature can be gradually decreased to attain 18 °C or even 15 °C on day 10 of lactation as long as a draft-free heated creep area is provided for the piglets (Farmer et al. 1998b). Overheating of the mammary glands by inappropriate placement of heating lamps decreases milk production (Muirhead and

Alexander 1997). Supplementary heat should be directed away from the sow and her udder and be removed when not needed to favor the sow's well-being and maximize her milk production.

Providing plenty of space and adequate enrichment material for nest building may reduce the incidence of PDS compared with housing sows in farrowing crates (Bäckström et al. 1984; Oliviero et al. 2009). Improving the level of hygiene of the farrowing unit was reported to be associated with lower preweaning mortality (Ravel et al. 1996).

Nutrition, feed, and feeding

Reducing feed consumption in the first days of lactation decreased the incidence of lactation failure, and gradually increasing feed intake of sows in the first week postpartum (rather than ad libitum) decreased the risk of PDS (Moser et al. 1987; Neil et al. 1996; Papadopoulos et al. 2010), but feeding ad libitum peripartally resulted in greater colostrum yield (Decaluwé et al. 2014b).

The inclusion of fiber in the feed is an often cited nutritional factor to reduce PDS. However, if the basic feed contains sufficient fiber, no extra beneficial effects on lactation are expected by adding extra fiber, whether as fermentable or inert carbohydrates (Guillou et al. 2016). Further research on the inclusion level of fibers and on the effects of different types of fibers (soluble vs. insoluble, degree of fermentability) in sow diets is needed. According to Papadopoulos (2008), a diet with a low $n-6:n-3$ ratio (fish diet) provided 8 days before expected farrowing improved feed intake during the first days postpartum and was associated with a better metabolic adaptation and inflammatory profile in periparturient sows.

The relationship between dietary selenium or vitamin E and sow lactational problems was explored because these antioxidant micronutrients can alleviate the effects of endotoxins (Elmore and Martin 1986). A low level of vitamin E (16 or 33 IU/kg vs. 66 IU/kg) was reported as a risk factor for PDS (Mahan 1991), but Mahan et al. (2000) later reported that increasing dietary levels of vitamin E from 30 to 60 IU did not decrease the prevalence of MMA diagnosed at parturition. In another study, injections of vitamin E (400 IU) and selenium (3 mg) to sows three times during gestation, on days 30, 60, and 100 of gestation, while fed normal levels of these nutrients, increased the survival rate of piglets but did not affect litter weights at weaning (Chavez and Patton 1986).

Mycotoxins are often suspected by swine veterinarians as a risk factor for PDS, yet the only well-recognized mycotoxin contributor is ergot (Kopinski et al. 2007). Grains contaminated with ergot derivatives of *Claviceps purpurea* hinder milk production in sows. Indeed, sows fed 1.5% ergot for 6–10 days preceding farrowing produced no milk, while ergot inclusions of 0.6–1.2% caused lesser problems in milk release and neonatal piglet mortality (Kopinski et al. 2007). The effects are likely due to suppressed prolactin secretion by ergot toxins. Böhmer et al. (2006) reported that diets containing probiotics at the end of gestation and around parturition may lead to a reduction in the incidence of PDS (6 vs. 13%) and to greater feed intake during lactation.

Both the sow and piglets should have easy access to fresh, good quality water. Slippery and dirty floors are one of the main causes of low activity of lactating sows and may lead to many health problems including PDS or reduced feed and water intakes.

Management

In a survey done by Papadopoulos (2008), two of the four significant risk factors for PDS were related to management: (1) moving pregnant sows to the farrowing unit 4 days instead of 7 days or earlier before expected farrowing and (2) farrowing induction, the latter with conflicting results. In some herds with a significant percentage of gilts and sows showing PDS, induction of parturition with prostaglandins was effective in reducing the incidence of PDS (Cerne and Jochle 1981; Holtz et al. 1983), whereas it had no effect in other herds (Ehnvall et al. 1977; Hansen 1979). Prostaglandins could be effective in treating PDS caused by retarded lactogenesis since incomplete luteolysis of corpora lutea can lead to high progesterone concentrations that could potentially inhibit lactogenesis. However, farrowing induction at 113 days of pregnancy did not modify the chronology of endocrine changes taking place during the peripartum period, nor did it affect colostrum yield and IgG concentrations (Foisnet et al. 2010b).

Postpartum administration of prostaglandins may have a beneficial effect on uterine involution and prevention of severe clinical endometritis (Waldmann and Heide 1996) because they are luteolytic agents causing a prepartum decline in progesterone and the release of relaxin from the corpora lutea. Vanderhaeghe et al. (2008) saw no beneficial effects of administering a prostaglandin F2α analogue to sows within 24–48 hours after farrowing on preweaning mortality and litter ADG, but litter size was greater the next parity in older sows.

Papadopoulos et al. (2010) reported that frequent farrowing supervision and neonatal care given by the stockperson compared with no farrowing supervision decreased the risk of PDS. Obstetric intervention, especially if not done properly (e.g. unhygienic), is, however, among the infectious factors increasing the occurrence of CM in sows (Bostedt et al. 1998). Depending on the criteria used to define the problem of PDS (mastitis or dysgalactia), interpretation of observations may differ. Assisting a sow showing signs of dystocia may reduce the occurrence of PDS. Similarly, sow productivity was positively correlated with procedures associated with enhanced supervision, including frequent manual

assistance, frequent use of pharmaceuticals, and alternative options for cross-fostering piglets, such as using sows from other farrowing batches and artificial suckling machines (Martel et al. 2008). With new technology such as photosensors, prediction of the onset of parturition becomes feasible (Oliviero et al. 2008b).

Treatment of the diseased sow

Treatment of affected sows needs to be done early and given a high priority while also considering risk factors and preventative measures for PDS. Since the disease is a multifactorial clinical condition with changing environmental and management contributors, treatment protocols need critical and frequent updating. In any case of PDS, the use of nonsteroidal anti-inflammatory drugs (NSAID) is indicated, as is the use of oxytocin for milk letdown. Antimicrobials may be warranted, depending on individual situations.

NSAID are used to alleviate the effects of inflammation and endotoxemia and show benefits for affected sows. Drugs used include flunixin (2 mg/kg) (Cerne et al. 1984), tolfenamic acid (2–4 mg/kg) (Rose et al. 1996), and meloxicam (0.4 mg/kg) (Hirsch et al. 2003). Treatment strategies usually consist of one treatment on the day of parturition and sometimes a second treatment one or two days later. With a 1% improvement in piglet mortality expected from the treatment, the benefits outweigh costs of the NSAID treatment (Hirsch et al. 2003).

Initiation of antimicrobial treatment should be based on the clinical picture as a whole rather than relying on increased body temperature, which may reflect simply physiological or normal variations. Use of antimicrobials is indicated if generalized signs are present. The selection of an antibiotic should be based on its spectrum of activity against those bacterial organisms identified or thought to be responsible for the problem. Identifying the most important bacteria in the diseased sows is difficult.

Another important objective in treatment is to stimulate milk flow to minimize the consequence of PDS. Repeated use of oxytocin in clinical cases of PDS is likely to be the most frequent treatment administered to stimulate milk production. Oxytocin may be given at 5–10 IU/sow (IV) for 4–5 times at 2–3 hourly intervals (Martineau 2005). There should be at least 30 minutes between two subsequent injections. In fact, in most cases, 5 IU appears to be enough to induce milk letdown, and when using this concentration, repeated doses may be given without side effects. Parenteral administration of synthetic oxytocin is a very efficient way to trigger milk ejection. The intramuscular route of administration is most common, but using the intravenous route may further improve the effect. Although efficient and considered as safe, repeated use of oxytocin might have some detrimental effects on sows. Overuse may be associated with poor piglet growth (Bilkei Papp 1994; Ravel et al. 1996) and increased SCC in milk (Garst et al. 1999).

Vaccination for PDS is not widely practiced. *Escherichia coli* strains causing neonatal diarrhea are not those usually detected in sows with PDS, but at least one report found vaccination to be useful (Fairbrother 2006). The variation in bacteria found in farrowing units as well as the lack of randomized, blinded controlled studies brings into question the use of vaccination for PDS.

Supportive treatment for the sow and piglets, especially fluid therapy, is an important part of treatment in serious cases of PDS. The main target of supportive therapy is to alleviate dehydration, common in sows with endotoxemia (Reiner et al. 2009). If monitoring determines that the sow is not drinking, then fluids can be administered intravenously or per rectum (Peltoniemi and Hälli 2004). Daily fluid requirement is considered to be approximately 7% of the body weight. If given per rectum, a backflow of about 50% is expected, and therefore volumes need to be larger than for intravenous administration.

In the case of piglets, the first step is to provide an alternative source of energy and/or to transfer piglets to another healthy sow. Piglets of heavier birth weights are cross-fostered with greater success because they have some energy reserves. Piglets may drink appreciable amounts of water on the first day following birth, particularly if milk supply is limited (Fraser et al. 1988). Water with electrolyte and glucose supplementation may help to prevent dehydration and promote survival of the piglets. Intraperitoneal infusion of approximately 15 mL of 5% glucose will temporarily alleviate dehydration and starvation (Fairbrother 2006). The piglets may thereafter be alert enough to find a teat and start suckling. Piglets may also be raised with a combination of milk replacers and highly digestible adapted feed. However, immunoglobulins included in some milk replacers are unlikely to contain antibodies specific for a farm and cannot be considered as replacement for colostrum. Providing additional heat for piglets suffering from hypothermia is always of utmost importance.

References

Algers B. 1993. *J Anim Sci* 71:2826–2831.
Algers B, de Passillé AMB. 1991. *Sven Vet Tidn* 43:659–663.
Algers B, Jensen P. 1991. *Can J Anim Sci* 71:51–60.
Algers B, Uvnäs-Moberg K. 2007. *Horm Behav* 52:78–85.
Algers B, Rojanasthien S, Uvnas Moberg K. 1990. *Appl Anim Behav Sci* 26:267–276.

Armstrong JD, Coffey MT, Esbenshade KL, et al. 1994. *J Anim Sci* 72:1570–1577.
Atwood CS, Hartmann PE. 1995. *J Dairy Res* 62:221–236.
Auldist DE, Morrish L, Eason P, et al. 1998. *Anim Sci* 67:333–337.
Auldist DE, Carlson D, Morrish L, et al. 2000. *J Anim Sci* 78:2026–2031.
Awad M, Baumgartner W, Passering A, et al. 1990. *Tierarztl Umsch* 45:526–535.
Bäckström L, Morkoc AC, Connor J, et al. 1984. *J Am Vet Med Assoc* 185:70–73.
Balzani A, Cordell HJ, Edwards SA. 2016a. *Theriogenology* 86:1913–1920.
Balzani A, Cordell HJ, Sutcliffe E, et al. 2016b. *J Anim Sci* 94:394–400.
Barone R. 1978. École nationale vétérinaire, Lyon.
Bauman DE, Currie WB. 1980. *J Dairy Sci* 63:1514–1529.
Benjaminsen E. 1981. *Acta Vet Scand* 22:67–77.
Beyer M, Jentsch W, Hoffmann L, et al. 1994. *Arch Anim Nutr* 46:7–36.
Biermann J, Jourquin J, van Gelderen R, et al. 2010. In Proceedings of the International Pig Veterinary Society Congress, p. 1187.
Bilkei Papp G. 1994. *Magy Allatorv Lap* 49:680–683.
Bilkei G, Goos T, Bolcskei A. 1994. *Prakt Tierarzt* 75:16–21.
Björkman S, Oliviero C, Hasan S, et al. 2017. ESDAR 2017, August 24–26, Bern, Switzerland.
Böhmer B, Kramer W, Roth-Maier D. 2006. *J Anim Physiol Anim Nutr* 90:309–315.
Bolet G, Etienne M. 1982. In Proceedings of 14èmes Journées du Grenier de Theix, Clermont-Ferrand, France.
Boren CA, Carlson MS. 2006. *Albeitar* 100:14–16.
Bories P, Vautrin F, Boulot S, et al. 2010. *J Rech Porc France* 42:233–239.
Bostedt H, Maier G, Herfen K, et al. 1998. *Tierarztl Prax* 26:332–338.
Boulot S, Quesnel H, Quiniou N. 2008. *Adv Pork Prod* 19:1–8.
Bourne FJ. 1976. *Vet Rec* 98:499–501.
Bourne FJ, Curtis J. 1973. *Immunology* 24:157–162.
Boyd RD, Kensinger RS, Harrell RJ, et al. 1995. *J Anim Sci* 73:36–56.
Calhoun ML, Stinson AW. 1987. Integuments. In Texbook of Veterinary Histology. Philadelphia: Lea & Febiger, p. 351.
Campbell RG, Dunkin AC. 1982. *Anim Prod* 35:193–197.
Campos PHRF, Silva BAN, Donzele JL, et al. 2012. *Animal* 6:797–806.
Cariolet R. 1991. *J Rech Porc France* 23:189–194.
Castren H, Algers B, Jensen P, et al. 1989. *Appl Anim Behav Sci* 24:227–238.
Castren H, Algers B, Saloniemi H. 1991. *Livest Prod Sci* 28:321–330.
Castren H, Algers B, de Passillé AMB, et al. 1993. *Anim Prod* 57:465–471.
Cerne F, Jochle W. 1981. *Theriogenology* 16:459–467.
Cerne F, Jerkovic I, Debeljak C. 1984. In Proceedings of the International Pig Veterinary Society Congress, p. 290.
Chaloupková H, Illmann G, Neuhauserová K, et al. 2007. *J Anim Sci* 85:1741–1749.
Charette R, Bigras-Poulin M, Martineau G-P. 1996. *Livest Prod Sci* 46:107–115.
Chavez ER, Patton KL. 1986. *Can J Anim Sci* 66:1065–1074.
Chertkov DD. 1986. *Zhivotnovodstvo* 8:55–56.
Cools A, Maes D, Decaluwé R. 2013. *Domest Anim Endocrinol* 45:22–27.
Cools A, Maes D, Decaluwé R. 2014. *Anim Reprod Sci* 145:130–140.
Cowart RP. 2007. Parturition and dystocia in swine. In Youngquist RS, Threlfall WR, eds. Large Animal Theriogenology. St. Louis, MI: Saunders, pp. 778–784.
Crenshaw TD, Grieshop CM, McMurtry JP, et al. 1989. *J Dairy Sci* 72:258–259.
Cronin GM, Leeson E, Cronin JG, et al. 2001. *J Anim Sci* 14:1019–1023.
Decaluwé R, Maes D, Declerck I, et al. 2013. *Animal* 7:1999–2007.
Decaluwé R, Maes D, Cools A, et al. 2014a. *Livest Sci* 162:185–192.
Decaluwé R, Maes D, Cools A, et al. 2014b. *J Anim Sci* 92:3557–3567.
Declerck I, Dewulf J, Piepers S, et al. 2015. *J Anim Sci* 93:1309–1317.
Declerck I, Dewulf J, Sarrazin S, et al. 2016a. Long-term effects of colostrum intake in piglet mortality and performance. *J Anim Sci* 94:1–11.
Declerck I, Dewulf J, Decaluwé R, et al. 2016b. *Livest Sci* 183:48–53.
Delouis C. 1986. Lactation. In: Perez JM, Mornet P, Rérat A, eds. Le porc et son élevage bases scientifiques et techniques. Paris: Maloine, pp. 55–63.
van der Peet-Schwering CM, Kemp B, Plagge JG, et al. 2004. *J Anim Sci* 82:1246–1257.
Devillers N, van Milgen J, Prunier A, et al. 2004. *Anim Sci* 78:305–313.
Devillers N, Le Dividich J, Prunier A. 2006. *INRA Prod Anim* 19(1):29–38.
Devillers N, Farmer C, Le Dividich J, et al. 2007. *Animal* 1:1033–1041.
Dobson H. 1988. Softening and dilation of the uterine cervix. In Clarke JR, ed.Oxford Reviews of Reproductive Biology. Toronto: Oxford University Press, pp. 491–513.
Dourmad JY, Noblet J, Etienne M. 1998. *J Anim Sci* 76:542–550.
Drendel C, Wendt K. 1993. *Monatsh Veterinarmed* 48:413–417.
Dubreuil P, Pelletier G, Petitclerc D, et al. 1990. *Can J Anim Sci* 70:821–832.
Dusza L, Sobczak J, Jana B, et al. 1991. *Med Weter* 47:418–421.

Dyck GE, Swierstra EE. 1987. *Can J Anim Sci* 67:543–547.
Edwards SA, Baxter EM. 2015. Piglet mortality: Causes and prevention. Chapter 11. In Farmer C, ed. The gestating and lactating sow. Wageningen, The Netherlands: Wageningen Academic Publishers, pp. 253–278.
Ehnvall R, Einarsson S, Larson K, et al. 1977. *Nord Vet Med* 29:376–380.
Einarsson S, Rojkittikhun T. 1993. *J Reprod Fertil Suppl* 48:229–239.
Ellendorf F, Forsling ML, Poulain DA. 1982. *J Physiol* 333:577–579.
Elmore RG, Martin CE. 1986. Mammary glands. In Leman AD, Straw BE, Glock WL, et al., eds. Diseases of Swine, 6th ed. Ames, IA: Iowa State University Press.
Elmore RG, Martin CE, Riley JL, et al. 1979. *J Am Vet Med Assoc* 174:620–622.
Elsley FWH. 1971. Nutrition and Lactation in the Sow. London, UK: Butter Worth.
English PR, Morrison V. 1984. *Pig News Info* 5:369–376.
Evans PA, Newby TJ, Stokes CR, et al. 1982. *Vet Immunol Immunopathol* 3:515–527.
Fahmy MH, Bernard C. 1971. *Can J Anim Sci* 51:351–359.
Fairbrother JM. 2006. Coliform mastitis. In Straw B, Immermann JJ, D'Allaire S, et al., eds. Diseases of Swine, 9th ed. Ames, IA: Blackwell Publishing, pp. 665–671.
Farmer C. 2001. *Livest Prod Sci* 70:105–113.
Farmer C. 2013. *Animal* 7:1964–1968.
Farmer C, Hurley WL. 2015. Mammary development. Chapter 4. In Farmer C, ed. The Gestating and Lactating Sow. Wageningen, the Netherlands: Wageningen Academic Publishers, pp. 73–94.
Farmer C, Palin MF. 2005. *J Anim Sci* 83:825–832.
Farmer C, Palin MF. 2008. *Can J Anim Sci* 88:585–590.
Farmer C, Petitclerc D. 2003. *J Anim Sci* 81:1823–1829.
Farmer C, Quesnel H. 2009. *J Anim Sci* 87(Suppl 1):56–65.
Farmer C, Robert S, Matte JJ. 1996. *J Anim Sci* 74:1298–1306.
Farmer C, Robert S, Rushen J. 1998a. *J Anim Sci* 76:750–757.
Farmer C, Robert S, Choiniere Y. 1998b. *Can J Anim Sci* 78:23–28.
Farmer C, Sorensen MT, Robert S, et al. 1999. *J Anim Sci* 77:1851–1859.
Farmer C, Sorensen MT, Petitclerc D. 2000. *J Anim Sci* 78:1303–1309.
Farmer C, Palin MF, Sorensen MT, et al. 2001. *Can J Anim Sci* 81:487–493.
Farmer C, Petitclerc D, Sorensen MT, et al. 2004. *J Anim Sci* 82:2343–2351.
Farmer C, Devillers N, Rooke JA, et al. 2006. Colostrum production in swine: From the mammary glands to the piglets. *CAB Rev Perspect Agric Vet Sci Nutr Nat Resour* 3:1–16.
Farmer C, Knight C, Flint D. 2007. *Can J Anim Sci* 87:35–43.
Farmer C, Palin MF, Hovey R. 2010a. *Can J Anim Sci* 90:379–388.
Farmer C, Palin M-F, Gilani GS, et al. 2010b. *Animal* 4:454–465.
Farmer C, Palin MF, Theil PK, et al. 2012. *J Anim Sci* 90:3743–3751.
Farmer C, Comi M, Duarte CRA, et al. 2016a. *J Anim Sci* 94:3206–3214.
Farmer C, Duarte CRA, Vignola M, et al. 2016b. *J Anim Sci* 94:1897–1905.
Farmer C, Amezcua M, Bruckmaier R, et al. 2017. *J Anim Sci* 95:681–687.
Fisette K, Laforest JP, Robert S, et al. 2004. *Can J Anim Sci* 84:573–579.
Flint DJ, Gardner M. 1994. *Endocrinology* 135:1119–1124.
Foisnet A. 2010. Variabilité de la production de colostrum par la truie: implication des changements endocriniens et métaboliques en période péripartum. PhD thesis. Université Européenne de Bretagne, France.
Foisnet A, Farmer C, David C, et al. 2010a. *J Anim Sci* 88:1672–1683.
Foisnet A, Farmer C, David C, et al. 2010b. *J Anim Sci* 88:1684–1693.
Ford JA Jr, Kim SW, Rodriguez-Zas SL, et al. 2003. *J Anim Sci* 81:2583–2589.
Foxcroft GR, Vinsky MD, Paradis F, et al. 2007. *Theriogenology* 68:30–39.
Fraser D. 1976. *Br Vet J* 129:324–336.
Fraser D. 1977. *Br Vet J* 133:126–133.
Fraser D. 1980. *Appl Anim Ethol* 6:247–255.
Fraser D. 1984. *Anim Prod* 39:115–123.
Fraser D, Jones RM. 1975. *J Agric Sci* 84:387–391.
Fraser D, Phillips PA. 1989. *Appl Anim Behav Sci* 24:13–22.
Fraser D, Nicholls C, Fagan W. 1985. *J Agric Eng Res* 31:371–376.
Fraser D, Phillips PA, Thompson BK, et al. 1988. *Can J Anim Sci* 68:603–610.
Gandhi SS, Getty R. 1969a. *Iowa State J Sci* 44:15–30.
Gandhi SS, Getty R. 1969b. *Iowa State J Sci* 44:31–43.
Garst AS, Ball SF, Williams BL, et al. 1999. *J Anim Sci* 77:1624–1630.
Genest M, D'Allaire S. 1995. *Can J Anim Sci* 75:461–467.
Gerjets I, Kemper N. 2009. *J Swine Health Prod* 17(2):97–105.
Gerjets I, Traulsen I, Reiners K, et al. 2011. Assessing individual sow risk factors for coliform mastitis: A case-control study. *Prev Vet Med* 100:248–251.
Ghoshal NG. 1975. Porcine nervous system. Spina nerves. In Getty R, ed. Sisson and Grossmann's the Anatomy of the Domestic Animal, 5th ed. Toronto: W.B. Saunders, pp. 1383–1396.
Goransson L. 1989. *J Am Vet Med Assoc* 36:505–513.
Grun D, Reiner G, Dzapo V. 1993a. *Reprod Domest Anim* 28:14–21.

Grun D, Reiner G, Dzapo V. 1993b. *Reprod Domest Anim* 28:22–27.
Guillou D, de Grave X, van der Aar, et al. 2016. *J Anim Sci* 94(Suppl 3):146–149.
Guthrie HD, Meckley PE, Young EP, et al. 1987. *J Anim Sci* 65:203–211.
Hacker RR. 1970. Studies on the development and function of porcine mammary glands. PhD thesis, Purdue University, West Lafayette, Indiana.
Hacker RR, Hill DL. 1972. *J Dairy Sci* 55:1295–1299.
Hansen LH. 1979. *Nord Vet Med* 31:122–128.
Hansen AV, Strathe AB, Kebreab E, et al. 2012. *J Anim Sci* 90:2285–2298.
Harkins M, Boyd RD, Bauman DE. 1989. *J Anim Sci* 67:1997–2008.
Harmon RJ. 1994. *J Dairy Sci* 77:2103–2112.
Harrell RJ, Thomas MJ, Boyd RD. 1993. In Proceedings of Cornell Nutrition Conference Feed Manufacturers, pp. 156–164.
Hartmann PE, Holmes MA. 1989. Sow lactation. In Barnett JL, Hennessy DD, eds. Manipulating Pig Production II. Albury, Australia: Australasian Pig Science Association, pp. 72–97.
Hartmann PE, Whitely JL, Willcox DL. 1984. *J Physiol* 347:453–463.
Hartmann PE, Thompson MJ, Kennaugh LM, et al. 1995. Metabolic regulation of sow lactation. In Hennessy DP, Cranwell PD, eds. Manipulating Pig Production V. Werribee, Australia: Australasian Pig Science Association. pp. 26–29.
Hasan SMK, Junnikkala S, Valros A, et al. 2016. *Animal* 10:1728–1733.
Head RH, Williams IH. 1991. Mammogenesis is influenced by pregnancy nutrition. In Hennessy DP, Cranwell PD, eds. Manipulating Pig Production III. Werribee, Australia: Australasian Pig Science Association, p. 33.
Hemsworth PH, Winfield CG. 1976. *Anim Prod* 22(3):351–357.
Hendrix WF, Kelley KW, Gaskins CT. 1978. Porcine neonatal survival and serum gamma globulins. *J Anim Sci* 47:1281–1286.
Hermansson I, Einarsson S, Ekman L, et al. 1978a. *Nord Vet Med* 30:474–481.
Hermansson I, Einarsson S, Larson K, et al. 1978b. *Nord Vet Med* 30:465–473.
Herpin P, Louveau I, Damon M, et al. 2005. Environmental and hormonal regulation of energy metabolism in early development of the pig. In Burrin DG, Mersmann H, eds. Biology of Metabolism in Growing Animals. Amsterdam: Elsevier, pp. 353–374.
Hirsch AC, Philipp H, Kleemann R. 2003. *J Vet Pharm Ther* 26:355–360.
Holtz W, Hartmann JF, Welp C. 1983. *Theriogenology* 19:583–592.
Hoofs AIJ, Elst-Ter Wahle L. 1993. Report Praktijkonderzoek, Rosmalen, The Netherlands.
Howard KA, Nelson DA, Garcia-Sirera J, et al. 1994. *J Anim Sci* 72(Suppl 1):334.
Hoy S. 2004. In Proceedings of the International Pig Veterinary Society Congress, p. 849.
Hughes PE, Varley MA. 1980. Reproduction in the Pig. London: Butterworths.
Hultén F, Valros A, Rundgren M, et al. 2002. *Theriogenology* 58:1503–1517.
Hultén F, Persson A, Eliasson-Selling L, et al. 2003. *Am J Vet Res* 64:463–469.
Hultén F, Persson A, Eliasson-Selling L, et al. 2004. *Am J Vet Res* 65:1398–1403.
Hurley WL. 1989. *J Dairy Sci* 72:1637–1646.
Hurley WL. 2001. *Livest Prod Sci* 70:149–157.
Hurley WL. 2015. Chapter 9: Composition of sow colostrum and milk. In Farmer C, ed. The Gestating and Lactating Sow. Wageningen, the Netherlands: Wageningen Academic Publishers, pp. 13–26.
Hurley WL, Grieve RCJ. 1988. *Vet Res Commun* 12:149–153.
Hurley WL, Doane RM, O'Day Bowman MB, et al. 1991. *Endocrinology* 128:1285–1290.
Illmann G, Madlafousek J. 1995. *Appl Anim Behav Sci* 44:9–18.
Jensen P. 1986. *Appl Anim Behav Sci* 16:131–142.
Jensen P. 1988. *Appl Anim Behav Sci* 20:297–308.
Jensen P, Stangel G, Algers B. 1991. *Appl Anim Behav Sci* 31:195–209.
Jensen P, Vestergaard K, Algers B. 1993. *Appl Anim Behav Sci* 38:245–255.
Ji F, Wu G, Blanton JR, Kim SW. 2005. *J Anim Sci* 83:366–375.
Ji F, Hurley WL, Kim SW. 2006. *J Anim Sci* 84:579–587.
Johnson RW. 2002. *Vet Immunol Immunopathol* 87:443–450.
Jorsal SE. 1986. In Proceedings of the International Pig Veterinary Society Congress, p. 93.
Kemper N, Gerjets I. 2009. *Acta Vet Scand* 51:26–33.
Kensinger RS, Collier RJ, Bazer FW, et al. 1982. *J Anim Sci* 54:1297–1308.
Kensinger RS, Collier RJ, Bazer FW. 1986. *Domest Anim Endocrinol* 3:237–245.
Kent JC, Kennaugh LM, Hartmann PE. 2003. *J Dairy Res* 70:131–138.
Kerr BJ. 1997. Amino acid nutrition of lactating sows. Blokyova Technical Review-10, St-Louis, Missouri.
Kim SW, Hurley WL, Han IK, et al. 1999a. *J Anim Sci* 77:2510–2516.
Kim SW, Hurley WL, Han IK, et al. 1999b. *J Anim Sci* 77:3304–3315.
Kim SW, Osaka I, Hurley WL, et al. 1999c. *J Anim Sci* 77:3316–3321.

Kim SW, Hurley WL, Han IK, et al. 2000. *J Anim Sci* 78:1313–1318.
Kim SW, Easter RA, Hurley WL. 2001. *J Anim Sci* 79:2659–2668.
King RH, Toner MS, Dove H, et al. 1993. *J Anim Sci* 71:2457–2463.
King RH, Pettigrew JE, McNamara JP, et al. 1996. *Anim Reprod Sci* 41:37–50.
King RH, Mullan BP, Dunshea FR, et al. 1997. *Livest Prod Sci* 47:169–174.
Klobasa F, Werhahn E, Butler JE. 1987. *J Anim Sci* 64:1458–1466.
Klobasa F, Habe F, Werhahn E. 1990. *Berl Munch Tierarztl Wochenschr* 103:335–340.
Klobasa F, Werhahn E, Habe F. 1991. *Berl Munch Tierarztl Wochenschr* 104:223–227.
Klobasa F, Schroder C, Stroot C, et al. 2004. *Berl Munch Tierarztl Wochenschr* 117:19–23.
Klopfenstein C. 2003. Variation temporelle des caractéristiques comportementales et physiologiques des truies qui allaitent les portées à croissance faible et normale en période du péri-partum. PhD thesis, Université de Montréal, Montréal.
Klopfenstein C, D'Allaire S, Martineau GP. 1995. *Livest Prod Sci* 43:243–252.
Klopfenstein C, Bigras Poulin M, et al. 1997. *J Rech Porc France* 29:53–58.
Klopfenstein C, Farmer C, Martineau GP. 2006. Mammary glands and lactation problems. In Straw BE, Mengeling WL, D'Allaire S, et al., eds. Diseases of Swine, 9th ed. Ames, IA: Iowa State University Press, pp. 833–860.
Koketsu Y, Dial GD, Pettigrew JE, et al. 1996a. *J Anim Sci* 74:1036–1046.
Koketsu Y, Dial GD, Pettigrew JE, et al. 1996b. *J Anim Sci* 74:1202–1210.
Koketsu Y, Takenobu S, Nakamura R. 2006. *J Vet Med Sci* 68:821–826.
Konar A, Thomas PC, Rook JAF. 1971. *J Dairy Res* 38:333–341.
Kopinski J, Blaney B, Downing J, et al. 2007. *Aust Vet J* 85:169–176.
Kusina J, Pettigrew JE, Sower AF, et al. 1995. *J Anim Sci* 73:189.
Labroue F, Caugant A, Ligonesche B, et al. 2001. *J Rech Porcine France* 33:145–150.
Larsen I, Thorup F. 2006. The diagnosis of MMA. *Proc Int Pig Vet Soc* O.52-02:256.
Le Cozler Y, David C, Beaumal V, et al. 1998. *Reprod Nutr Dev* 38:377–390.
Le Cozler Y, Pichodo X, Roy H, et al. 2004. *J Rech Porc France* 36:443–450.
Le Dividich J, Herpin P, Mourot J, et al. 1994a. Comp *Biochem Physiol* 108A(4):663–671.
Le Dividich J, Herpin P, Rosario-Ludovino RM. 1994b. *J Anim Sci* 72:2082–2089.
Le Dividich J, Martineau GP, Thomas F, et al. 2004. *J Rech Porc France* 36:451–459.
Le Dividich J, Rooke JA, Herpin P. 2005. *J Agric Sci* 143:469–485.
Le Dividich J, Marion J, Thomas F. 2007. *Can J Anim Sci* 87:571–577.
Lewis NJ, Hurnik FJ. 1985. *Appl Anim Behav Sci* 14:225–232.
Lewis AJ, Speer VC, Haught DG. 1978. *J Anim Sci* 47:634–638.
Lignereux Y, Rossel R, Jouglar JY. 1996. *Rev Med Vet* 147:191–194.
Loisel F, Farmer C, Ramaekers P, et al. 2014. *J Anim Sci* 92:2931–2941.
Lyvers-Peffer PA, Rozeboom DW. 2001. *Livest Prod Sci* 70:167–173.
Madec F, Leon E. 1992. *J Vet Med A* 39:433–444.
Maes D, Janssens G, Delputte P, et al. 2004. *Livest Prod Sci* 91:57–67.
Maes D, Papadopoulos G, Cools A, et al. 2010. *Tierarztl Prax* 1:15–20.
Magnusson U, Rodriguez Martinez H, Einarsson S. 1991. *Vet Rec* 129:485–490.
Mahan D. 1991. *J Anim Sci* 69:2904–2917.
Mahan DC, Becker DE, Norton HW, et al. 1971. *J Anim Sci* 33:35–37.
Mahan DC, Kim YY, Stuart RL. 2000. *J Anim Sci* 78:110–119.
Manjarin R, Trottier NL, Weber PS, et al. 2011. *J Dairy Sci* 94:4950–4961.
Marchant J. 1997. *Pig Farming* April:36–37.
Marnell L, Mold C, Du Clos TW. 2005. *Clin Immunol* 17:104–111.
Martel G, Dourmad JY, Dedieu B. 2008. *Livest Sci* 116:96–107.
Martineau GP. 1990. Body building syndrome in sows. In: Proceedings of American Association of Swine Veterinarians Annual Meeting, pp. 345–348.
Martineau GP, 2005. Postpartum dysgalactia syndrome and mastitis in sows. In Kahn CM, ed. Reproduction. The Merck Veterinary Manual, 9th ed. Whitehouse Station, NJ: Merck Co, Inc., pp. 1134–1137.
Martineau GP, Klopfenstein C. 1996. *J Rech Porc France* 28:331–338.
Martineau GP, Smith BB, Doizé B. 1992. *Vet Clin N Am Food Anim Pract* 8:661–684.
Martineau GP, Le Treut Y, Guillou D, et al. 2013. *J Swine Health Prod* 21:85–93.
Matzat PD, Hogberg MG, Fogwell RL, et al. 1990. Report of Swine Research, Michigan State University AS-SW-8904, pp. 36–40.
Meisner M. 2005. *Curr Opin Crit Care* 11:473–480.
Menrath A, Gerjets I, Reiners K, et al. 2010. Bacterial pathogens present in the milk of healthy sows and sows with PDS (Postpartum Dysgalactia Syndrome): Detailed analysis of isolated Aerococcus spp. In Proceedings

of the International Pig Veterinary Society Congress, p. 1186.
Messias de Bragança M, Prunier A. 1999. *Domest Anim Endocrinol* 16:89–101.
Messias de Bragança M, Mounier AM, Hulin JC, et al. 1997. *J Rech Porc France* 29:81–88.
Michelchen G, Ender K. 1991. *Arch Tierzucht* 34:313–322.
Miquet J, Viana G.2010. In Proceedings of the International Pig Veterinary Society Congress, p. 160.
Mitchell AD, Scholz AM. 2001. Techniques for measuring body composition of swine. In Lewis AL, Southern L, eds. Swine Nutrition. Boca Raton, FL: CRC Press, pp. 32–37.
Molenat M, Thibeault L. 1977. *L'Élevage Porcin* 108:33–36.
Moser RL, Cornelius SG, Pettigrew JE, et al. 1987. *Livest Prod Sci* 16:91–99.
Mroz Z, Jongbloed AW, Lenis NP, et al. 1995. *Nutr Res Rev* 8:137–164.
Muirhead M. 1991. *Int Pigletter* 10:22–23.
Muirhead M, Alexander T. 1997. Mastitis, lactation failure. In Muirhead MR, Alexander TJL, eds. Managing Pig Health and the Treatment of Disease. Sheffield, UK: 5M Enterprises Limited, pp. 236–239.
Mullan BP, Williams IH. 1989. *Anim Prod* 48:449–457.
Munsterhjelm C, Valros A, Heinonen M, et al. 2008: *Reprod Domest Anim* 43:584–591.
Neil M, Ogle B, Annér K. 1996. *J Anim Sci* 62:337–347.
Nielsen OL, Sorensen MT. 1998. *J Anim Sci* 76(Suppl. 1):377 (Abstr).
Noblet J, Etienne M. 1986. *J Anim Sci* 63:1888–1896.
Noblet J, Etienne M. 1989. *J Anim Sci* 67:3352–3359.
Oliviero C, Pastell M, Heinonen M, et al. 2008a. *Biosyst Eng* 100:281–285.
Oliviero C, Heinonen M, Valros A, et al. 2008b. *Anim Reprod Sci* 105(3–4):365–377.
Oliviero C, Heinonen M, Valros A, et al. 2009. *Anim Reprod Sci* 119:85–91.
Oliviero C, Kokkonen T, Heinonen M, et al. 2010a. *Res Vet Sci* 86(2):314–319.
Oliviero C, Heinonen A, Valros A, et al. 2010b. *Anim Reprod Sci* 119:85–91.
Papadopoulos GA. 2008. Lactation physiology in sows: impact of feeding strategies and risk factors for postpartum dysgalactia syndrome. PhD thesis, Ghent University, p. 229.
Papadopoulos GA, Vanderhaeghe C, Janssens GPJ, et al. 2010. *Vet J* 184:167–171.
de Passillé AMB, Rushen J. 1989a. *Appl Anim Behav Sci* 22:23–38.
de Passillé AMB, Rushen J. 1989b. *Can J Anim Sci* 69:535–544.
de Passillé AMB, Rushen J, Hartsock TG. 1988. *Can J Anim Sci* 68:325–338.
Peltoniemi OAT, Hälli O. 2004. *Suomen eläinlääkärilehti* 11: 573–576.
Peltoniemi OAT, Tast A, Heinonen M, et al. 2009. *Reprod Domest Anim*. doi: 10.1111/j.1439-0531.2009.01477.x.
Pepys MB, Hirschfield GM. 2001. *Ital Heart* 2:804–806.
Perrin DR. 1955. *J Dairy Sci* 12:103–107.
Persson A, Pedersen A, Goransson L. 1983. In Proceedings of International Conference on Production Disease in Farm Animals, Uppsala, Sweden, pp. 220–223.
Persson A, Pedersen E, Gorensen L, et al. 1989. *Acta Vet Scand* 30:9–17.
Persson A, Pedersen Morner A, Kuhl W. 1996. *Acta Vet Scand* 37:293–313.
Pettigrew JE. 1995. The influence of substrate supply on milk production in the sow. In Hennessy DP, Cranwell PD, eds. Manipulating Pig Production V. Werribbee, Australia: Australasian Pig Science Association, p. 129.
Plaut KI, Kensinger RS, Griel LC Jr, et al. 1989. *J Anim Sci* 67:1509–1519.
Pluske JR, Williams IH, Aherne FX. 1995a. Nutrition of the neonatal pig. In Varley MA, ed. The Neonatal Pig-Development and Survival. Wallingford, UK: CAB International, pp. 187–235.
Pluske JR, Williams IH, Cegielski AC, et al. 1995b. Super-alimentation of first litter sows during lactation. In Hennessy DP, Cranwell PD, eds. Manipulating Pig Production V. Werribbee, Australia: Australasian Pig Science Association.
Pluske JR, Williams IH, Zak LJ, et al. 1998. *J Anim Sci* 76:1165–1171.
Preissler R, Hinrichs D, Reiners K et al. 2012. *J Anim Breed Genet* 129:98–102.
Puppe B, Tuchscherer A. 2000. *Anim Sci* 71:273–279.
Quesnel H. 2011. *Animal* 5:1546–1553.
Quesnel H, Brossard L, Valancogne A, et al. 2008. *Animal* 2:1842–1849.
Quesnel H, Meunier-Salaun M-C, Hamard A, et al. 2009. *J Anim Sci* 87:532–543.
Quesnel H, Farmer C, Devillers N. 2012. *Livest Sci* 146:105–114.
Quesnel H, Farmer C, Theil PK. 2015. Colostrum and milk production. Chapter 8. In Farmer C, ed. The Gestating and Lactating Sow. Wageningen, the Netherlands: Wageningen Academic Publishers, pp. 173–192.
Quiniou N, Noblet J. 1999. *J Anim Sci* 77:2124–2134.
Ravel A, D'Allaire S, Bigras Poulin M. 1996. *Prev Vet Med* 29:37–57.
Reale TA. 1987. Supplemental liquid diets and feed flavours for young pigs. MSc thesis, University of Melbourne, Melbourne, Australia.
Reiner G, Hertampf B, Richard HR. 2009. *Tierarztl Prax* 37: 305–318.
Robertson HA, King GJ. 1974. *J Reprod Fertil* 40:133–141.
Rooke JA, Bland IM. 2002. *Livest Prod Sci* 78:13–23.
Rose M, Schnurrbusch U, Heinrotzi H. 1996. In Proceedings of the International Pig Veterinary Society Congress, p. 317.

Rozeboom DW. 2015. Conditioning of the gilt for optimal reproductive performance. Chapter 1. In Farmer C, ed. The Gestating and Lactating Sow. Wageningen, The Netherlands: Wageningen Academic Publishers, pp. 13–26.
Ruan WF, Monaco ME, Kleinberg DL. 2005. *Endocrinology* 146:1170–1178.
Rushen J, Foxcroft GR, de Passillé AMB. 1993. *Physiol Behav Sci* 53:265–270.
Rushen J, Ladewig J, de Passillé AMB. 1995. *Appl Anim Behav Sci* 45:53–61.
Salmon H, Berri M, Gerdts V, et al. 2009. *Dev Comp Immunol* 33:384–393.
Salmon-Legagneur E. 1965. Quelques aspects des relations nutritionnelles entre la gestation et la lactation chez la truie. Thèse de doctorat. Université de Paris, Paris, France.
Sauber TE, Stahly TS, Ewan RC, Williams NH. 1994. *J Anim Sci* 72:364–364.
Scheel DE, Graves HB, Sherritt GW. 1977. *J Anim Sci* 45:219–229.
Schnulle PM, Hurley WL. 2003. *Vet Immunol Immunopathol* 91:227–231.
Schollenberger A, Degorski A, Frymus T, et al. 1986. *J Vet Med A* 33:31–38.
Schummer A, Wilkens H, Vollmerhaus B, et al. 1981. The Circulatory System, the Skin, and the Cutaneous Organs of the Domestic Mammals, Vol. 3. New York: Springer-Verlag, p. 630.
Shoenherr WD, Stahly TS, Cromvell GL. 1989. *J Anim Sci* 67:482–488.
Sialelli J-N, Vautrin F, Bories P, Boulot S, Pere M-C, Martineau G-P. 2010. In Proceedings of the International Pig Veterinary Society Congress, p. 125.
Smith BB. 1985a. *Theriogenology* 3:283–296.
Smith BB. 1985b. *Am J Vet Res* 46:175–180.
Smith VG, Leman AD, Seaman WJ, et al. 1991. *J Anim Sci* 69:3501–3510.
Smith BB, Martineau GP, Bisaillon A. 1992. Mammary glands and lactation problems. In Leman AD, Straw BE, Mengeling WL, et al., eds. Diseases of Swine, 7th ed. Ames, IA: Iowa State University Press, pp. 40–61.
Smith KL, Hillerton JE, Harmon RJ. 2001. Guidelines on Normal and Abnormal Raw Milk Based on Somatic Cell Counts and Signs of Clinical Mastitis. National Mastitis Council.
Smits RJ, Henman DJ, King RH. 2012. *Anim Prod Sci* 53:23–29.
Soede NM, Kemp B. 2015. Best practices in the lactating and weaned sow to optimize reproductive physiology and performance. Chapter 17. In Farmer C,ed. The Gestating and Lactating Sow. Wageningen, The Netherlands: Wageningen Academic Publishers, pp. 377–407.
Sohn KS, Maxwell CV. 1999. *Asian-Aust J Anim Sci* 12:956–965.
Solignac T. 2008. *Porc Mag* 424:133–135.
Solignac T, Keita A, Pagot E, et al. 2010. In: Proceedings of the International Pig Veterinary Society Congress, p. 124.
Sorensen MT, Sejrsen K, Purup S. 2002. *Livest Prod Sci* 75:143–148.
Sorensen MT, Farmer C, Vestergaard M, et al. 2006. *Livest Prod Sci* 99:249–255.
Spinka M, Illmann G. 2015. Nursing behavior. Chapter 13. In Farmer C, ed. The Gestating and Lactating Sow. Wageningen, The Netherlands: Wageningen Academic Publishers, pp. 297–317.
Spinka M, Illmann G, Algers B, et al. 1997. *J Anim Sci* 75:1223–1228.
Spinka M, Illmann G, Stetkova Z, et al. 1999. *Domest Anim Endocrinol* 17:53–64.
Stangel G, Jensen P. 1991. *Appl Anim Behav Sci* 31:211–227.
Stevenson JS, Cox NM, Britt JH. 1981. *Biol Reprod* 24:341–353.
Stone CC, Brown MS, Waring GH. 1974. *J Anim Sci* 39:137.
Taverne M, Bevers M, Bradshaw JMC, et al. 1982. *J Reprod Fertil* 65:85–96.
Theil PK, Jørgensen H, Jakobsen K. 2002. *J Anim Physiol Anim Nutr* 86:399–413.
Theil PK, Labouriau R, Sejrsen K, et al. 2005. *J Anim Sci* 83: 2349–2356.
Theil PK, Kristensen NB, Jørgensen H, et al. 2007. *Animal* 1:881–888.
Theil PK, Lauridsen C, Quesnel H. 2014a. *Animal* 8:1021–1030.
Theil PK, Flummer C, Hurley W, et al. 2014b. *J Anim Sci* 92:5507–5519.
Thomsson O, Sjunnesson Y, Magnusson U, et al. 2016. *PLoS One* 11(6):e0156581. doi: 10.1371/journal.pone.0156581.
Threlfall WR, Martin CE. 1973. *Vet Med Small Anim Clin* 68:423–426.
Toner MS, King RH, Dunshea FR, et al. 1996. *J Anim Sci* 74:167–172.
Tseng S, Jourquin J, Goossens L. 2010. In Proceedings of the International Pig Veterinary Society Congress, p. 1188.
Tuchscherer M, Puppe B, Tuchscherer A, et al. 2000. *Theriogenology* 54:371–388.
Tucker HA. 1985. In Larson BL, ed. Lactation. Ames, IA: Iowa State University Press, pp. 39–79.
Turner CW. 1952. The Anatomy of the Udder of Cattle and Domestic Animals. Missouri: Lucas Brothers, pp. 279–314.
Tyler JW, Cullor JS, Thurmond MC, et al. 1990. *Am J Vet Res* 51:1400–1406.
Vaillancourt JP, Stein TE, Marsh WE, et al. 1990. *Prev Vet Med* 10:119–130.
Valros AE, Rundgren M, Špinka M, et al. 2002. *Appl Anim Behav Sci* 76:93–104.

Van den Brand H, Kemp B. 2006. In Proceedings of Seventh International Conference on Pig Reproduction, Netherlands, pp. 177–189.
Van den Brand H, Heetkamp MJW, Soede NM, et al. 2000. *J Anim Sci* 78:1520–1528.
Van den Brand H, Schouten WGP, Kemp B. 2004. *Appl Anim Behav Sci* 86:41–49.
Van der Lende T, de Jager D. 1991. *Livest Prod Sci* 28: 73–84.
Van Gelder KN, Bilkei G. 2005. *Tijdschr Diergeneeskd* 130:38–41.
Vanderhaeghe C, Dewulf J, Daems A, et al. 2008. *Reprod Domest Anim* 43:484–489.
VanKlompenbeg MK, Manjarin R, Trot JF, et al. 2013. *J Anim Sci* 91:1102–1111.
Vasdal G, Østensen I, Melisová M, et al. 2011. *Livest Prod Sci* 136:225–231.
Waldmann KH, Heide J. 1996. In: Proceedings of the International Pig Veterinary Society Congress, p. 614.
Wallace HD, Thieu DD, Combs GE. 1974. Alfalfa meal as a special bulky ingredient in the sow diet at farrowing and during lactation. Research Report-Department of Animal Science Gainesville Florida.
Weary DM, Pajor EA, Thompson BK, et al. 1996. *Anim Behav* 51:619–624.
Weary DM, Huzzey JM, von Keyserlingk MAG. 2009. *J Anim Sci* 87:770–777.
Wechsler B, Brodmann N. 1996. *Appl Anim Behav Sci* 47:191–199.
Weldon WC, Thulin AJ, MacDougald OA, et al. 1991. *J Anim Sci* 69:194–200.
Whitacre MD, Threlfall WR. 1981. *Am J Vet Res* 42:1538–1541.
Whitely JL, Hartmann PE, Willcox DL, et al. 1990. *J Endocrinol* 124:465–484.
Whittemore C. 1998. The Science and Practice of Pig Production, 2nd ed. London, UK: Blackwell Science Ltd., pp. 91–130 and 421–454.
Wientjes JGM. 2013. Piglet birth weight and litter uniformity. Importance of pre-mating nutritional and metabolic conditions. PhD dissertation, University of Wageningen, Wageningen, The Netherlands.
Williams IH. 1995. Sow's milk as a major nutrient source before weaning. In Hennessy DP, Cranwell PD, eds. Manipulating Pig Production V. Werribee, Australia: Australasian Pig Science Association, pp. 107–113.
Wolter BF, Ellis M, Corrigan BP, et al. 2002. *J Anim Sci* 80:301–308.
Wu WZ, Wang XQ, Wu GY, et al. 2010. *J Anim Sci* 88:2657–2664.
Xu RJ. 2003. Composition of porcine milk. In Xu RJ, Cramwell P, eds. Nutrition. Nottingham, UK: Nottingham University Press, pp. 213–246.

19

Nervous and Locomotor System

Darin M. Madson, Paulo H.E. Arruda, and Bailey L. Arruda

Introduction

Disorders, diseases, and deficits related to mentation and locomotion are both production and welfare concerns. Clinical diseases linked to the muscular, skeletal, and nervous systems are not as prevalent as those affecting the respiratory or enteric system; however, when they do occur, there is often substantial economic impact.

Potential etiologies of clinical and subclinical locomotor or nervous disorders are numerous and occur as either sporadic individual animal afflictions or affecting large populations. Etiologies can be infectious, noninfectious, or multifactorial and hence can be difficult to differentiate without a detailed history, proper clinical assessment, and proper diagnostic investigation, including sample selection and laboratory submission. Nervous disorders commonly occur with sudden onset or acute outbreaks, whereas most disorders of the skeletal system can be insidious, with faulty bone formation, structural loss, poor conformation, protracted inflammation, or trauma all contributing. Locomotor disorders contribute to losses in all stages of production with considerable variation between individual farms and are associated with numerous contributing risk factors (D'Allaire 1987; D'Allaire et al. 1987; Jones 1967; Smith and Robertson 1971; Svendsen et al. 1979).

Normal structure and function

The fundamental resources for the anatomy, physiology, and pathology necessary for the effective study of the four body systems presented in this chapter are listed in Table 19.1. The combined systems (nervous and locomotor) are responsible for perception, locomotion, reflex, and voluntary movement.

Nervous system components

The brain is normally white to light yellow in color and weighs about 35 g in the newborn piglet and 110–120 g in the adult (Widdowson and Crabb 1976). The spinal cord weighs about 4 g in the newborn and 30–40 g in the adult. The wild pig may have a brain that is 20% heavier (Rohrs and Kruska 1969). The brain grows rapidly from 5 weeks before birth to 8 weeks after birth; thereafter the ratio of brain weight to total body weight decreases.

Myelination begins about 55–60 days of gestation and peaks around birth. There is a surge in the myelination rate at about 3 weeks of age (Dickerson and Dobbing 1967; Patterson and Done 1977; Sweasey et al. 1976), and myelination is usually complete by around 6 weeks. The neuronal complement of the central nervous system (CNS) is complete by about 6 weeks of age; neurons lost after this are not replaced. Once mobilized, Schwann cells can provide limited repair of myelin.

Bone

Bone is metabolically active in mineral metabolism and is dynamically changing as a result of the mechanical forces of muscles and tendons as well as the homeostatic process of remodeling (Goff 2010). In the pig, skeletal growth is still visible at three to three and a half years of age because the growth plates continue to be functional (Getty 1975). The physeal closure times are provided in Table 19.2.

Growth plates are the site of endochondral ossification and composed of layers of maturing chondrocytes. There are resting, proliferating, hypertrophic, and mineralizing zones that ultimately form a lattice called the primary spongiosa within which mineralization occurs. The secondary spongiosa forms nearer to the diaphysis where the trabeculae are remodeled to produce the medullary cavity.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 19.1 References for anatomy, physiology, and pathology of the neurolocomotory system.

Subject	References
Anatomy	Sack (1982)
Neuroanatomy and nerves	Dellmann and McClure (1975)
Autonomic nervous system	Swenson (1977), De Lahunta (1983)
Brain of the wild pig	Rohrs and Kruska (1969)
Cerebrospinal fluid	D'Allaire and de Rota (1980), Fankhauser (1962)
Neuroanatomy and behavior	Signoret et al. (1975)
Brain development	Marrable (1971), Larsell (1954), Done and Hebert (1968), Dickerson and Dobbing (1967), Widdowson and Crabb (1976)
Eye	Prince and Diesem (1960), Diesen et al. (1975)
Inner ear	Dellmann (1971)
Bones	Ham and Cormack (1979), Sisson (1975)
Ossification	Ham and Cormack (1979)
Muscles	Handel and Stickland (1986), Ham (1979),Sack (1982), Sisson and Gandhi (1975)
Muscle tone	Simpson (1972), Palmer (1976)
Neuromuscular junction	McComas (1977)
Joint	Ghadially (1983), Ham and Cormack (1979), Doige and Horowitz (1975)
Synovial fluid	Getty (1975), Sokoloff (1978), Van Pelt (1974)
Sites of disease in the CNS	Kornegay and Seim (1996), Lorenz and Kornegay (2004)
Pathological reactions	Innes and Saunders (1962), Fankhauser and Luginbuhl (1968), Jubb and Kennedy (1970), Blackwood and Corsellis (1976)
Neuropathology taxonomy	Done (1957, 1968), O'Hara and Shortridge (1966)
Patterns of neurobiology	Done (1976a), Nietfield (2010)
Locomotor disorders	Dewey (1996), Doige (1988), Hilley (1982), Hill (1998)
CNS disorders	Done and Wells (2005)

Table 19.2 Physeal closure times in bones of the thoracic and pelvic limbs of the pig.

Thoracic limb			Pelvic limb		
Bone	Physis	Closure time (years)	Bone	Physis	Closure time (years)
Humerus	Proximal	3.5	Femur	Proximal	3
	Distal	1		Distal	3.5
Radius	Proximal	1	Tibia	Proximal	3.5
	Distal	3.5		Distal	2
Ulna	Proximal	3.5	Fibula	Proximal	3.5
	Distal	3		Distal	2–2.5
Metacarpal III	Proximal	Before birth	Metatarsal	Proximal	Before birth
	Distal	2		Distal	2
Phalanx I	Proximal	2	Phalanx I	Proximal	2
	Distal	Before birth		Distal	Before birth
Phalanx II	Proximal	1	Phalanx II	Proximal	1
	Distal	Before birth		Distal	Before birth

Growth plates have many shapes and sizes; some bones have a single physis, while others have multiple. The epiphyseal growth component of the articulo-epiphyseal cartilage complex is responsible for the increase in size of the secondary centers of ossification such as the head and greater trochanter of the humerus. Not all long bones have epiphyses.

The metaphysis is mainly cancellous bone and surrounded by a thin shell of cortical bone. The metaphysis merges with the diaphysis seen grossly as thicker area in cortical bones. In the diaphysis, cancellous bone is virtually absent, creating a medullary or marrow cavity filled with marrow. In older animals, bone marrow is replaced by fat except in the ribs, vertebrae, and pelvis. The surface of bone is covered by the periosteum except on the articular surfaces and sites where tendons are inserted.

Joint and muscle

The joints are composed of articular cartilage supported by bone, synovial membrane that secretes synovial fluid, joint capsule, and insertions of tendons and ligaments and may communicate with tendon sheaths. The joints incur the most stress in the locomotor system and are prone to infection following local trauma or bacterial septicemia.

The muscles of the pig are described in Sisson (1975). There are no special features of porcine mycology compared with the other species regarding pathology and medicine. Muscle mass is influenced by genetics and factors in fetal development as well as postpartum nutrition. Well-fed animals without deficiencies or overdoses of essential nutrients have greater muscle mass than those fed incorrectly. Importantly, many myopathies of skeletal muscle can affect cardiac muscle as well as the smooth muscle of other organ systems.

Clinical examination of the locomotor systems

Assessing the animal(s) within their environment is essential to identifying the root cause(s) of locomotor deficits. A complete clinical history and objective determination of all body systems affected are a major part of a locomotor clinical investigation. The six sites of the locomotor system (brain, spinal cord, peripheral nerves, bones, muscles, and joints) each should be considered as potential sites of involvement. The objectives of a clinical investigation are to record all clinical signs or lesions present in the group; quantify the number or percentage of animals affected with each specified clinical sign, lesions, or limb deficit; determine the age and age range of group(s) affected; determine onset, duration, and prevalence of the predominant clinical signs; and inform the course of action. This is best accomplished by systematic observation and asking questions of those providing the animal care. Determine if disease onset in the group was abrupt or gradual, as well as assess progression of disease in individual pigs. Quantify the number and percentage of animals with a particular limb or joint involvement. Determine the impact of the affliction on individual animals and the group, including productivity, mobility to acquire water and feed, and welfare. Animals may appear lame and reluctant to move yet improve with exercise and perform normally. For growing animals, determine the range in severity of clinical signs, examine animals that are either acutely or chronically affected, observe their movement, and palpate the joints and then each leg looking for heat, swelling, and response to pain. Careful observation for pain, wounds, visible swellings, muscle tone, fasciculation tremor, gait abnormalities, proprioceptive deficits, or altered mentation requires patience and practice. Assessment of the brain and spinal cord as part of a neurologic examination is discussed in the section below on neurologic examination.

Treatments and medication history as well as the response to specific treatments are important considerations when investigating potential causes of lameness. An assessment of overall impact is useful to determine if the condition is a sporadic individual animal affliction, involves multiple animals as a herd issue, or is creating an animal welfare concern.

Floor type and housing are important risk factors. Slick, rough, wet, or damaged floors as well as flooring type (solid vs. slatted floors), flooring material, flooring texture, and the overall environment in which pigs are housed are important risks factors to consider when investigating locomotor compromise.

The likelihood of specific etiologies of locomotor disorders can vary depending on the age, weight, or production stage of pigs affected. As pigs get heavier, body weight can exacerbate lameness etiologies. Infectious disease risk is higher when pigs of different sources are commingled or new animals are introduced to the group or herd. Abrupt onset of unique clinical disease is sometimes the result of a recent diet change or new feed delivery. If commercial milling is used, it is useful to know if the mill prepares feed for other species that may serve as source of error or intoxication. A thorough clinical assessment and history, along with necropsy of affected animals, should generate a list of possible causes or differential diagnosis. For confirmation of diagnosis, collection and preservation of proper samples for appropriate testing will continue the diagnostic investigation.

Evaluating locomotory issues in the breeding herd can be more challenging depending on how the animals are

housed. Often, subtle early changes can go unnoticed until there is substantial involvement. Assessing the adult herd starts by examining animals in all stages of gestation, lactation, and pre-breeding with close attention to hoof cracks, hoof wall damage, sole lesions, foot rot, long toes, dewclaw damage, and soft tissue damage. Hoof and claw disorders are discussed in Chapter 17. Note the number of sows that are lame, the severity, whether the clinical signs involve one or more limbs, and the parity and stage of gestation of the sows affected. Palpate limbs, joints, and soft tissue of the affected limbs, and take the sows out of the crates to observe their gait. Specifically look for reluctance to move, degree of difficulty in standing, stiffness, stance with legs under the body, and weight bearing on all limbs.

Weight distribution of forelimb and hind limb is approximately 60 and 40%, respectively. Changes in posture such as an arched back, slow to rise, or reluctance to move are indications that joint or bone diseases are present. Clinical examination should be performed in all age groups and not be limited to the most severely affected animals. Importantly, not all causes of lameness are infectious; environment factors can play an important role in the manifestation of clinical locomotor issues.

Bone examination

Clinical examination of bones is difficult because they are covered by muscle or other soft tissue. Obvious fractures and separation of epiphyseal heads are detectable, but other changes may depend on history, progression, and postmortem examination with detailed dissection. Many bone abnormalities are a result of nutritional inadequacies or developmental compromise magnified by cumulative trauma and specific deficiencies in later life, particularly in the sow. Common diseases affecting bone are listed in Table 19.3.

Alterations in bone shape or size are not uncommon in swine. Reduced bone length can be associated with oversupplementation of vitamin A and premature closure of growth plates. There may be enlarged ends to the long bones as seen with rickets, enlarged costochondral junctions may indicate fluorine toxicity, or rickets and localized enlargements can result from osteomyelitis or partially healed fracture (callus) sites.

Bone strength is reduced in the most common metabolic bone disease disorders, including rickets, osteomalacia, fibrous osteodystrophy, and osteoporosis. Pathological fractures of fragile bones are found in osteoporosis, maternal copper deficiency, and *osteogenesis*

Table 19.3 Selected diseases affecting bones.

Insult/disease	Clinical/comment
Osteomyelitis	Bacterial infections, abscesses
Fracture	Traumatic or nutritional
	Acute: sharp edges with crepitus, hemorrhage (long bones, ribs, vertebrae)
	Chronic: calluses/enlargement, with fibrosis (ribs, long bones)
Electrocution	Fractures (e.g. lumbosacral vertebrae, femur)
Osteochondrosis (includes related epiphysiolysis or apophysiolysis)	Primary effect on growth plates. These conditions can be manifested as joint disease, including physeal separation and hemorrhage
Metabolic bone disease	Rickets, osteomalacia, osteoporosis, fibrous osteodystrophy variably present depending on age and stage; caused by interactions of Ca, P, vitamin D_3, and other dietary contributors or risk factors. Enlarged metaphyses and growth plates; decreased bone strength; pathological fractures; soft, brittle, or friable bones. Deaths with acute hypocalcemia and tremors
Vitamin A toxicity	Shortened bones; congenital deformities
Vitamin C deficiency	Subperiosteal hemorrhages
Copper deficiency	Fragile bones
Manganese deficiency	Limb deformity, decreased length
Fluorine toxicity	Enlarged costochondral junctions
Chondrodystrophic dwarf	Shortened bones; genetic
Congenital hyperostosis	Thickened limb bones, heritable, fatal
Congenital porphyria	Brown discoloration of bones
Vitamin D toxicity; Solanum	Vitamin D_3 or analogues: excessive calcification of soft tissues and bones
Classical swine fever virus	Lines of discoloration in metaphysis parallel to physis

imperfecta. Fractures of long bones and vertebrae can also be caused by trauma, electrocution, or stunning, but the likelihood of such fractures increases when there is inadequate mineralized bone present. Antemortem physeal separation seen in epiphysiolysis and apophysiolysis will have associated hemorrhage. Ascorbic acid (vitamin C) deficiency can present with bone hemorrhages, both at subperiosteal and metaphyseal locations. Limb deformity and reduced bone length in pigs can result from manganese deficiency. Classical swine fever may cause lines of discoloration in the metaphysis parallel to the physis.

Joint examination

Joint swelling, enlargement, or dysfunction has various causes. The nature of the capillary bed in joints allows for trapping of circulating organisms, especially in young animals. In older heavier muscled animals, joint enlargement can be difficult to discern. Increased time spent in recumbency because of locomotor deficits can result in adventitious bursa formation over bony protuberances on the lower limbs, mainly the hock. The presence of an adventitious bursa is commonly misinterpreted as joint swelling, but bursae do not communicate with joint spaces. Prevalence of adventitious bursae can be present in up to 85% of animals within a particular barn (Mouttotou et al. 1998a,b) and is most common on the lateroplantar aspect of the hock. Prevalence varies considerable based on floor type, availability of bedding, and breed.

Synovial fluid supplies nutrients to the articular cartilage, lubricates joint surfaces, and is normally clear to slightly yellow and viscous. The amount of fluid within the joint can vary by location and does increase following injury to the joint. Normal synovial fluid contains few cells; however, neutrophils and hemorrhage are common following insults, either infectious or noninfectious. In disease states the synovial fluid increases, has less viscosity, and can become turbid with fibrin or inflammatory cells, depending on the insult type.

The synovial membrane is accessible to bacteria via bacteremia, sepsis, or penetrating surface abrasion. Synovitis follows and can progress to suppurative arthritis and osteomyelitis. If not resolved, villous hypertrophy of the synovial membranes and degenerative joint disease with articular cartilage roughing or erosion can occur. Pannus formation, which is vascular granulation tissue spreading across the surface of the articular cartilage, can be a sequel and often follows chronic suppurative arthritis with pyogenic bacteria or *Erysipelothrix* or, occasionally, in chronic *Mycoplasma hyosynoviae* infections.

Common joint insults are provided in Table 19.4. Useful specimens for laboratory analysis include aseptically collected smears, swabs, joint fluids, and synovium used for microscopy, PCR, or bacterial culture. Examination of joints and collection of laboratory specimens require aseptic technique to prevent contamination. Collection of these samples should have priority and performed first when performing a necropsy or pathological examination.

Muscle Examination

Examination of muscle is by visual inspection and palpation to give an overall impression of size, tone, strength, normal contours, symmetry, and normal motion or function. Generally, clinical abnormalities of muscle involve excess muscle or muscle bundles, paucity of muscle, trauma, denervation, inflammation, degenerative disease, metabolic dysfunction, and fatty degeneration that may be the result of traumatic, infectious, toxic, or inherited insults.

Table 19.4 Selected joint diseases.

Disease/insult type	Clinical/comment
Osteochondrosis	The result of defective bone and growth plate; shifting joint pain
Bacterial infections *Erysipelothrix* *Haemophilus parasuis* *Streptococcus* spp. *Actinobacillus suis* *Mycoplasma hyosynoviae* *Mycoplasma hyorhinis* *Trueperella pyogenes* Other sporadic bacteria	Most are localization of septic bacteria. Usually abrupt onset of joint pain, joint swelling, fever, lethargy, reluctant to move
Trauma: acute athletic injuries, chronic insults	Limb lameness with or without joint swelling; afebrile
Anticoagulant/vitamin K deficiency	Joint hemorrhage
Viruses, toxins, unknowns	Congenital arthrogryposis; bone deformities

Neurological examination

The primary purpose of the neurological examination is to determine the extent of the neurologic deficits and the potential anatomical location(s) affected. First observe affected animals within pens, and segregate them from their normal environment to determine visual impairment, behavior changes, proprioceptive deficits, purposeful movements, mental status, gait, posture, and evidence of trauma (Kornegay and Seim 1996). Detailed examination of multiple acutely affected animals is important to determine the range and consistency of the clinical signs. A normal gait requires proper neural networking between the cerebrum, cerebellum, brain stem, spinal cord, and peripheral nerves. Descriptive terms used to communicate clinical signs of CNS disease are listed in Table 19.5.

Cerebral cortex and forebrain

The cerebrum is responsible for voluntary movement, consciousness, and behavior. Diseases affecting the cerebral cortex or thalamus (forebrain) cause altered mental behaviors, seizures, blindness with a pupillary light reflex, circling, compulsive walking, or head pressing (Kornegay and Seim 1996). The four types of altered mental attitude are depression or a decreased responsiveness, stupor where animal is unresponsive but arousable, coma where animal is unresponsive and not arousable, and mania or delirium with excessive motor activity. Stupor and coma are often associated with cerebral cortex dysfunction. Mania can be due to diseases of the cerebral cortex, particularly the limbic system. Depression may be due to any systemic disease and may not involve the brain.

Cerebellum

The cerebellum controls unconscious proprioception with dysfunction seen clinically as truncal ataxia, broad-based stance, dysmetria, or intention tremor (Kornegay and Seim 1996). Intention tremor is the involuntary trembling caused by intentional movement. Dysmetria is the improper measuring of distance in muscular acts, either overstepping (hypermetria) or underreaching (hypometria).

Spinal cord

The degree of spinal cord damage can be determined by severity of functional loss. Functional loss from spinal cord injuries follow a sequential progression: proprioception deficit is followed by loss of voluntary motor function, then loss of superficial pain reflex, and finally loss of deep pain sensation. The return of function follows the reverse order.

Spinal or myotatic reflexes test the sensory and motor components of the reflex arc and the descending motor pathways. It is easier to localize spinal reflexes to the hind limbs than to the thoracic limbs (Table 19.6). Lower motor neuron (LMN) disease is an interruption of the reflex arc from neurons of the ventral horn to effector muscles with signs that include poor strength, flaccid muscle tone, muscle fasciculation, early neurogenic muscle atrophy, and easy bladder expression. Spinal reflexes can be normal, absent, or depressed with LMN disease. Reflexes often are exaggerated with upper motor neuron (UMN) compromise because of the compromise in the descending pathways from the brain and spinal cord that would normally inhibit the reflex.

Deep pain perception tests the functional integrity of the spinal cord. It is the most important prognostic test but should be applied at the end of the physical examination to get reliable responses to the prior tests. Apply painful stimuli to each limb and the tail. The pig will vocalize, look, or attempt to move away. The withdrawal of the limb is not a behavioral response. Loss of deep pain sensation indicates a poor prognosis.

Eyes

Visual assessment is often limited to observations of behavior indicating blindness and testing of simple reflexes. The blink and fixating reflexes are both dependent on the integrity of pathways from the retina to the visual cortex. These reflexes are all lost in conditions affecting the cerebrum or in arsanilic acid poisoning, which specifically damages the optic nerve and tract. Some of the clinical signs related to the eye are listed in Table 19.7.

Diagnostic investigation

A complete history and clinical assessment, usually accompanied by necropsy and differential diagnosis, will determine appropriate samples for laboratory testing to confirm a specific etiology for the disease process at hand. Steps in animal selection and sample collection that will improve diagnostic accuracy include the following: (1) Sample acutely affected pig(s) that accurately represent the predominant clinical signs. (2) Sample only pigs that have received no medication by any route. (3). Accurately assess, record, and aseptically collect samples from likely locations of lesions, including specific limbs, joints, bones, muscle, and portions of the nervous system (cerebrum, cerebellum, brain stem, spinal cord, peripheral nerves). (4) Collect and rapidly chill fresh samples as well as collect and preserve tissues in 10% neutral buffered formalin.

Table 19.5 Descriptive terms and clinical signs affecting the nervous system.

Descriptive terms	Clinical signs	Possible location of lesion
Blind, loss of smell, loss of control of eye movement, pupil reflex	Impaired smell, vision, eye reflexes, eye control	Cranial nerve deficits (CN1–4, 6)
Loss of sensation of the face	Facial sensory deficit, mastication muscle deficits	Cranial nerve deficit (CN5)
Facial paralysis	Facial muscle deficits, expression	Cranial nerve deficit (CN7)
Seizure	Episodic uncontrolled brain activity, convulsion	Cerebrum
Depression, stupor, coma, mania	States of mental awareness	Cerebrum
Tremors	Involuntary rhythmic muscle contraction, shaking	Cerebrum, cerebellum, spinal cord, peripheral nerve; cold/pain
Fasciculation	Muscle twitch, involuntary muscle contractions	CNS, muscle, systemic, metabolic, or toxin
Nystagmus	Involuntary, rapid and repetitive movement of the eyes	CN6–8, brain stem, meningitis
Vestibular syndrome	Circling, head tilt, lateralizing signs, recumbent, and paddling	Brain stem, CN8, otitis
Pain	Reluctant to move, vocalization	Report location
Incoordination	Abnormal gait, hypermetria, postural defects	Cerebellum, spinal cord
Proprioceptive deficits	Improper positioning of legs and feet, knuckling	Cerebellum, spinal cord, peripheral nerves
Flaccidity	Decreased muscle tone	Spinal cord
Spasticity	Increased muscle tone	Cerebrum, spinal cord
Placing deficits	Abnormal foot placement due to visual or tactile deficits	Reposition foot based on tactile or visual queues
Proprioceptive deficit	Inaccurate foot placement; abnormal foot position; scuffed hoof	Any level of CNS
Ataxia	Lack of coordination	Cerebellum, vestibular system, or spinal cord
Plegia/paralysis	Complete loss of motor function, failure of withdrawal reflexes	Spinal cord
Paresis	Complete or partial loss of voluntary motor function	Cerebrum, vestibular system, spinal cord
Hypermetria	Exaggerated movement; "high stepping"	Cervical cord, brain stem, or cerebral cortex
Tetraplegia	All four limbs paralyzed	Spinal cord
Paraplegia	Both hind limbs paralyzed	Spinal cord
Hemiplegia	Front and hind limbs on one side	Spinal cord, brain stem
Monoparesis	A single limb paralyzed	Peripheral nerve, spinal cord
Anal tone	No sphincter response, unable to pass feces or urine	Spinal cord
Incoordination	Lack of coordination of muscle movement	Cerebellum, spinal cord, muscle
Truncal ataxia	Broad-based stance, dysmetria, intention tremor	Cerebellum

Table 19.6 Influence of location of spinal cord lesion on expected reflex response.

Approximate location of lesion (vertebra number)	Expected neurologic reflex
Craniocervical: Cl–C4	Forelimbs and hind limbs: UMN reflex deficits to all limbs
Caudocervical: CS–Tl	Forelimbs: LMN reflex deficits
	Hind limbs: UMN reflex deficits
Thoracolumbar: T2–L3	Forelimbs: normal
	Hind limbs: UMN reflex deficits
Lumbosacral: L4–L6	Forelimbs: normal
	Hind limbs: LMN reflex deficits
Sacral: L5	Forelimbs: normal reflexes
	Hind limbs: normal reflexes
	Anus/tail: LMN reflex deficits

Source: Adapted from Kornegay and Seim (1996).
UMN, upper motor neuron problem; LMN, lower motor neuron problem.

Antemortem sampling

Serum collected from affected and non-affected animals can be useful to determine antibody status and the presence of viremia/bacteremia for certain infectious agents or for serum chemistry and nutritional assays for metabolic or nutritional parameters of concern. Joint fluid can be collected antemortem via surgical preparation of the site and aspiration, usually with benefit of chemical restraint. Synovial fluid is useful for bacterial culture, cytological microscopic examination, or PCR detection of specific pathogens of interest. Urine is another antemortem sample. Urine calcium, phosphorus, and creatinine values reported are useful to estimate calcium and phosphorus dietary status related to bone disease (Hagemoser et al. 2000).

Postmortem sampling

Postmortem sampling from typically affected euthanized or dead animals is common in swine production settings. Collection of serum from euthanized pigs as well as normal cohorts can be quite useful samples. In cases of suspected joint or CNS disease, it is recommended to collect samples from joints or neural tissue first to reduce contamination. Alternatively, intact but rapidly chilled limbs, joints, heads, and vertebral columns can be submitted to a diagnostic laboratory for a thorough investigation. Whether performed in the field or in the laboratory, gross examination of bone, joint surfaces, synovium, and muscle is essential as part of the sample collection process. Tips for examination and collection of samples to improve accuracy of diagnosis of suspected joint disease include:

Clean affected joint regions, carefully remove skin, and then tap the joint with a sterile needle and syringe.

A synovial swab is collected after aseptically opening the joint.

Larger joints (stifle and elbow) have more synovial fluid and commonly have infectious pathogens if this is the cause of lameness.

Changes in synovial fluid color (i.e. red tinged from the normal clear to yellow) is an indication of disease along with changes in viscosity (normal synovium has high viscosity).

Table 19.7 Clinical signs and potential causes of diseases related to the eye.

Observed signs	Possible cause
Discharges: serous, mucoid, purulent	Ammonia, other gases, rhinitis, atrophic rhinitis, PCMV, *Staphylococcus hyicus*, streptococci, pseudorabies
Swelling (edema) of the eyelids	ASF, CSF, PRV, PRRS, edema disease, *Haemophilus parasuis*
Cloudy conjunctiva	Expected change by 24 hours after death
White conjunctiva	Anemia: blood loss, aplastic (iron deficiency)
Yellow conjunctiva	Jaundice: liver diseases (toxin, bacteria, virus)
Dehydration/starvation: globe sinks into orbit	Reflection of systemic illness, dehydration, diarrhea, starvation
Hemorrhagic conjunctiva	ASF, CSF, septicemia, coagulopathy, trauma
Facial staining (tears)	PRV, CSF, blocked tear ducts, atrophic rhinitis, irritating gases (ammonia)
Conjunctivitis (reddened, inflamed)	CSF, IAV, PRV, PRRS, PCMV, other viruses *Chlamydia*, *Mycoplasma,* other bacteria
Cataracts	Riboflavin deficiency
Blindness	Toxic (organic As, Pb, Hg, Na); PRV; botulism; vitamin A deficiency

Carefully examine the articular cartilage noting roughened, indented, or lost regions. Elbow, stifle, and tibiotarsal joints are the most common location of osteochondrosis.

Collect both fresh and formalin-fixed synovium from affected joints. Synovium can be difficult to visualize at times, but collection of the soft, often fatty tissue, directly adjacent to the joint, will help ensure inclusion of the synovium.

Collection of the brain and spinal cord can be difficult without the proper tools. If the brain and spinal cord are collected during a field necropsy, the following should be considered:

Cerebrospinal fluid can provide basic information via cytology, culture, or PCR if collected with a needle and syringe first, prior to disarticulating the head.

Aseptic collection of multiple sections of brain stem and spinal cord is essential given the endemic nature of multiple viral or bacterial pathogens. Swabbing sites or lesions of interest prior to removal reduces contamination. Spinal cord sampling should include fresh and formalin-fixed sections from cervical, thoracic, and lumbar areas.

Once removed, the brain and spinal cord can be examined for hemorrhage, defects, or gross indication of infection. Examine the calvarium and spinal canal after organ removal for gross pathology or palpable masses.

The brain can be sliced in half longitudinally, with one half placed in formalin and the other chilled for laboratory testing. Be sure to collect brain stem and cerebellum as well as cerebrum.

Continue with a complete and systematic necropsy, collecting fresh and fixed samples of major internal organs. The viscera can be removed from the body to visualize the ribs and ventral vertebral bodies. Examine ribs and vertebral bodies for fractures or fracture calluses, and collect those if needed. A simple assessment technique is to isolate individual ribs and access breaking strength. The second rib has recently been used as a standard for assessing bone density, ash, and mineral content (Madson et al. 2012). Growth plate alterations can be assessed by microscopic examination of sections of costochondral junctions.

Muscle samples should be collected soon after death when suspecting primary myopathy with source or name of the muscle noted. When possible, clamp ends of the sample before excision to reduce artifacts and to assure there are both a longitudinal section and a cross section of the muscle fibers since diagonal sections are impossible to interpret (Bradley 1978). For investigation of a specific disorder, it may be necessary to target specific muscles for typical lesions or fiber-type variations. For example, the diaphragm, the *gastrocnemius*, and the *supraspinatus* muscles are useful to assess type I fibers and the *psoas* and *longissimus* muscles to assess type II fibers (Ruth and van Fleet 1974). The *psoas* is an excellent muscle to use as a control as it is deep in the abdomen protected by the spinal column and is therefore unlikely to be affected by trauma and surface infection.

Samples useful for investigation of locomotor disease include serum; urine; affected limb(s) or synovial fluid and fresh and formalin-fixed synovium; brain and spinal cord (fresh and formalin-fixed brain stem, cerebellum, cerebrum; cervical cord, thoracic cord, lumbar cord); second rib and costochondral junctions; and fresh and formalin-fixed tissue representing other organ systems including skeletal muscle from multiple different locations. And lastly retain a feed sample from the site for possible future investigation. More details regarding formulation of the diagnostic question, diagnostic sample selection and collection, selection of diagnosis assays, and general guideline for diagnostic interpretation are in Chapters 6 and 7.

Mere detection of endemic agents should not necessarily indicate causation; pathogen detection should be associated with compatible clinical signs and identification of histopathologic lesions.

Congenital conditions

Congenital and newborn locomotor diseases can result from myriad insults affecting gestating sows or newborn pigs (Table 19.8.).

Splayleg

Splayleg is a congenital condition characterized by posterior paresis in newborn pigs. In severe cases the forelimbs may also be involved. Splayleg is usually found at or within hours of birth (Figure 19.1.). Although sporadic on individual farms, the problem is widespread, is common, and typically affects 1–4 pigs per litter and just a few litters at a time. Usually individual herd prevalence is less than 1%; however, prevalence of 8% or higher has been reported (Ward and Bradley 1980). Males may be predisposed based on reports where incidence in males was 174–233% greater than in females (Holl and Johnson 2005; Vogt et al. 1984). As many as half of piglets affected with splayleg die due to starvation or crushing by the sow. Piglets have a difficulty competing with their littermates for milk and are less able to move out of the sow's lying space. With nursing care and interventions, affected pigs can survive if provided with supplemental colostrum, milk, and heat or are assisted to nurse or cross-fostered to reduce competition. Often, piglets benefit from having their limbs taped or supported in a natural standing pose. Pigs that survive the first week of life can recover completely.

Table 19.8 Congenital and neonatal diseases affecting the locomotor system.

Viral	Clinical sign/lesion
Atypical porcine pestivirus	Intentional tremors at birth, variable severity (Table 19.9)
Classical swine fever virus	Tremors at birth, cerebellar hypoplasia, microencephaly
Japanese B virus	Cerebellar hypoplasia, hydrocephalus
Menangle virus	Arthrogryposis, craniofacial defects, mummies, stillbirths,
Feed, toxin, other	Clinical sign/lesion
Congenital tremors	Tremors at birth (see Table 19.9)
Splayleg	Posterior paresis, muscle hypoplasia, hypomyelinogenesis
Hypoglycemia	Vocalization, lethargy, recumbent, convulsions, coma
Hypoxia	Huddle, fail to suckle, "squawking" vocalization
Actinobacillus suis	Lameness, found dead, serositis, arthritis; acute sepsis
Arthritis (bacterial)	Lameness, swollen joints, arthritis
Vitamin A deficiency or toxicity	Deformities, cerebellar hypoplasia, stillbirths, blindness, arthrogryposis, flaccid paralysis
Trichlorfon toxicity	Cerebellar hypoplasia
Manganese deficiency	Arthrogryposis
Conium (hemlock)	Cleft palate, arthrogryposis
Nicotiana (tobacco)	Cleft palate, arthrogryposis
Datum (jimsonweed)	Arthrogryposis
Heat stress	Microencephaly
Genetic	References
Congenital tremors	Tremors at birth (see Table 19.8)
Kinky tail, fused vertebrae	Donald (1949), Nordby (1934)
Limbless pig	Johnson (1940)
Clubfoot, absence of fibula, extra toes	Palludan (1961), Nordby (1939)
Polydactyly	Hughes (1935), Malynicz (1982)
Syndactyly	Detiefsen and Carmichael (1921), Leopold and Dennis (1972), Ross et al. (1944)
Chondrodysplasia	Jensen et al. (1984)
Congenital hyperostosis	Doige and Martineau (1984), Roels et al. (1996)
Hydrocephalus	Hereditary, multiple causes
Meningocele, meningoencephalocele	Multiple causes
Hemivertebrae	Done et al. (1998)
Hyperostosis	Dalton (1992), Gibson and Rogers (1980), Kaye (1962)
Cleft palate	Painter et al. (1985)
Congenital muscle hyperplasia	Done et al. (1990)

The condition has been associated with low birth weight, slippery floors, congenital tremor, *Fusarium* toxins, certain genotypes, and short gestation lengths (Ward 1978 a,b). More recently, it was found that increased litter size can significantly increase the likelihood of splayleg pigs (Holl and Johnson 2005). Farrowing induction may result in a higher prevalence of splayleg especially if producers do not first determine the average gestation length in their herd (Sellier et al. 1999). Some older studies suggested that splayleg incidence was higher when sow diets were deficient in choline or methionine, which are essential for normal myelin production (Cunha 1968; Kornegay and Meacham 1973). However, in clinical trials on two farms, Dobson (1971) showed that adding 3 g choline and 5 g methionine to the sows' daily ration had no effect on the occurrence of splayleg.

Some suggest muscle hypoplasia is a feature of splayleg; however myofibrillar hypoplasia is common if not normal in newborn pigs. At 1 day of age, myofibrils do not fill the muscle cells completely in splayleg or in normal pigs (Ward and Bradley 1980), and no differences in vasculature, nervous supply, neuromuscular bundles, and the myelination of the intramuscular nerves were observed between healthy and affected pigs over the

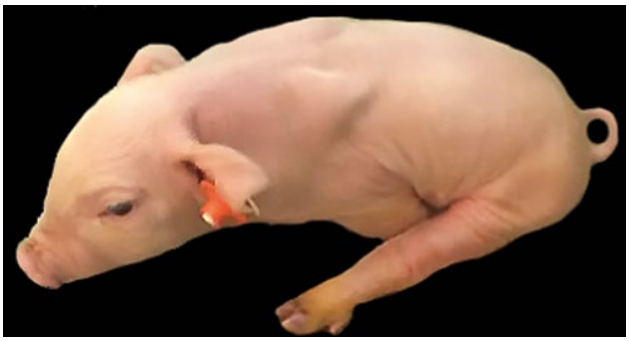


Figure 19.1 Splayleg; newborn piglet. *Source:* Image courtesy of the Iowa State University Veterinary Diagnostic Laboratory.

clinical course of disease. Szalay et al. (2001) reported a reduction in the axonal diameter and myelin sheath thickness of the fibers that innervate the hind limb adductors of piglets affected by splayleg. Myelination is required for conduction of normal impulses along nerve fibers and appears to occur in two phases. The first peak occurs 2 weeks prior to birth and the second 3 weeks after birth. If pigs live past the first couple of weeks, the myelin sheath is properly developed during this second phase. It is unknown why this problem with myelination occurs in splaylegged pigs.

Congenital tremor

Congenital tremor (CT), also known as "myoclonia congenita," "trembling pig syndrome," or "jumpy pig disease," is a disease of newborn pigs that is characterized by bilateral clonic contractions of skeletal muscle that cease when piglets are at rest (Done 1968). Early reports of this clinical malady were by Kinsley (1922) in the United States, Payen and Fournier (1934) in France, and Hindmarsh (1937) in Australia. Although sporadic, CT is widely distributed and reported in most swine-producing countries and likely occurs globally. Tremors vary in intensity from mild to severe repetitive contractions that can completely impair the piglets' ability to stand or walk; vision, reflexes, and pain response are normal. Gross examination and histopathology of affected piglets is unremarkable, with an absence of inflammation in the CNS. Hypomyelination and dysmyelination have been described in some affected piglets, but lesions are subtle and not consistent and often do not correlate with the severity of clinical signs.

Survival of severely affected piglets is decreased due to nursing and ambulation difficulties, and deaths may result from starvation, inadequate colostrum intake, or crushing by the sow. Pigs born with CT may also have features of splayleg. Treatment for CT is aimed at reducing mortality. Affected pigs may be provided with additional heat and assisted to feed. The severity of the tremors decreases over time (Schwarz et al. 2017) and is usually resolved by 4 weeks of age.

Historically, clinical signs of CT occurred with fetal infections with classical swine fever virus (CSF) (Harding et al. 1966), inherited disorders (Harding et al. 1973; Patterson et al. 1973), and metrifonate/trichlorfon toxicity (Knox et al. 1978). Based on the eradication of CSF, genetic variability in swine herds, and current production practices, a majority of outbreaks of CT have been attributed to an unidentified virus (Table 19.9).

Clinical signs consistent with CT occurred in newborn piglets by intramuscular or intrauterine inoculation of pregnant sows with brain suspensions from piglets with CT (Done et al. 1986; Vandekerckhove et al. 1989), yet the viral etiology of CT type AII remained elusive. Through the use of next-generation sequencing, a divergent lineage pestivirus provisionally identified as atypical porcine pestivirus (APPV) was detected in pigs with CT in the United States (Arruda et al. 2016), Germany (Postel et al. 2016), the Netherlands (de Groof et al. 2016), Spain (de Groof et al. 2016), and Austria (Schwarz et al. 2017). Clinical signs consistent with CT have been experimentally reproduced by independent research groups through inoculation of gilts or sows and fetuses with APPV-containing inocula (Arruda et al. 2016; de Groof et al. 2016). There is limited information concerning the transmission, pathogenesis, carriage, and epidemiology of APPV. APPV was detected in the serum of one male and one female with CT out to 14 weeks of age at which time clinical signs had resolved (Schwarz et al. 2017). This finding suggests the likelihood of chronically and/or persistently infected animals. APPV was also detected in the semen of the male (Schwarz et al. 2017).

Hypoxia

Hypoxia is probably more common than recognized and vaguely characterized clinically as depression, inability to nurse, inappetence, huddling, lethargy, shivering, squealing or peculiar "squawk" when moved, coma, convulsions, and death. It is often associated with starvation or/and chilling, but, currently, it is not possible to definitively differentiate hypoxia or hypoglycemia. Umbilical cord rupture, cord occlusion during birth, dystocia, slow farrowing, or porcine reproductive and respiratory syndrome virus (PRRSV) lesions in the umbilical cord may be associated with the condition. The last pigs born in the litter may be more likely affected. Heated buildings with high levels of carbon monoxide may also cause hypoxia.

Hypoglycemia

Hypoglycemia is a common problem, both in weak newborn pigs who do not nurse regularly and in sows with limited functional mammary glands. Early clinical

Table 19.9 Causes, key features, and reference for types of congenital tremors described.

Type	Cause	Key features	References
A1	Classical swine fever virus (CSF)	Dysgenesis, cerebellar hypoplasia, small cord, demyelination	Harding et al. (1966), Bradley et al. (1985), Done (1976b), Done et al. (1984)
AII	Atypical porcine pestivirus (APPV)	Endemic globally; few lesions; sporadic	Done (1986), Arruda et al. (2016), deGroof et al. (2016)
AIII	Genetic: sex-linked recessive (Landrace and males)	Demyelination, hypoplasia of the spinal cord	Harding et al. (1973)
AIV	Genetic: autosomal recessive (Saddleback and Landrace/Saddleback)	Demyelination; CNS hypoplasia	Berge et al. (1987), Kidd et al. (1986)
AV	Trichlorfon toxicity	Cerebellar hypoplasia at 65–79 days' gestation	Pope (1986)
B	Unknown	No special features	Gedde-Dahl and Standal (1970)

signs include tachycardia, tremor, nervousness, vocalization, and irritability. As the hypoglycemia progresses, hypothermia, visual disturbances, mental dullness, confusion, depression, and seizures occur. Causes of hypoglycemia include sow factors such as nutrition, presence of disease, fewer functioning mammary glands than piglets, and poor presentation of both rows of mammary glands. Cold or wet creep areas and poor crate design will exacerbate hypoglycemia by increasing the demand for energy.

Hydrocephalus

Hydrocephalus is an accumulation of cerebrospinal fluid within the brain. It may be congenital or acquired – secondary to encephalitis or meningoencephalitis. Congenital hydrocephalus may be caused by vitamin A deficiency or a lethal defect that is inherited as an autosomal recessive trait in Durocs (O'Hara and Shortridge 1966). Clinical signs vary from compulsive walking, head pressing, blindness, and seizures to a more mild form indicated by strabismus and stunted growth. Animals with a primary encephalitis will have signs of multifocal brain involvement.

Cerebellar hypoplasia

Cerebellar hypoplasia causes pigs to sit with their forelimbs extended or to assume a tripod sitting position. The clinical signs are nonprogressive, and other CNS signs are not present. Cerebellar hypoplasia can occur with viral (CSF and possibly other pestiviruses) infection in the sow and is reported as a sequel to trichlorfon poisoning (Pope 1986). Once common, vitamin A deficiency has serious effects on the nervous system. In pregnancy, low vitamin A levels caused herniation and constriction of dorsal and ventral nerve roots. It results in abortion with stillbirths and small or absent eyes. Other abnormalities include cleft palate, edema, high morbidity, or high mortality.

Cerebellar abiotrophy

Cerebellar abiotrophy is a condition where pigs are normal at birth but develop ataxia and a fine tremor by 3–8 weeks of age. This condition, found in Yorkshire and Large White pigs, is probably an autosomal recessive trait. It is progressive over days to weeks until affected pigs are recumbent and unable to rise. It is a condition diagnosed on histopathology as premature degeneration of neurons (Purkinje cells).

Vitamin A

Vitamin A deficiency can manifest as a generalized flaccid congenital paralysis of all four limbs, head tilt, incoordination, stiff gait, lordosis, excitability, muscle spasms, night blindness, progressive dysfunction with incoordination, swaying gait, restlessness, dog-sitting, reduced growth or spasm, and perhaps paralysis due to degeneration of portions of the spinal cord and sciatic and femoral nerves. At postmortem there may be hydrocephalus, a small cranium, and herniation of spinal cord in the lumbar region. Deficiency can also be associated with eye lesions including anophthalmia, microphthalmia, and entropion.

Miscellaneous joint, muscle, and bone disorders

Bone changes are unusual with infectious diseases but can occur with Menangle virus and CSF. Abnormalities of endochondral ossification include "bullhead," bent legs, deformed feet, congenital carpal flexion, and

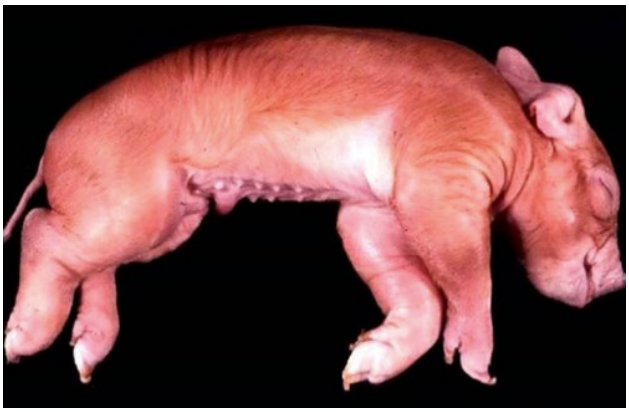


Figure 19.2 Hyperostosis; newborn piglet. *Source:* Image courtesy of the Iowa State University Veterinary Diagnostic Laboratory.

kyphosis associated with a congenital hemivertebrae. A second group causing fetal or neonatal abnormalities is associated with the consumption of toxic plants during pregnancy. Consumption of *Conium maculatum* will produce bone abnormalities, particularly cleft palate, as will tobacco poisoning. Arthrogryposis is characterized by rigid and hyperflexed or hyperextended limbs. Congenital hyperostosis (Figure 19.2) is a lethal, rare autosomal recessive condition where leg bones are thickened (Kaye 1962). Congenital abnormalities affecting muscles are rare in pigs.

Neurological conditions

The list of agents or insults that can affect neural and locomotor function is long yet not complete. Common infectious agents, nutritional deficiencies, and toxicities are discussed in respective chapters. APPV associated with CT is discussed above.

Viral pathogens

Swine are the natural host of porcine herpesvirus 1 (PHV-1), also known as pseudorabies virus (PRV) or Aujeszky's disease, but can infect a wide range of species, often fatally. Pseudorabies is eradicated in some swine-producing countries, whereas in others it may be notifiable as a controlled disease or may simply be endemic. Although eradication at local or national level is successful within commercial herds, PRV can remain widespread in populations of non-domesticated swine. Neurological signs are observed predominantly in young piglets, with clinical signs including ataxia and convulsions, whereas infection of pregnant sows can result in fetal death and reabsorption, mummification, and/or abortion.

Classical swine fever is a highly contagious viral disease of major worldwide importance; the disease belongs to the World Organization for Animal Health (OIE)-listed diseases. Swine are the only known reservoir. CSF is endemic in parts of Eastern Europe, Southeast Asia, and Central and South America. The domestic swine population in Australia, New Zealand, North America, and Western Europe are considered CSF-free. Severe systemic illnesses including depression, fever, anorexia, conjunctivitis, death, weak staggering gait, skin hyperemia, watery diarrhea, convulsions, and death have been described 1–3 weeks after infection. Mortality can reach up to 90%; mortality associated with less virulent strains range from 1 to 60% with nonspecific clinical signs and lesions. Petechiae in kidneys, larynx, urinary bladder, and mucous membranes, infarcts in spleen, and hemorrhage of lymph nodes are gross lesions observed in affected animals.

Eastern equine encephalomyelitis is infrequently reported in domesticated swine; clinical signs are age dependent with younger pigs being more susceptible to develop clinical disease. Infection of pigs older than 2 months of age is usually subclinical but causes incoordination, depression, seizures, and death in pigs of less than 2 weeks of age (Elvinger et al. 1994). Pathologic lesions include focal necrosis of the cerebral cortex and multifocal myocardial necrosis. Diagnosis is made after a history of mosquito swarming and positive EEEV serum virus neutralization assay.

Japanese encephalitis virus (JEV) has historically been associated with reproductive failure including increased rates of stillborn, mummies, and weak neonatal piglets (Daniels et al. 2002). Recent evidence has suggested that JEV may lead to a wasting syndrome with associated lymphoplasmacytic encephalitis with varying degrees of depression and hind limb tremors (Yamada et al. 2004). The virus is amplified in the pig and then transmitted via mosquitoes to other species.

Malignant catarrhal fever (MCF)-like condition is sporadically described in swine. The condition has been reported in Germany (Pohlenz et al. 1974), Norway (Loken et al. 1998; Okkenhave and Kjelvik 1995), Finland (Syrjala et al. 2006), the United States (Alcaraz et al. 2009; Gauger et al. 2010), and Brazil (Azevedo Costa et al. 2010). Clinical signs resemble those described in ruminants affected with MCF (lethargy, anorexia, fever, recovery, or death) and are associated with ovine herpesvirus type 2 (OHV2). Histologic lesions include mucopurulent tracheitis, interstitial nephritis and pyelitis, necrotizing arteritis, and lymphocytic meningoencephalitis with perivasculitis and vasculitis (Li et al. 2012). Diagnosis should be based on a combination of clinical signs, histologic lesions, and detection of the virus by PCR or virus-specific antibodies in the blood.

Encephalomyocarditis (EMC) virus infection is not uncommon; however, clinical disease is rare. Acute

myocarditis in young pigs and reproductive failure in sows are the most common clinical signs described; however, lymphoplasmacytic meningoencephalitis has been described (Maurice et al. 2005).

Blue eye disease is caused by the blue eye paramyxovirus (BEP). To date this virus has only been reported in Mexico. The disease is primarily described as causing a sharp increase in preweaning mortality and CNS signs (Ramirez-Herrera et al. 2001). Clinically affected pigs show incoordination, hind limb paralysis, and convulsions. Corneal opacity (edema) and conjunctivitis are described in pigs of all ages and can be observed without other clinical signs; corneal lesions usually resolve spontaneously. Histologic lesions are primarily concentrated in the brain and spinal cord and have been described (Stephano et al. 1988). Diagnosis should be made based on clinical signs, macroscopic (corneal opacity highly suggestive) and histologic lesions, serological assays, and/or detection of the virus by PCR.

Teschovirus A is one cause of polioencephalomyelitis. The virus has a worldwide distribution and is considered endemic in domestic pigs with swine the only known host. Despite the endemic nature of this virus, clinical disease is sporadic. Clinical signs vary but can include fever, depression, anorexia to more severe muscle tremor, nystagmus, opisthotonos, ataxia of the rear legs, paralytic symptoms, trembling, recumbency, paddling of the rear legs, and convulsions (Mills and Nielsen 1968). Histologic lesions are predominately found in the spinal cord. Diagnosis is by typical clinical signs, characterization of histologic lesions, and detection of viral RNA by PCR within affected spinal cord segments. Spinal cord used for viral RNA detection should be collected aseptically given the ubiquitous nature of this virus in feces. More recently, sapelovirus A has been detected in the CNS of pigs with similar clinical signs and histopathologic lesions (Schock et al. 2014; Arruda et al. 2017).

PRRSV will occasionally produce CNS signs with associated encephalitis, myelitis, or meningitis (Cao et al. 2012; Rossow 1998) or can exacerbate neurologic signs in pigs coinfected with pseudorabies (Narita and Ishii 2004). PCV2 does not typically cause encephalitis; however, Seeliger et al. (2007) have identified PCV2 as a potential etiologic cause of neurologic disease.

Rabies is rare in swine but when occurs is usually manifested as the "dumb" form with posterior paresis and prostration.

Bacterial agents

Major diseases caused by bacteria or their toxins are discussed in respective chapters. Edema disease is an acute, often fatal, enterotoxemia of recently weaned pigs. It is associated with multiple genotypes of *Escherichia coli* (see Chapter 52) that usually possess F18 or F4 (K88) fimbriae and elaborate Shiga-like verotoxin SLT-IIE (STx2e).

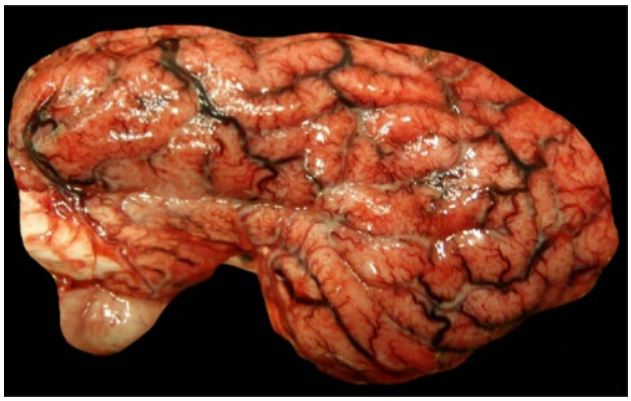


Figure 19.3 Suppurative meningitis (leptomeninges); bacterial sepsis. Note the injected meningeal vessels and the white exudate in sulci. *Source:* Image courtesy of the Iowa State University Veterinary Diagnostic Laboratory.

Systemic bacterial infections are common. *Haemophilus parasuis* is particularly common following PRRSV or PCV2 infections, with leptomeningitis (Figure 19.3) and polyserositis typical of Glässer's disease. *Streptococcus suis* septicemia is very common in young piglets but can occur in growing pigs as well. Streptococcal septicemia can lead to similar clinical signs and macroscopic and histologic lesions as Glässer's disease.

A variety of other opportunistic bacteria can cause sporadic disease in the nervous system in individual animals. Erysipelas can cause acute deaths in naïve pigs or when first introduced to a system or when vaccination is discontinued. It produces fever, difficulty in rising, and lameness due to arthritis and pain. *Actinobacillus suis* can cause septicemia, arthritis, and subsequent nervous infections as does *Trueperella pyogenes. E. coli* is a common cause of septicemia in neonates. Stachybotryotoxicosis, caused by the toxin of *Stachybotrys atra* acquired from hay or straw, could potentially cause toxemia but is rarely reported in swine. Moldy hay or straw can result in depression, vomiting, tremors, sudden death, and abortion. *Listeria monocytogenes* can cause encephalitis, but disease is rare. Many cases are diagnosed solely on brain culture or through histopathology. Lymphoplasmacytic meningitis in addition to encephalitis with perivascular cuffing and microabscessation is reported (Lopez and Bildfell 1989).

Spongiform encephalopathy

There is no evidence for naturally occurring transmissible spongiform encephalopathies (TSE) in swine (Ryder et al. 2000). Despite the lack of natural cases, typical histologic lesions and intracellular and neuropil-associated

pathological prion protein (PrPSc) deposition corroborated by Western blot (WB) have been described in pigs challenged with BSE-derived material via three simultaneous distinct routes such as intraperitoneal, intravenous, and intracerebral (Hedman et al. 2016; Konold et al. 2009; Ryder et al. 2000). A recent study demonstrated that pigs can become infected when intracranial and orally inoculated with scrapie isolates. At 6 months' postchallenge, no evidence of scrapie infection was noted by any diagnostic method. However, after 51 months swine were positive by either immunohistochemistry (IHC), WB, or enzyme immunoassay (EIA). No neurological signs or histologic spongiform changes were observed in experimental animals (Greenlee et al. 2016).

Parasitic

Toxoplasma gondii infection can cause stillborn piglets and prolonged gestation. Toxoplasmosis rarely may produce tremors, weakness, staggering, and mortality, and survivors may be ataxic and blind.

Toxins and deficiencies

Salt poisoning (water deprivation, sodium ion intoxication)

Risk factors for sodium intoxication occur with excess sodium chloride ingestion (e.g. brines, whey, salted fish, feed mixing error) or water deprivation (e.g. not available, frozen) and are exacerbated by water engorgement when water becomes available. Clinical signs vary by severity and stage of disease. Animals may be found dead, while others have signs that include epileptiform seizures, snout twitching, contraction of neck muscles, head pressing, stepwise movements of head, walking backward, dog-sitting with nose upward, twitching of face/ears, and blindness. Signs can progress to tremors, lateral recumbency, running movements, prostration, coma, and death. Eosinophilic meningoencephalitis is a transient microscopic lesion in the brain quite useful for diagnosis when present. Polioencephalomalacia may be present in chronic or nonfatal cases. Sequelae include blindness and polioencephalomalacia. The most important intervention when water deprivation has occurred is to limit water intake, with gradual introduction of water, gradually increasing to ad libitum over a several hour period.

Hepatic encephalopathy

Hepatic encephalopathy may have multifocal lesions in the CNS, but the clinical signs are primarily due to forebrain involvement. Hepatic encephalopathy occurs when neurotoxic substances (e.g. ammonia) build up in the blood due to liver dysfunction. Clinical signs include blindness, ataxia, head pressing, seizures, and aggression. There will also be signs referable to liver disease, such as anorexia, weight loss, or diarrhea.

Micronutrient deficiencies

Several mineral deficiencies will produce signs in the CNS, peripheral nerves, and special senses. Calcium and phosphorus deficiency can produce hyperesthesia, tremors, tetany, or posterior paralysis depending on magnitude and duration. Magnesium deficiency will produce hyperirritability and tetany. Copper deficiency can cause swayback, ataxia, posterior paralysis, and paraplegia in pigs (Bennetts and Beck 1942; Fletcher and Banting 1982; McGavin et al. 1962; Wilkie 1959). Histopathology reveals marked spinal demyelination affecting the dorsal spinocerebellar tracts (Pritchard et al. 1985).

Vitamin B_6 (pyridoxine) deficiency produces a mincing gait, hyperexcitability, ataxia, waddling, "goose-stepping," and epileptiform convulsions. Naturally occurring pantothenic acid deficiency was described (Doyle 1937; Goodwin 1962) as incoordination, poor growth, and "goose-stepping." Nicotinic acid deficiency has been induced in pigs by feeding the antimetabolite 6-aminonicotinamide (O'Sullivan and Blakemore 1980) when maize is low in tryptophan or high in antimetabolites.

Micronutrient toxicities

The risk of selenium toxicity (see Chapter 68) from mixing errors is common because the toxic dose in feed is low despite a 10× safety margin from the legal fortification limit of 0.3 ppm (Stowe et al. 1992; Stowe and Herdt 1992). Doses of >10 ppm are usually required to cause demyelination or ambulatory dysfunction. Acute toxicity occurred when 25 g/kg was accidently fed (Nathues et al. 2010), with clinical signs of apathy, paralysis, and death within 36–72 hours after exposure. Bilateral symmetrical focal myelomalacia particularly in the cervical and lumbar spinal intumescence (Casteel et al. 1985; Harrison 1983; Penrith and Robinson 1996; Stave et al. 1992) and in the nuclei of the facial nerve in the medulla oblongata is seen histologically. Polioencephalomalacia is suggestive of selenium toxicosis, but it can also follow nicotinamide deficiency (Wilson and Rake 1972) or other conditions.

Arsanilic acid has been fed to pigs to promote growth or treat swine dysentery. Overdoses of arsanilic acid can decrease growth rate and cause head tremor, progressive blindness, ataxia, and paresis. Other clinical signs are seen in pigs poisoned with 3-nitro-4-hydroxy phenyl arsonic acid (Rice et al. 1980). After exercise there is trembling of the muscles of the shoulders, hams, and backs followed by violent tremors, incoordination, and extreme agitation and screaming. Diagnosis is by the

detection of arsenicals in the urine and observation of degeneration of the optic nerves, optic tracts, and peripheral nerves (Harding et al. 1968).

Insecticide

Organophosphate and organochlorine poisoning occurs in pigs due to inadvertent mixing or spilling of insecticides (Frank et al. 1991). Most pigs (70%) consuming contaminated feed die within hours of ingesting these products. Organophosphates cause pulmonary edema, myocardial hemorrhage, and cholinesterase inhibition. Organochlorines cause convulsions. Chemical analysis of the feed is needed for the definitive diagnosis. Orthocresyl phenols are organophosphates with delayed effect of demyelination and posterior paresis and paralysis, also known as delayed organophosphate toxicity.

Plant poisoning

Majority of plant poisonings are diagnosed by finding plants in the environment or in the digestive tract. They often occur when adult swine are raised in derelict land or woodland. *Cassia* spp. seeds cause incoordination, ataxia, staggering, decreased weight gain, and increased mortality in grower pigs (Colvin et al. 1986; Flory et al. 1992). Only a few seedlings of cocklebur (*Xanthium strumarium*) may cause depression, nausea, weakness, ataxia and spasms of neck muscles, convulsions, and death. Mortality is sporadic and limited to when plants are emerging numerously. Solanaceae (nightshade) causes stupefaction, depression, ataxia, muscle tremors, loss of appetite, convulsions, and coma with dilation of pupils. Hyperexcitement can be observed initially; animals may lie on their side and kick their feet as the clinical signs progress. Water hemlock ingestion causes nervousness, pawing, spasmodic twitching and contraction of muscles, paralysis, and death. The pigs squeal before and during the convulsions (Barlow 2006).

Poison hemlock ingestion produces rapid trembling of the flank muscles followed by paralysis caused by damage to ends of the motor nerves (Hayashi and Muto 1901). Bracken poisoning results in muscle weakness and can lead to death in 4–5 days. Buttercups will cause muscle twitching in the ears, nervousness, and paralysis in two to three days followed by death. Pigweed (*Amaranthus* or redroot) poisoning is typically observed in the late summer and fall, with clinical signs 5–10 days after exposure. Signs include trembling, weakness, incoordination, kicking, almost complete rear leg paralysis, and sternal recumbency. The morbidity is variable and the mortality can be high. Death is usually within 48 hours of onset of clinical signs. Red squill ingestion causes hyperesthesia, depression, weakness, ataxia, paralysis, and death usually within three days. Ergot affecting the nervous system was described by Chennells et al. (2006).

Gases

Carbon monoxide poisoning will produce lethargy, incoordination, coma, and death due to hypoxia; abortion has also been reported (Dominick and Carson 1983; Jennings 2001; Wood 1978). Carbon dioxide produces anxiety, staggering, coma, and death. Hydrogen sulfide produced listlessness, incoordination, spasms, coma, and death. All are discussed in respective chapters.

Vestibular disease

Vestibular disease causes a head tilt toward the affected side, horizontal nystagmus, and ataxia, which may include circling or falling. The condition is widespread but sporadic. Peripheral lesions are not associated with the ascending or descending tracts, so there is no paresis or proprioceptive deficit, animals are not depressed, and nystagmus is always rotatory or horizontal with the fast phase opposite the head tilt and does not vary with head tilt. The animals remain bright and the appetite is normal (Buddle 1987). Alternatively, central lesions frequently cause depression, tetraparesis, and nystagmus that varies with head position and may cause other cranial nerve deficits. Most lesions cause ipsilateral clinical signs, but the paradoxical vestibular disease causes contralateral signs. Rupture of the tympanic membrane usually follows inflammation of the middle ear.

Otitis media (peripheral vestibular disease) is usually due to progressive external otitis (Kornegay and Seim 1996). The external ear is inflamed and contains debris, and the pig may be sensitive to manipulation of the ear. Various bacterial species have been recovered from infected ears (Shimada et al. 1992), yet otitis media and interna may be common sequelae to *Mycoplasma hyorhinis* (MHR) and *S. suis* infections. Multiple reports have isolated MHR in approximately 80% of young swine with otitis media (Friis et al. 2002, Kazama et al. 1994), whereas MHR was recovered in over 50% of normal swine without otitis media (Friis et al. 2002), suggesting this bacterium may be an opportunistic invader. A majority of pigs (20 out of 28) diagnosed with meningitis due to *S. suis* had either otitis media or otitis interna (Madsen et al. 2001). Exudative otitis interna with a positive IHC for *S. suis* was found in 71% of the pigs. Most of these pigs had a perineuritis along the vestibulocochlear nerve. This nerve is likely a conduit to the CNS. Half of the pigs with both ears available for evaluation were affected bilaterally. Otitis interna can be the result of infection via either meningeal or auditory canal via tympanic membrane breach. Pigs recovering from *S. suis* meningitis may have residual hearing and vestibular dysfunction due to chronic otitis interna.

Eyes and vision

In the day-to-day life of the pig, excessive lacrimation and conjunctivitis may be associated with dust, ammonia, hydrogen sulfide, or obstruction of nasolacrimal duct secondary to rhinitis. Important infectious causes of conjunctivitis include CSF and ASF. Pseudorabies is another primary cause of conjunctivitis, retinitis, and optic neuritis. Other agents sometimes associated with outbreaks of conjunctivitis include *Chlamydia*, *Mycoplasma*, porcine cytomegalovirus (PCMV), influenza A virus (IAV), and PRRSV, among other viruses. PCMV (Edington et al. 1988) can cause a severe rhinitis with nasal discharge, conjunctivitis, and sneezing. Rarely, occasional fractures of the orbital bones may distort the eyes.

Gross lesions are rare in the optic nerve and globe; histopathology is needed for conditions affecting these structures. Hypopyon, rare in pigs, is suppuration in the anterior chamber of the eye. Corneal edema is associated with blue eye disease (Stephano et al. 1988) or following trauma. There are other unusual conditions such as riboflavin deficiency that will cause cataracts. Enteroviral encephalomyelitis can cause retinitis and neuritis, and swine vesicular disease can cause optic neuritis.

Histologically, organic arsenical poisoning produces degeneration of the optic nerve, optic tracts, and peripheral nerves. Mercury poisoning will produce blindness, and organophosphate poisoning will produce lacrimation. Hygromycin B toxicity will produce cataracts. Lead poisoning is rare but will cause blindness. Excess vitamin A to sows will produce cataracts in piglets.

Keratosis is rare in the pig but reported following trauma, noxious gas exposure, pseudorabies, and *Chlamydia* infection. Pigs with GM2 gangliosidosis may have multiple white foci in the retina.

Bone disorders

Metabolic bone diseases

Metabolic bone disease broadly categorizes disturbances related to bone formation and remodeling. Disease is associated with inadequate calcium, phosphorus, vitamin D, or problems related to parathyroid hormone. Clinical manifestations within a population are generally gradual but can be abrupt in individual animals depending on the mechanism and degree of the insult.

Common mechanisms related to disease development include inadequate dietary supplementation of vitamin D_3 (Pepper et al. 1978), inadequate available phosphorus in the diet, inadequate dietary calcium, or an imbalance of available calcium and phosphorus ratio, which should be roughly 1.2 : 1. Calcium uptake and utilization is dependent on adequate levels of vitamin D; therefore the consequence of vitamin D deficiency is calcium deficiency.

Rickets and osteomalacia are the disease names associated with metabolic bone disease in growing animals and adult animals, respectively, and characterized by decreased mineralization of bone. The mechanisms are similar for these two diseases, the difference being that rickets involves growing bones and osteomalacia occurs by remodeling of mature bone. If metabolic demands for calcium or phosphorus are greater than intake, reduction and retardation of bone growth or remodeling follows.

Metabolic bone disease in swine is usually related to ration formulation or feed mixing errors, but a rare hereditary form of rickets has been described where the enzyme to convert D_2 to D_3 is absent in the kidney (Fox et al. 1985).

Growing pigs with rickets will have weak bones that bend before breaking with weak snap, have increased propensity for bone fractures, and have enlarged growth plates giving a clinical appearance of swollen joints. Since the bones bend or fracture easily, there often is evidence of recent or healing fractures and calluses. The joint surfaces may be folded, and growth plates are thickened and irregular. Hemorrhages occur in the growth plate cartilage or adjacent bone. Lameness can be a common occurrence, and conformation may be abnormal because of bending or bowing of bones. Although nonspecific, long hair and decreased appetite have also been reported for vitamin D-deficient pigs. Rortvedt and Crenshaw (2012) experimentally reproduced and reported kyphosis or "humpback" in pigs as a manifestation of rickets.

Gross lesions can take months to develop in swine fed deficient diets; however, the onset, progression, and prevalence of disease are dependent on the severity of the deficiency. Chronic nutrient imbalances can have acute clinical presentation as pathologic fractures, rubbery, weak bones, or less frequently acute hypocalcemia (Madson et al. 2012). Individual or multiple growing pigs with acute hypocalcemia can unexpectedly develop one or more of the following clinical signs: tremors, tetany, seizure-like muscle fasciculation, weakness, lameness, painful gait with reluctance to move, or bone fractures (macroscopic and/or microscopic). Sometimes the first clinical sign observed is animals reported with central nervous signs or simply found dead. In a large population of pigs, many of these signs may be present simultaneously.

Osteomalacia can be present in late finishing or adult swine (Doige 1982; Gayle and Schwartz 1980) since this is a condition of increased absorption of previously formed bone. Fractured femurs, vertebrae, or ribs at load-out or at slaughter occur with increased frequency when there is osteomalacia.

Table 19.10 Metabolic bone diseases.

Term	Abnormality	Age	Cause	Clinical signs	Lesions
Rickets	Abnormal endochondral ossification, defective bone mineralization	Growing pigs	Deficiency of available vitamin D, P, Ca; Ca/P ratio discrepancy	Lameness, pathological fractures, sudden death, tremors, kyphosis	Soft rubbery bones (ribs), enlarged epiphyses, and growth plates; fractures and calluses (ribs)
Osteomalacia	Abnormal bone remodeling, defective bone mineralization	Postpubertal and adult pigs	Deficiency of available vitamin D, P, Ca; Ca/P ratio discrepancy	Pathological fractures, lameness	Cortices thin, spongy, soft with deposition of matrix at sites of mechanical stress
Fibrous osteodystrophy	Excessive bone resorption, defective bone mineralization	Growing and adult pigs	Calcium and/or vitamin D deficiency; excess P	Lameness, stiff, reluctant to rise, fractures, jaw swelling, movable teeth	Soft bone
Osteoporosis	Reduced bone mass	Growing and adult pigs	Starvation; Ca deficiency; more resorption than bone formation	Pathological fractures, possible tremors, tetany, death	Brittle bones with thin cortices and reduced cancellous bone; bones easily break
Lactational osteoporosis	Reduced bone mass	Adult pigs, weaned sows	Inadequate Ca; intake or bone reserves during lactation	Hind limb weakness and fractures; slow farrowing, uterine prolapse, tremors, death	Brittle bones with thin cortices; bones easily break

Osteoporosis is a disorder of bone classified under the umbrella of metabolic bone disease. This term is a lesion rather than a process as in rickets or osteomalacia and denotes overall decreased bone mass. Osteoporosis is caused by an excess resorption of bone and results in endosteal thinning of the trabeculae and cortices (Spencer 1979). Bones are structurally normal but become increasingly brittle (easily snap). Osteoporosis results from decreased caloric intake, decreased dietary calcium, lactation, disuse, copper deficiency, vitamin A toxicity, or increased stress (Thompson 2007). With lactational osteoporosis (Spencer 1979), large amounts of calcium and phosphorus are preferentially resorbed from the skeleton to provide high levels of calcium and phosphorus in milk. This process typically occurs in mid- to late lactation or the early post weaning period (Gayle and Schwartz 1980). This can manifest clinically as lameness or "downer" sow syndrome. Complicating factors include absence of sunlight and periods of restricting exercise (sow stall, farrow crates), particularly for first litter gilts that are still growing.

Osteomalacia and osteoporosis have been described in diagnostic surveys of paretic or paralyzed sows (Doige 1982; Gayle and Schwartz 1980). Sows may become lame or unable to stand and may have fractures (particularly of the humerus, femur, and vertebrae) and paraplegia. Crepitus can be heard and felt when clinically examined. At necropsy most frequent sites for lesions are the proximal one-third of the humerus and proximal one-third of the femur. Comminuted spiral fractures extend from the metaphysis to the diaphysis. It is a result of an imbalance between bone formation (osteoblast activity) and bone resorption (osteoclast activity). There is usually due to calcium deficiency (Spencer 1979). Metabolic bone disease conditions are summarized in Table 19.10.

Micronutrient disorders affecting bone

Copper is required for linking collagen molecules. Deficiencies have been associated with skeletal development, osteoporosis, and osteochondrosis. Suboptimal levels can result in widened growth plates, mimicking rickets-like changes in growing pigs, or spontaneous fractures.

Vitamin A toxicity is a well-recognized problem associated with lameness in swine. Toxicity is most often associated with diet formulation/mixing errors and is associated with osteoporosis, exophyte formation, and premature growth plate closure in growth. Affected growing pigs typically lay down more and can have an arched back appearance when standing and hoof placement is altered to reduce pain. Long bones can be reduced in length with flaring of the physeal region.

Jensen et al. (1983) described inherited vitamin C deficiency in a group of 9–10-week-old pigs with radiodense metaphyses of the long bones and ribs, widening of the metaphyses, and lipping over the epiphyseal plates. There were also subperiosteal hemorrhages around the edge of the shafts of the long bones, and histopathology revealed reduced osteoid in the growth plates. It is

Table 19.11 Nutritional contributors to locomotor dysfunction.

Insult/disease	Clinical signs	Comment
Vitamin D deficiency	Rickets, enlarged joints, lameness, weak bones, brittle bones, fractures, "humpbacks"	Interactions with calcium, phosphorus, phytase, sunlight, growth rate, and age can complicate identification of cause
Vitamin E deficiency	Nutritional myopathy	Mulberry heart disease
Selenium deficiency	Nutritional myopathies	Mulberry heart disease
Calcium and phosphorus	Metabolic bone disease	See text
Biotin deficiency	Lameness, hoof lesions	Hamilton and Veum (1984), Simmins and Brooks (1988), Penny et al. (1980)
Calcium – hypocalcemia	Abrupt onset of tremors, prostration, low serum calcium	Chapman et al. (1962), Storts and Koestner (1965), Madson et al. (2012)
Copper deficiency	Incoordination, paralysis, aortic rupture	Teague and Carpenter (1951), Follis et al. (1955), Fletcher and Banting (1982), Pritchard et al. (1985)
Manganese deficiency	Weak, incoordination, lameness, bowed legs, reproductive effects	Rare; Neher et al. (1956)
Magnesium deficiency	Hyperirritable, tetany	Miller et al. (1940), Plumlee et al. (1954)
Zinc deficiency	Parakeratosis, lameness	Brink et al. (1959)
Vitamin C deficiency	Joint hemorrhage	Wegger and Palludan (1994)
Vitamin A deficiency	Stillbirth, deformities, incoordination, blind	Rare; Dobson (1969), Pryor et al. (1969)

probably an autosomal recessive single-gene deletion mutation, leading to a deficiency of L-gulonolactone oxidase.

Table 19.11 defines nutritional disorders related to locomotion. See Chapter 68 for additional information regarding nutrient-related conditions.

Fractures of bone

Fractures can occur in all ages but are more common in gilts and sows during late lactation or post weaning. Highly productive sows are at risk for osteomalacia, and subsequent fractures, if feed intake is not adequate during lactation (Tubbs 1988). Piglets often have fractures if laid on by sows if they are hypoglycemic or weak. Older piglets may fracture bones when they are stuck in gates or equipment or during transport (Vaughan 1977). An increased rate of fractures in a particular age group should elicit a broad investigation of risk factors, including dietary factors for metabolic bone condition, housing and flooring effects, mechanical sources of trauma, transport trauma, and handling practices by caretakers. Fractures related to improperly formulated rations may become evident at slaughter; electrical stunning may increase the frequency of fractures in femurs and spine, leading to hemorrhage and subsequent increase trimming of meat.

Fractures found in several animals at the same time may be a result of accidental electrocution or outdoor lightning strike (Van Alstine and Widmer 2003). Lumbosacral vertebra and thoracic vertebrae, femur, neck of the femur, humerus, neck of the scapula, or pelvis are the usual sites. Sometimes the fractures in the lumbosacral junction result in separation of spinal cord and nerves and, if sublethal, cause posterior paresis, retroperitoneal and perirenal hemorrhage, and distension of urinary bladders from inability to urinate. Fractures were described in the outbreaks of osteoporosis (Douglas and Mackinnon 1993) that occurred in gilts when they were moved from the farrowing quarters and involved pelvis, spine, femur (Blowey 1994b), and other bones. This was considered a multifactorial occurrence with early mating, rapid growth, high milking yield, large litters, and insufficient nutrients in the diet to provide both milk and sow growth being potential risk factors.

Kyphosis/lordosis

Kyphosis/lordosis is a disease process that results in curvature of the spinal column. The condition occurs sporadically within herds and mainly affects individual animals. However, reports have indicated there can be outbreak scenarios in which 30% of pigs are affected. The disease is idiopathic, but congenital, heredity, or nutritional implications are suggested. The condition is not readily apparent at birth, but typically develops 8–16 weeks of age (Straw et al. 2009). The curvature is most prevalent at the 14–16th thoracic vertebrae and associated with abnormal ossification of the epiphysis (Nielsen et al. 2005). Recent genetic investigations have

indicated moderate hereditable and multiple loci affecting development (Holl et al. 2008, Lindholm-Perry et al. 2010). Lordosis or kyphosis has also been associated with precocious behavior causing relaxation of the spinal ligaments (Done and Gresham 1998). Genetic selection for an extra vertebrae in the spinal column (Pearson and Done 2004) resulted in too much muscle weight for the skeleton.

Kyphosis also occurs as a metabolic bone disease, particularly vitamin D deficiency (Madson et al. 2012; Rortvedt and Crenshaw 2012). In a controlled experiment, the latter group reproduced kyphosis with 20–30% incidence by omitting vitamin D from gestating sow and/or weaned pig diets. In field outbreaks, affected piglets are sometimes apparent in the first 3 weeks of life, suggesting dietary compromise in dams.

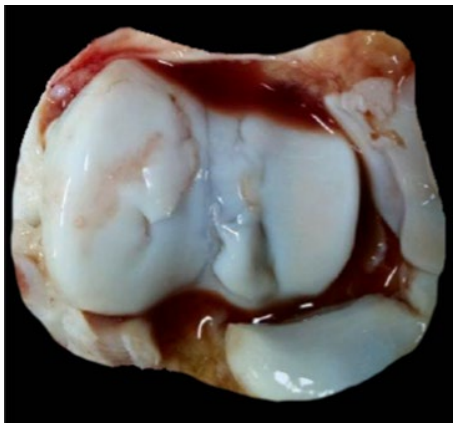


Figure 19.4 Osteochondrosis dissecans; articular cartilage thickening, necrosis, folding, and flap formation with serosanguinous joint fluid. *Source:* Image courtesy of the Iowa State University Veterinary Diagnostic Laboratory.

Spondylitis

Ankylosing spondylitis was identified by Grondalen (1974a, b) in culled sows and boars at abattoirs, but it is thought that the condition starts as early as the first year of life. Pigs have a painful lumbar region and may develop kyphosis and waddle when walking or drag the hind feet. The cause is probably multiple effects of wear and tear, spine trauma, poor nutrition, genetics, arthritis of spinal joints, etc. (Grabell et al. 1962; Grondalen 1974a, b). Vertebrae may eventually fuse and alleviation occurs.

Osteochondrosis

Osteochondrosis is a noninfectious and degenerative disorder affecting growth cartilage, previously referred to as osteochondritis or osteochondrosis dissecans (OCD). The disorder is most accurately defined as a dyschondroplasia affecting either the articular epiphyseal cartilage complex (AECC) or the physeal growth cartilage (Olsson 1978; Ytrehus et al. 2007) and can result in subsequent bone lesions.

Disease is usually progressive lameness affecting one or more limbs (Hill 1990a,b; Reiland 1975), due to localized failure of endochondral ossification and necrosis of cartilage. The condition can also be stratified based on the lesion advancement; osteochondrosis *latens* is a focal microscopic region of epiphyseal necrosis, osteochondrosis *manifesta* is macroscopic endochondral failure, and OCD is applied when there is fissure and cartilage flap formation (Ytrehus et al. 2007).

Osteochondrosis typically affects pigs from 6 to 20 weeks of age but can extend to 18 months or older, and microscopic changes have been reported in neonatal pigs. The growth plates that close last are the ones that are most susceptible, which includes the medial condyles of humerus and femur, ulna, costochondral junctions, and the sixth to eighth lumbar vertebrae. The medial condyle of the humerus appears to be the most consistent anatomic location for detecting or confirming lesions (Jorgensen 1995). Visible articular lesions start as cartilage thickening and then advance to wrinkling followed by flap formation or even detachment (Busch and Wachmann 2011). These gross changes are frequently bilateral and symmetrical (Figure 19.4). The disorder occurred in 10% of breeding animals (Grondalen 1974a) and 40% of boars in a testing station (Reiland 1978b), 47% of culled sows in Canada (Dewey 1996), and 100% of commercial pigs by 6 months (Walker and Aherne 1987). Recent investigations show that the presence of osteochondrosis *latens* and *manifesta* were about 65% but OCD was much lower at 7% (Busch et al. 2007) and 14% (Ytrehus et al. 2004).

Some features of osteochondrosis are summarized in Table 19.12. There remains confusion as to extent of the association between osteochondrosis and lameness (Brennan and Aherne 1986; Farnum et al. 1984; Fredeen and Sather 1978; Hill et al. 1984a,b; Hill 1998; Jorgensen et al. 1995; Jorgensen and Nielsen 2005; Lundeheim 1987; Reiland et al. 1978a,b). Most animals with lesions of osteochondrosis are subclinically affected, and lesions may resolve with time; however, in some animals, the pathologic changes can lead to synovial proliferation, even when lesions are small at the growth cartilage (Busch and Wachmann 2011). There is generalized belief that clinical lameness is dependent on multiple factors and can be complicated or enhanced by secondary processes or infections such as osteomyelitis, fractures, and damage to greater trochanter and tubercle (Blowey 1992, 1994a).

In the past, many of the lesions affecting the AECC have been examined at a stage when degenerative joint disease has become established. However, examination of early lesions shows microscopic foci of chondrolysis at

Table 19.12 Classification and gross pathology of osteochondrosis.

Definitions	
Osteochondrosis	Failure of endochondral bone formation; articular and/or physeal cartilage
Osteochondritis *dissecans*(OCD)	Cleft or fissures in articular cartilage that protrude into underlying bone; grossly visible
Osteochondrosis *latens*	Lesion confined to epiphyseal cartilage; visible microscopically but not grossly
Osteochondrosis *manifesta*	Lesions accompanied by delay in endochondral ossification; visible by radiographic or gross examination
Epiphysiolysis	Separation of proximal femoral epiphysis
Apophysiolysis	Bilateral separation of physes of ischiatic tuberosities; sitting with hind legs extended forward
Distribution of lesions	Can be unilateral or bilateral and symmetrical
Joint locations	More common in sites with later epiphyseal closure; humerus, femur, distal ulna and thoracolumbar vertebrae, costochondral junctions, glenoid of scapula
Joint surface	Lesions particularly found on medial surfaces (greater weight bearing)
Additional changes	Increased synovial fluid; serosanguinous joint fluid, ruptured ligaments; hemorrhages in joint capsule; thickened joint capsule

or near the interface of the articular cartilage and epiphyseal growth cartilage. The lesions may progress at this site, and lysed cartilage persists in the deeper layers of the AECC at the chondro-osseous interface and within the bone of the epiphysis. The recently replicated cells die, and there is either failure of matrix production, or formed matrix is disrupted. Clusters of chondrocytes develop at the periphery of the lesion in an attempt to repair. The soft denatured cartilage is further damage during joint movement so that flaps, fissures, and craters develop. When the AECC is breached, subchondral bone is in contact with the joint space, the joint becomes painful, and lameness develops. There is a consensus that vascular injury within cartilage canals initiates the disease process (Bullough and Heard 1967; Carlson et al. 1986, 1989, 1990; Kincaid and Lidwall 1982; Kincaid et al. 1985; Visco et al. 1991; Woodard et al. 1987b; Olstad et al. 2015). Without normal vascularization, there is no subsequent ossification.

Numerous risk factors for disease development include high growth rates, body conformation, hereditary factors, dietary stress, vascular defects, and trauma or athletic injuries. There is widespread belief that trauma may initiate the process of vascular necrosis, yet no single factor or etiology has been identified or confirmed as causal. Increased growth rate is commonly thought to be a risk factor. Multiple large epidemiological investigations have associated increased incidence of OCD association with growth rate (Lundeheim 1987; Busch and Wachmann 2011); however, there are also conflicting studies that show no association (Ytrehus et al. 2004; Stern et al. 1995). Dietary factors such as calcium and phosphorus, vitamin D, vitamin C, improper nutrient balance (energy and protein), and feeding practices have been investigated but are not found to have consistent roles in disease development. Deficient copper levels increase disease incidence in horses, but this has not been demonstrated in swine. Current information concerning swine osteochondrosis suggest that hereditary and anatomic characteristics could be important factors for disease progression as there are differences in prevalence between lines of pigs (Jorgensen and Andersen 2000) and certain conformational traits such as joint shape. The heritability of osteochondrosis in swine is moderate (Stern et al. 1995; Lundeheim 1987).

Joint disorders

Arthritis is very common in swine with multiple risk factors associated with both infectious and noninfectious causes. Many of the infectious causes are opportunistic resident microflora; however commingling pig sources increases the likelihood of introduction of new or variant strains of those potential pathogens. Effects of flooring, trauma, handling, management, and nutrition as risk factors greatly influence the occurrence and magnitude of joint diseases in modern production systems.

Infectious arthritis is often caused by bacteremic organisms capable of causing septicemia with tissue localizations in joints (synovitis and arthritis), meninges, serosal surfaces (polyserositis), lung, or other organs. Infectious arthritis can occur at any age but is most common in pigs 4–12 weeks of age, perhaps more prevalent in autumn and winter (Miniats et al. 1986). Clinical signs include pyrexia, lameness, inability to rise or move, and death. Joint lesions include increased synovial fluid, inflamed synovial membranes, fibrinous periarteritis, and joint swelling due to the exudation, sometimes with abscesses. Excess synovial fluid with color change, cloudiness, or change in viscosity is an important indicator of pathology (Figure 19.5). The hock, stifle, carpal, elbow, and hip joints are the joints most often affected.

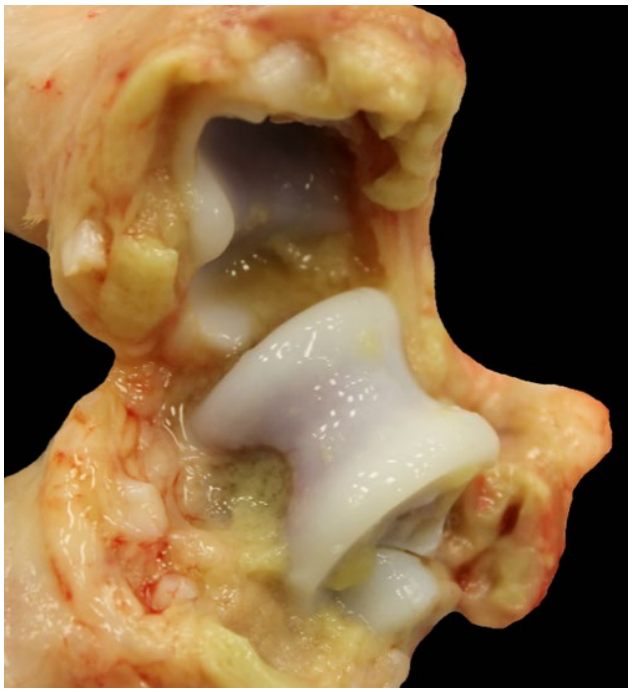


Figure 19.5 Acute fibrinous arthritis; bacterial infection. *Source:* Image courtesy of the Iowa State University Veterinary Diagnostic Laboratory.

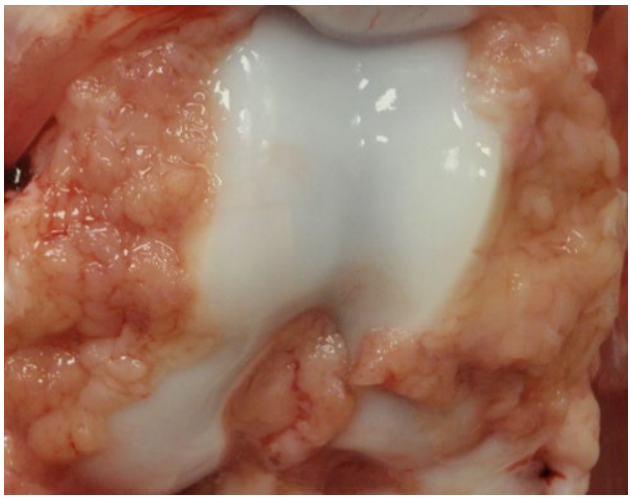


Figure 19.6 Proliferative synovitis; chronic joint irritation, multiple etiologies. Most often seen with *Mycoplasma hyosynoviae*, *Erysipelothrix rhusiopathiae*, or osteochondrosis. *Source:* Image courtesy of the Iowa State University Veterinary Diagnostic Laboratory.

The most common organisms of herd significance associated with arthritis will vary with age but include *S. suis, H. parasuis, Mycoplasma* spp., *Erysipelothrix rhusiopathiae,* and *A. suis* and are discussed in respective chapters. A potpourri of other organisms, especially pyogenic *Streptococcus* spp., *T. pyogenes*, and coliforms, are opportunists that affect individual pigs or are a result of poor hygienic practices in suckling piglets.

M. hyosynoviae is widespread in pig populations; disease is usually an uncomplicated, nonsuppurative arthritis of stifles with hocks, shoulder, and elbow in pigs greater than 30 kg and after 12–14 weeks of age (Hagedoorn-Olsen et al. 1998, 1999a, b; Gomes Neto et al. 2012). Reluctance to rise or inability to stand occurs within 24 hours of infection (Ross et al. 1971; Ross and Duncan 1970; Ross and Spear 1973) and is difficult to differentiate from other causes of severe lameness, particularly *E. rhusiopathiae*. Diagnosis is observation of typical gross and microscopic lesions, usually with excess serosanguinous synovial fluid (Figure 19.6), with agent confirmed present by culture or PCR (Platts et al. 2008; Gomes Neto et al. 2012).

MHR typically affects pigs younger than 10 weeks of age (Ross and Spear 1973; Friis and Feenstra 1994; Gomes Neto et al. 2012). Most pigs are colonized, but disease is relatively uncommon, usually with low morbidity (5–15%) and mortality (<10%) (Buddle 1987). Organisms acquired from dams or other pigs colonize in the nasopharynx and can produce bacteremia. Occasionally the organism localizes, with a strong predilection for serosal surfaces or synovium of tarsal, carpal, shoulder, stifle, hock, or atlanto-occipital joints (Ross et al. 1973; Ross and Spear 1973, Roberts et al. 1963a,b). More detail can be found in Chapter 56 or review by Rosales and Nicholas (2010).

Haemophilus parasuis is a widespread infection and resident of the nasopharynx of pigs (Oliveira and Pijoan 2004). It is a common cause of multisystemic disease manifested as acute lameness, depression, fever, dyspnea, hot swollen joints, reluctant to stand or move, tremor, paralysis, meningitis, or sudden death (Hoefling 1994; Nielsen and Danielsen 1975; Smart et al. 1986). Experimental infections produce severe lesions within 60 hours (Vahle et al. 1997). The bacteria are fastidious, and therefore, PCR assays are useful to improve the sensitivity of detecting bacteria in clinical cases (Oliveira et al. 2003), preferably from a site of a compatible lesion. Disease control has been difficult due to serovar diversity and the lack of cross-protection between strains (Oliveira and Pijoan 2004).

Streptococcus suis are ubiquitous early colonizers of the nasopharynx of suckling pigs (Sanford and Tilker 1962) and persist on mucosal surfaces indefinitely (Torremorell and Pijoan 1998; Walsh et al. 1992). Organism diversity includes at least 33 serovars, which confounds efforts to establish solid immunity. Consequently, this opportunist is a very common yet sporadic cause of polysystemic diseases, including arthritis, meningitis, serositis, and pneumonia.

Erysipelothrix rhusiopathiae affects all ages of swine as the disease erysipelas, as well as causes carcass condemnations at abattoirs. Acutely affected animals are

pyrexic, prefer to lie down because of pain, have a stiff gait, and shift their weight from foot to foot (Grabell et al. 1962). Chronic erysipelas results in a progressively increasing lameness and weight loss as a rheumatoid-like proliferative arthritis in the joints of the long bones or vertebrae. A hypersensitivity reaction with pannus formation, periarticular fibrosis, and exostoses (Vaughan 1969) occurs in chronically affected joints. Granulation tissue forms in the articular cavity, and there is proliferation of the connective tissues with elongated tags attached to the synovial membrane (Grabell et al. 1962). Diagnosis is by culture, PCR, or IHC and is enhanced with samples from acutely affected and nontreated animals.

Polyarthritis

Polyarthritis is a common reason for locomotor dysfunction prior to weaning. Routes for bacterial infection are via the tonsil, small intestine, navel ill, or abrasions of the integument. Navel infections are also a risk factor for development of umbilical hernia. Management and hygiene in farrowing including trauma from competition for nipple, abrasions, and clipping of teeth, tail docking, or ear notching are risk factors. There is a lower incidence of polyarthritis in female pigs and in pigs from multiparous sows, small litters, and closed herds (Nielsen et al. 1975; Smith and Mitchell 1976). *Streptococcus* spp., particularly *S. suis and S. equisimilis*, have been reported to cause 63% of the cases, but *T. pyogenes*, *Staphylococcus* sp., and *E. coli* are also frequent causes (Nielsen et al. 1975; Smith and Mitchell 1976).

Skin and hoof abrasions commonly occur bilaterally on hind limbs and forelegs and can be evident within a few hours of birth. Skin of the neonate is very soft with minimum keratin and is easily eroded over the joints. Floor abrasiveness increases the chance of skin lesions and therefore the opportunity for the invasion of microorganisms. Lesions are more common in the forelimbs (Furniss et al. 1986). Depending on environmental conditions, 98% of 3-day-old pigs can have skin abrasions, but only 11% of pigs are severely affected, and most lesions heal within 2–3 weeks of life (Svendsen et al. 1979; Furniss et al. 1986). The incidence of skin abrasions is highest on old cement floors, intermediate on punch metal and new cement floors, and lowest on plastic-coated woven wire. The incidence increases if the size and shape of the slot is large compared to the piglet's foot size and in litters of hypogalactic sows (Smith and Mitchell 1976). Joints involved in polyarthritis are often swollen and contain fibrinous exudates, with carpal, elbow, and hock joints the most frequently affected. *Trueperella pyogenes* is also associated with abscesses, tenosynovitis, and vertebral infections, which are suppurative and which can cause complete collapse of the vertebral body, often following tail biting.

Muscle disorders

Myopathies can be developmental (congenital, hereditary) or acquired. Acquired myopathies are by a variety of mechanisms including toxins, autoimmune mechanisms, endocrine dysfunction, parasite insult, neurogenic atrophy, cachexia, or metabolic dysfunction. Muscle atrophy is rapid and severe in cases of denervation but can be protracted with repair by fibrosis in cases of nerve injury. In slow progressive muscle disorders, muscle changes are progressive and irreversible. All structures of the lower limb (nerves, tendons, ligaments, bones, and muscles) are liable to ascending infections from the foot because of the permanent exposure to bacteria and trauma.

Primary myopathies are uncommon since porcine stress syndrome (PSS) has largely been mitigated. Myopathies may affect different groups of muscles. The most common signs are muscular weakness, trembling while standing, pain, muscle swelling, or muscle wasting. All manifest as locomotor or postural disorders. There is usually a rise in the plasma CPK levels that indicates muscle damage, followed by a transient myoglobinuria. Elevation in serum troponin is also a prognostic indicator of myocardial degeneration or necrosis. Table 19.13 summarizes some conditions primarily affecting muscle.

Porcine stress syndrome

PSS and pale, soft exudative (PSE) is a complex of conditions associated with an autosomal recessive gene of variable penetrance (Bradley and Wells 1978; Bradley and Fell 1981; Swatland 1974). Positive steps by breeders to reduce the prevalence of the gene have reduced its incidence and prevalence that was once at 95% in Pietrain and 35% of Landrace. Recent data from dead or nonambulatory animals at slaughter facilities revealed a prevalence of approximately 5% (Ritter et al. 2008). Well-muscled pigs die suddenly from a variety of stressors under natural conditions. Susceptible animals must have a hereditary predisposition that is then triggered by an exertional stress in the form of transportation, mating, excessive preslaughter tension, high environmental tension, or excitement. Exposure to halothane is a particularly effective stimulant that can be used to test for the predisposition.

Clinically, pigs have abrupt onset of respiratory distress, staggering, rigid firm muscles, and discolored skin. Pyrexia, muscle rigidity, and increased lactate in muscles cause rapid death. The pig enters a premature rigor with pyrexia that persists for an hour or so after death. If pigs have high glycogen levels at death, the meat particularly from the loin and ham take on the appearance of PSE muscle (Briskey 1964). There is an excess of watery leakage from the muscle (drip). If there is little

Table 19.13 Diseases and insults of muscle.

Insult/disease	Clinical signs/lesions	Common age
Splayleg	Hind legs adducted; myofibrillar hypoplasia	Congenital/neonate
Nutritional myopathy (vitamin E and/or selenium deficiency)	Myocardial hemorrhages with pericardial fluid; hepatosis dietetica; less common, gritty pale white streaks in muscle	Early post weaning (3–8 weeks of age usually)
Trauma/injection sites granuloma or abscess	Bruising, hemorrhage, inflammation, fibrosis, abscess	Any age
Bacterial infections	Usually initiated by trauma or by sepsis	Any age
Clostridium spp.	Necrosis, hemorrhage, edema; poor injection techniques and/or trauma	Any age
Porcine stress syndrome (PSS/PSE/DFD)	Muscle rigidity, skin flushed, pyrexia, rapid rigor, lactic acidosis, malignant hyperthermia	Any age but more common in finisher and young adult
Hemorrhages from stunning at slaughter	Muscle petechiae and hemorrhages in carcasses at slaughter	At slaughter; euthanasia by electrocution
Hemorrhages: ASF, CSF	Focal hemorrhages in muscle	Any age
Acute hypocalcemia	Weakness, tremors, muscle fasciculation	Post weaning to adult
Steatosis	Excess fat in the muscle; heritable	Adult
Gossypol toxicity	Cardiomyopathy: toxin found in cottonseed meal	Any age
Ionophore toxicity	Myopathy: synergism with tiamulin or salinomycin	Any age
Congenital muscle hypertrophy	Ataxia at birth with very large hams; heritable	Congenital
Parasites (*Taenia*, *Trichina*)	Myalgia, inflammatory nodules	Post weaning to adult

glycogen, the pH rises, and the muscle does not store well and ends up as dry, firm, and dark (DFD) meat. Essentially the stress has induced an accelerated glycolysis.

This complex is an inherited defect in the uptake, storage, and release of calcium in the muscle fibers that is triggered by exertional stress. Bonca (2009) has recently described the small changes in the normal RYR1 gene encoding for the protein ryanodine that is responsible for the calcium channel control in the skeletal muscle fibers. Animals with the normal gene can properly adjust the ion transfer, but those with the mutation cannot.

Back muscle necrosis seems to be a special manifestation of the PSS (Bradley et al. 1979). The condition is sporadic and affects predominately pigs over 50 kg. There is pain, difficulty in moving, and swelling of the back muscles (*Mm multifidi* and *longissimus*) with heat and pain on palpation. There is loss of sensation over the affected area, and pigs may be reluctant to stand and eventually dog-sit. The body curves toward the affected side in unilaterally affected animals, but bilaterally affected animals cannot stand and may die. There may be knuckling of the lower limb joints. Muscle damage is pronounced with high enzyme levels, and diagnosis is easily performed by sectioning the spine across the longissimus muscles, which appear dark and hemorrhagic.

A novel stress syndrome in pigs was recently reported and associated with a dystrophin gene defect (Nonneman et al. 2012). Affected 12-week-old pigs were from a single herd and exhibited open-mouth breathing, loss of mobility, vocalization, and skin discoloration following transport-induced stress. The clinical condition was similar to RYR1 mutation pigs with elevation of CPK, acute death, and an anesthetic trigger. The dystrophin protein was reduced when muscle tissue was analyzed and microscopic changes could be seen in skeletal and cardiac muscle (Nonneman et al. 2012).

Nutritional myopathy

Vitamin E and selenium are major antioxidants, and deficiency may lead to a generalized myopathy in skeletal muscle (Lannek and Lindberg 1975; Mortimer 1983; Nafstad and Tollersrud 1970; Trapp et al. 1970) but more likely manifests as mulberry heart disease or hepatosis dietetica. Previous disease names include microangiopathy and white muscle disease. Affected animals are typically 3–7 weeks of age and found dead with transmural myocardial hemorrhage, gelatinous pericardial material, and pulmonary edema (Pallares et al. 2002). There does not appear to be sex, breed, time of year, or known viral infection associated with the process (Rogers et al. 2017; Shen et al. 2011). Disease can occur when diets are constructed from plants that are grown in selenium-deficient soils or soils containing selenium antagonists. It may occur with increased frequency with ingestion of plants high in inhibitors of vitamin E, diets high in polyunsaturated fatty

acids or copper, low vitamin A, or possibly mycotoxins that can destroy vitamin E or make it less available. Previous investigations have also suggested that iron, calcium, magnesium, or sodium may have a role in disease development, but this appears to be inconsistent (Korpela 1990, 1991; Shen et al. 2011).

Swine appear to be less susceptible to nutritional muscular dystrophy in skeletal muscle than other domestic species. If it does occur, it is seen as pallor in the skeletal muscles with white streaks (gritty, calcified myofibrils, and muscle bundles) particularly in the *longissimus dorsi*. It is usually in pigs of 50–60 kg and clinically presented as ataxia, stiff gait, staggering gait, weakness, paralysis, depression, anorexia, and recumbency prior to death. Chronic cases have lameness and shedding of hooves.

Grossly, the muscle is edematous with white streaks, and histologically there is loss of myocyte structure with vacuolation, fragmentation, and mineral deposition in individual fibers. The primary change is selective destruction of type I fibers and lack of phosphorylase activity in type II fibers (Ruth and van Fleet 1974). Gorham et al. (1951) described yellow fat disease where there was pale skeletal and cardiac muscle in animals fed fish or fish products, and it was thought that these rations were deficient in vitamin E.

Other myopathies

Pigs are sensitive to ionophore toxicity, and this is enhanced with concurrent tiamulin administration. Affected pigs develop anorexia or feed refusal within days of consuming contaminated feed and progressively have diarrhea, lethargy, dyspnea, stiffness and painful gait, posterior ataxia or reluctance to stand, trembling, myoglobinuria, and sudden death (Umemara et al. 1985; Plumlee et al. 1995; Van Vleet et al. 1983). Skeletal muscle fibers are preferentially affected with myocardial fibers less so. Muscle enzymes, particularly CPK and AST, are elevated and provide a method of diagnosis. Serum troponin levels can also aid in a diagnosis when cardiac muscle is affected. The highly active muscles are more severely affected (tongues, diaphragm, thigh muscles, intercostal, longissimus, and the triceps), and these may show areas of pallor corresponding to the areas of myodegeneration. Microscopically there is hyaline necrosis of myofibrils with macrophage infiltration in acute stages that chronically may have muscle regeneration and fibrosis.

Asymmetric hindquarter syndrome is principally a disorder of slaughter weight pigs, though recognized as early as two to three months (Bradley and Wells 1978; Done et al. 1975). Viewed from behind, the affected leg has reduced muscle mass, particularly in semimembranosus, semitendinosus, and biceps femoris, and the other leg is larger. The discrepancy is due to a reduction in the reduced number of myocytes in the affected limb. The cause is unknown.

Pietrain creeper syndrome was first described by Bradley and Wells (1978, 1980; Wells et al. 1980) and is a familial disease characterized by progressive muscular weakness. It starts at about 3 weeks and ends with permanent recumbency at about 12 weeks of age. It may be an autosomal recessive gene with about one-third to one-fourth of pigs affected in each affected litter. There is tremor, followed by collapse to sternal recumbency, and then tremor stops. Progressively there is an increased reluctance to stand, muscular weakness, loss of condition, and finally a creeping type of gait using flexed limbs.

There is a very low incidence of *Trichinella spiralis* (Chapter 67) when there is a proper food inspection (Kapel et al. 1998). It has a predilection for the active striated muscles such as the diaphragm, intercostal, masseter, and ocular muscles. The clinical signs include intense muscle pain, decreased weight gain, itching, loss of appetite, paralysis of the hind limbs, and stiff muscles. At postmortem examination, the encysted parasites are easily observed. Viable cysts are able to remain intact for years, but calcification begins gradually to destroy the larvae and capsules. The subject has recently been reviewed by Gottstein et al. (2009) and a description of a diagnostic test using PCR by Guenther et al. (2008). *Taenia solium* may cause "measly pork" where there are cysts in the heart, tongue, diaphragm, and generalized body muscles. *Sarcocystis* sp. can be found in any striated muscle. Each cyst is double walled and contains many spores, given the name Rainey's corpuscles. Most infections are asymptomatic, but pyrexia, weakness in the loins, and posterior paralysis are sporadically described. At postmortem examination there may be watery, light-colored, small white cysts in the muscle. Avargal et al. (2004) have recently described the infection in wild boars.

An injection administered with poor hygiene, improperly placed, or with an inappropriate hypodermic needle size may damage muscle as well as vital nerves or the spinal cord. Injections in leg (ham) muscles are discouraged, with cervical muscles the preferred injection site. Injection sites can be secondarily infected with opportunists, particularly if hygiene is poor, needles are reused, vials are contaminated from previous use, or injected animals are wet or soiled. The outcome of these infections depends on the nature of the contributing organisms. Most common infections are the pyogenic (abscess-forming) bacteria such as streptococci, staphylococci, and *T. pyogenes* but may include gram-negative bacteria and *Clostridium* spp.

Clostridium septicum, C. chauvoei, and other *Clostridium* spp. can infect traumatic sites and produce gross swelling with crepitation and necrosis of the skin and underlying muscle (Figure 19.7). Lesions are typically

Figure 19.7 Necrotizing myositis and dermatitis; *Clostridium* spp. *Source:* Image courtesy of the Iowa State University Veterinary Diagnostic Laboratory.

on ventral abdomen, head, and ventral cervical regions. While usually sporadic in occurrence, epidemics of clostridial myositis and cellulitis can occur. *Bacillus anthracis*, although unusual in swine, can cause marked swelling, edema, and hemorrhage in sub-pharyngeal connective tissues. A myositis affecting the masseter muscles in association with *H. parasuis* has also been described, resulting in the swelling of the head.

Tetanus (*Clostridium tetani*) is rare but was often associated with castration or tail docking in poor hygienic conditions. Affected pigs have a rapid clinical course to death with signs that include a stiff gait, muscle rigidity, erect ears, straight tail, lateral recumbency, opisthotonos, and legs extended backward as "extensor rigidity syndrome." Loud noise will often produce tetanic spasms. The actual site of the lesion is difficult to detect; signs are an elaboration of a potent neurotoxin.

Botulism (*Clostridium botulinum*) rarely affects pigs as they are fairly resistant by oral ingestion. Type C strains have caused death in pigs after eating dead fish. Doutre (1967) described botulism in pigs fed swill with decomposing brewers' waste. Affected pigs have a progressive flaccid paralysis of voluntary muscles.

Muscle atrophy is not uncommon. Focal or extensive atrophy may follow nerve damage, disuse, senility, and undernutrition and usually will require microscopic evaluation for diagnosis. Focal myopathy of individual muscle cells is seen under the microscope (Bradley and Wells 1978) and occurs in less than 1% of muscle cells. Wasting with generalized muscle atrophy is commonly associated with PCV2 or other specific diseases, but wasting can be linked to over 30 different disorders, so care still has to be made in making a specific diagnosis.

Myositis ossificans was described by Hulland (1974), and it is a generalized familial disease in which bony lesions develop in the muscles adjacent to the spinal column, ribs, and tarsal bones at around 2 months of age. The muscles atrophy near the lesions, and the cause is unknown.

Cassia plant seeds in grain sorghum cause decreased appetite, incoordination, ataxia, staggering, decreased weight gain, and increased mortality in grower pigs (Flory et al. 1992). The plant causes muscle atrophy and pallor.

Many pigs are born with or acquire hernias that involve muscle or connective tissues. These are natural openings that have not formed correctly. There are four possibilities for these – umbilical, inguinal or scrotal, perineal, and diaphragmatic – but only inguinal and umbilical are common. Umbilical hernias are generally related to umbilical infections acquired in the early postnatal period, perhaps exacerbated by trauma, whereas an inguinal hernia has good evidence for hereditary predisposition. Perineal hernia is likely of traumatic origin. Epidemics of diaphragmatic hernias have been reported (Schwartz 1991).

Unusual is muscular steatosis (Bradley and Wells 1978), which is found incidentally at slaughter. It is replacement of muscle by fat. Inflammation of fat, steatitis, was described by Kirby (1981). Very old animals may have deposits of lipofuscin in the muscles, but often this can only be detected by histopathology and using special stains.

Lameness in sows and boars

Lameness is a major welfare concern and the second most common cause of culling for breeding animals within a herd, representing 10–20% of all culled sows (Dagorn and Aumaitre 1978; Dewey et al. 1992; Friendship et al. 1986; Heinonen et al. 2006; Karlberg 1979; Kilbride et al. 2009; Reiland 1975; Walker et al.1966) and 8–24% of boars (D'Allaire and Leman 1990; Koketsu and Sasaki 2009). Leg weakness is nonspecific term used to describe lameness associated with osteochondrosis, arthrosis, epiphysiolysis, apophysiolysis, or poor conformation. These diseases as well as foot lesions, leg injuries, fractures, and osteomalacia are common reasons for removal of breeding animals from the herd (Penny 1979, Wells 1984). Diagnosis is by clinical examination, necropsy of cull animals, and appropriate testing to rule out other causes of lameness.

Culling for lameness is more common for gilts and parity-one sows than in older animals (D'Allaire 1987; Dagorn and Aumaitre 1978), important because these animals are removed from the herd before peak reproductive performance is achieved (Ritter et al. 1999). Lameness between 30 and 40% at a performance station and approximately 24% of boars at artificial insemination

units are culled because of leg weakness; 75% of the latter are less than 18 months of age (Grondalen 1974e; Reiland 1975).

Lameness is frequently a cause of herd culling or euthanasia in sows (D'Allaire et al. 1987) and is more common during the lactation period and winter months (Anil et al. 2005). In one study, locomotor problems caused 9% of the culling and 28% of sow deaths (D'Allaire et al. 1987). Sow herds in France with a lameness prevalence of at least 15% had higher mortality rates than herds with less lameness (Abiven et al. 1998). Reiland (1975) examined 230 boars and sows culled for lameness and found few cases of foot rot and concluded that hoof lesions were of secondary importance in leg problems.

The culling rates due to lameness varies from farm to farm, implying leg weakness is a concern on certain farms. In a survey of Ontario farms, it ranged from 0 to 38%, with an average of 11%, and was associated with a high culling rate for sows and high proportions of gilts to sows in the breeding herd (Dewey et al. 1992). The culling rate due to lameness in start-up herds (26 ± 13%) was higher than in established herds (8 ± 6%) (Dewey et al. 1992). Herds that are repopulating have a larger proportion of young breeding-age animals and a higher level of culling for lameness. Farms in which stockman were responsible for higher numbers of breeding animals had an increased risk for lameness and subsequent culling (Willgert et al. 2014).

The housing factors associated with high levels of culling due to lameness were slatted floors for finisher pigs and sows (Kilbride et al. 2009), the use of individual sow stalls, and a high density of pigs in the finishing area. These findings suggest that a change of housing design may result in reducing the culling rate due to lameness and that attention to the way young replacement stock are housed and handled may be important with respect to subsequent longevity and soundness. Floor type and quality, the size of the space between the slats, the width of the slat, the flooring material, the slipperiness, and the type of ground for outdoor housed pigs all impact the prevalence and cause of lameness. Housing pigs indoors on concrete is associated with lameness in pigs (Barnett et al. 2001), and sows are more prone to develop lameness with slatted versus solid concrete flooring (Kilbride 2009).

Group housing of gestating sows is now required in some countries due to welfare concerns, and this trend extends globally. The prevalence of lameness in group-housed sows has been reported to be 8–27% (Bonde et al. 2004; Cador et al. 2014; Heinonen et al. 2006; Kilbride et al. 2009; Pluym et al. 2011); lameness prevalence can be higher in grouped scenarios compared with individual housing systems. Lameness development in pens typically occurs shortly after grouping. A recent study reported that 13.1% of penned sows developed lameness within 3–5 days of grouping and that fecal soiling of the animal may be an indicator of lameness (Pluym et al. 2017; Zurbrigg and Blackwell 2006). Sows housed in pens for a portion of gestation are expected to have reduced farrowing time (Ferket and Hacker 1985), reduced lameness in gilts (Hale et al. 1984), and less joint damage (Fredeen and Sather 1978).

Many sows culled for lameness have more than one cause for the clinical signs. Lameness has associations with genotype, type of feed ingredients, housing type (specifically, intensive versus extensive), floor type, and effluent drainage. Associations between the culling rate due to lameness in sows and various housing factors involving finisher pigs indicate that the environment of the young growing animal may have an impact on the skeletal system that becomes apparent only later in life. This would suggest that managers of herds with higher than acceptable levels of lameness in the sow herd should examine the flooring, management, and housing systems used for the young replacement animals in addition to factors in the sow herd.

Epiphysiolysis and apophysiolysis

Epiphysiolysis and apophysiolysis are conditions related to osteochondrosis and associated with abnormalities of the AECC. These conditions result in separation of weakened epiphyseal sites or physes. Epiphysiolysis is separation of the proximal femoral epiphysis (femoral head) from the remaining bone. It generally occurs between 5 months and 3 years of age because epiphyses fuse at three to seven and a half years of age (Cunningham 1966; Duthie and Lancaster 1964; Grondalen 1974c; Nemeth and van der Valk 1976; Reiland 1975). The cause is a combination of excess tension in the hip joint across a weakened physeal region in the femur that then separates. Clinical lameness is often severe and sudden but is occasionally insidious. It may be unilateral or bilateral and manipulation reveals crepitation.

Apophysiolysis is the bilateral separation of the ischiatic tuberosities along their physes. It has been recognized in young sows (Done et al. 1979; Petterson and Reiland 1967; Van Alstine and Toben 1989). Most affected animals are heavily pregnant, most dog-sit with hind limbs forward, and palpation elicits crepitus. It is associated with slippery floors excessively pulling the *biceps femoris* tendons from the *tuber ischidicum*. Unilateral lesions cause a moderate to severe lameness, but bilateral separation may prevent the sow from rising or walking (Done et al. 1979).

Osteomyelitis

Depending on location, osteomyelitis may result in lameness or cause pathological fractures, affecting long bones, physes, or vertebrae with subsequent compression

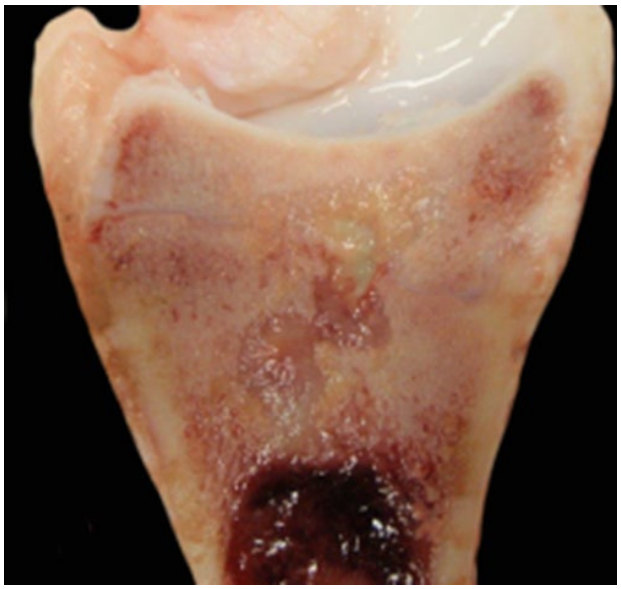


Figure 19.8 Purulent osteomyelitis with physitis (growth plate involvement); bacterial sepsis, multiple possible etiologies. *Source:* Image courtesy of the Iowa State University Veterinary Diagnostic Laboratory.

of the spinal cord. It can follow bacterial septicemia or be a local progression as in ascending myelitis from tail biting. It may be associated with apophysiolysis, with the marrow and metaphysis of a long bone (Figure 19.8) or with pathological fractures. Sows are prone to arthritis that is often by extension from septic feet (Reiland 1975) or other surface abrasions such as shoulder sores. It complicates spondylitis and may develop into osteoarthritis.

Infectious arthritis

Infectious arthritis is a modest cause of sow culling and affects 2.5% of culled boars (Grondalen 1974a, d; Grondalen and Vangen 1974; Nakano et al. 1979a). In Reiland's (1975) study of animals culled for lameness, the clinical signs were due to infectious arthritis in 18% of the sows that were less than 18 months of age and in 64% of the sows that were older than 18 months. Most of these animals had spondylitis, osteomyelitis, and/or arthritis of the hock joint associated with the presence of *E. rhusiopathiae,* streptococci, or *T. pyogenes*. In the vertebral column, the chronic proliferative arthritis and discospondylitis were secondary to lesions of osteochondrosis.

When lesions such as fractures or epiphysiolysis are coinfected with osteotropic bacteria such as streptococci or *T. pyogenes,* primary insult often cannot be determined (Reiland 1975). Suppurative arthritis of single joints is more likely to be the result of bacteria acquired by direct penetration, whereas bacteria from a source of sepsis, such as infected hoof lesions, fight wounds, skin abrasions, or uterine infections, can often cause polyarthritis. The clinical signs of bacterial arthritis are heat, swelling, pain, refusal to bear weight on the leg, pyrexia, and anorexia.

Proliferative osteitis

Proliferative osteitis is a lesion described at the femoral greater trochanter and medial epicondyle of the humerus (Blowey 1992), usually affecting gilts after the first weaning. Affected animals are found "dog-sitting" with obvious pain and discomfort when attempting to stand. Locally extensive hemorrhage within muscle is reported.

Arthrosis

Arthrosis, sometimes called arthropathy, osteoarthrosis, or osteoarthritis, is a nonspecific degenerative condition of cartilage that develops in chronic joint disease (Palmer 1985). In one report, animals less than 18 months had 7% prevalence, but those over 18 months had 82% (Reiland 1975), suggesting increased prevalence with age. This condition is generally considered a result of joint instability from osteochondrosis whereby the surface lesions in the joint fill with osseous repair tissue (Grondalen 1974a; Nakano et al. 1979a; Palmer 1985). Pathologically, the lesions include fibrillation of joint cartilage, ulceration of the articular surface, osteophyte production, and thickened synovial membrane and joint capsule (Palmer 1985).

Hoof and claw

A discussion of hoof and claw lesions, common in sows and a risk factor for lameness (Cador et al. 2014), is found in Chapter 17.

Neoplasms

Neoplasms are not common but include osteosarcoma of the maxilla occluding the nasal cavity (S. Done, personal observation), osteogenic sarcoma (Harcourt 1973), metastasis from malignant melanoma, congenital melanoma, glioblastoma, and multiple myeloma (Fisher and Olander 1978; Rintisch et al. 2010).

Acknowledgments

We are grateful for contributions from all preceding authors and contributors from previous editions of *Diseases of Swine*, including Jack Done, Ray Bradley, Mike Hill, Cate Dewey, Stan Done, Susanna Williamson, and Ben Strugnell.

References

Abiven N, Seegers H, Beaudeau F, et al. 1998. *Prev Vet Med* 33:109–119.

Alcaraz A, Warren A, Jackson C, et al. 2009. *J Vet Diagn Invest* 21:250–253.

Anil SS, Anil L, Deen J. 2005. *J Am Vet Med Assoc* 226:956–961.

Arruda BL, Arruda PH, Magstadt DR, et al. 2016. *Plos One* 11.2: e0150104.

Arruda PHE, Arruda BL, Schwartz KJ, et al. 2017. *Transbound Emerg Dis* 4(2): 311–315.

Avargal RS, Sharma JK, Juyal PD. 2004. *Vet J* 168:358–361.

Azevedo Costa E, de Marco Viott A, de Souza Machado G. 2010. *Emerg Infect Dis* 16:2011–2012.

Barlow A. 2006. *Pig J* 57:254–258.

Barnett JL, Hemsworth PH, Cronin GM, et al. 2001. *Aust J Agric Res* 52:1–28.

Bennetts HW, Beck AB. 1942. *Bull Council Sci Ind Res*: 147. https://www.cabdirect.org/cabdirect/abstract/19421404024.

Berge GN, Fonnum F, Brodal P. 1987. *Acta Vet Scand* 28:321–332.

Blackwood W, Corsellis JAN. 1976. Greenfields Neuropathology, 3rd ed. London: Edward Arnold.

Blowey RW. 1992. *Vet Rec* 131:312–315.

Blowey RW. 1994a. *Pig J* 32:88–90.

Blowey RW. 1994b. *Vet Rec* 134:601–603.

Bonca G. 2009. *Rev Rom Med Vet* 19:21–28.

Bonde M, Rousing T, Badsberg JH, et al. 2004. *Livest Prod Sci* 87:179–187.

Bradley R. 1978. *Br Vet J* 134:434–443.

Bradley R, Fell BF. 1981. Myopathies in animals. In Walton J, ed. Disorders of Voluntary Muscle, 4th ed. London: Churchill-Livingstone, pp 824–872.

Bradley R, Wells GAH.1978. *Vet Ann* 18:144–157.

Bradley R, Wells GAH. 1980. The "Pietrain Creeper" pig a primary myopathy. In Rose FG, Behrens FC, eds. Animal Models of Human Diseases, Tunbridge Wells: Pitman Medical Ltd.

Bradley R, Wells GAH, Gray LJ. 1979. *Vet Rec* 104:183–187.

Bradley R, Done JT, Hebert CN, et al. 1985. *J Comp Pathol* 93:43–59.

Brennan JJ, Aherne FX. 1986. *Can J Anim Sci* 66:777–790.

Brink MF, Becker DE, Terrill SW, et al. 1959. *J Anim Sci* 18 836–840.

Briskey EJ. 1964. *Adv Food Res* 13:89–103.

Buddle JR. 1987. The T. G. Hungerford VADE MECUM Series for Domestic Animals (Differential Diagnosis). The Diagnosis of the Diseases of Pigs. Series B, Number 8, November. Sydney: University of Sydney Post-graduate Foundation in Veterinary Science.

Bullough PG, Heard TW. 1967. *Br Vet J* 123:305–310.

Busch ME, Wachmann H. 2011. *Vet J* 188:197–203.

Busch ME, Christensen G, Wachmann H, et al. 2007. *Dansk Veterinartidsskrift* 90:20–25.

Cador C, Pol F, Hamoniaux M, et al. 2014. *Prev Vet Med* 116:102–110.

Cao J, Li B, Fang L, et al. 2012. *J Vet Diagn Invest* 24:767–771.

Carlson CS, Hilley HD, Henriikson CA. 1986. *Calcif Tissue Int* 38:44–51.

Carlson CS, Hillley HD, Meuten DJ. 1989. *Vet Pathol* 26:47–54.

Carlson CS, Meuten DJ, Richardson DC. 1990. In Proceedings: Orthopaedic Research Society, p. 347.

Casteel SW, Osweiler GD, Cook WO. 1985. *J Am Vet Med Assoc* 186:1084–1085.

Chapman HL, Kastelic CY, Caron DV, et al. 1962. *J Anim Sci* 21:112–118.

Chennells DC, Nelson K, Penlington N, et al. 2006. *Pig Vet J* 57:259–270

Colvin BM, Harrison LR, Sangster LT. 1986. *J Am Vet Med Assoc* 189:423–426.

Cunha TJ. 1968. *Feedstuffs* 40:25.

Cunningham B. 1966. *Irish Vet J* 20:66–68.

D'Allaire S. 1987. *Compend Cont Ed Pract Vet* 9:F187–F191.

D'Allaire A, de Rota L. 1980. *Can J Comp Med* 45:205–206.

D'Allaire S, Stein TE, Leman AD. 1987. *Can J Vet Med* 51:506–512.

Dagorn J, Aumaitre A. 1978. *Livest Prod Sci* 6:167–177.

D'Allaire S, Leman AD. 1990. *Can Vet J* 31:581–583.

Dalton JR. 1992. *Vet Rec* 131:107–108.

Daniels PW, Williams DT, Mackenzie JS. 2002. Japanese encephalitis virus. In Morilla, Antonio, Kyoung-Jin Yoon, and Jeffrey J. Zimmerman eds. Trends in Emerging Viral Infections of Swine. Ames, IA: Iowa State Press, pp. 249–263.

De Lahunta A. 1983. Veterinary Neuroanatomy and Clinical Neurology, 2nd ed. Philadelphia: Saunders.

Dellmann H-D. 1971. Veterinary Histology: An Outline Text Atlas. Philadelphia: Lea and Febiger.

Dellmann H-D, McClure RC. 1975. The CNS. In Getty R. ed. Anatomy of the Domestic Animals. 5th ed. Philadelphia: WB Saunders and Co.

Detiefsen JA, Carmichael WT. 1921. *J Agric Res* 20:595–604.

Dewey CE. 1996. Diseases of the nervous and locomotory systems. In Barbara E. Straw, Jeffrey J. Zimmerman, Sylvie D'Allaire, et al. ed. Diseases of Swine, 9th ed. Oxford: Blackwell Scientific Publishing.

Dewey CE, Friendship RM, Wilson MR. 1992. *Can Vet J* 33:747–748.

Dickerson JWT, Dobbing J. 1967. *Proc R Soc Lond (Biol)* 166:384–395.

Diesen CD, Gandhi SS, Ellenport CR. 1975. Sense organs and common integument. In Getty R, ed. Anatomy of

the Domestic Animals, 5th ed. Philadelphia: WB Saunders Co, pp. 1409–1417.
Dobson JJ. 1969. *Aust Vet J* 45:570–573.
Dobson KJ. 1971. *Aust Vet J* 47:587–590.
Doige CE. 1982. *Can J Comp Med* 46:1–6.
Doige CE. 1988. Skeletal system. In Thomson RG, ed. Special Veterinary Pathology. Toronto, BC: Decker Inc, pp. 467–507.
Doige CE, Horowitz A. 1975. *Can J Comp Med* 39:7–16.
Doige CE, Martineau GP. 1984. *Can J Comp Med* 48:414–419.
Dominick MA, Carson TL. 1983. *Am J Vet Res* 44:35–40.
Donald HP. 1949. *J Agric Sci* 39:164–165.
Done JT. 1957. *Vet Rec* 69:1341–1349.
Done JT. 1968. *Lab Anim* 2:207–217.
Done JT. 1976a. Developmental disorders of the CNS in animals. In: Brandly CH, Jungherr EL, eds. Advances in Veterinary Science and Comparative Medicine, Vol. 20. New York: Academic Press, pp. 69–114.
Done JT. 1976b. *Vet Ann* 16:98–102.
Done SH, Gresham AC. 1998. *Pig Vet J* 41:134–141
Done JT, Hebert CN. 1968. *Res Vet Sci* 9:143–148.
Done SH, Wells GAH. 2005. *Pig J* 55:146–166.
Done JT, Allen WM, de Gruchy PM. 1975. *Vet Rec* 96:482–488.
Done SH, Meredith MJ, Ashdown RR. 1979. *Vet Rec* 105:520–523.
Done JT, Woolley J, Upcott DH, et al. 1984. *Zentral Vet Med* 31:81–90.
Done JT, Woolley J, Upcott DH, et al. 1986. *Br Vet J* 142:145–150.
Done SH, Walton JR, Carr J. 1990. In Proc Cong Int Pig Vet Soc, p. 282.
Done SH, Potter RA, Courtenay A, et al. 1998. *Pig J* 43:148–153.
Douglas RGA, Mackinnon JD.1993. *Proc Pig Vet Soc* 30:77–80.
Doutre MP. 1967. *Bull Off Int Epiz* 67:1497–1501.
Doyle LP. 1937. *J Am Vet Med Assoc* 90:656–662.
Duthie IF, Lancaster MC. 1964. *Vet Rec* 76:263–273.
Edington N, Broad S, Wrathall AE, et al. 1988. *Vet Microbiol* 16:189–193.
Elvinger F, Liggett AD, Tang ICN, et al. 1994. *J Am Vet Med Assoc* 205:1014–1016.
Fankhauser R. 1962. The cerebrospinal fluid. In Innes JRM, Saunders LZ, eds. Comparative Neuropathology. New York: Academic Press, pp. 21–54.
Fankhauser R, Luginbuhl H. 1968. Pathological Anatomy of the Central and Peripheral Nervous Systems of the Domestic Animals. Berlin and Hamburg: Paul Parey, p. 272.
Farnum CE, Wisman NJ, Hiley HD. 1984. *Vet Pathol* 21:141–151.
Ferket SL, Hacker RR. 1985. *Can J Anim Sci* 65:851–859.
Fisher LF, Olander HJ. 1978. *J Comp Pathol* 88:505–517.
Fletcher JM, Banting LF. 1982. *J S Afr Vet Med Assoc* 54:43–46.
Flory W, Spainhour CB, Colvin B, et al. 1992. *J Vet Diagn Invest* 4:65–69.
Follis RH, Bush JA, Cartwright GE. 1955. *Bull Johns Hopkins Hosp* 97:405–410.
Fox J, Maunder EMW, Randal VA, et al. 1985. *Clin Sci* 69:541–548.
Frank R, Braun HE, Wilkie J, et al. 1991. *Can Vet J* 32:219–226.
Fredeen HT, Sather AP. 1978. *Can J Anim Sci* 58:759–773.
Friendship RM, Wilson MR, Almond GW, et al. 1986. *Can J Vet Res* 50:205–208.
Friis NF, Feenstra HH. 1994. *Acta Vet Scand* 35:93–98.
Friis NF, Kokotovic B, Svensmark B. 2002. *Acta Vet Scand* 43:191–193.
Furniss SJ, Edwards SA, Lightfoot AL, et al. 1986. *Br Vet J* 142:434–440.
Gauger PC, Patterson AR, Kim WI, et al. 2010. *J Swine Health Prod* 18:244–248.
Gayle LG, Schwartz WL. 1980. *S West Vet* 33:69–71.
Gedde-Dahl TW, Standal N. 1970. *Anim Prod* 12:665–668.
Getty R. (1975) In Sisson and Grossman's The Anatomy of the Domestic Animals, Vol. 2, 5th ed.. Philadelphia: WB Saunders.
Ghadially FN. 1983. The Fine Structure of Synovial Joints. London: Butterworths, pp. 1–4.
Gibson JA, Rogers RJ. 1980. *Aust Vet J* 56:254–255.
Goff J. 2010. Overview of bone physiology. In Proceedings of the Swine Disease Conference for Swine Practitioners, Iowa, 4–5 November 2010, pp. 33–40.
Gomes Neto JC, Strait EL, Strait EL. et al. 2012. *J Swine Health Prod* 20:82–86.
Goodwin RFW. 1962. *J Comp Pathol* 72:214–232.
Gorham JR, Boe N, Baker GA. 1951. *Cornell Vet* 41:332–338.
Gottstein B, Pozio E, Nockler K. 2009. *Clin Microbiol Rev* 22:127–145.
Grabell I, Hansen HJ, Olsson S, et al. 1962. *Acta Vet Scand* 3:33–50.
Greenlee JJ, Kunkle RA, Smith JD, et al. 2016. *Prion* 10:S16–S16.
Grondalen T. 1974a. Osteochondrosis and arthrosis in pigs.II. Incidence in breeding animals. *Acta Vet Scand* 15:26–42.
Grondalen T. 1974b. Osteochondrosis and arthrosis in pigs.III. A comparison of the incidence in young animals of the Norwegian Landrace and Yorkshire breeds. *Acta Vet Scand* 15:43–52.
Grondalen T. 1974c. Osteochondrosis and arthrosis in pigs.IV. Effect of overloading on the distal epiphyseal plate of the ulna *Acta Vet Scand* 15:53–60.
Grondalen T. 1974d. Osteochondrosis and arthrosis in pigs. VI. Relationship to feed level and calcium,

phosphorus and protein levels in the ration. *Acta Vet Scand* 15:147–149.
Grondalen T. 1974e. Leg weakness in pigs. II. Incidence and relationship to skeletal lesions, feed level, protein amd mineral supply, exercise and exterior conformation. *Acta Vet Scand* 15:553–573.
Grondalen T, Vangen O. 1974. Osteochondrosis and arthrosis in pigs. V. A comparison of the incidence in three different lines in the Norwegian Landrace. *Acta Vet Scand* 15:61–79.
de Groof A, Deijs M, Guelen L, et al. 2016. *Viruses* 8(10).
Guenther S, Nockler K, Nikisch-Rosenegk M von, et al. 2008. *J Microbiol Methods* 75:278–282.
Hagedoorn-Olsen T, Friis NF, Nielsen NC. 1998. *M. synoviae* infection profiles in three pig herds. *Proc Cong Int Pig Vet Soc* 15(2):203.
Hagedoorn-Olsen T, Basse O, Jensen TC, et al. 1999a. *Acta Pathol Microbiol Immunol Scand* 107:201–210.
Hagedoorn-Olsen T, Nielsen NC, Friis NF. 1999b. *J Vet Med A* 46:317–325.
Hagemoser WA, Goff JP, Sanderson TP, et al. 2000. *J Vet Diagn Invest* 12:525–534.
Hale OM, Newton GL, Cleveland ER. 1984. *J Anim Sci* 58:541–544.
Ham AW. 1979. Histology, 8th ed. Philadelphia: WB Saunders.
Ham AW, Cormack DH (1979). Bones and joints. In Ham AW, ed. Histology, 8th ed. Philadelphia Co, pp 380–387.
Hamilton CR, Veum TL. 1984. *J Anim Sci* 59:151–157.
Handel S, Stickland NC. 1986. *J Comp Pathol* 96:447–457.
Harcourt RA. 1973. *Vet Rec* 93:159–161.
Harding DJD, Done JT, Derbyshire JB.1966. *Vet Rec* 79:388–390.
Harding DJD, Lewis G, Done JT. 1968. *Vet Rec* 83:560–564.
Harding DJD, Done JT, Harbourne JF, et al. 1973. *Vet Rec* 92:527–529.
Harrison LH. 1983. *Vet Pathol* 20:268–273.
Hayashi M, Muto H. 1901. *Arch Exp Pathol* 48:96–103.
Hedman C, Bolea R, Marin B, et al. 2016. *Vet Res* 47:14.
Heinonen M, Oravainen J, Orro T, et al. 2006. *Vet Rec* 159:383–387.
Hill MA. 1990a. *J Am Vet Med Assoc* 157:107–112.
Hill MA. 1990b. *J Am Vet Med Assoc* 197:181–194.
Hill MA. 1998. *Proc Cong Int Pig Vet Soc* 15(1):254–259.
Hill MA, Ruth GR, Hilley HD, et al. 1984a. *Am J Vet Res* 45:903–906.
Hill MA, Hilley HD, Feeney DA, et al. 1984b. *Am J Vet Res* 45:917–925.
Hilley HD. 1982. *Vet Clin North Am Large Anim Pract* 4:225–258.
Hindmarsh WL. 1937. Trembling in young pigs. *Aust Vet J* 1'3:13:249–251.
Hoefling DC. 1994. *Swine Health Prod* 2:19–23.
Holl JW, Johnson RK. 2005. *J Anim Sci* 83:34–40.
Holl JW, Rohrer GA, Shackelford SD, et al. 2008. *J Anim Sci* 86:1765–1769.
Hughes EH. 1935. *J Hered* 26:415–427.
Hulland TJ. 1974. Muscle. In Jubb KVF, Kennedy Peter C eds. Pathology of the Domestic Animals, 2nd ed. New York: Academic Press, pp. 453–494.
Innes JRM, Saunders LZ. 1962. Comparative Neuropathology. New York: Academic Press, p. 839.
Jennings DS. 2001. *Pig J* 48:150–151.
Jensen PT, Basse A, Nielsen DH. 1983. *Acta Vet Scand* 24:392–402.
Jensen PT, Nielsen DH, Jensen P, et al. 1984. *Nord Vet Med* 36:32–37.
Johnson LE. 1940. *J Hered* 31:229
Jones JET. 1967. *Br Vet J* 12:327–339.
Jorgensen B. 1995. *Live Prod Sci* 41:171–181.
Jorgensen B, Andersen S. 2000. *Anim Sci* 71:427–434.
Jorgensen B, Nielsen B. 2005. *Anim Sci* 81:319–324.
Jorgensen B, Ambjerg J, Aaslyng M. 1995. *J Vet Med A* 42:489–504.
Jubb and Kennedy.1970. Pathology of the Domestic Animals, vol. 1. New York: Academic Press, pp. 117–119.
Kapel CMO, Webster P, Pozio E, et al. 1998. *Parasitol Res* 84:264–271.
Karlberg K. 1979. *Nordsk Tidsskr* 91:423–426.
Kaye MM. 1962. *Can J Comp Med* 26:218–221.
Kazama AS, Yagihashi T, Morita T, et al. 1994. *Res Vet Sci* 56:108–110.
Kidd ARM, Done JT, Wrathall AE, et al. 1986. *Br Vet J* 142:275–285.
Kilbride AL, Gillman CE, Green LE. 2009. *Anim Welf* 18:215–224.
Kincaid SA, Lidwall ER. 1982. *Am J Vet Res* 43:938–944.
Kincaid JA, Allhands RV, Pijanowski GJ. 1985. *Am J Vet Res* 46:726–732.
Kinsley AT. 1922. *Vet Med* 17:123.
Kirby PS. 1981. *Vet Rec* 109:385.
Knox B, Askaa J, Basse A, et al. 1978. *Nord Vet Med* 30:538–545.
Koketsu Y, Sasaki Y. 2009. *Theriogenology* 71:1186–1191.
Konold T, Spiropoulos J, Chaplin M, et al. 2009. *BMC Vet Res* 5:35.
Kornegay ET, Meacham TN. 1973. Livestock Research Report, 1972–73, 32–33.
Kornegay JN, Seim HB. 1996. Neurology and neurosurgery. In TG Hungerford Course for Veterinarians, Proceedings 266, 12–16 February, University of Sydney, Australia.
Korpela H. 1990. *Ann Nutr Metab* 34:193–197.
Korpela H. 1991. *J Am Coll Nutr* 10:127–131.
Lannek N, Lindberg P. 1975 *Adv Vet Sci Comp Med* 19:127–164.
Larsell O. 1954. *Anat Rec* 118:73–107.
Leopold WH, Dennis SM. 1972. *Cornell Vet* 62:269–272.

Li H, Brooking A, Cunha CW, et al. 2012. *Vet Microbiol* 159:485–489.
Lindholm–Perry AK, Rohrer GA, Kuehn LA, et al. 2010. *BMC Genet* 11:21.
Loken T, Aleksanderson M, Reid H, et al. 1998. *Vet Rec* 143:464–467.
Lopez A, Bildfell R. 1989. *Can Vet J* 30:828–829.
Lorenz MD, Kornegay JN. 2004. Localisation of the lesions in the nervous system. In Handbook of Veterinary Neurology, 4th ed. St Louis. USA: Saunders, pp. 45–74.
Lundeheim N. 1987. *Acta Agric Scand* 37:159–173.
Madsen LW, Svensmark B, Elvestad K, et al. 2001. *Vet Pathol* 38:190–195.
Madson DM, Ensley SM, Schwartz KJ, et al. 2012. *J Vet Diagn Invest* 24:1137–1144.
Malynicz GL. 1982. *Ann Genet Select Anim* 14:415–420.
Marrable AW. 1971. The Embryonic Pig; A Chronological Account. London: Pitman Medical.
Maurice H, Nielsen M, Brocchi E, et al. 2005. *Epidemiol Infect* 133:547–557.
McComas AJ. 1977. Neuromuscular Function and Disorders. London: Butterworths, p. 364.
McGavin MD, Ranby PD, Tammemagi L. 1962. *Aust Vet J* 38:8–14.
Miller RC, Keith TB, McCarthy MA, et al. 1940. *Proc Soc Exp Biol Med* 45:50–51
Mills JHL, Nielsen SW. 1968. *Adv Vet Sci* 12:33–104.
Miniats OP, Smart NL, Meyzger K. 1986. *Proc Cong Int Pig Vet Soc* 9:279.
Mortimer DT. 1983. *Vet Rec* 112:278–279.
Mouttotou N, Hatchell FM, Green LE. 1998a. *Vet Rec* 142:109–114.
Mouttotou N, Sterry J, Green LE. 1998b. *Vet Rec* 142:52–55.
Nafstad I, Tollersrud S. 1970. *Acta Vet Scand* 11:452–463.
Nakano T, Aherne FX, Thompson JR. 1979a. *Can J Anim Sci* 59:491–502.
Narita M, Ishii M. 2004. *J Comp Pathol* 1312:277–284.
Nathues H, Boehne I, Beilage TG, et al. 2010. *Can Vet J* 51:515–518.
Neher GM, Doyle LP, Thrasher DM.1956. *Am J Vet Res* 17:121–128.
Nemeth F, van der Valk PC. 1976. In Proc Int Cong Prod Dis Farm Anim, pp. 226–228.
Nielsen R, Danielsen V. 1975. *Nord Vet Med* 27:20–25.
Nielsen NC, Bille N, Larsen JL, et al. 1975. *Nord Vet Med* 27:529–543.
Nielsen LWD, Hogedal P, Arnbjerg J, et al. 2005. *APMIS* 113:702–707.
Nietfield J. 2010. In Proc Sw Dis Conf Sw Pract, Iowa, 4–5 November 2010, pp. 63–66.
Nonneman, D.J., Brown–Brandl, T., Jones, S.A., et al. 2012. *BMC Genomics* 13:233.
Nordby JE. 1934. *J Hered* 25:171–174.
Nordby JE. 1939. *J Hered* 30:307–310.
O'Hara PJ, Shortridge EN. 1966. *NZ Vet J* 14:13–18.
O'Sullivan BM, Blakemore WF. 1980. *Vet Pathol* 7:748–758.
Okkenhave H, Kjelvik O. 1995. *Nord Vet Med* 107:199–203.
Oliveira S, Pijoan C. 2004. *Vet Microbiol* 99:1–12.
Oliveira S, Blackall PJ, Pijoan C. 2003. *Am J Vet Res* 64:435–442.
Olsson SE.1978. *Acta Radiol Suppl* 358:9–12.
Olstad K, Ekman S, Carlson CS. 2015. *Vet Pathol* 52:785–802.
Painter KE, Keeler RF, Buck WB. 1985. *Am J Vet Res* 46:1368–1371.
Pallares FJ, Yaeger MJ, Janke BH, et al. 2002. *J Vet Diagn Invest* 14:412–414.
Palludan B. 1961. *Acta Vet Scand* 2:32–59.
Palmer, A.C. (1976). Introduction to Animal Neurology. 2nd ed. Oxford: Blackwell Scientific.
Palmer NC. 1985. Bones and joints. In Jubb, KVF, Kennedy PC, Palmer NC, eds. Pathology of Domestic Animals, Vol 1, 3rd ed. New York: Academic Press, pp. 1–138.
Patterson DSP, Done JT. 1977. *Br Vet J* 133:111–119.
Patterson DS, Sweasey D, Brush PJ, et al. 1973. *J Neurochem* 21:397–406.
Payen B, Fournier P. 1934. *Recueil Med Vet* 110:84–86.
Pearson R, Done SH. 2004. *Pig J* 53:207–219.
Penny RHC. 1979. *Proc Pig Vet Soc* 4:85–96.
Penny RHC, Cameron RDA, Johnson S, et al. 1980. *Vet Rec* 107:350–351.
Penrith ML, Robinson JTR. 1996. *Onderstepoort J Vet Res* 63:171–179.
Pepper TA, Bennett D, Taylor D. 1978. *Vet Rec* 103:4–8.
Petterson K, Reiland S. 1967. *Svensk Vet Tidn* 19:648–651.
Platts J, Strait E, Erickson B, et al. 2008. *Proc Am Assoc Swine Vet* 39:295–296.
Plumlee MP, Thrasher DM, Beeson WM, et al. 1954. *J Anim Sci* 13:996–1003.
Plumlee KH, Johnson B, Galey FD. 1995. *J Vet Diagn Invest* 7:419–420.
Pluym L, Van Nuffel A, Dewulf J, et al. 2011. *Vet Med* 56:101–109.
Pluym LM, Maes D, Van Weyenberg S, et al. 2017. *Vet J* 220:28–33.
Pohlenz J, Bertschinger HU, Koch W. 1974. In Proc Int Pig Vet Soc, Lyon:1–3.
Pope AM. 1986. *J Am Vet Med Assoc* 189:761–763.
Postel A, Hansmann F, Baechlein C, et al, 2016. *Sci Rep* 6:27735.
Prince JH, Diesem CD. 1960. The Pig. In Anatomy and Physiology of the Eye and Orbit in Domestic animals. Springfield: Charles C Thomas, pp. 210–215.
Pritchard GC, Lewis G, Wells GAH, et al. 1985. *Vet Rec* 117:545–548.
Pryor WJ, Seawright AA, McCosker PJ. 1969. *Aust Vet J* 45:563–569.

Ramirez–Herrera MA, Mendoza–Magna ML, Duenas–Jiminez JM, et al. 2001. *J Vet Med B* 48:477–487.
Reiland S. 1975. Osteochondrosis in the Pig-A Morphologic and Experimental Investigation with Special Reference to the Leg Weakness Syndrome Stockholm, Sweden: Akademisk Avhandling. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C16&q=Osteochondrosis+in+the+pig%3A+A+morphologic+and+experimental+investigation+with+special+reference+to+the+leg+weakness+syndrome.&btnG=
Reiland S. 1978a. *Acta Radiol Suppl* 358:14–22.
Reiland S. 1978b. *Acta Radiol Suppl* 358:23–44.
Rice DA, McMurray CH, McCracken RM, et al. 1980. *Vet Rec* 106:312–313.
Rintisch V, Munzel B, Klopfleisch R, et al. 2010. *Berl Munch Tierarztl Wschr* 123:70–73.
Ritter LA, Xue J, Dial GD, et al. 1999. *J Am Vet Med Assoc* 214:525–528.
Ritter MJ, Ellis M, Hollis GR, et al. 2008. *J Anim Sci* 86:511–514.
Roberts ED, Switzer WP, Ramsey FK. 1963a. *Am J Vet Res* 24:9–18.
Roberts ED, Switzer WP, Ramsey FK. 1963b. *Am J Vet Res* 24:19–31.
Roels S, Simoens P, Ducatelle R. 1996. *Vet Rec* 139:446–447.
Rogers AJ, Huang YW, Heffron CL, et al. 2017. *Transbound Emerg Dis*
Rohrs M, Kruska D. 1969. *Deutsch Tierartzl Wschr* 76:514–518.
Rortvedt LA, Crenshaw TD. 2012. *J Anim Sci* 90:4905–4915.
Rosales RS, Nicholas RJ. 2010. *Pig J* 63:68–72.
Ross RF, Duncan JR. 1970. *J Am Vet Med Assoc* 157:1515–1518.
Ross RF, Spear ML. 1973. *Am J Vet Res* 34:373–378.
Ross RF, Switzer WP, Duncan JR. 1971. Experimental production of *M.hyosynoviae* arthritis in swine. *Am J Vet Res* 32:1743–1749.
Ross RF, Dale JE, Duncan JR. 1973. Experimentally induced *M. hyorhinis* arthritis in swine. *Am J Vet Res* 34:367–374.
Rossow KD. 1998. *Vet Pathol* 35, 1–20.
Ruth GR, van Fleet JF. 1974. *Am J Vet Res* 35:237–244.
Ryder SJ, Hawkins SAC, Dawson M, et al. 2000. *J Comp Pathol* 22:131–143.
Sack WO. 1982. Horowitz/Kramer Atlas of musculoskeletal anatomy of the pig. In Sack WO, Pig Anatomy and Atlas. Ithaca, Veterinary Textbooks, pp. 61–187.
Sanford SE, Tilker AME.1962. *J Am Vet Med Assoc* 181:673–676.
Schock A, Gurrala R, Fuller H, et al. 2014. *Vet Microbiol* 172:381–389.
Schwartz KJ. 1991. *J Vet Diagn Invest* 3:362–364.
Schwarz L, Riedel C, Hogler S, et al. 2017. *Vet Res* 48:1.
Seeliger FA, Brugmann ML, Kruger L, et al. 2007. *Vet Pathol* 44:621–634.
Sellier P, Dando E, Dando P. 1999. *Ann Zootechnie* 48:153–161.
Shen H, Thomas PR, Ensley SM, et al. 2011. *Transbound Emerg Dis* 58:483–491.
Shimada A, Adachi T, Umemura T, et al. 1992. *Vet Pathol* 29:337–342.
Signoret JP, Baldwin BA, Fraser D, et al. 1975. The behaviour of swine. In Hafez ESE, ed. The Behaviour of Domestic Animals, 3rd ed. London. Bailliere Tindall, pp. 295–329.
Simmins PH, Brooks PH. 1988. *Vet Rec* 122:431–435.
Simpson JA. 1972. Muscle. In Critchley, Macdonald, James Lee O'Leary, and Bryan Jennett. Scientific Foundations of Neurology. Heinemann Medical.
Sisson S. 1975. Porcine osteology. In Getty R, ed. The Anatomy of the Domestic Animals, 5th ed. Philadelphia: WB Saunders. pp. 1222–1227.
Sisson S, Gandhi SS. Porcine mycology. (1975) In Getty R, ed. The Anatomy of the Domestic Animals, 5th ed. Philadelphia: WB Saunders Co., pp. 1256–1282.
Smart NI, Miniats OP, Friendship RM, et al. 1986. *Proc Cong Int Pig Vet Soc* 9:280.
Smith WJ, Mitchell CD. 1976. *Proc Pig Vet Soc* 1:91–104.
Smith WJ, Robertson AM.1971. *Vet Rec* 89:531–533.
Sokoloff L. 1978. The Joints and Synovial Fluid. New York: Academic Press.
Spencer GR. 1979. *Am J Pathol* 95:277–280.
Stave HD, Eavey RJ, Granger L, et al. 1992. *J Am Vet Med Assoc* 201:242–245.
Stephano HA, Gay GM, Ramirez TC. 1988. *Vet Rec* 122:6–10.
Stern S, Lundeheim N, Johansson K, et al. 1995. *Livest Prod Sci* 44:45–52.
Storts RW, Koestner A. 1965. *Am J Vet Res* 26:280–294.
Stowe HD, Herdt TH. 1992. *J Anim Sci* 70:3928–3933.
Stowe HD, Every AJ, Halstead S, et al. 1992. *J Am Vet Med Assoc* 201:292–295.
Straw B, Bates R, May G. 2009. *J Swine Health Prod* 17:28–31.
Svendsen J, Olsson O, Nilsson C. 1979. *Nord Vet Med* 31:49–61.
Swatland HJ. 1974. *Vet Bull* 44:179–202.
Sweasey D. Patterson DSP, Glancy EM. 1976. *J Neurochem* 27:375–380.
Swenson MJ. 1977. Editor Dukes Physiology of Domestic Animals, 9th ed. Ithaca: Cornell University Press.
Syrjala A, Saarinen H, Laing T, et al. 2006. *Vet Rec* 159:406–409.
Szalay F, Zsarnovszky A, Fekete S, et al. 2001. *Anat Embryol* 2003:53–59.
Teague HS, Carpenter LE. 1951. *J Nutr* 43:389–399.

Thompson K. 2007. Bones and joints. In Maxie M. ed. Pathology of Domestic Animals. Philadelphia, PA: Elsevier, pp. 1–184.
Torremorell MP, Pijoan C. 1998. *Vet Rec* 134: 394–395.
Trapp AL, Keahey KK, Whitenack DL, et al. 1970. *J Am Vet Med Assoc* 157:289–295.
Tubbs RC. 1988. *Agri–Practice* 9:9–13.
Umemara T, Nakamura H, Goryo M, et al. 1985. *Vet Pathol* 22:409–414.
Vahle JL, Haynew JS, Andrews JJ.1997. *J Vet Diagn Invest* 7:476–480.
Van Alstine WG, Toben CG.1989. *Cont Educ* 11:874–879.
Van Alstine WG, Widmer R. 2003. *J Vet Diagn Invest* 15:289–291.
Van Pelt RW. 1974. *J Am Vet Med Assoc* 164:91–95.
Van Vleet JF, Amstutz HE, Weirich WE, et al. 1983. *Am J Vet Res* 44:1469–1475.
Vanderkerckhove P, Maenhout D, Curvers P, et al. 1989. *J Vet Med A* 36:763–771.
Vaughan LC. 1969. Locomotory disturbances in pigs. *Br Vet J* 125:354–365.
Vaughan LC. 1977. *Vet Rec* 79:2–8.
Visco DM, Hill MA, Van Sickle DC, et al. 1991. *Vet Rec* 128:221–228.
Vogt DW, Gipson TA, Akremi B, et al. 1984. *Am J Vet Res* 45:2408–2409.
Walker B, AHerne FX.1987. 1987 Special Issue, pp. 20–22.
Walker T, Fell BF, Jones AS, et al. 1966. *Vet Rec* 79:472–479.
Walsh B, Williams AE, Satsangi J. 1992. *Rev Med Microbiol* 3:65–71.
Ward PS. 1978a. *Vet Bull* 48:279–285.
Ward PS. 1978b. *Vet Bull* 48:381–399.
Ward PS, Bradley R. 1980. *J Comp Pathol* 90:421–431.
Wegger I, Palludan B. 1994. Vit C deficiency causes haematological and skeletal abnormalities during foetal development in swine. *J Nutr* 124:241–248.
Wells GAH. 1984. *In Pract* 6:43–53.
Wells GAH, Pinsent PJN, Todd JN. 1980. *Vet Rec* 106:556–558.
Widdowson EM, Crabb DE. 1976. *Biol Neonate* 28:261–271.
Wilkie WJ. 1959. *Aust Vet J* 35:209–216.
Willgert KJE, Brewster V, Wright AJ, et al. 2014. *Prev Vet Med* 113:268–272.
Wilson TM, Rake TR. 1972. *Can J Comp Med* 46:218–220.
Wood EN. 1978. *Proc Pig Vet Soc* 3:117–118.
Woodard JC, Becker HN, Poulos PW. 1987b. *Vet Pathol* 24:118–123.
Yamada M, Nakamura K, Yoshi M, et al. 2004. *Vet Pathol* 41:62–67.
Ytrehus B, Grindflek E, Teige J, et al. 2004. *J Vet Med A* 51:188–195.
Ytrehus B, Carlson CS, Ekman S. 2007. *Vet Pathol* 44:429–448.
Zurbrigg K, Blackwell T. 2006. *J Swine Health Prod* 14:202–206.

20

Diseases of the Reproductive System

Gary C. Althouse, Johannes Kauffold, and Stephanie Rossow

Female reproduction

Diseases of the ovaries

Primary ovarian diseases are rather uncommon in swine. The vast majority of ovarian disorders are secondary to risk factors related to management or environment. Cystic ovaries are less common than currently believed (Weitze et al. 1998; Kauffold et al. 2004a) but certainly can be of higher or lower incidence on different farms. Ovarian cysts can be found as single or multiple cysts with oligocystic ovarian degeneration (OOD) or without normal ovarian bodies, known as polycystic ovarian degeneration (POD) as described by Kauffold et al. (2004a). For cysts to develop, a partial or complete lack of preovulatory luteinizing hormone (LH) and/or follicle LH receptors must occur. This disruption can be caused by several extrinsic factors such as stress (i.e. high ambient temperature) or incorrect therapeutic hormonal treatments (e.g. gonadotropins at times when corpora lutea [CL] are present). Mycotoxins, specifically from the family of zearalenone produced by *Fusarium* spp., have estrogen-like activity, thus acting as endocrine disruptors. Lactational estrus has also been associated with the development of ovarian cysts, presumably due to an insufficient preovulatory LH secretion.

The presence of a single ovarian cyst generally has negligible effects on reproduction. Females with conditions such as OOD may experience higher return rates and/or lower litter size. Females with POD generally exhibit clinical signs of anestrus and infertility.

Other ovarian disturbances include inactive ovaries and pseudopersistent CL (pCL), both of which are associated with anestrus and/or pseudopregnancy. Inactive ovaries are most often seen in first-parity sows after weaning, especially when sow body condition is poor, but may also be observed in pregnancy-checked negative females, usually caused by an insufficient gonadotropin secretion (Kauffold et al. 2004a). Both gilts and sows can develop pCL, usually precluded with events such as late embryonic death, endometritis, or zearalenone exposure.

Intersexuality may occur in single pigs and is likely a multigene-based outcome. Pseudohermaphrodites have gonads of one sex with the physical presence of genital organs from the opposite sex, which is a rare condition. More commonly seen are true intersex (hermaphrodite) animals that have both testicular and ovarian tissues. The external genitalia of intersex pigs are phenotypically female, albeit variable in appearance (e.g. normal vulva or prepuce-like or underdeveloped; variable enlargement of the clitoris). Some individuals show male behavior, while others may show estrus and ovulation and even become pregnant.

The diagnosis of ovarian disorders in the live animal is by using B-mode, real-time ultrasonography (RTU). From a production standpoint, postmortem examination is also an option. Currently, the best decision is still to cull animals with POD upon detection; however, a treatment with a gonadotropin-releasing hormone (GnRH) analogue twice at a 12 hour interval can be applied with reported success (Cech and Dolezel 2007). There is preliminary evidence that a multi-day regimen using altrenogest may aid in resolving POD with a complete reestablishment of fertility (Kauffold et al. 2014a).

Females with inactive ovaries can be treated with gonadotropins either in combination with 400 IU equine chorionic gonadotropin (eCG)/200 IU human chorionic gonadotropin (hCG) or, where available, with pure eCG preferably at a dosage of 1000 IU eCG. Resolution of pCL may be tried using prostaglandin F2α (PGF2α) or similar analogue, with estrus exhibited within one-week post-treatment; daily boar contact can be beneficial with the success of this treatment. Importantly, normal CL observed during diestrus will not respond to prostaglandin treatment until around day 12 of the estrous cycle, making it of very limited use in "short-cycling" sows.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Diseases of the oviducts

The diseased oviduct has received little attention in the scientific literature. From early studies, tubal disorders such as hydrosalpinx and pyosalpinx can occur in swine (Wilson et al. 1949). Salpingitis can occur in the sow and is quite often the result of an ascending bacterial infection ascending from the uterus (Kauffold et al. 2006). Bacteria that cause salpingitis have not been well defined but in general are similar to those pathogens that can cause endometritis and myometritis in the sow. *Chlamydia* spp. have been detected in the pig oviducts (Kauffold et al. 2006) that, while they may not necessarily cause morphological damage, may alter the tubal secretory profile, leading to an undesired milieu for gametes and early embryo development (Debattista et al. 2003). Zearalenone mycotoxicosis may also affect tubal function, with preliminary data indicating that it can change gene expression of tubal epithelial cells (Kauffold and Wehrend 2014b).

Cysts of the mesosalpinx (i.e. paraovarian cysts) can be a frequent finding at necropsy. These cysts are residual to remnants of the Wolffian ducts and are considered negligible in causing disturbances to reproduction.

The clinical relevance of tubal diseases can be quite straightforward. If bilateral tubal occlusions are present, females will be unable to become pregnant, leading to regular returns and, thus, infertility. A definitive diagnosis of a tubal disease can only be done through a postmortem examination. Although appropriate antimicrobial treatment may be of value when treating a salpingitis, treatment for tubal occlusions carries a poor prognosis. Antimicrobials may help clear an infection caused by susceptible bacteria, but occlusions cannot be resolved.

Non-puerperal diseases of the vagina, cervix, and the uterus

Diseases specific to the vagina and the cervix occurring outside of the window of parturition are sparse. A study from Finland reported cervicitis/vaginitis in 19/824 (2.3%) of discharging sows (Oravainen et al. 2006). The most common non-puerperal genital disease in the pig is endometritis and usually is the result of an infection with gram-negative (e.g. *Escherichia coli* spp., *Klebsiella* spp., *Proteus* spp.) or gram-positive bacteria (*Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Trueperella pyogenes*). Other bacteria, such as *Chlamydia* spp., anaerobic microbes (i.e. *Fusobacterium necrophorum, Prevotella* spp.), and fungi (i.e. *Candida* spp.) may be involved (Oravainen et al. 2006). Viral pathogens typically do not cause uterine inflammation, although their presence in the endometrium has been documented (Olanratmanee et al. 2011). Bacteria usually enter the uterus at breeding or in the periparturient period via the lower portion of the genital tract. Ascending infections from the urinary bladder are also possible. A systemic infection through the bloodstream may occur for some bacteria but is considered the main route of infection for viruses. In the majority of cases, infections are cleared by the local immune system, but in some circumstances when the immune system is compromised, such as during deoxynivalenol exposure or hormonal downregulation (e.g. diestrus when progesterone is at high concentrations or exogenously with altrenogest administration to animals already subclinically infected), an ensuing inflammation may develop.

Non-puerperal endometritis, not associated with the aforementioned, can occur with varying severity and can be an acute, subacute, or chronic situation. Acute and subacute conditions are commonly associated with the presence of a vulvar discharge. Chronic endometritis, however, can be more clinically evasive, with regular returns most often being the only clinical sign (Dalin et al. 1997, 2004; Kauffold et al. 2005). Besides infectious agents, zearalenone exposure can also elicit uterine disease that presents (but not always) as an endometrial and gross edema.

Other diseases of the uterus such as atrophy, degeneration, or adenomyosis can occur, but their incidence in swine is unknown. Uterine neoplasms are possible and may include leiomyoma, fibroma, cystadenoma, fibroleiomyoma, and carcinoma (Akkermans and van Beusekom 1984), but these findings tend to be geriatric conditions reported in older potbelly or miniature pigs (Golbar et al. 2010; Höltig et al. 2015). Congenital disorders of the uterus, vagina, and cervix (i.e. duplex uteri, segmental or complete aplasia) may also occur.

The clinical relevance of uterine diseases will be infertility in a clear majority of cases, but the clinical presentation can vary. For instance, cases of endometritis may or may not be associated with vulvar discharge or with returns and largely depend on the severity of inflammation, including the innate ability of the endometrial tissue to produce sufficient PGF2α.

Uterine diseases, particularly inflammation, typically present for diagnosis based on clinical signs such as pathological vulvar discharge. A vaginal examination is performed to determine the origin of discharge. In cases of reproductive irregularities in the absence of vulvar discharge, return to estrus can be of value in a diagnostic workup, for example, for anestrus associated with zearalenone exposure. In other still inconclusive cases, a postmortem examination will be necessary. If findings suggest a bacterial component to uterine disease, then identification and antimicrobial susceptibility testing is useful if deemed a herd problem.

A treatment decision clearly depends on the type of disease present. For example, animals with congenital

disorders should be culled immediately as well as those animals with a proven, non-recoverable zearalenone exposure. In contrast, a non-puerperal genital endometritis can be treated with antimicrobials systemically and/or locally; however, an intrauterine infusion using a post cervical artificial insemination (PCAI) catheter may be difficult to perform due to the usually tight cervical closure. Drugs that may help to evacuate uterine contents (e.g. oxytocin or PGF2α) can be ineffective due to a presumably limited responsiveness of the myometrium at the time of treatment.

Reproductive problems on a herd level

General diagnostic approach

The diagnostic procedure addressing herd fertility problems is comprehensive and must include a critical assessment of the tripartite contributors to herd reproductive performance: (1) farrowing personnel, (2) breeding personnel, and (3) sow and semen management. Diagnosticians must be aware of the multitude of factors and their interrelatedness that might be causative of the problem, including facility type, the building environment, seasonality, nutrition, sanitation/hygiene, and disease-related occurrences or interventions. While different types of reproductive failure would need different differential diagnoses, there can certainly be overlap. For instance, if conception rates are low, breeding may have been performed at improper times or poor quality semen has been used. Similar factors can also be associated with cases of low farrowing rate, with added differentials driven by clinical signs such as abortion or sows confirmed pregnant but fail to farrow (irregular returns to estrus). Critical evaluation of herd performance data, including flow diagrams and in-depth anamnestic investigations, is part of any diagnostic approach and has priority ahead of other diagnostic procedures (Almond et al. 2006; Carr 2008).

Numerous software programs are currently available for assessing herd performance data. While all provide summaries of most relevant production data related to breeding, farrowing, and lactation, they may be different in terms of data entry, report format, and content, as well as the accessibility to specific data that could be of value in some troubleshooting processes. Current software development is being driven to capture real-time production- and veterinary-related data in order to quickly recognize performance disruptions that then allow for immediate investigation or intervention. A proper analysis of the recorded data relies on correct and comprehensive data entry, but the level of detail desired may not be available. For instance, when investigating sow removals or late pregnancy losses ("fallouts"), it is critical to have not only the day of fallout available but also the correct cause inputted (i.e. death, cull, abortion, etc.).

After records analysis, a farm walk-through and clinical examination of the animals and their surroundings are performed. The execution of the actual examinations required is greatly influenced by the problem presented. For example, pregnancy losses in late gestation require examining the late gestating sows, whereas in losses from poor conception rate, the assessment of both semen quality and ovulation patterns is a logical starting point given how crucial both are in terms of proper mating times and conceiving. If still undiagnosed, attention should next focus on diagnostic assessment of the reproductive tract in females exhibiting inciting clinical signs. This can be performed using RTU or postmortem examination and sample collection. As uterine infections are often related to immunosuppression along with sufficient bacterial pressure, diagnostic procedures may also include investigations for causes of immunosuppression (i.e. mycotoxins, systemic pathogens such as porcine reproductive and respiratory syndrome virus [PRRSV], porcine circovirus type 2 [PCV2], porcine parvovirus [PPV], and bacteria).

Individual pig examination

Examination of representative individual female pigs exhibiting reproductive disorders is often warranted. The examination should start with assessment of estrus activity, body condition, and an overall physical examination with focus on genital and mammary gland health. It is important to use a boar for estrus determination, but single animals may not immediately respond to the presence of a boar, but rather may need additional duration and frequency of boar exposure to elicit a sufficient stimulation. A vaginal inspection may follow using a metal, plastic, or disposable cardboard speculum (Oravainen et al. 2008), which can be particularly useful in cases where discharges of the urinary tract have to be differentiated from uterine discharges. Although uncommonly performed today given the wide availability of RTU, a rectal examination may be of diagnostic value for determining pregnancy and ovarian functional status (Meredith 1977). From a go-to diagnostic tool, RTU generates the most conclusive results in the live animal. The examination can be performed transrectally and transcutaneously. Manual transrectal RTU is more applicable when examining sows, but not in gilts due to the narrow pelvis. When performing transrectal RTU, probe guidance is facilitated using a transducer stabilizing rod. Transcutaneous RTU is approached via the inguinal area dorsally to the last pair of teats. Linear, sector, and convex probes are all feasible, with sector and convex being the preferred probes. Frequencies of 3.5–7.5 MHz are most commonly used; however, a 5.0 MHz transducer provides the most versatility when it comes to penetration depth and image resolution.

RTU can be used to assess for both physiological and pathological conditions. Tissues frequently examined include gravid and nongravid uteri, the ovaries, and the urinary bladder (Table 20.1). Additionally, RTU is utilized to measure backfat thickness as a more objective measure of body condition and nutrition impact in females suffering fertility issues (Quesnel 2009).

Pregnancy can be diagnosed with most commercially available ultrasound units starting on day 20/21 postbreeding and, when using superior quality units, as early as day 16 by a skilled practitioner. Accuracy of diagnosis increases as pregnancy progresses due to conceptus growth. Pregnancy failures to be detected by RTU include embryonic/fetal deaths, resorptions, and mummified fetuses. Fetal viability testing can also be performed by visually assessing the heart beating in the developing conceptus as early as day 24 of pregnancy.

Parameters currently used by RTU to define uterine functionality are fluid echogenicity, echotexture, and size. For example, a flocculent fluid echogenicity or extremely heterogeneous echotexture is indicative of a severe acute or subacute endometritis. Similarly, an extremely heterogeneous echotexture may also be expected after zearalenone mycotoxicosis. Interpretation of uterine echotexture should be performed in concert with ovarian findings as the uterine architecture physiologically depends on endogenous steroids and thus is related to the ovarian status. For instance, if the uterine echotexture is heterogeneous at a time when it should be homogeneous (i.e. *in situ*ations where CL or small follicles are present and endogenous estrogens are expected to be low), the uterus is likely to be pathologic and, thus, fertility decreased (Kauffold and Althouse 2007). The determination of the uterine size provides valuable information for the assessment of puberty in gilts, with prepubertal gilts having a smaller uterus than pubertal gilts (cross-sectional areas of the uterine horns of $\leq 1.0\,cm^2$ and $\geq 1.2\,cm^2$ in prepubertal versus pubertal gilts) (Kauffold et al. 2004b).

All typical ovarian structures are readily imageable, including follicles of different sizes, corpora hemorrhagica (CH), and CL (Figure 20.1). Also, pathological conditions such as ovarian cysts are clearly visible, so are cases of intersexuality (i.e. ovotestes).

A complete RTU examination includes the assessment of the urinary bladder for uniformity, thickness, and the presence of bladder sediment. Presence of bladder sediment in sows and gilts can be a prognostic indicator of animals predisposed for urogenital infections (Kauffold et al. 2010).

If bacterial infections are suspected, a specimen obtained directly from the site of interest should be collected using a disposable double-guarded swab (commonly used in mares) to avoid inadvertent bacterial contamination.

Table 20.1 Applicability of ultrasonography in female swine reproduction and for troubleshooting of "on-farm" fertility problems.

Applicability in female swine reproduction[a]	Applicability for troubleshooting of "on-farm" fertility problems[a]
Diagnosis of pregnancy and pregnancy failure	Low conception rate
Assessment of health of the nonpregnant uterus	Low farrowing rate
Monitoring of ovulation and ovulation failure	Late fallouts
Assessment of puberty and failure to attain puberty	High rate of returns
Assessment of follicle growth and failure to grow	Delayed puberty/high cull rate of gilts for "no heat"
(Measurement of backfat depth)	Vulval discharges
(Evaluation of health of the urinary bladder)	Reduced litter size
	Long wean–estrus interval
	(Body condition monitoring)

[a] Non-fertility-associated applications are in parentheses.

Problems associated with puberty attainment

In general, attainment of puberty in gilts is expected to occur around 180 days of age. If ≥5% of the gilts in a group show delay or failure to cycle, an investigation is warranted. Typically, investigations reveal these scenarios as multifactorial, frequently including numerous intrinsic as well as extrinsic factors.

Gilt birth weights may have an effect on puberty. Gilts weighing 0.9–1.3 kg at birth had delayed puberty compared with gilts with a birth weight of approximately 1.6 kg. However, in another study heavier gilts at birth (i.e. ≥1.8 kg) were less often found to have been pubertal at 200 days of age via RTU when compared with lower birth weight gilts (Kauffold et al. 2010), presumably due to relatively lower body fat. While there might be breed-specific recommendations for a desired backfat thickness at breeding, it should at minimum be 14 mm across breeds. To achieve this target, proper feeding strategies will facilitate more fat deposition and less muscle while achieving optimum daily gain. For instance, gilts with a growth rate of 724 versus 577 g/day between birth and 144 days of age reached puberty earlier and to a higher percentage by day 190 and were culled less often for anestrus (Kummer et al. 2009). Seasonality may also have an effect on puberty attainment. During long-day periods such as during summer and early autumn in the Northern Hemisphere, puberty is typically delayed (Peltoniemi and Virolainen 2006). In addition, high ambient temperatures during that time will lower voluntary feed intake that may additionally contribute to this delay. Field evidence suggests that diseases and toxin

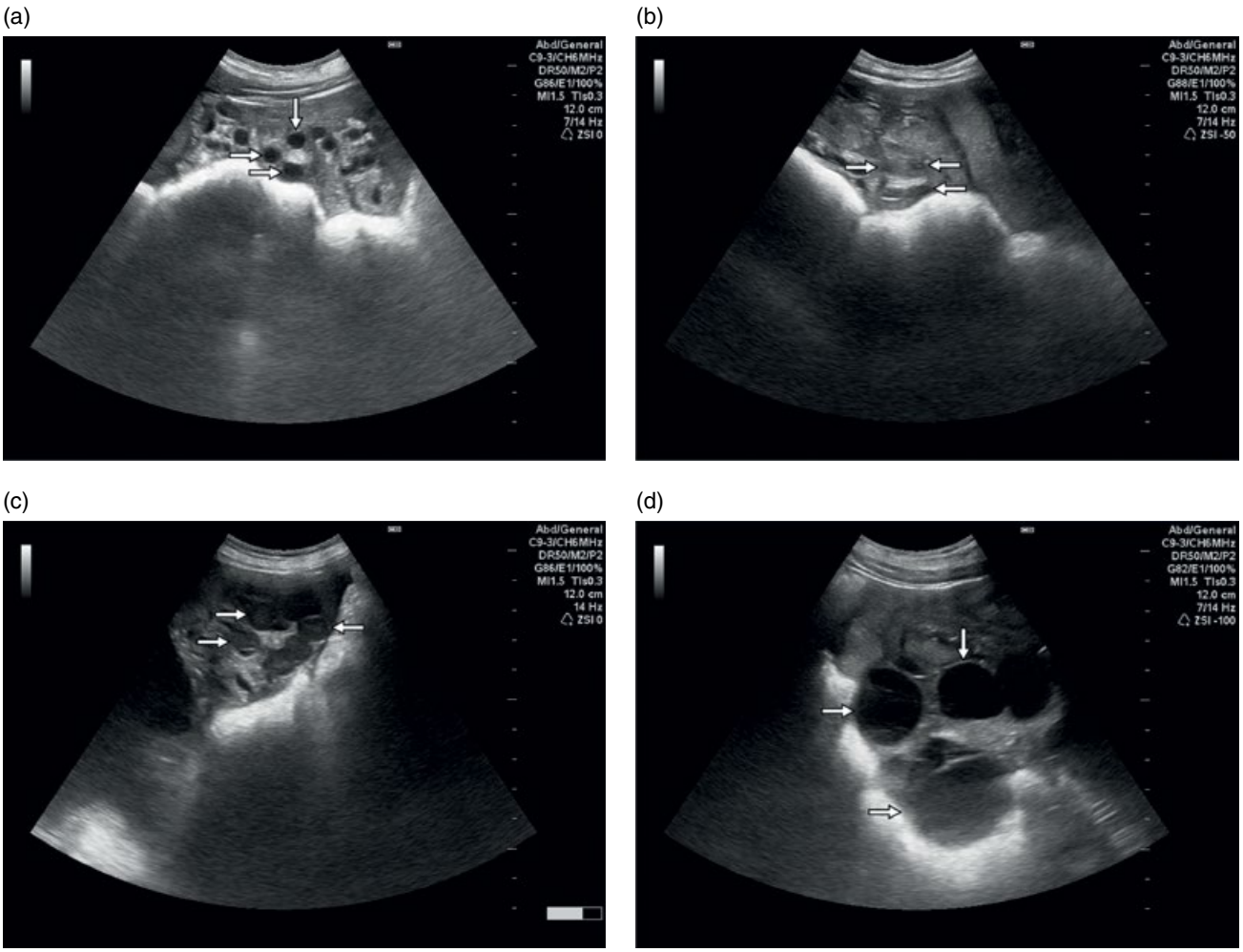


Figure 20.1 Ultrasound images of ovaries from gilts and sows with medium-sized follicles (a), corpora hemorrhagica (b), corpora lutea (c), and follicle cysts (polycystic ovarian degeneration) (d). Example ovarian bodies are marked with arrows, respectively. Scanning was performed with a Fujifilm Fazone CB and a convex probe (FZT C9-3) at a background frequency of 6 MHz and a penetration depth of 12 cm.

exposure (such as with zearalenone) during any time of gilt development have a detrimental effect on puberty.

If done properly, boar exposure is an effective means of inducing early puberty in gilts. Adequate stimulation of estrus generally requires direct physical contact, while estrus detection may only need fence-line contact. If the efficacy of boar exposure appears inadequate, it is important to evaluate whether the rules of boar contact are being followed (Kirkwood 1999). The rules are related to age of gilts and boars as well as the contact and environment provided for gilts and boars.

Gilts must be at least 160 days of age with maximal response when contact starts at 180 days (see Figure 20.2). There is evidence that gilts exhibiting a more rapid response to boar exposure are innately more fertile. The corollary is that gilts not pubertal within 28 days of boar contact are likely less fertile and may be culled. However, any culling policy has to be carefully leveraged, with cost

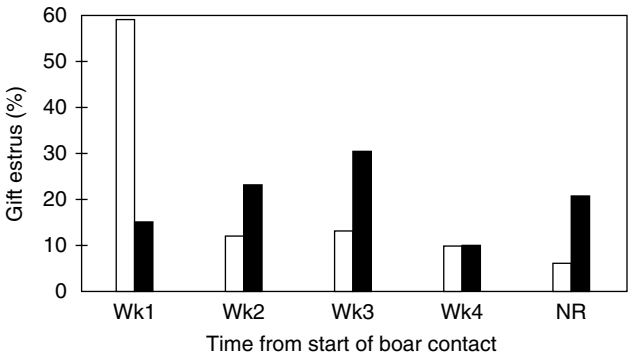


Figure 20.2 Influence of starting boar contact at a gilt age of 180 days (open bars) or 160 days (black bars) on pubertal responses. Increasing the age of gilts at first boar contact improves the timing and synchrony of the pubertal response but does not affect potential litter size. *Source:* Adapted from Van Wettere et al. (2006). Reproduced with permission of Elsevier.

for new purchases as well as gilt acclimation/vaccination put against losses through a potentially reduced productivity and longevity being part of the equation.

Boars must be mature enough (≥10 months of age) in order to adequately stimulate the female. The stimulus value of a boar depends on his ability to produce sufficient stimulatory pheromones from his submaxillary salivary gland that achieves adult proportions by 10 months of age. While this physiology may argue that it not be necessary to use the oldest and smelliest boar, there is evidence to suggest that boars may be different in their olfactory-stimulating ability. Minipig boars, for instance, seem to stimulate more effectively than commonly used production boars, presumably because of much higher concentrations of testosterone and pheromones in their saliva (Dehnhard et al. 2013).

Gilts should be in physical contact with the boar for at least 15–20 minutes per day to allow enough time for all gilts to interact with the boar. If gilts are housed in large groups, use at least 1 boar per 12 gilts. Since a fertile mating at puberty is likely not desired, boar contact should be supervised, or sterile boars (vasectomized or epididymectomized) should be used and mating allowed (Althouse and Evans 1997a, b). Indeed, a sterile breeding at puberty may enhance fertility to a fertile breeding at the subsequent estrus, with a 5–10% increase in farrowing rate and a 0.5–1.5 increase in litter size.

For boar exposure, gilts should be housed at least one meter away from potential stimulus boars and preferably downwind. Gilts housed adjacent to boars are stimulated to an earlier puberty. Understand though that if further separation postpuberty is not implemented, low estrus detection rates can occur as gilts will have stood previously and are, thus, refractory at the time of supervised boar contact.

Gilts should be taken to the boar and not vice versa. If this is not manageable, than fence-line stimulation can be a practical alternative. In the event of poor estrus detection management, the use of a separate detection-mating area should be considered.

Other variations, such as using different boars on different days or housing gilts with a boar continuously, will not usually improve the response over that obtained by daily contact with a single mature boar. However, if the pubertal response is considered inadequate, consider twice-daily boar exposure and/or rotating stimulus boars every 2–3 days. This is because some boars have a low stimulus value that may be compensated by rotating boars. After puberty, boar exposure should continue at least every other day in order to promote a regular estrous cycle.

If appropriate boar exposure does not appear to be effective, hormonal treatment of either 750–1000 IU of eCG or a combination of 400 IU eCG/200 IU hCG may be considered. In some studies, the eCG/hCG combination has proven more effective at inducing estrus in gilts than has eCG alone (Manjarin et al. 2009), suggesting that LH-like activity may be required for more robust follicle development. If a group of gilts is administered the 400 IU eCG/200 IU hCG combination and the response is poor (e.g. 10–15% exhibit estrus), it is likely that the gilts were already cyclic and their prior estrus was missed. If after treatment up to 30% do not exhibit behavioral estrus, and of those about 30% fail to recycle, then gilts may have been treated while immature. Although gilt fertility will improve if bred at a subsequent natural estrus, it is generally recommended to breed gilts at the induced estrus because of the relative unpredictability of cyclic estrous behavior after exogenous gonadotropin use. However, if experience on an individual farm indicates that gilts have a high regular return rate (e.g. >90%), then delaying breeding to the following natural estrus is the preferred option.

If puberty is delayed, a diagnostic workup may start with scanning gilts using RTU in order to determine their pubertal status starting at around 180 days of age and continuing at 20-day intervals for at least 2 months. Older gilts with reported "no heat" can be imaged using RTU. This approach may allow in-depth understanding of the magnitude of the problems and to aid in ruling in/out poor estrus detection management. It can also be of value in determining the effectiveness of different stimulation interventions.

Within a diagnostic workup, the entire gilt's life between birth and puberty has to be evaluated for factors that may have had a negative effect on puberty. This includes diseases as well as toxins, but most importantly nutrition. Gilts of known age should be weighed periodically, and backfat thicknesses measured to ensure that daily gain and body composition are optimum at the time of desired puberty age.

Problems associated with wean–estrus interval (WEI)

Ideally, weaned sows are expected to exhibit estrus any time before 5 days post weaning. If no estrus is observed, then one needs to consider whether they are a "missed heat" or "truly not in heat" sows. Estrus may not be detected in sows exhibiting a silent heat or if heat detection was done incorrectly, hence not observed. Errors in heat detection include use of heavy boars (especially for gilts that could be intimidated), reduced boar libido, incorrect placement of the boar, rushing to perform heat checking, and/or incorrect testing by personnel. Sows found "truly not in heat" hence with no estrus activity can be caused by absence of ovarian inactivity or having previously had a lactational estrus. Ovarian inactivity may be the result of insufficient gonadotropins during late lactation and around weaning. This has been linked to an

excessive body mass loss during lactation due to a combination of insufficient feed intake and excessive milk production and is mediated by the insulin/IGF system (Quesnel 2009). Other factors for ovarian inactivity may include parity (as it is observed predominantly in first-parity sows), seasonality/high ambient temperature (as it is observed more often during the summer period), and insufficient boar stimulation post weaning. In contrast, for a lactational estrus to occur, gonadotropins must have been sufficiently available while lactating for follicles to grow and ovulate. In theory, gonadotropin secretion and follicle growth in lactation are inhibited through mechanisms induced by piglet suckling (Quesnel 2009). At the end of lactation and post weaning, when the frequency of suckling decreases and suckling finally ceases, gonadotropin secretion reemerges, leading to follicle growth and ovulation (Quesnel 2009). In fact, lactational estrus is most often seen in late lactation at the end of the third and into the fourth week. Moreover, in nurse sows or sows submitted to intermittent suckling (e.g. suckling continuity is interrupted), lactational estrus is more often seen than in continuously lactating sows (Soede et al. 2012). Also, management factors that unintentionally lead to temporary cessation of suckling, such as piglet vaccination in late lactation, are also suspected to facilitate lactational estrus. Observations from the field suggest that lactational estrus increasingly occurs in the Northern Hemisphere in late autumn and lasts until early spring (i.e. during times when the ambient temperature is lower and feed intake higher than during the hot summer season).

RTU of the ovaries of sows not exhibiting estrus by day 7 post weaning can help to accurately direct diagnostic efforts and treatment strategies. For instance, in cases of missed heats, all procedures surrounding heat detection should be carefully evaluated, while in cases of ovarian inactivity, major diagnostic emphasis needs to be placed on nutrition in lactation.

Treatment options depend upon the outcomes of RTU imaging. Sows with observed inactive ovaries may be treated with gonadotropins. If CL are observed, an initial treatment with PGF2α can be used. If no response within 7 days is observed upon this initial PGF2α treatment, the CL may have been early in diestrus when treated. A second PGF2α treatment approximately 7 days later is therefore recommended. If RTU imaging is unavailable, a random treatment approach may consist of a PGF2α injection followed by a gonadotropin application 24 hours later, with the intention of consecutively addressing both ovarian conditions (i.e. presence of CL and inactive ovaries). This option is not without consequences; however, the risk of inducing cysts in sows may occur if gonadotropin treatment is performed while normal cyclic CL are present. Preventative measures to consider in these cases are supplementation of carbohydrates (e.g. dextrose or sugar) pre- and post weaning in order to stimulate follicle growth (Chen et al. 2016). If lactational estrus is observed, sows can be bred. Data indicates that fertility results from lactational estrus breedings are similar to those in normally weaned/bred sows (Soede et al. 2012). Alternatively, sows may be bred upon their next estrus, but the number of sows available per week and the expected dates of their estrus are unpredictable.

Problems associated with conception and farrowing rates

In modern production systems, conception (pregnancy) and farrowing rates should be at a minimum of 90 and 88% rate, respectively. Conception failures and loss of pregnancy in either early or late in gestation can be caused through both infectious and noninfectious mechanisms, the latter of which usually involves management issues related to the female as well as the male and semen qualities. For an animal to conceive and maintain pregnancy beyond day 25, there must be a minimum number of conceptuses, starting with matured oocytes that have ovulated and were fertilized by capacitated sperm. Embryos enter the uterus approximately 48 hours after ovulation. Intrauterine migration of the embryos is essential to maternal recognition of pregnancy. For this process, a minimum number of embryos must be present in the uterus to adequately migrate throughout the tract while releasing sufficient amounts of estrogens (E2), which is the embryonic signal in the process of pregnancy recognition. E2 is produced biphasically in two signals with the first appearing at around day 10 and the second at days 14–18 of pregnancy (Geisert et al. 1990). Both signals are required for pregnancy to be maintained. If there is conception failure, early embryonic death, or less than 4 viable embryos present before the first signal at day 10, animals will exhibit a regular return (e.g. 17–24 post breeding). If significant embryonic deaths occur between the first and second signals, the animal will be classified as an irregular return to estrus after post breeding day 24. Conceptus deaths after the second signal but before day 35 of pregnancy may result in late returns. Most but not all conception failures associated with a regular return to estrus are usually due to noninfectious reasons, while later returns (>35 days' gestation) are more likely to be related to infectious insults. If there is an intrinsic or extrinsic pathophysiological disruption in the reproductive hormonal cascade, pseudopregnancy may result. For instance, short- or long-term exogenous treatment with estradiol starting at day 11 of the estrous cycle can induce pseudopregnancy for up to several weeks. Also, zearalenone is capable of not only interfering with early embryonic development but also inducing pseudopregnancy (Tiemann and Dänicke 2007) when provided in biologically relevant mycotoxicotic doses.

Ovulation failure (no or delayed ovulation) will curtail conception efforts with the animal showing a regular return to estrus. Appropriate insemination timing is also critical to achieving acceptable conception rates. It is not uncommon to find farms implementing AI programs where insemination timing with respect to ovulation is inappropriate. Usually, inseminations are being performed post ovulatory, when oocytes are aged and have reduced viability; oocyte viability only lasts for approximately 8 hours after ovulation. The goal of an AI program is that insemination occurs within 24 hours (preferably within 12 hours) prior to ovulation (Waberski et al. 1994; Nissen et al. 1997). Within an existing breeding program, it is hard to anticipate a sudden change in the herd's ovulation patterns, which is typically caused by alterations to pre and post weaning follicle growth. Typical situations that cause this are reduced feed intake during lactation (possibly confounded with hotter times of the year), breed/genetic line changes, and changes to existing hormone programs and products. Lastly, insemination with poorly handled, aged, or poor quality semen can interfere with conception rates.

Uterine disease and tubal disorders may contribute to conception failure; however, their incidence is unknown. Other aspects to consider when diagnosing low conception rates include seasonality (due to increased melatonin and its suppressive effect on GnRH/LH), high ambient temperature (reduced voluntary feed intake), or moving sows from breeding into pen gestation at critical times, especially during the phase of maternal recognition of pregnancy that occurs between 9 and 18 days of gestation.

If pregnant, anything that can disrupt pregnancy will cause reduced farrowing rates. The most likely cause of pregnancy loss is infectious insults (see abortion). Clinical signs associated with reduced farrowing rates due to pregnancy loss are abortions, the passage of stillbirths, or mummified fetuses as well as pseudopregnancy and fetal resorptions.

Incorrect pregnancy diagnosis by personnel when performing heat detection can lead to low conception and farrowing. False positives for pregnancy can occur with respect to POD, inactive ovaries, or pseudopregnancy. A false-negative pregnancy diagnosis is possible when truly pregnant animals display estrus-like behavior at the time of regular estrus return. If pregnancy diagnosis is being performed with Doppler-mode ultrasound, false-positive results may occur in sows tested during proestrus/estrus or when animals have a significant endometritis. False-negative diagnosis using Doppler may occur when examinations are conducted in a noisy environment or if feces become packed around the probe. Amplitude-depth instruments (A-mode or pulse echo) may yield false-positive results in cases of a fluid-filled urinary bladder, pyometra, hydrometra, or cystic ovaries. False-negative results can occur if animals are examined before 28 days of gestation or after day 80. Although B-mode ultrasound (RTU) is very reliable for pregnancy testing, false-positive and false-negative diagnoses have been occasionally noticed, with causes associated with technician skill, day of gestation, equipment, and probe type (e.g. frequency) being the main influencing factors.

When initiating a herd reproductive investigation, a first step is to assess production records, paying particular attention to the ratio of regular versus irregular returns (suggested target 4 : 1) to obtain initial insight as to whether the causes are likely infectious or noninfectious. On-site, ovarian activity should be assessed using RTU. Multiple scanning sessions at intervals of approximately 24 hours are usually necessary for an accurate assessment of ovulation patterns in sows. These results can be used in conjunction with assessing the farm's estrus detection programs. Appropriateness of semen handling, semen quality, insemination technique, and timing should be assessed.

Very early pregnancy using RTU can be performed as early as day 16 when using a higher-quality diagnostic ultrasound unit. Such early assessment may be helpful in objectively diagnosing early embryonic deaths. Open animals should be scanned for pathological conditions (i.e. cystic ovaries or endometritis) and, along with suspected animals typical of the clinical signs being observed, should be considered for further postmortem examination. If there is indication for infectious disease or mycotoxicosis, respective analyses should be performed. Treatment or management decisions clearly depend on the nature of the problem. Those issues related to semen, insemination procedures (including breeding times), and pregnancy testing are relatively easy to correct. If ovulation patterns are wide and, thus, difficult to capture in a breeding protocol, exogenous hormonal use should be strongly considered in order to simplify an insemination protocol that will provide timely deposition of semen in concert with ovulation. Successful programs have included single or double fixed-time insemination programs with or without the use of follicle-stimulating substances (i.e. eCG, combinations of eCG/hCG, select GnRH analogues) given peri-weaning, followed by a timed ovulation-inducing hormone (i.e. GnRH analogues, pLH, hCG) given afterward (Brüssow et al. 2001; Kirkwood and Kauffold 2015).

Problems associated with vulvar discharge

Vulvar discharges are typically observed for up to 48 hours post farrowing as the sow attempts to expel placental remnants and debris from the uterus. A scant mucopurulent vulvar discharge can be observed in pregnant sows during the last 2–3 weeks of gestation. Slight amounts of a usually nontransparent peri-estrous

discharge are also considered normal. Milky to creamy discharges can occasionally be seen immediately post-insemination in up to 50% of inseminated animals on a farm. These discharges can be considered a normal mechanism of evacuating fluids post-insemination. Using ultrasonography, the uteri in these discharging sows appear perfectly normal. These discharges usually disappear shortly after occurrence and do not affect an animal's fertility. Along with evacuating the uterus, other possible inciting factors for discharge may be as a response to certain components in the extended semen and/or the insemination equipment.

After ruling out the aforementioned, all other vulvar discharges are classified as abnormal. A fresh, bloody discharge is usually indicative of vulvar lacerations from biting by sows (particularly in pen housing), trauma, natural mating, or improper AI procedures.

Purulent vulvar discharges that occur several days post breeding are indicative of a pathological condition. It is not uncommon to see some abnormal discharging on a farm. If, however, discharging rates are present in ≥3% of the breeding group, an investigation is warranted. Discharges may originate from the urinary tract in the case of cystitis and pyelonephritis or, more commonly, from the genital tract due to an endometritis or myometritis. Discharges from the urinary tract are usually viscous, contain mucus and occasionally blood, and are associated with urination, especially at the end of the stream. Discharges from an inflamed uterus are usually of high volume, are milky or creamy, and usually are observed as a passive discharge in recumbent animals. These type discharges are usually associated with a bacterial infection that could have been introduced via improper breeding hygiene and technique. Other predisposing factors that could contribute to bacterial infections are building cleanliness, solid versus partly slatted concrete floors, excessive drafts, high ambient temperatures, overcrowding, or any factor that could lead to an immunosuppressed animal. Sows with a previous puerperal endometritis may be at higher risk of discharging at their next post breeding, usually due to a subclinical situation that is then exacerbated by the breeding process.

Although the characteristics of the discharge itself may be indicative toward a urinary versus a genital tract infection, a vaginal examination and urine analysis should be performed in order to confirm the diagnosis. Discharges from the uterus can be clearly diagnosed by RTU based on observation of flocculent uterine echogenicity. If inappropriate insemination timing (i.e. post ovulatory inseminations) is a differential diagnosis, ovulation should be monitored by RTU in animals that were recently identified in estrus and bred. Farm hygiene and sanitation during the breeding process should be evaluated as well. If puerperal discharge is a problem, procedures around farrowing should be critically addressed (e.g. hygiene, vaginal examination, treatments, etc.).

If untreated animals with abnormal discharge express estrus, it is best to not breed the animal, but to wait until their next estrus. As far as treatment options, both medicated feed and injectable antibiotics are commonly used. To obtain the best effect with antimicrobials, accurate identification of pathogens involved and their antimicrobial sensitivity patterns should be determined prior to selecting the product and treatment regime. When treating a single animal with discharge, provide therapeutic levels of antimicrobials for at least 3 continuous days. If discharges are a herd problem, a common protocol is to prophylactically treat all animals at risk at the most likely time that bacterial entry occurs, i.e. around breeding, for an interval of at least 5 continuous days. This preventative therapeutic approach should be considered short term until identification and resolution of the underlying causes can be performed.

Problems associated with parturition

A solid knowledge of the normal process of parturition is essential to determine when to intervene. Swelling of the vulva occurs within 4 days prepartum. The mammary glands become more turbid and tense during the last 2 days before farrowing, with mammary secretions being serous approximately 48 hours prior to parturition and then becoming milky within 24 hours of farrowing. Restlessness and nesting behavior usually start 24 hours pre-farrowing, with the sow becoming less active in the last hour before the first piglet. Intermittent abdominal straining occurs before the birth of the first piglet, but straining is usually mild thereafter except at the moment of fetal expulsion. A prelude to farrowing is the presence of a viscous, blood-tinged secretion from the vulva that appears within 30 minutes of the first piglet. Once farrowing commences, the average interval between the births of piglets is 15–20 minutes. The entire parturition process is usually less than 3 hours. Fetal membranes are typically expulsed from 20 minutes to 12 hours after birth of the last piglet. Piglets can be presented cranially or caudally.

Signs of dystocia in a sow can include an abnormally prolonged gestation, blood-tinged vulvar discharges, meconium without straining, straining without delivery of piglets, cessation of labor after straining, cessation of labor after delivery of one or more piglets, exhaustion of the sow, and foul-smelling and discolored vulvar discharge. Primary uterine inertia associated with a decreased contractile activity of the myometrium is probably not as common as secondary uterine inertia resulting from uterine and maternal exhaustion associated with fetal malposition, fetopelvic disproportion, or obstruction.

From an obstetrical point of view, the causes of dystocia are classified into two categories, fetal and maternal, based upon the origin. Fetal causes include fetal malpositions such as a breech, simultaneous presentation of two fetuses into the birth canal, and fetal congenital malformations. Maternal causes include uterine inertia, deviation of the uterus, fetopelvic disproportion, and maternal excitement. Dystocia may also occur as a result of the misuse of exogenous prostaglandin and oxytocin use to induce and/or control farrowing.

Intervention for dystocia must be in a timely fashion. In a successful farrowing program, sows are optimally assessed at 30 minute intervals to be aware of the sow's interval between the births of piglets once parturition has commenced. If over 1 hour has lapsed since the last piglet has farrowed (and no placenta has been passed), then a clean vaginal examination should be performed. Correction of dystocia due to obstructions or malpositioned fetuses is achieved by manual vaginal examination and requires strict hygiene, obstetrical gloves, and lubricant. Manual extraction is usually the safest technique to extract fetuses. Caution must be exercised when using forceps, blunt hooks, or cable snares due to the risk of trauma to the fetus and the sow's reproductive tract. After removal of the malpositioned piglet, the birth canal should be digitally reexamined prior to the administration of oxytocin. High doses (>20 IU) of oxytocin may create a refractory period (3 hours) in which endogenous and exogenous oxytocin fails to stimulate contractions. In some cases where oxytocin does not stimulate contractions, calcium preparations may be used successfully prior to oxytocin treatment. In other cases, animals may continue farrowing after being treated with a combination of an analgesic and spasmolytic drug. Farrowing should be completed as soon as possible, as a prolonged farrowing can have negative effects on colostrum yield, involution of the uterus, and subsequent fertility and predisposes the animal for the development of postpartum dysgalactia syndrome (Peltoniemi et al. 2016).

Injectable antibiotics are warranted if sufficient contamination of the uterus occurs during farrowing management. Intrauterine infusions of antibiotics or iodine solutions are usually not effective in promoting uterine involution or preventing uterine infections in swine.

Hemorrhage may occur postpartum as a result of uterine, vaginal, or vulvar lacerations. Lacerations of the vagina and vulva can be sutured externally, whereas severe uterine lacerations or uterine ruptures are difficult to repair without conducting a laparotomy. Oxytocin treatment promotes uterine contractions and may be beneficial with minor uterine lacerations. Hematomas of the vulva are resolved as the blood and fluid are resorbed; however, sharp projections in farrowing crates increase the danger of lacerating the hematomas.

Vaginal and uterine prolapses are often seen shortly before, during, or up to several days after farrowing. Factors that have been suspected to increase the risk of vaginal or uterine prolapse in sows are genetics, housing, physical trauma to the genital tract following parturition, older parity, nutrition, and exposure to estrogenic mycotoxins, but neither etiology nor pathogenesis is documented. Vaginal prolapses can be surgically repaired using a purse-string suture with good success. Uterine prolapse repair typically has a very poor prognosis.

Reproductive problems in the boar

Reproductive disease in a boar can have a direct effect on the animal's well-being, breeding behavior, semen quality, and subsequent herd fecundity. The broad use of AI in the global swine industry today further facilitates a boar's impact on herd health through the dissemination of infectious pathogens through extended semen into the sow herd. As with other body systems, disease can be noninfectious or infectious in origin.

In today's commercial herds a large percentage of reproductive problems appear to be secondary to issues related to improper management, irrespective of whether boars are housed with the sow herd or in dedicated boar studs (Althouse 2014). Management-related diseases in the boar negatively impact their semen quality, whose breeding outcomes may be high return rates, low farrowing rates, decreases in average total born per litter, and/or increases in litter scatter.

Heat stress or pyrexia

One of the more common insults associated with reproductive failure in confined boars is exposure to high ambient or "heat stress" temperatures. Under heat stress-induced conditions, normal thermoregulatory mechanisms for the boar's testis are compromised, leading to increased testis temperatures and subsequent disruptions in spermatogenesis (McNitt and First 1970; Wettemann et al. 1976; Althouse 1992). Considerable variation also appears to exist between boars in their individual susceptibility and response to heat stress. Variables such as the duration and intensity of the elevated temperatures, relative humidity (RH), and adequacy of housing ventilation can further complicate the issue. In general, the risk of heat stress in boars is present when ambient temperatures are at or above 27 °C with ≥50% RH (Figure 20.3). The detrimental effects of heat stress on the spermiogram (i.e. reduced sperm viability, low sperm motility, increased abnormal sperm morphology, decrease in total sperm output) begin to appear 7–14 days post-insult (Figure 20.4) and usually remain present for 5 weeks or longer based upon the scope of the heat stress event (Althouse 1992).

Relative humidity (%)

Air temperature (°c)	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
41	37	37	38	39	41	43	45	48	51	54	58	62	67	Boar heat index							
38	35	35	35	36	37	38	39	41	43	46	49	52	55	59	63						
35	33	32	32	33	33	34	35	36	37	39	41	43	45	48	50	53	57				
32	30	30	30	30	30	30	31	31	32	33	34	36	37	39	40	42	44	47	49		
29	28	27	27	27	27	28	28	28	29	29	30	30	31	32	33	34	35	36	37	38	40
26	25	25	25	25	26	26	26	26	26	26	27	27	27	27	27	28	28	28	28	28	28
23	22	22	23	24	24	24	25	25	25	25	25	25	25	24	24	24	23	23	22	21	20
20	19	20	21	22	23	24	25	25	25	25	25	25	25	24	23	22	21	20	18	17	15

Extreme heat Stress | Probable heat Stress | Possible heat Stress

Figure 20.3 Risk of a heat stress event in boars based upon ambient temperature and relative humidity.

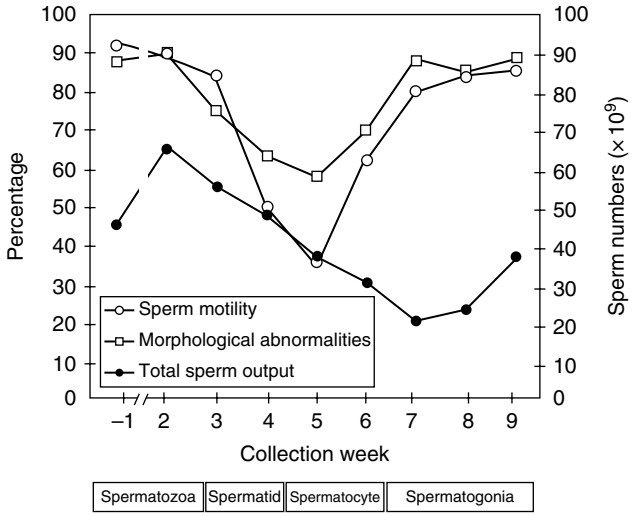


Figure 20.4 Effect of heat stress on boar sperm quality and time for recovery. Open circle points and line = sperm motility. Open box points and line = morphological abnormalities; Blackened round points and line = Total sperm output. *Source:* Adapted from Althouse (1992).

In similar mode to a heat stress event, an infectious challenge or even a routine vaccination to a boar can elicit an increase in core body temperature (e.g. pyrexia). The increased body temperature causes a concomitant increase in testis temperature that disrupts the spermatogenetic process. In addition and because of the critical role the scrotum plays in regulating testis temperature in most mammals, any trauma or abnormal condition to the scrotal area (and underlying testis) can have an adverse effect on spermatogenesis. In general, the changes to semen quality after scrotal/testis trauma or pyrexia are similar to those that occur after boars are exposed to environmental heat stress conditions. To reduce the negative semen quality effects on a boar exhibiting fever, antipyretic medication should be administered to keep body temperature below 39.5 °C.

Mycotoxins

Cereal grains contribute substantially to a typical boar diet; hence continued vigilance in minimizing the risk of mycotoxicosis from mycotoxins in feedstuffs is critical to boar health and normal spermatogenesis. Mycotoxins are products of fungi and mold that colonize cereal crops and can be produced in crops either pre- or postharvest. Factors that favor mycotoxin formation include crop stress, weather conditions, and production practices. Mycotoxicosis in a boar is dependent upon the type of mycotoxin(s), amount consumed, and duration of exposure. Specific mechanisms of mycotoxin impact on boars are not well understood, but general loss of vigor and reproductive compromise can be associated with their presence. Mycotoxins of current concern to boar health include aflatoxins, ergot, fumonisin, trichothecene toxins (e.g. ochratoxin, T-2 toxin), vomitoxin (deoxynivalenol), and zearalenone (Althouse 2014). Prudent mycotoxin screening of boar diets is recommended with a strong preference not to use grains in which mycotoxins are present. If exclusion is not an option, then selective inclusion of effective mold inhibitors, binders, absorbents, and denaturants, specific to the mycotoxin(s) found, to the diet may be of benefit.

Boar overuse

Many factors such as available boar power, genotype, libido, season, animal age, and health may drive how frequently a boar is used in a breeding program. If a boar's ejaculate frequency is too high (e.g. overuse) over several weeks, a concomitant decrease in total sperm numbers,

Table 20.2 Suggested semen collection frequencies by age for AI boars used at stud.

Boar age	Ejaculate collection frequency
<8 mo	1×/7–10 days
8–10 mo	1×/7 days
10–12 mo	≤3×/2 weeks
12+ mo	≤2×/week

Table 20.3 Minimum requirements for use of fresh boar semen for artificial insemination.

Semen variable	Descriptor/value
Appearance	Milky to creamy consistency
Color	Gray – white to white
Gross motility (unextended)	≥70% (if used by 48 hours)
	≥80% (if used after 72 hours)
Abnormal morphology	<25% (includes cytoplasmic droplets)
Cytoplasmic droplets	<15% (proximal and distal droplets)

Source: Adapted from Althouse (2007). Reproduced with permission of Elsevier.

semen quality, and subsequent fertility can be expected (Swierstra and Dyck 1976; Hemsworth et al. 1983). Young boars (<8 months of age) exposed to overuse conditions early in their careers tend to develop chronic subfertility problems, leading to premature culling (Leman and Rodeffer 1976). To avoid boar overuse, active boar management and ejaculate monitoring is essential. Table 20.2 provides suggested semen collection frequencies based upon boar age for boars used in AI programs and standing at stud. For boars used in natural mating conditions, a male–female ratio of 1 : 15–1 : 25 should be available. To avoid semen quality issues related to boar underuse, breeding boars should be ejaculated at least once every 10–14 days.

Semen quality

Achievement of herd fecundity goals is dependent, in part, upon the delivery of good quality extended boar semen. Standard tests in evaluating boar semen include a subjective visual inspection for normal appearance and color, along with more detailed objective analysis of sperm motility, sperm morphology, sperm concentration, volume, and total sperm numbers (Althouse 2007). Minimum requirements of fresh boar semen for intended AI use are listed in Table 20.3. In addition to acceptable spermiogram parameters, the source stud should confirm that any materials used in the direct production of extended semen (i.e. semen extender, plastics, gloves, etc.) are obtained from AI material suppliers that abide by acceptable supply chain practices that support product safety, quality, consistency, and traceability.

Infectious pathogens

Numerous infectious pathogens can cause reproductive problems in the boar and/or appear to play an epidemiological role in the transmission of the disease in swine (Guerin and Pozzi 2005; Althouse and Rossow 2011; Maes et al. 2016). Certain infectious agents (e.g. *Brucella*, *Chlamydophila/Chlamydia*, cytomegalovirus, Japanese B encephalitis virus [JEV], rubulavirus) can directly infect the testis and/or epididymis, eliciting an inflammatory response that disrupts sperm cell development in addition to promoting the shedding of the pathogen through the semen. Other pathogens (i.e. African swine fever [ASF], Aujeszky's/pseudorabies, classical swine fever [CSF], FMD, *Leptospira*, parvovirus, PCV2, PRRS) appear to disseminate and infect tissues throughout the body, including the boar's genital tract. Once seeded, the pathogen is shed in the seminal fluids of the boar ejaculate, facilitating the transmission of the disease to the female bred with infected semen. Currently known pathogens that have been identified in boar semen and have been transmitted through AI are listed in Table 20.4. Lastly, any pathogen in the boar that elicits a febrile response has the undesirable side effect of increasing core body and testis temperature, leading to disruption in spermatogenesis with a similar outcome to that which occurs with heat stress exposure.

Many bacterial species have been identified in fresh boar ejaculates (Althouse et al. 2000; Althouse and Lu 2005; Gączarzewicz et al. 2016). Contribution of other bacterial contaminants can occur to semen during processing for use in AI programs (Althouse and Lu 2005; Payne et al. 2008). Several bacterial species present as contaminants have been found to thrive under the cooling and storage conditions of extended boar semen in the days prior to insemination use (Althouse 2008), with the effect of damaging the sperm and affecting sow reproductive performance upon use (Althouse et al. 2000; Maroto-Martin et al. 2010; Kuster and Althouse 2016). To minimize this risk, implementation of effective bacterial control strategies during semen collection and processing should be followed (Table 20.5).

Diagnosis of abortion and reproductive diseases

The root cause of abortion lies in the loss of progesterone provided by the CL. Loss of the CL terminates pregnancy. The release of cortisol by sows and/or fetuses

Table 20.4 Selected swine diseases that have been identified in boar semen and spread through artificial insemination (AI).

Disease	Demonstrated transmission via AI
African swine fever	
Aujeszky's disease (pseudorabies)	
Brucellosis	
Chlamydophilosis/ chlamydiosis	
Circovirus type 2 (PCV2)	Yes (Madson et al. 2009; Sarli et al. 2012)
Classical swine fever (hog cholera)	Yes (De Smit et al. 1999; Hennecken et al. 2000)
Foot-and-mouth disease	
Japanese encephalitis	
Leptospirosis	
Malignant catarrhal fever	
Parvovirus	Yes (Lucas et al. 1974)
Reproductive and respiratory syndrome	Yes (Prieto et al. 1997; Nathues et al. 2016)
Rubulavirus	
Vesicular disease	

initiates a hormonal cascade, resulting in prostaglandin release from the pregnant uterus, lysis of the CL, and pregnancy loss. Infectious agents and noninfectious events can interrupt the endocrine equilibrium of pregnancy.

Diagnosis of fetal death is challenging due to the broad array of processes that have been associated with *in utero* mortality, including genetic, hormonal, nutritional, toxic, traumatic, metabolic, hypoxic, and infectious causes (Christianson 1992).

The goal of abortion diagnosis is the same as for any diagnostic investigation, which is to identify the etiology and associated epidemiology. The diagnostic process differs because clinical signs (abortion) may occur well after the inciting cause; therefore the veterinarian and producer, blinded to the initiating event, must try to retrospectively determine the event or events resulting in pregnancy loss. Infectious agents may cause fetal death shortly before the abortion or well before the abortion. An intimate knowledge of the pathogenesis of the agent or agents causing abortion is required. For example, knowing that the viremia of PRRSV in sows with preexisting immunity is much shorter than in PRRSV, naïve sows will influence the types of samples to collect, the best time for sample collection, and the number of samples required to have confidence in results of virus detection tests. The detailed pathogenesis of the specific pathogenic agents is discussed in their respective chapters.

Table 20.5 Boar and laboratory management to minimize semen contamination.

Boar preparation/semen collection

1. Hair should be kept trimmed around the preputial opening.
2. Use double gloving, with the outer glove discarded after preparation of the boar, allowing for a clean gloved hand for grasping of the penis.
3. Disposable gloves or a hand disinfectant should be used during semen collection to minimize contamination of semen and reduce risk of cross-contamination between boars.
4. Clean preputial opening and surrounding area (if needed) with a single- use disposable wipe.
5. Manually evacuate preputial fluids prior to exteriorizing penis for semen collection.
6. Hold penis horizontally to minimize preputial fluid contamination of the semen and semen collection vessel.
7. Divert initial jets of an ejaculate (i.e. presperm fraction) and gel fraction from the semen collection vessel.
8. Dispose of rubber band and filter/gauze before passing collected semen to the laboratory for further processing.

Semen processing/laboratory and barn sanitation

1. Encourage single-use disposable products when economically feasible to minimize cross-contamination.
2. If using reusable laboratory materials that cannot be heat/gas sterilized or boiled, clean initially using a laboratory-grade detergent (residue free) with water, followed by a distilled water rinse, and lastly, a 70% alcohol (nondenatured) rinse. Allow enough time and proper ventilation for complete evaporation of residual alcohol. Rinse with semen extender prior to their first use of the day.
3. Disinfect countertops and contaminated lab equipment at the end of the processing day with a moist residue-free detergent and rinse.
4. Floor should be mopped at the end of the day with a disinfectant.
5. Break down bulk products into smaller, daily use quantities immediately after opening.
6. Ultraviolet lighting can be installed to aid in sanitizing reusables and lab surfaces; however, safety precautions need to be integrated to prevent exposure of personnel.
7. Boar housing should be on a maintenance schedule that minimizes buildup of organic material and surface moisture.
8. The semen collection area and collection dummy should be thoroughly cleaned and disinfected at the end of the collection day.

In swine, the most common mechanisms of infectious abortion are infection of one or more fetal–placental units or the systemic effects of maternal illness. Clinical signs observed and the selection of appropriate specimens and diagnostic tests differ depending on the mechanism of abortion. In abortions due to infection of the fetus or placenta, the sow may have seroconverted by the time of abortion and may no longer be viremic or bacteremic, but will be seropositive for the offending

agent. The organism may or may not be detected in placenta or in one or more of the aborted fetuses. Diagnostic efforts need to focus on the placenta, fetuses, and seroconversion of dams. Abortion due to maternal illness usually happens during the acute phase of the disease at which time the sows typically exhibit signs of systemic illness such as fever, inappetence, lethargy, respiratory disease, or enteric disease. The sows usually have not had time to seroconvert or have an anamnestic response to the causative infectious agent. With acute maternal infections, there is an opportunity to detect the causative agent in clinical or postmortem specimens (i.e. serum, nasal swab, tonsil scraping, tissues) from the sow. Fetal infection may or may not be present; therefore collection of specimens from sows and fetuses from acute maternal illness will optimize the likelihood of diagnosing the causative agent.

The initial step in investigating an abortion outbreak is to assess whether aborting sows have clinical signs of systemic illness either at or near the time of abortion. If the aborting dam is clinically ill, then specimens from both the dam and fetuses should be submitted for diagnostic testing. The nature of the sow's illness will dictate the appropriate specimen collection strategy. For example, influenza A virus (IAV) can cause sows to become febrile, lethargic, and inappetent and develop signs of respiratory disease such as coughing and dyspnea. Since IAV infection does not cause viremia, abortions result from the maternal inflammatory response that then initiates the disruption of the pregnancy endocrine equilibrium. A fetus is not an acceptable specimen for identifying IAV but may prove valuable in identifying a secondary sow bacteremia. A diagnosis of IAV as the initiating cause of the abortion is then dependent on identifying the virus in nasal swabs, bronchial swabs, and lung or oral fluids of sick or dead sows. Demonstrating seroconversion to SIV with paired serum samples may be problematic considering the myriad of influenza viruses circulating in pigs.

Infectious agents that can infect and replicate in fetuses are detectable in fetal tissues and/or placenta if a sufficient number of specimens are examined and the diagnostic tests are capable of detecting live pathogens or their residual specific antigens or nucleic acids. Studies have demonstrated that maternal bacteremia or viremia may result in infection of one to several fetuses, with later *in utero* spread from fetus to fetus (Nielsen et al. 1991). What is important about this observation is that not all fetuses in a litter are infected at the time of abortion; therefore diagnosis of the causative agent may be missed if only a few fetuses are evaluated. A general recommendation is to sample 4–6 fetuses per litter from at least 3 litters. This is based on a calculation to achieve 90–95% confidence that at least one infected fetus is represented in a submission, assuming a fetal infection rate of 50% and a litter size of 12 (Benson et al. 2002). For most diagnostic tests, pooling of samples from several fetuses generally has limited impact on diagnostic sensitivity. The stage of gestation and *in utero* spread of pathogens influence the clinical presentation of reproductive disease with fetal or placental infection. Infection of a fetus can result in a range of outcomes, including embryonic death and resorption, fetal death and mummification, abortion of fresh or autolyzed fetuses, stillborn pigs, or weak-born pigs with increased neonatal mortality (Christianson 1992). Infection early in gestation may lead to embryonic death and resorption of some but not all fetuses, resulting in decreased litter size. Complete resorption of fetuses indicates fetal death occurred prior to 35 days' gestation. After 35 days, fetal bone mineralization occurs; therefore fetuses that die become mummified. Progressive *in utero* spread of a pathogen between fetuses may lead to fetal death and mummification, with variation in the size of mummified fetuses. Fetuses infected after 65–70 days of gestation can mount an immune response to a number of agents. These fetuses may be live born yet seropositive to the pathogen (Nielsen et al. 1991). Clinically, this sequential infection scenario may manifest as multiple variable-sized mummified fetuses with a few apparently normal live-born piglets at parturition.

Porcine abortion: Serology

Serology remains a useful tool in diagnosing infectious agents of abortion. The greatest value of serology is either to confirm the presence of an agent previously absent from the herd or to verify that the agent in question is still not present in the herd. Accumulating negative results for different agents is a valuable part of the diagnostic effort. Diagnosis by opinion and strict reliance on agent detection tests or on single test types (e.g. polymerase chain reaction [PCR], IHC) rather than pursuing a complete, proper, and objective diagnostic investigation leads to prolonged clinical disease and inappropriate or delayed interventions.

With an acute infectious event, the affected sow likely has not yet seroconverted. Specific antibody detection via acute and convalescent samples from several acutely affected or sick sows is quite valuable to demonstrate seroconversion and/or increasing antibody titer. Serum samples from acutely affected animals can be split, with one portion used for agent detection testing and the remainder retained for paired serology if needed. These samples can later be discarded if they are not needed, but they are invaluable for investigation of abortion events that continue to be undiagnosed over time. Seroconversion to a known pathogen and temporal association with an abortion event is strong evidence for a role in the clinical process. If abortion rate is increasing and sows are not

clinically ill, then serum collected from sows that have aborted and a few herd mates can identify seroconversion to the causative agent. Samples consistently negative for antibody for the suspected agent will help rule out a role for that agent in abortion.

The detection of serum antibodies to known endemic infectious agents can be difficult to interpret. That frustration is a common reason serology has become less a part of abortion diagnostic investigations. However, antibody detection with appropriate serology tests remains an important diagnostic tool. Serology performed on a few animals of known clinical status for diagnostic reasons can be very insightful, and sampling utilized for this type of diagnostic investigation should not be confused with the more generic epidemiology-based sampling protocols used for detection and prevalence studies in subclinical populations or animals of unknown clinical status.

Agent-specific antibody detection performed on fetal thoracic fluid is not generally reliable due to both a lack of sensitivity and specificity. IgG/IgM identified in fetal thoracic by agar-gel immunodiffusion (AGID) is a reliable method to indicate *in utero* antigen exposure after 65 days' gestation. A positive result is not specific for a causative agent but is an indication of an infectious event prompting additional submissions.

Tissue sampling guidelines

The most important part of a diagnostic investigation is a thorough review and evaluation of the clinical problem by the veterinarian and producer prior to sample selection and submission. The decision may be straightforward in cases of acute abortion events. Chronic or recurring events causing different stages of fetal death or pregnancy loss can be especially challenging. These events may require multiple submissions or the collection of samples from multiple animals over time with a single submission. In the absence of lesions or detection of offending etiology, the diagnosis is idiopathic with cause unknown. The relative frequency of identified causes reported from diagnostic laboratories should not be considered as true prevalence or a complete data set, as that data is a tiny fraction of the abortions occurring day to day and is strongly influenced by selection bias in sampling and test selection.

A summary of preferred specimens for porcine abortion laboratory examinations is listed in Table 20.6. Collection and submission of chilled or frozen fetuses and placenta, along with dam sera, is the most common type of diagnostic specimen submitted to laboratories as caretakers can then collect and submit directly from the farm with guidance from the herd veterinarian. Diagnosticians can then sample and select appropriate tests using hygienic techniques. Intact fetuses are expected to be sterile or devoid of infectious agents, so identification of an agent is usually a significant finding. Whole fetus submissions give the pathologist or diagnostician the opportunity for gross examination (gross lesions are infrequent), crown-to-rump measurements for determining fetal age, and separation of fetuses used for testing by size/age and condition (mummified, partially autolyzed, or fresh). Contamination of samples with agents normally found in feces such as PPV, PCV2, and a host of endemic bacterial flora is not uncommon; mere detection is not causation, so care in interpretation is warranted before assigning cause. Histopathology is a very useful tool for routine diagnostic investigation as it can link an agent to a typical or expected lesion. Histopathology can also identify tissue lesions of unknown origin, leading to more in-depth analysis.

Table 20.6 Fetal tissue sampling guidelines in cases of porcine abortion.

Formalin-fixed tissues	Fresh/chilled tissues
Brain	Brain
Heart	Heart
Lung	Lung
Liver	Liver
Spleen	Spleen
Kidney	Kidney
Placenta	Placenta
Skeletal muscle	Fetal thoracic fluid
	Fetal stomach contents[a]
	Serum ± nasal swab, other specimens from aborting sows

[a] Fetal stomach contents are a minimally contaminated specimen for bacterial culture and can easily be harvested by puncturing a syringed needle through the wall of the stomach and aspirating.

Viruses affecting reproduction

Viruses, of which many are known, are considered the most economically significant infectious cause of pregnancy loss in swine. A large portion of viral reproductive losses is associated with maternal infections rather than direct fetal infection; hence careful observation of sows and appropriate sampling strategies are a critical part of all abortion investigations. Viruses found in the United States related to pregnancy loss include PRRSV, PCV2, PPV1, PPV2, IAV, encephalomyocarditis virus (EMCV), porcine enteroviruses (PEV), porcine teschoviruses (PTV), atypical porcine pestiviruses including bovine viral diarrhea virus (BVDV)/border disease virus (BDV), swine vesicular disease, porcine cytomegalovirus (PCMV), and the potential for pseudorabies virus (PRV)

from feral pigs. Outside of the United States, pregnancy loss occurs by infection with JEV, CSF, ASF, Menangle virus, and rubulavirus, which is also known as blue-eyed pig disease.

Diagnosis of viral abortion

Gross lesions that may indicate viral-induced reproductive disease include an increased number of mummified fetuses, the presence of fetal anomalies in multiple litters (e.g. cerebellar hypoplasia, arthrogryposis), and multiple pinpoint foci of necrosis (PRV). The most common observation with fetal viral infection is "no gross lesions." Microscopic lesions are valuable indicators of viral abortion and are present more frequently than gross lesions. Microscopic lesions that suggest viral involvement include nonsuppurative encephalitis, myocarditis, vasculitis, and necrotizing lesions in multiple tissues.

The advent of PCR for the identification of fetal viral infections has remarkably enhanced abortion diagnostic investigations. PCR tests can be designed to identify a broad range of genetic variations within a group of viruses such as PRRSV and to also have applications across a broad range of sample types including autolyzed tissues or tissues from mummified fetuses. The application of next-generation sequencing (NGS), a method to screen for and identify unknown RNA or DNA in a sample for abortion diagnosis, will increase the number of viruses detected in fetal tissues and be able to rule out the presence of infectious agents so that negative results will have diagnostic value as well. No longer are diagnosticians reliant on insensitive use of virus isolation for the discovery of unknown viruses. Submission of whole fetuses gives the pathologist or diagnostician the option of specific tissue selection for NGS investigations.

Bacterial and fungal abortion

A wide variety of bacteria and fungi have been isolated from porcine abortions (Kirkbride and McAdaragh 1978; Eustis et al. 1981), but most are not commonly thought of as herd problems. Fungal infections are only reported to account for roughly 0.3% of porcine abortions (Kirkbride and McAdaragh 1978). A maternal bacteremia that causes fetal and/or placental infection may originate from exposure to newly introduced bacteria or more commonly from endemic opportunists that may also be involved in pneumonia, pericarditis, and toe or joint infections. The herd impact of various bacterial species causing abortion varies considerably with the size of the herd (large herds increase opportunities for expansion/spread and can provide an increasing challenge dose) and the degree of preexisting individual immunity to the bacteria. Abortions associated with the most common maternal bacterial infections occur in clusters lasting several weeks or possibly longer (i.e. *Erysipelothrix rhusiopathiae, Actinobacillus* spp., *E. coli*) and are occasionally of economic significance.

Leptospira spp. and *Brucella suis* are bacteria in swine that can contribute to higher morbidity, mortality, and herd persistence. *Leptospira* spp. pregnancy loss in sows is currently uncommon in the United States because of vaccination and diminished exposure to the organism (in addition to eliminating access to wildlife reservoirs and untreated surface waters). Regulatory programs using serologic testing and animal removal in infected herds have led to the virtual elimination of *B. suis* from domestic populations in the United States, Northern Europe, and Australia. However, there is the potential for reintroduction of *B. suis* into commercial populations from feral swine.

Diagnosis of bacterial abortion

Gross lesions indicative of bacterial abortion are uncommon but may include fetal icterus, splenomegaly, fibrinous exudate on the surface of abdominal or thoracic viscera, or gross placental hemorrhage, necrosis, or exudates. Gross placental lesions are difficult to identify as most are fecal contaminated. Microscopic lesions identified are most commonly pneumonia and placentitis. Preferred fetal tissues for bacterial culture are lung and stomach contents. Assigning causal roles of bacteria isolated from the placenta as the causative agent of abortion is dependent on both confirming a compatible lesion (e.g. acute placentitis) by microscopy and by a bacterial culture result indicating a relatively heavy growth.

Toxins associated with abortion and infertility

Abortion and infertility associated with exposure to toxins can impact multiple animals in breeding herds and, to a degree, mimic infectious and contagious abortions. The two most common toxins associated with reproductive wastage in swine are carbon monoxide and zearalenone.

Carbon monoxide (CO) poisoning is usually the result of incomplete combustion and inadequate removal of fossil fuel by-products in heated facilities. Cherry red discoloration of fetal subcutaneous tissue, muscle, abdominal, and thoracic viscera is a hallmark for carbon monoxide poisoning, confirmed by detection of carboxyhemoglobin levels >2% in fetal thoracic fluid (Carson 1990). A carbon monoxide detector would give a more rapid diagnosis.

Experimental evidence does not support a direct role for any particular mycotoxin as cause of abortion in swine; abortion as a direct result of mycotoxin ingestion in an otherwise asymptomatic sow is rare. On the

Table 20.7 Some infectious and toxic diseases causing abortion, stillbirth, and mummification in swine.

Disease	Signs in sow	Reproductive manifestations	Gross fetal lesions	Diagnosis
PRRSV	Mild depression, anorexia, fever	Late-term abortion, stillbirths, weak-born pigs	Meconium staining of fetal skin; umbilical cord edema and segmental hemorrhage	PCR: pooled fetal thoracic fluid and/or tissues; PCR on serum from acutely ill dams
PCV2	None	Weak births, stillbirths, fetal mummification	Dilated and hypertrophied heart; fluid in the body cavities; enlarged, congested liver	PCR on fetal heart/lung; IHC on fetal heart
Parvovirus (PPV)	None	Embryonic death and resorption (small litter size), fetal mummification	Fetal mummification (3–16 cm crown-rump length)	FA/PCR mummified fetal tissues (lung)
Psuedorabies virus (PRV); Aujeszky's disease	Generally none	Embryonic death, fetal mummification, abortion, stillborn, and weak-born pigs	Multifocal; random; pinpoint white foci (necrosis) in liver, spleen, and possibly lung	FA, PCR, VI on fetal lung, liver, spleen, brain, and kidney
Influenza virus A (IAV)	Fever, lethargy, coughing, dyspnea	Infertility, decreased litter size, abortion, and stillbirths	None	IAV not found in fetuses; PCR, VI, AgELISA on nasal swabs or lung from sick sows; paired serology
Encephalomyocarditis virus (EMCV)	None	Decreased farrowing rates, abortion, mummification, stillbirths, weak-born pigs	Chalky white foci in the heart, hydrothorax, hydropericardium, ascites	VI, PCR on heart, lung, spleen, kidney, and brain
Enterovirus, teschovirus	None	Infertility, embryonic death, mummification, stillbirths, neonatal mortality	None	VI, FA, PCR on lung, spleen, kidney, and heart
Classical swine fever (CSF); Hog cholera	Fever, reduced feed intake, depression, ataxia, conjunctivitis, constipation, cachexia, and cutaneous erythema	Embryonic death and resorption, abortion, mummification, stillbirths, fetal malformations, and increased neonatal mortality	Ascites, widespread petechiation, pulmonary hypoplasia, malformations, micrognathia, cerebellar hypoplasia, microcephaly	PCR on tonsil, kidney, spleen, lung, and placenta
Atypical pestiviruses (APPV, BVD, BDV)	None	Decreased conception, abortions, stillbirths, congenital anomalies, increased neonatal mortality	Cerebellar hypoplasia	VI, FA, PCR on brain, spleen, lung, and kidney
Japanese encephalitis virus (JEV)	None	Abortion, fetal mummification, stillborn or weak piglets	Subcutaneous edema, hydrocephalus, cerebellar hypoplasia, cavitary effusions, serosal petechia	VI, FA, IHC, PCR brain, liver, spleen, lung, and placenta
Porcine cytomegalovirus (PCMV)	None	Decreased litter size, fetal mummification, stillbirths, neonatal mortality	Interlobular pulmonary edema	VI, FA, PCR on fetal lung, spleen, and kidney
Rubulavirus	Transient anorexia, occasional corneal opacity	Infertility, stillbirths, fetal mummification, infrequent abortion	Fetal mummification	VI, FA, PCR on brain, lung, liver, and placenta
Menangle virus	None	Infertility, decrease live-born piglets, fetal mummification, stillborn fetuses, congenital anomalies	Malformation: brachygnathia arthrogryposis, kyphosis, pulmonary hypoplasia, CNS abnormalities	VI on brain, lung, and heart; serology

(*Continued*)

Table 20.7 (Continued)

Disease	Signs in sow	Reproductive manifestations	Gross fetal lesions	Diagnosis
Leptospira	Transient fever, anorexia, and depression	Infertility, fetal mummification, abortion, stillbirths, and weak-born pigs	Occasional fetal jaundice	PCR, IHC, FAT on fetal kidney; dam serology
Brucella suis	None	Infertility, abortion, stillborn and weak-born pigs	Placentitis	Bacterial culture: liver, placenta, stomach content, lung; dam serology
Chlamydia spp.	None	Abortion, infertility	None	IHC, PCR, antigen-capture ELISA on placenta, liver
Carbon monoxide	Typically none	Abortions, stillbirths, weak-born piglets	Cherry red subcutaneous tissue, muscle, abdominal and thoracic viscera	Carboxyhemoglobin levels on fetal thoracic fluid
Zearalenone	Infertility, anestrus, pseudopregnancy, swollen mammae and vulvas, anestrus	Infertility, embryonic death, small litter size, small pigs, piglets with hyperestrogenism	None	Feed analysis

other hand, zearalenone is an estrogenic mycotoxin produced by *Fusarium roseum*, and to a lesser extent *Fusarium moniliforme*, that has a well-known influence on reproductive function. Zearalenone infection occurs most commonly in corn, but other feedstuffs may also be infected. Zearalenone fed during pregnancy may interfere with early fetal development, resulting in reduced litter size (Kirkbride and McAdaragh 1978; Osweiler et al. 1985) or pseudopregnancy, but not abortion. A summary of infectious and toxic causes of reproductive wastage in swine is found in Table 20.7.

References

Akkermans JP, van Beusekom WJ. 1984. *Vet Q* 6(2):90–96.

Almond GW, Flowers WL, Batista L, et al. 2006. Diseases of the reproductive system. In Straw BE, Zimmerman JJ, A'llaire D, et al. eds. *Diseases of Swine*, 9th ed. Ames, IA: Blackwell Publishing, pp. 113–147.

Althouse GC. 1992. Biochemical composition of the spermatozoal plasma membrane in normal and heat-stressed boars. PhD thesis, Iowa State University.

Althouse GC. 2007. Artificial Insemination in Swine: Boar Stud Management. In *Large Animal Theriogenology*, 2nd ed. Missouri: Saunders, pp. 731–738.

Althouse GC, 2008. *Reprod Domest Anim* 43:374–378.

Althouse GC. 2014. Applied Andrology in Swine. In Chenoweth PJ, Lorton SP, eds. *Animal Andrology*. Oxfordshire: CABI, pp. 404–417.

Althouse GC, Evans LE. 1997a. *JAVMA* 210:675–677.

Althouse GC, Evans LE. 1997b. *JAVMA* 210:678–680.

Althouse GC, Lu K. 2005. *Theriogenology* 63:573–584.

Althouse GC, Rossow K. 2011. *Reprod Domest Anim* 46:64–67.

Althouse GC, Kuster CE, Clark SG, et al. 2000. *Theriogenology* 53:1167–1176.

Benson JE, Yaeger MJ, Christopher-Hennings J, et al. 2002. *J Vet Diagn Invest* 14:8–14.

Brüssow KP, Schneider F, Kanitz W, et al. 2001. *Soc Reprod Fertil Suppl* 66:187–195.

Carr J. 2008. Bench-marking pig production breeding herd performance. *Proc Pig Vet Soc Suppl*:29–40.

Carson TL. 1990. Carbon Monoxide-induced stillbirth. In: Kirkbride CA, ed. *Laboratory Diagnosis of Livestock Abortion*. 3rd ed. Ames, IA: Iowa State University Press, pp. 186–190.

Cech S, Dolezel R. 2007. *Vet Med (Praha)* 52:413–418.

Chen TY, Lines D, Dickson C, et al. 2016. *Reprod Domest Anim* 51(5):813–818.

Christianson WT. 1992. *Vet Clin North Am Food Anim Pract* 8:623–639.

Dalin AM, Gidlund K, Eliasson-Selling L. 1997. *Acta Vet Scand* 38(3):253–262.

Dalin AM, Kaeoket K, Persson E. 2004. *Anim Reprod Sci* 82–83:401–413.

De Smit AJ, Bouma A, Terpstra C, et al. 1999. *Vet Microbiol* 67:239–249.

Debattista J, Timms P, Allan M, et al. 2003. *Fertil Steril* 79(6):1273–1287.

Dehnhard M, Rohrmann H, Kauffold J. 2013. In Proceedings of the Joint Meeting of the 5th European Symposium of Porcine Health Management and 50th Anniversary Meeting if the Pig Veterinary Society of Great Britain, Edinburgh, UK, p. 113.

Eustis SL, Kirkbride CA, Gates C, et al. 1981. *Vet Pathol* 18:608–613.

Gączarzewicz1 D, Udała J, Piasecka M, et al. 2016. *Pol J Vet Sci* 19(3):451–459.

Geisert RD, Zavy MT, Moffatt RJ, et al. 1990. *J Reprod Fertil Suppl* 40:293–305.

Golbar H, Izawa T, Kuwamura M, et al. 2010. *J Vet Med Sci* 2:253–266.

Guerin B, Pozzi N. 2005. *Theriogenology* 63:556–572.

Hemsworth PH, Winfield CG, Hansen C. 1983. *Anim Prod* 37:409–413.

Hennecken M, Stegeman JA, Elbers AR, et al. 2000. *Vet Q* 22(4):228–233.

Höltig D, Nicoll I, Kauffold J, et al. 2015. *Tierärztl Prax (K)* 43:427–433.

Kauffold J, Althouse GC. 2007. *Theriogenology* 67(5):901–911.

Kauffold J, Wehrend A. 2014b. *Tierärztl Prax (G)* 42(3):179–186.

Kauffold J, Rautenberg T, Gutjahr S, et al. 2004a. *Theriogenology* 61:1407–1417.

Kauffold J, Rautenberg T, Richter A, et al. 2004b. *Theriogenology* 61(9):1635–1648.

Kauffold J, Rautenberg T, Hoffmann G, et al. 2005. *Theriogenology* 64(7):1546–1558.

Kauffold J, Melzer M, Berndt A, et al. 2006. *Theriogenology* 66:1816–1823.

Kauffold J, Gmeiner K, Sobiraj A, et al. 2010. *Vet J* 183(1):103–108.

Kauffold J, Peltoniemi O, Knöppel H, et al. 2014a. Proceedings of the 6th European Symposium of Porcine Health Management, May 7–9, 2014, Sorrento, Italy, p. 256.

Kirkbride CA, McAdaragh JP. 1978. *JAVMA* 172:480–483.

Kirkwood RN. 1999. *Cont Prof Dev* 2:52–55.

Kirkwood RN, Kauffold J. 2015. *Reprod Domest Anim* 50 (Suppl 2):85–89.

Kummer R, Bernardi ML, Schenkel AC, et al. 2009. *Reprod Domest Anim* 44(2):255–259.

Kuster CE, Althouse GC. 2016. *Theriogenology* 85(1):21–26.

Leman AD, Rodeffer HE. 1976. *Vet Rec* 98:457–459.

Lucas MH, Cartwright SF, Wrathall AE 1974. *J Comp Pathol* 84:347–350.

Madson DM, Patterson AR, Ramamoorthy S, et al. 2009. *Vet Pathol* 46:707–716.

Maes D, Soom AV, Appeltant R, et al. 2016. *Theriogenology* 85:27–38.
Manjarin R, Cassar G, Sprecher DJ, et al. 2009. *Reprod Domest Anim* 44:411–413.
Maroto Martín LO, Muñoz EC, De Cupere F, et al. 2010. *Anim Reprod Sci* 120:95–104.
McNitt JI, First NL. 1970. *Int J Biometeorol* 14:373–380.
Meredith MJ. 1977. *Vet Rec* 101 (4):70–74.
Nathues C, Perler L, Bruhn S, et al. 2016. *Transbound Emerg Dis* 63(2):251–261.
Nielsen J, Rønsholt L, Sørensen KJ. 1991. *Vet Microbiol* 28(1):1–11.
Nissen AK, Soede NM, Hyttel P, et al. 1997. *Theriogenology* 47:1571–1582.
Olanratmanee EO, Wangnaitham S, Thanawongnuwech R, et al. 2011. *Tropl Anim Health Prod* 43(2):451–457.
Oravainen J, Heinonen M, Seppä-Lassila L, et al. 2006. *Reprod Domest Anim* 41(6):549–554.
Oravainen J, Heinonen M, Tast A, et al. 2008. *Reprod Domest Anim* 43(1):42–47.
Osweiler G, Carson T, Buck W, et al. 1985. *Clinical and Diagnostic Veterinary Toxicology*, 3rd ed. Kendal Hunt Publishing Company.
Payne BJ, Clark S, Maddox C, et al. 2008. *J Swine Health Prod* 16:316–322.
Peltoniemi O, Virolainen JV. 2006. *Soc Reprod Fertil Suppl* 62:205–218.
Peltoniemi O, Björkman S, Oliviero C. 2016. *Reprod Domest Anim Suppl* 51(2):36–47.
Prieto C, Suarez P, Simarro I, et al. 1997. *Theriogenology* 47:647–654.
Quesnel H. 2009. *Soc Reprod Fertil Suppl* 66:121–134.
Sarli G, Morandi F, Panarese S, et al. 2012. *Acta Vet Scand* 54:51.
Soede NM, Laurenssen B, Abrahamse-Berkeveld M, et al. 2012. *Anim Reprod Sci* 130(1–2):74–81.
Swierstra EE, Dyck GW. 1976. *J Anim Sci* 42:455–460.
Tiemann U, Dänicke S. 2007. *Food Addit Contam.* 24(3):306–314.
Waberski D, Weitze KF, Gleumes T, et al. 1994. *Theriogenology* 42:831–840.
Weitze KF, Schmidt A, Waberski D, et al. 1998. *Reprod Domest Anim Suppl*:26.
Wettemann RP, Wells ME, Omtvedt IT, et al. 1976. *J Anim Sci* 42:664–669.
van Wettere WH, Revell DK, Mitchell M, Hughes PE. 2006. *Anim Reprod Sci.* 95(1-2):97–106. Epub 2005 October 25.
Wilson RF, Nalbandov AV, Krider JL. 1949. *J Anim Sci* 8:558–568.

21

Respiratory System

Michael J. Yaeger and William G. Van Alstine

Introduction

The average adult pig-sized mammal breathes 7,000–12,000 L of air every day. The combined area of the air passages and alveolar surface of the respiratory tract represents the largest epithelial surface exposed to the outside environment in the mammalian body. The interaction of the massive respiratory surface with the enormous volumes of air, containing potentially harmful gases, particles, and pathogenic microorganisms, has necessitated the development of robust respiratory defenses.

The increased pressures of rearing pigs in modern-day confinement systems have the potential to overcome these robust defenses and lead to the development of respiratory disease. In most swine-producing areas, large groups of pigs are housed under intensive conditions, often in geographical regions with a dense pig population. Respiratory disease may result when the respiratory defenses are compromised by exposure to fine particulates, such as dust, and volatile chemicals, such as ammonia from animal waste (Michiels et al. 2015). The high stocking density in a closed environment also facilitates transmission of airborne pathogens within a herd (Buddle et al. 1997; Donham 1991) and between herds (Christensen et al. 1993; Stark et al. 1992). Many of these primary respiratory pathogens adversely impact respiratory defenses, leading to the development of costly secondary bacterial bronchopneumonia.

Worldwide, respiratory disease continues to cause substantial losses to the swine industry (Bak et al. 2008; Grandia et al. 2010; Jager et al. 2010; Neumann et al. 2005; Sales et al. 2010). Results from the 2012 National Animal Health Monitoring System (NAHMS) study showed that respiratory infections were the main cause of nursery deaths (47.3%) and grower/finisher pig mortality (75.1%) (United States Department of Agriculture 2015). Two large surveys identified pneumonia lesions in an average of 55.9–59.6% of slaughtered pigs (Hillen et al. 2014; Ostanello et al. 2007). Financial losses associated with respiratory disease are due to increased mortality, decreased weight gain, increased feed costs, increased condemnation at slaughter, and increased costs for treatments, vaccination, and labor. Consequently, respiratory disease is regarded as one of the most serious health problems in modern swine production.

Normal structure and function

The respiratory system is broadly divided into the upper and lower respiratory tract. The upper respiratory tract is the portion of the respiratory tract external to the chest and consists of the nasal cavity, sinuses, nasopharynx, larynx, and trachea. The primary functions of the upper respiratory tract are to condition incoming air (warm, humidify, cleanse), sample antigens to initiate an adaptive immune response, and conduct air to the lower respiratory tract. Many of these functions also occur in the lower respiratory tract, where air is ultimately delivered to the alveoli for efficient gas exchange.

Upper respiratory tract

The nasal cavity has a large, moist, highly vascularized, and convoluted mucosal surface that conditions incoming air by removing particulates, warming, humidifying, and uniformly mixing the air (Figure 21.1). The upper respiratory tract, including the majority of the nasal cavity and continuing through the pharynx, larynx, trachea, and bronchi, is lined by a mucus-coated ciliated epithelium that forms the mucociliary apparatus – one of the most important respiratory defenses.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Lower respiratory tract

The lungs are divided into seven lobes: the right lung is composed of the cranial, middle, and caudal and accessory lobes; the left lung is composed of the cranial, middle, and caudal lobes (Figure 21.2). Lobes vary in size. The lobes are subdivided by solid interlobular septa into separate lobules, each served by a bronchiole. Collateral ventilation between lobules is minimal. As a result of this complete lobulation, exudate within alveoli, as seen with a bronchopneumonia, is often retained within lobules, giving the lungs a sharp line of demarcation between affected and unaffected lobules.

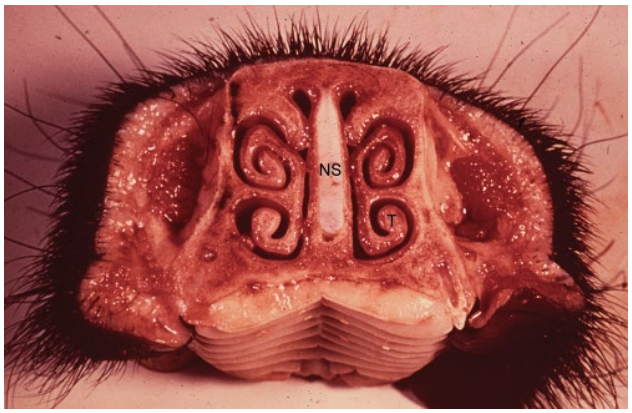


Figure 21.1 Normal turbinates from a normal pig. The nasal septum (NS) is straight, and the turbinates (T) fill the nasal cavity.

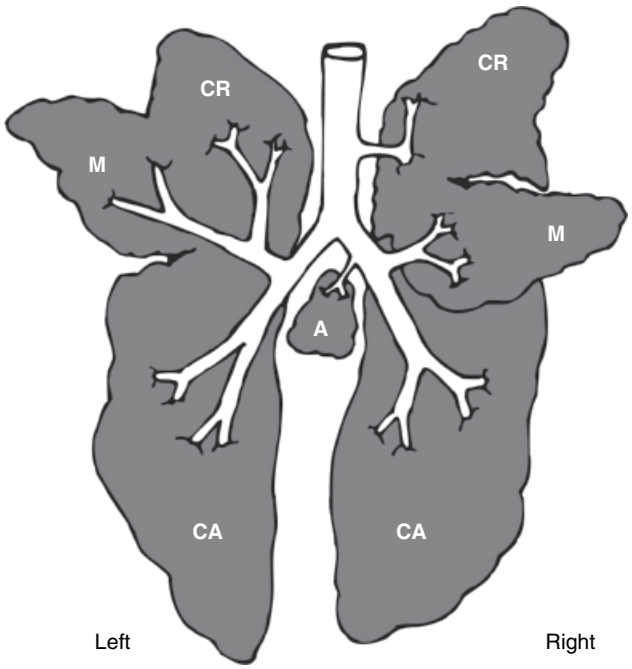


Figure 21.2 Schematic diagram of the lung lobation with branches of the bronchial tree. CR, cranial lobe; M, middle lobe; CA, caudal lobe; A, accessory lobe.

The lower respiratory tract consists of the bronchi, bronchioles, terminal and respiratory bronchioles, and alveoli. At the level of the terminal and respiratory bronchioles, lung function transitions from cleansing and conducting incoming air to the region where gas is exchanged. The terminal air passages and alveoli are no longer protected by a mucus layer, since mucus would slow the diffusion of oxygen and carbon dioxide. Blood in alveolar capillaries is separated from luminal air by three extremely compact tissue layers measuring only 0.22–0.55 µm in thickness (Bradley and Wrathall 1977), allowing for efficient gas exchange. A drawback of this design is that this very large and fragile surface lacks the protective components of the mucociliary apparatus and is vulnerable to infectious agents should they penetrate deep into the lung.

Respiratory defenses

Mucociliary apparatus

Throughout much of the respiratory tract, extending from the nasal cavity to the bronchioles, the mucosal surface is protected by the mucociliary apparatus. In this system, mucus coats the epithelial surface. High air velocity in the nasal cavity coupled with marked air turbulence around nasal turbinates results in impaction and trapping of larger airborne particles (>30 µm) onto the mucus bilayer lining the nasal mucosa (Bustamante-Marin and Ostrowski 2017). Particles greater than 10 µm diameter are mostly removed before reaching the bronchial tree (Baskerville 1981). Particles are trapped in the mucus, and the rhythmic beating of the cilia results in the delivery of mucus from the nasal cavity, trachea, and bronchi to the pharynx where it is subsequently swallowed.

The particle-trapping surface mucus of the mucociliary apparatus is not simply a passive layer of viscous liquid. The mucus gel acts as a mechanical barrier for bacteria. The surface mucus also contains specific immunoglobulins, primarily immunoglobulin A (IgA), and many antimicrobial substances, including antimicrobial peptides (defensins), lysozyme, surfactant, opsonins, lactoferrins, and complement factors, all of which aid in the neutralization and removal of pathogens, further bolstering the effectiveness of the mucociliary apparatus (Nicod 2005).

Alveolar defenses

Particles less than 5 µm in diameter may be able to reach the alveoli. The primary defense against particulates in the alveolus is the alveolar macrophage. Additionally, pneumocytes produce surfactant proteins, which are

members of the collective family of mammalian lectins that enhance the phagocytosis and killing of microbes by alveolar macrophages (Nicod 2005).

Phagocytes

Phagocytic cells in the respiratory tract include dendritic cells (DCs), macrophages, and neutrophils. In general, macrophages and neutrophils are key components of the innate immune system, while the primary role of DCs is to interface with the adaptive immune system and modulate immune responses.

The basal layers of the respiratory mucosa in the nose and the conducting airways contain a tight network of phagocytic DCs that capture invading organisms and present them to the draining lymph nodes (Nicod 2005). They form a network optimally situated to sample inhaled antigens. After antigen uptake, airway DCs migrate to the paracortical T-cell zone of the draining lymph nodes of the lung, where they interact with naive T cells to generate adaptive immunity (Nicod 2005).

Macrophages play a key role in defense against respiratory infection. They are important in both innate and acquired immunity of the respiratory tract. Pulmonary macrophages remove foreign material that escapes the mucociliary defense mechanism, process antigens, and modulate the immune response. Alveolar macrophages provide the first line of defense against organisms or particles reaching the lower airways. They either neutralize the invading pathogens or recruit neutrophils and other mononuclear cells. Nonpathogenic particles and microbes are phagocytized and removed in the mucus flow or through the lymphatic system. Pigs also have intravascular macrophages that reside within the vascular lumen and likely contribute to defense against pathogens through uptake of pathogen products and enhancement of immune responses (Ohgami et al. 1989).

In healthy pigs the normal ratio between the cellular elements in the bronchoalveolar mucus is 70–90% alveolar macrophages, 5–18% lymphocytes, 4–12% neutrophils, and up to 5% eosinophilic granulocytes (Jolie et al. 2000; Neumann et al. 1985). Inflammation can lead to the recruitment of neutrophils into the alveolus to augment the phagocytic activity of macrophages. Neutrophils are phagocytic and have a potent array of pathogen killing enzymes. The recruitment of neutrophils is a major component of the protective host response to acute bacterial infections (Mizgerd 2002).

If the invading agents are not neutralized by the alveolar macrophages, the activity of the phagocytes is highly accelerated and inflammation or tissue damage can result. Proinflammatory cytokines produced by macrophages play an important role in porcine respiratory disease by coordinating and activating the adaptive immune response, which enables the host to eliminate pathogens (Thanawongnuwech et al. 2004). This adaptive immune response ideally ends in cell-mediated immunity and local and systemic production of specific antibodies.

Immunoglobulins

The production of specific antibodies is crucial in the defense of the respiratory tract. IgA is the predominant antibody in mucus of the conducting airways. Immunoglobulins in the mucus bilayer act primarily to prevent the initial establishment and penetration of pathogens. IgG antibodies, which originate in the blood, are the predominant immunoglobulin in mucus of the lower respiratory tract near the alveoli. Regardless of location, acute inflammation leads to serum transudation and allows circulating antibodies to aid in protecting the respiratory mucosa. IgE antibodies contribute to the immune response against parasites such as lungworms (*Metastrongylus* sp.) and migrating ascarid larvae.

Cell-Mediated immune response

Immunity is divided into a humoral immune response, in which the immunoglobulins play an essential role, and a cell-mediated immune (CMI) response based on antibody-independent components. However, immunity cannot be separated into two distinct components because many mechanisms are interdependent. The CMI response is particularly important in viral infections such as influenza A virus (IAV), porcine reproductive and respiratory syndrome (PRRS), porcine circovirus type 2 (PCV2), and pseudorabies (PRV) (Aujeszky's disease), but is also assumed to play an important role in *Mycoplasma hyopneumoniae* (MHP) infection (Fort et al. 2008; Lowe et al. 2005; Maes 2010; Tsai et al. 2010). Included in this response are interferons that activate antiviral responses in epithelial and other cell types.

Factors that impair or overwhelm respiratory defenses

The previously described respiratory defenses are robust. Respiratory disease in swine typically occurs when adverse environmental and management conditions or primary and opportunistic infectious agents conspire to impair and/or overwhelm these defenses.

Mucociliary apparatus impairment

The mucociliary apparatus is designed to trap, neutralize, and remove particulate material and infectious agents from the respiratory tree. Interference with this vital defense mechanism by adverse environmental

conditions or by primary respiratory pathogens has the potential to allow bacteria to reach the deeper, more vulnerable portions of the lung, leading to the development of bacterial bronchopneumonia.

Mucociliary clearance can be negatively impacted by a variety of environmental influences including temperature, dust, gases, pollutants, and dehydration. Excessive cold leads to increased viscosity of the mucus and the cilia becoming sluggish, resulting in reduced mucous velocity (Giesbrecht 1995). High temperatures in a low humidity environment, especially in an animal that is dehydrated, can lead to diminished hydration of the mucus layer and decreased clearance. Ammonia in concentrations of 50–100 ppm depresses normal mucociliary function (Curtis et al. 1975; Neumann et al. 1987), and epidemiologic studies demonstrate that higher ammonia concentrations are associated with a higher incidence of respiratory disease (Pointon et al. 1985). Dust decreases mucociliary clearance, resulting in increased mortality and an increased severity of lung lesions (Michiels et al. 2015; McClendon et al. 2015).

Several primary respiratory pathogens negatively impact the mucociliary apparatus. IAV, PCV2, PRV, and porcine respiratory coronavirus (PRCV) can all cause necrosis of the airway epithelium, destroying cells that produce and maintain the mucociliary apparatus (Brockmeier et al. 2002). *M. hyopneumoniae* damages the mucociliary apparatus by binding to cilia, causing ciliostasis, clumping and loss of cilia, and loss of airway epithelial cells (Brockmeier et al. 2002). The adverse effects of each of these primary pathogens on the mucociliary apparatus have the potential to increase the incidence and severity of secondary bacterial bronchopneumonia.

Pulmonary macrophages

Innate immunity, including the maintenance of pulmonary macrophage health and function, is an important defense mechanism against respiratory pathogens. Alveolar macrophage activity can be negatively impacted by both environmental influences and primary pathogens. Organic dust extract exposure negatively affects pig macrophage activation and function, potentially enhancing host susceptibility to a variety of respiratory infections (Knetter et al. 2014). PRRSV infection results in destruction and decreased function of pulmonary alveolar macrophages and pulmonary intravascular macrophages (Gómez-Laguna et al. 2013). PRRSV, *M. hyopneumoniae*, and PCV2 all adversely affect macrophage function, and there is abundant clinical evidence that all three are associated with the development of secondary bacterial bronchopneumonia (Brockmeier et al. 2002).

Stress

A variety of stresses, including excessive heat, cold, crowding, mixing, weaning, limit feeding, shipping, noise, restraint, nutritional status (macronutrients and micronutrients), and concurrent infections, can suppress innate and adaptive immune function and have the potential to contribute to the development of respiratory disease.

Overwhelming of respiratory defenses

Outbreaks of respiratory disease may be facilitated by overwhelming the respiratory defenses. For example, *Actinobacillus pleuropneumoniae* may be carried by pigs in their tonsil and upper respiratory tract asymptomatically because a fully functional mucociliary apparatus generally prevents this organism from reaching the alveoli and causing disease. Impairment of the mucociliary apparatus by excessive dust or *M. hyopneumoniae* infection can lead to the development of pleuropneumonia in an index animal (Gottschalk 2012). An outbreak of *A. pleuropneumoniae* may ensue when massive numbers of finely atomized bacteria are aerosolized into the environment of neighboring pigs overwhelming their respiratory defenses (Gottschalk 2012). This process is exacerbated by overcrowding, poor ventilation, and failure to utilize sick pens.

Primary and secondary pathogens in the lung

Pathogens can be categorized as either primary or opportunistic (secondary) pathogens. Although primary respiratory infectious agents can cause serious disease on their own, particularly when introduced to immunologically naïve herds, uncomplicated infections with these agents are more often mild and transient. More serious and economically important chronic respiratory disease results when these primary infections become complicated by concurrent infection with multiple agents, including opportunistic bacterial infections.

Primary respiratory pathogens include a number of viruses and several bacteria, including *Mycoplasma*, while secondary invaders are potential bacterial pathogens that frequently reside in the nasopharynx. Primary bacterial and viral pathogens have virulence factors that allow them to overcome natural defenses in the respiratory tract and to cause disease acting alone. Primary pathogens involved in porcine respiratory disease complex (PRDC) vary tremendously between countries, regions, and farms. The most common viral agents involved with PRDC include PRRSV, classical swine fever virus (CSFV), IAV, PRV, and PCV2 (Brockmeier

et al. 2002; Thacker et al. 2010). *M. hyopneumoniae* and *A. pleuropneumoniae* are the most common primary bacterial pathogens.

The commensal bacterial flora of the respiratory tract may have a favorable competitive effect for the host by outnumbering pathogenic agents, stimulating immunity, and inducing the production of innate defense factors. However, many of the bacteria that colonize the tonsil and nasal cavity of live healthy pigs are considered to be potential or secondary pathogens. Secondary bacterial pathogens readily colonize the tonsil and nasal cavity of healthy pigs and are in position to replicate to a greater extent and infect deeper tissues when adverse environmental conditions or primary pathogens adversely impact the respiratory defenses. Potential secondary bacteria include *Mycoplasma* spp., streptococci, staphylococci, *Escherichia coli*, *Klebsiella*, *Trueperella pyogenes*, *Bordetella bronchiseptica*, *Haemophilus parasuis*, and *Pasteurella multocida* (Amass et al. 1994; Castryck et al. 1990; Ganter et al. 1990; Hansen et al. 2010; Møller and Kilian 1990).

Interaction between infectious agents

PRDC was coined to emphasize the complexity of events leading to the development of pneumonia, including the involvement of viral and bacterial pathogens as well as the environmental, management, and genetic factors (Brockmeier et al. 2002). Four or more infectious agents are commonly identified in cases of PRDC, resulting in complex and potentially synergistic interactions that can increase the severity and duration of respiratory disease and lesions.

Diagnostic laboratory data, which typically represents pigs that died or were euthanized due to illness, provides insight into the most deleterious forms of respiratory disease in swine (Table 21.1). It is commonly recognized in such diagnostic investigations that severe disease is typically polymicrobial, opportunistic bacterial infections enhance the severity of primary disease insults, and opportunistic bacteria are rarely isolated alone, but are recovered in combination with one or more primary pathogens.

Because of the complicated nature of mixed infections, studying their interactions can be challenging, and the pathogenesis of mixed respiratory infections in swine is still somewhat speculative. Some of the known mechanisms by which primary pathogens can diminish respiratory defenses and lead to secondary bacterial infections include damaging the mucociliary apparatus (IAV, PCMV, PRCV, PCV2, PRV, *M. hyopneumoniae*, *B. bronchiseptica*) (Loving et al. 2010; Pol et al. 1997), inducing immunosuppression (PCV2, PRRSV, PRV) (Renukaradhya et al. 2010), altering cytokine responses (*M. hyopneumoniae*, PRRSV, IAV, PCV2) (Thanawongnuwech et al. 2004), or affecting macrophage function (*M. hyopneumoniae*, PRRSV, PCV2) (Chiou et al. 2000).

Table 21.1 Frequency (percent) of common agents of porcine pneumonia.

Agent	Frequency[a] (% of cases)
	1429 (40.8)
PRRS	1362 (38.9)
Pasteurella multocida	576 (16.5)
Mycoplasma hyopneumoniae	513 (14.7)
Streptococcus suis	456 (13.0)
Haemophilus parasuis	267 (7.6)
Actinobacillus suis	188 (5.4)
Trueperella pyogenes	101 (2.9)
Bordetella bronchiseptica	96 (2.7)
Actinobacillus pleuropneumoniae	94 (2.7)
PCV2	87 (2.5)
Salmonella spp.	44 (1.3)
Ascarid migration	37 (1.1)

Source: Adapted from Sorensen et al. (2006).
[a] Cases often have more than one diagnosis; mixed infections are common in outbreaks of respiratory disease.
Data from derived from microbiologic testing and histopathology of 3512 cases of porcine pneumonia submitted to the Iowa State University Veterinary Diagnostic Laboratory in 2016.

Bacteria including *P. multocida*, *Streptococcus suis*, *H. parasuis*, and *B. bronchiseptica* are some of the most common agents isolated from cases of swine pneumonia. Attempts to experimentally induce bronchopneumonia by aerosolizing these organisms into the respiratory tract of healthy pigs rarely result in pulmonary infection (Amass et al. 1994; Reams et al. 1994; Vahle et al. 1995). These agents typically colonize the tonsil and nasal cavity of pigs without causing disease until an insult allows them to replicate to a greater extent and infect deeper into the respiratory tract. Though both *S. suis* and *H. parasuis* can behave as primary pathogens resulting in septicemia and/or polyserositis, when limited to lung involvement, they are generally considered to be opportunistic or secondary causes of bacterial bronchopneumonia.

Respiratory pathology

Respiratory lesions can be categorized into three main disease entities: rhinitis, pneumonia, and pleuritis. For each of these entities, pathognomonic gross lesions (lesions uniquely associated with a specific disease or agent) are uncommon; however, the character of any

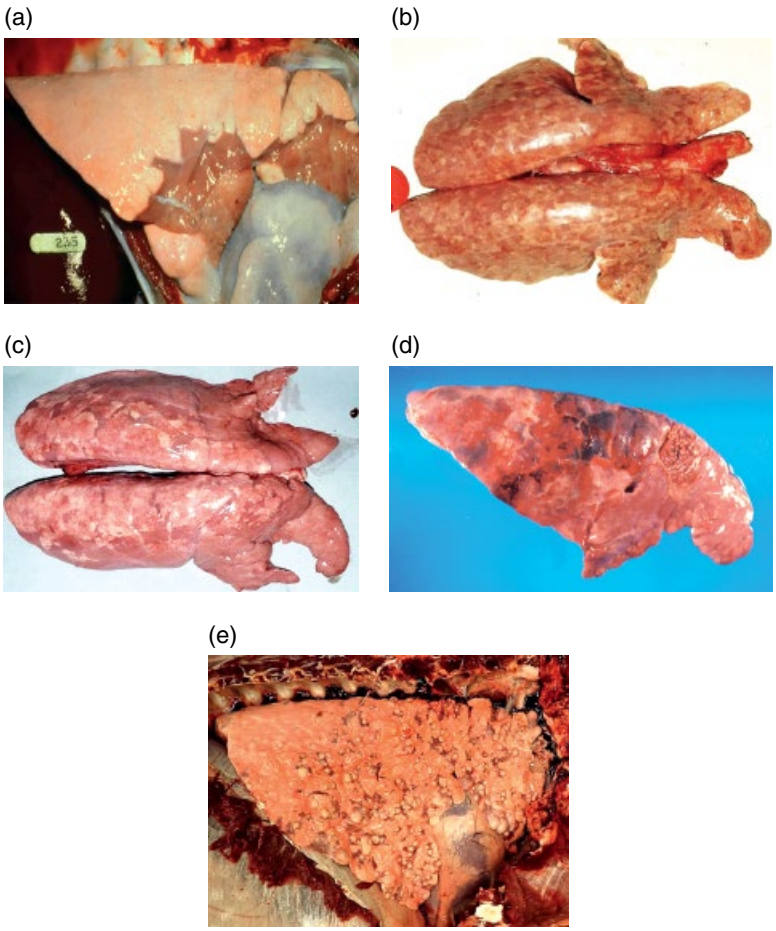


Figure 21.3 Patterns of pneumonia in swine. (a) Suppurative bronchopneumonia with cranioventral consolidation. (b) Diffuse interstitial pneumonia with diffusely tan mottled, non-collapsing lungs. (c) Bronchointerstitial pneumonia with diffusely tan mottled, non-collapsing lungs accompanied by cranioventral consolidation. (d) Hemorrhagic bronchopneumonia and fibrinous pleuritis. (e) Embolic pneumonia with variably sized abscesses in many lobes.

gross lesions that are present can assist in narrowing the list of differentials and aid in the selection of appropriate laboratory tests.

Pneumonia (inflammation of the lungs) can be categorized based on gross morphologic patterns to help predict cause or insult type (e.g. viral, bacterial, parasitic, intoxication, degenerative, or neoplastic) (López 2007). Figure 21.3 illustrates examples of the common grossly identifiable morphologic patterns of pneumonia. Table 21.2 lists some of the common respiratory pathogens associated with these patterns of pneumonia.

Rhinitis and tracheitis

Inflammation of the nasal mucosa is common in young animals. Many agents that affect the ciliated nasal epithelium can also affect the tracheal ciliated epithelium. Sneezing is a common clinical sign of rhinitis, along with serous or purulent discharge (Table 21.3). The cause is often infectious (PRV, IAV, cytomegalovirus, *B. bronchiseptica*, toxigenic strains of *P. multocida*, *Mycoplasma hyorhinis*), but air pollution, such as elevated ammonia and dust, can initiate nasal mucosal inflammation (Hamilton et al. 1998). *P. multocida* can colonize even a slightly damaged mucosa and induce progressive atrophic rhinitis with permanent distortion and shrinkage of turbinates (Figure 21.4). The structural changes in atrophic rhinitis are the result of the elaboration of cytotoxins by *P. multocida* types A and D, which disrupt osteogenesis of the turbinate bone (Foged 1992). *B. bronchiseptica* can also contribute to atrophic rhinitis through colonization of the ciliated epithelium with release of dermonecrotic and other toxins (Horiguchi 2012).

Bronchopneumonia

Bronchopneumonia results from the deposition of infectious agents, typically bacteria, at the level of the terminal bronchioles and/or alveoli, leading to the accumulation of exudate within the lumens of alveoli and small airways. This process results in several characteristic gross features. Because the alveoli and small airways are filled with an exudate, bronchopneumonia has the most firm/solid consistency of any of the broad classes of pneumonia, leading to the use of terms such as consolidation (the process of becoming solid) or hepatization (the consistency of liver) to describe their texture. When

Table 21.2 Classification of pneumonia based on gross morphology.

Type of pneumonia	Distribution of lesions	Texture of lungs	Grossly visible exudate	Potential causes[a]
Suppurative bronchopneumonia	Cranioventral consolidation	Affected portion of lung is firm	Exudate in bronchi of affected lung	Bacteria (Pm, Ss, Hps, Bb, Tpyo) often follow 1^0 insults (*M. hyopneumoniae*, IAV)
Interstitial pneumonia	Diffuse	Diffusely rubbery	Not visible	Viruses (PRRSV, PCV2), Gm– sepsis (Sal, Hps), ascarid migration
Bronchointerstitial pneumonia	Cranioventral consolidation while remaining lung is non-collapsing	Cranioventral lung is firm; caudal lung is rubbery	Exudate in bronchi of cranioventral lung	Combinations of bronchopneumonia and interstitial agents of pneumonia
Hemorrhagic bronchopneumonia and fibrinous pleuritis	Region of hilus or cranioventral	Areas of necrosis or hemorrhage are very firm	Fibrin on pleural surface; exudate in bronchi of affected lung	*A. pleuropneumoniae*, *A. suis*, *Bordetella* (in very young pigs)
Embolic pneumonia	Multifocal and random	Nodular	Abscesses or granulomas	Streptococci, Tpyo, pyogenic bacteria, tuberculosis

[a] Pm, *P. multocida*; Ss, *S. suis*; Hps, *H. parasuis*; Bb, *B. bronchiseptica*; Tpyo, *T. pyogenes*; Sal, *S. choleraesuis*.

the affected lung is sectioned, exudate can typically be expressed from small airways. Because alveolar air has been replaced by exudate, the affected lung will typically sink when placed in water or formalin. The vast majority of true bronchopneumonia cases will have a cranial and ventral pattern of distribution. *A. pleuropneumoniae* is a notable exception; often this bacterial bronchopneumonia is characterized by consolidation and hemorrhage involving the dorsal portions of the caudal lung lobe in the region of the hilus (Gottschalk 2012).

Bronchopneumonia is most commonly caused by bacteria that reside as normal flora in the upper respiratory tract, such as *P. multocida*, *S. suis*, *H. parasuis*, *T. pyogenes*, and *B. bronchiseptica*, all of which are typically secondary invaders that opportunistically colonize the lung as a result of adverse environmental influences or primary pathogens diminishing the respiratory defenses.

Table 21.3 Respiratory disease entities and agents associated with sneezing.

Disease	Agents
Rhinitis	*Bordetella bronchiseptica*, cytomegalovirus, IAV, PRV, hemagglutinating encephalomyelitis virus, dust, ammonia, and others
Progressive and atrophic rhinitis	*B. bronchiseptica* and toxigenic *Pasteurella multocida* (usually type D)

Source: Adapted from previous edition of Diseases of Swine (Sorensen et al. 2006).

Interstitial pneumonia

Interstitial pneumonia is characterized by an inflammatory process focused on the alveolar walls and adjacent interstitium. Interstitial pneumonias are typically diffuse because they commonly result from blood-borne dissemination of infectious agents including bacteria (e.g. *Salmonella choleraesuis*) and viruses (e.g. PRRSV, PCV2). Diffuse inflammation of the pulmonary interstitium results in several characteristic gross features. Lungs are typically diffusely tan mottled, heavy, and rubbery, do not fully collapse, and may have rib imprints on the pleural surfaces. The interstitium is expanded by inflammation, but alveoli still contain air, so affected lungs will be spongy, will float when placed in formalin, and are palpably less firm than lungs with a bronchopneumonia. Migration of *Ascaris suum* through the lung results in small hemorrhages in many lobes and, if sufficiently severe, will resemble an interstitial pneumonia (Liljegren et al. 2003). Certain intoxications (e.g. paraquat or fumonisin) as well as heart failure or valvular endocarditis can cause gross appearance similar to interstitial pneumonia.

Embolic pneumonia

Embolic pneumonia is characterized by randomly scattered hemorrhagic foci, discolored firm foci, or small abscesses in multiple lung lobes. These lesions result from hematogenous dissemination of bacteria from lesions elsewhere in the body. The presence of an embolic

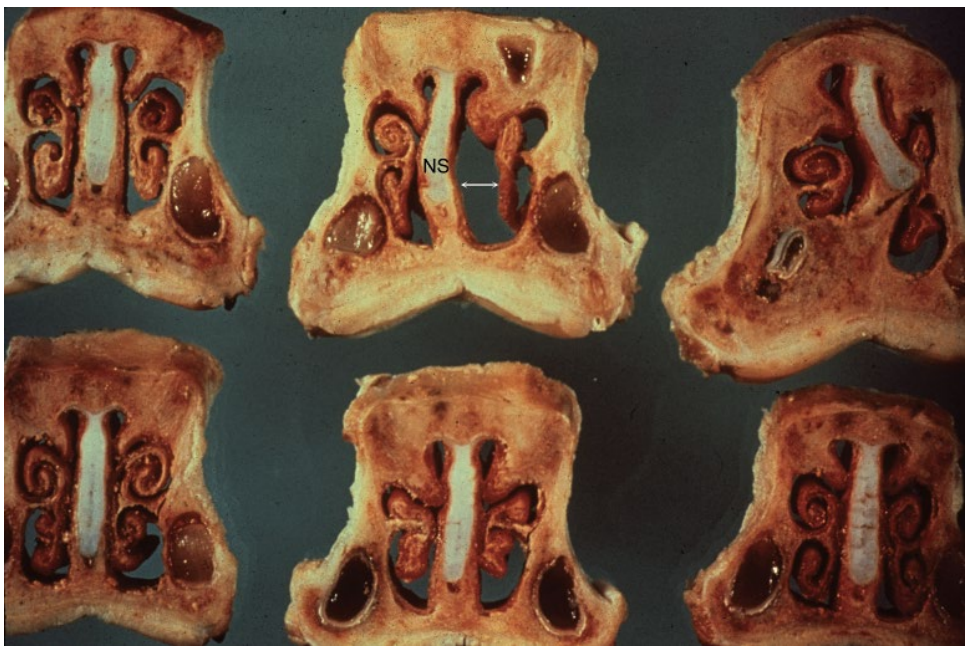


Figure 21.4 Gross lesions of atrophic rhinitis in swine. The nasal septum (NS) is distorted, and turbinates are shrunken and misshapen, resulting in increased airspace (arrow) in the nasal cavity.

pneumonia suggests that there is or was a bacterial septicemia. Acute sepsis is not uncommon with bacteria such as *Actinobacillus suis*, where multifocal hemorrhages and necrosis are suggestive of acute embolic pneumonia. Subacute and chronic lesions are often the result of bacterial infection and emboli arising from another location, and attempts should be made to identify the initial sources of infection, which may include navel infections, skin infections (ear or tail necrosis), abscesses from other sites (liver, subcutaneous, joints), and vegetative valvular endocarditis.

While most of the lung abscesses in pigs are due to pyogenic bacteria (e.g. streptococci, staphylococci, *Trueperella*), mycobacteriosis (*Mycobacterium tuberculosis* complex and *Mycobacterium avium* complex) is a recognized cause of nodular lesions in the lung and thoracic lymph nodes in some parts of the world (Gómez-Laguna et al. 2010; Mohamed et al. 2009).

Healing of pneumonia

The healing, repair, and restitution period, and probability of complete resolution, depends greatly on the agents involved. Uncomplicated viral diseases such as swine influenza can cause bronchiolitis and a mild interstitial pneumonia that can heal in 2–3 weeks with complete resolution (no visible lesions) remaining in the lung. Bronchopneumonia usually takes much longer to heal and may result in lung fibrosis or pleural adhesions to the chest wall or both. In specific-pathogen-free (SPF) pigs inoculated with *M. hyopneumoniae*, lung lesions were healed after 2 months, but pleural adhesions and fissures persisted for more than 3 months (Kobisch et al. 1993; Sørensen et al. 1997). Pattison (1956) identified lesions of pneumonia up to 25 weeks after inoculation with *M. hyopneumoniae*, presumably due to secondary bacterial infection.

Pleuritis

In swine, pleuritis is most commonly associated with hematogenous dissemination of bacteria to serosal surfaces, such as *H. parasuis*, *S. suis*, or *M. hyorhinis* (Kim et al. 2010; Palzer et al. 2015), or an extension from an underlying bacterial bronchopneumonia or abscess. Hematogenous dissemination of bacteria to serosal surfaces can lead to an acute diffuse fibrinous pleuritis, as well as polyserositis. Often, this can be without an accompanying frank pneumonia and may result in acute mortality. If the animal survives, fibrin deposited on serosal surfaces will gradually become organized, leading to the development of chronic fibrous adhesions between the lung and chest wall, which is one of the most frequent lesions observed in swine at slaughter. If fibrous adhesions are extensive to diffuse at slaughter and are accompanied by epicardial/pericardial fibrosis or fibrous adhesions between abdominal viscera, pleuritis/polyserositis likely resulted from hematogenous dissemination of bacteria and implicates agents such as *H. parasuis*, *S. suis*, *and M. hyorhinis*. If the chronic fibrous adhesions are confined to the cranioventral portions of the lung or are associated with abscesses or underlying sequestered or encapsulated foci of necrosis, these chronic lung lesions are likely secondary to a prior bronchopneumonia. Enoe et al. (2002) demonstrated that herds seropositive for *A. pleuropneumoniae* serotype 2 and *M. hyopneumoniae* had chronic pleuritis and pneumonia in 51 and 29% of the pigs at slaughter, respectively.

Control

PRDC results from a combination of primary and opportunistic infectious agents, often facilitated by adverse environmental and management conditions. The primary principles for herd-level control of respiratory diseases are to (1) eliminate or reduce pathogen load (diminish the infection pressure), (2) minimize negative influences on the pig's respiratory defenses, and (3) maximize the pigs' resistance. Issues to consider to accomplish these goals include (1) the environment; (2) pig flow, age segregation, and commingling; (3) biosecurity; (4) pathogen elimination; and (5) vaccination.

Environment

Dust and gases inside swine buildings can contribute to the development of respiratory disease by negatively impacting the respiratory defenses. Dust is made up primarily of feed particles, but dried fecal material, containing bacteria and bacterial toxins such as lipopolysaccharide (LPS), also contributes, along with hog dander, molds, pollen, insect parts, mineral ash, and bedding material in certain housing systems. Ammonia is released by bacterial action on urine and feces. Studies have documented that the level of air contaminants, including dust, ammonia, and microbes, correlated with pneumonia and pleuritis in swine (Donham 1991; Michiels et al. 2015).

Since dust is made up largely of feed particles, liquid feeding has the potential to reduce dust during handling and feeding. Removal of particulates by electrostatic particle ionization has been shown to reduce the quantity of airborne infectious agents, significantly improve average daily gain, and decrease mortality (Alonso et al. 2016; Rademacher et al. 2012). Good ventilation and robust cleaning practices can improve indoor air quality by reducing exposure to air pollutants and respiratory pathogens (McClendon et al. 2015). As expected, low ventilation rates are negatively correlated with the concentration of indoor gases, while gaseous contaminants decrease as

the ventilation rates increase (Kim and Ko 2007). Dust levels in excess of 3.7 mg m^{-3} for pigs are reported to have a negative health impact on both humans and pigs in swine production facilities (Donham et al. 2002; Schenker et al. 1998). Maximal safe concentrations of airborne pollutants have been proposed on the basis of dose–response correlation to swine and human health problems: dust 2.4 mg/m^3, ammonia 7 ppm, LPS (endotoxin) 0.08 mg/m^3, total microbes 105 cfu/m^3, and carbon dioxide 1540 ppm (Donham 1991).

Airflow and climate

The goal of good ventilation is to achieve an effective compromise between removal of airborne pollutants and pathogens without inducing stress caused by drafts and chilling. Cold drafts and wide temperature differentials stress the immune system, increasing susceptibility to disease. When weaned pigs were exposed to cold drafts, their immune response was significantly reduced (Scheepens et al. 1988). Prolonged cold stress in suckling piglets experimentally infected with *P. multocida* resulted in lowered levels of serum IgG, lowered phagocytic activity of the polymorphonuclear granulocytes, and delayed local cellular reaction in the lungs of some of the piglets compared with controls (Rafai et al. 1987).

A higher incidence of pneumonia occurs in pigs during cold seasons than summer months, indicating that climatic factors can influence respiratory disease (Agostini et al. 2014; Bille et al. 1975; Maes et al. 2001a). Season of the year can also influence the prevalence of specific primary pathogens. A low minimum outdoor temperature was positively associated with the probability of being *M. hyopneumoniae* positive at the piglet level (Segalés et al. 2012; Vangroenweghe et al. 2015).

Management

Management practices can influence the development of respiratory disease by impacting stress levels, pathogen exposure and dose, and the transmission of primary respiratory pathogens. Identification of potential environmental or management problems may by themselves control the transmission or susceptibility to disease. Problems with overcrowding, ventilation, or temperature control need to be addressed because they cause stress or damage the respiratory tract. Filling barns with pigs from a single source helps eliminate the introduction of new pathogens that often occurs when pigs from different sources are mixed. Optimally, herd management practices should be implemented that decrease the transmission of pathogens. Practices such as multisite production (separating pigs by stage of production), all-in/all-out production (moving pigs in and out of a building as a group with disinfection between groups), isolation and acclimatization of new breeding stock (isolation to avoid bringing new diseases in and acclimatization to expose animals to agents already on the farm), segregated early weaning with or without the addition of medication (diminish transfer of agents from dam to offspring), and basics in biosecurity have helped control infectious disease outbreaks and led to the establishment of higher health herds. Management practices that keep a herd relatively free from pathogens do carry an inherent risk. Lack of adaptive immunity makes these animals more vulnerable to the introduction of new diseases.

Management factors including increased herd size, increased stocking density, and farrow-to-finish operations have been associated with an increased risk of lung lesions at slaughter, while implementing an all-in-all-out system has been associated with a decreased risk of lung lesions at slaughter (Cleveland-Nielsen et al. 2002; Enoe et al. 2002; Meyns et al. 2011; Stärk et al. 1998).

Control of primary respiratory pathogens

Respiratory disease in pigs is a complex multifactorial disease associated with respiratory pathogens that are often found in combination in pneumonic lungs (Palzer et al. 2008). Methods used to reduce or eliminate primary respiratory pathogens vary widely and are covered in individual pathogen chapters. The spread of respiratory diseases from herd to herd involves two potentially distinct mechanisms. First, respiratory pathogens may be disseminated through infectious contacts (purchase of pigs, incoming and outgoing vehicles, birds, rodents, persons, flies, semen, etc.) (Schurrer et al. 2004; Swenson et al. 1994). Second, airborne pathogens can spread over long distances, resulting in rapid and widespread exposure among individual pigs and between farms.

Airborne transmission of respiratory diseases between herds

Respiratory pathogens in swine that can be transmitted over distances up to several kilometers include *M. hyopneumoniae* (Dee et al. 2010b; Goodwin 1985; Stark et al. 1992), PRCV (Henningsen et al. 1988), foot-and-mouth disease virus (Gloster et al. 2003), PRRS (Dee et al. 2010; Mortensen et al. 2002), and PRV (Mortensen et al. 1990). Airborne transmission between small pig units at close range can be experimentally induced with PRRSV, *A. pleuropneumoniae*, and *B. bronchiseptica* (Brockmeier and Lager 2002; Kristensen et al. 2004a,b; Torremorell et al. 1997). The typical pattern of simultaneous influenza outbreaks in many herds is highly suggestive of the airborne transmission of this virus (Madec et al. 1982). Factors affecting the risk of a

herd contracting an airborne infection are increasing herd size (Anderson et al. 1990; Flori et al. 1995; Mortensen et al. 1990), short distances between herds and large-sized neighboring herds (Flori et al. 1995; Stark et al. 1992), high regional pig density (Stark et al. 1992), and herds infected with *A. pleuropneumoniae* (Anderson et al. 1990).

Viruses can be labile and susceptible to varied environmental conditions (Jacobs et al. 2010; Van Alstine et al. 1993). However, airborne spread of disease between herds may be facilitated by several meteorological factors – most significantly the direction and velocity of the prevailing winds. Factors such as cloud cover, turbulence, and topography are also important. Overcast skies, night (when the turbulence is often low), and high relative humidity facilitate airborne transmission (Gloster et al. 1981).

Long-distance aerosol transmission of primary respiratory pathogens may render routine biosecurity measures ineffective. However, filtering incoming air has the potential to diminish this method of transmission. High-efficiency particulate air (HEPA) filtration has been shown to reduce or prevent long-distance transmission. Though airborne pathogens should be able to pass through HEPA filters, the particulates on which they are carried are removed by HEPA filtration, eliminating long-distance aerosol transmission (Dee et al. 2010).

Transmission of infection between age groups

Although most large swine production facilities use all-in/all-out and multisite systems, farrow-to-finish operations still exist. Commingling age groups, continuous flow of animals through the system, or even holding a few animals over between groups can lead to a steady transmission of primary respiratory pathogens from older to younger pigs. Replication of respiratory pathogens may concentrate in the facilities for growing pigs, which act as "pathogen generators" in the herd. From here the breeding animals can be periodically infected.

Commingling and new introductions to a herd

The mixing of two pig populations carrying different pathogens results in a sudden rise in infectious disease pressure, jeopardizing the established herd balance between infection and immunity. The risk of a herd contracting respiratory problems increases with the number of animal groups introduced and the number of different sources (Agostini et al. 2014; Castryck et al. 1990; Jorsal and Thomsen 1988). Documenting a herd's health status regarding respiratory pathogens, in particular the presence or absence of PRRSV, *A. pleuropneumoniae*, and *M. hyopneumoniae*, and assuring acquisition of pigs of a similar status can help prevent outbreaks of respiratory disease. Establishment of quarantine facilities for new breeding animals may help reduce pathogen exposure by identifying carrier or diseased animals prior to actual entry into the herd. Biosecurity is essential to prevent unwanted respiratory pathogens from entering the facilities by other routes.

Biosecurity

Biosecurity is paramount to sustaining healthy pigs (Amass 2005a,b). Strict hygiene and disinfection controls over vehicles, equipment, and personnel entering the farm will help reduce the introduction of pathogens and minimize transmission between herds. Barn workers should understand that some bacterial and viral diseases may spread from humans with respiratory illnesses to healthy pigs (Keenliside et al. 2010; Nielsen and Frederiksen 1990). Breeding stock should be quarantined, tested, and treated or vaccinated appropriately for respiratory pathogens prior to introduction to the herd.

Other factors that are important in maintaining a clean herd include personnel training to identify the earliest signs of respiratory disease, proper nutrition, reduction of nonrespiratory pathogens, separating pigs with solid barriers between different age groups if housed in the same airspace, maintaining appropriate stocking density to avoid overcrowding, maintaining proper temperature and airflow in the barn, minimizing ammonia and dust in the air, and ensuring appropriate sanitation between groups of pigs.

Pathogen elimination

Different techniques are used to eliminate specific diseases and can be found under specific disease sections. In general, methods include depopulation/repopulation or modified depopulation techniques; segregated early weaning; strict age segregation, especially with multisite separation of ages; medicated early weaning; test with removal of sick/infected pigs; and strategic vaccination and medication.

Vaccination

Studies have demonstrated that vaccination against a variety of primary respiratory pathogens, including *M. hyopneumoniae*, PRRSV, PCV2, and IAV, alone or in combination, can decrease the incidence and severity of gross and microscopic lung lesions, increase average daily gain, decrease tissue pathogen load, reduce mortality rate, and reduce time to market (Bourry et al. 2015; Fachinger et al. 2008; Jeong et al. 2016; Vincent et al. 2010). Harnessing the pigs' adaptive immune response to

enhance host defenses against these agents can diminish the negative impact of these organisms on respiratory defenses and decrease the incidence and severity of PRDC. Conversely, vaccination induces stress, which can be detrimental to the pig. A thorough understanding of the primary pathogens that are involved in each herd, when lactogenic immunity has waned sufficiently to allow for an active immune response, the time during which these pathogens impact the population, and the efficacy of various vaccines are vital in determining the most effective way to utilize vaccination to enhance pig health.

Diagnosis and monitoring of respiratory disease

The purpose of diagnosing and monitoring respiratory diseases is to identify clinical and subclinical infections that can affect production. Farm inspections, routine slaughter checks, serosurveys, and postmortem examinations of dead or euthanized animals are often the basis for diagnosing and estimating the severity of respiratory disease. A haphazard approach to diagnostics is typically inefficient and ineffective. Diagnostics should be undertaken with clear objectives, and a testing strategy developed to accomplish these goals.

If addressing underlying environment and management issues does not successfully bring respiratory disease under control, identifying the primary pathogens that are involved can assist in developing targeted control strategies. Since there is great overlap in clinical signs and pathologic lesions in PRDC, diagnosing the causes of respiratory disease is based on herd history, clinical observations, necropsies, gross and microscopic lesions, and laboratory testing (Andrews et al. 1986).

Monitoring of respiratory disease

Once the primary underlying agents have been identified and an intervention strategy devised (see individual disease sections), a monitoring plan should be implemented to measure the impact. This can be challenging as most systems are plagued by a high degree of normal variation, including seasonal variation. Statistical analysis, charting performance trends, and multiple replications are often necessary to properly determine the impact of an intervention strategy.

Slaughter checks of market weight pigs can identify chronic lung lesions or respiratory diseases that are active in the late stages of production. Labor concerns, including the fast line speed of modern slaughter facilities, have made slaughter inspections less practical. Cross-sectional and longitudinal serology and, sometimes, nasal swabs have increasingly replaced slaughter checks to determine the incidence, prevalence, and location of specific pathogens on farms (Andreasen et al. 2000, 2001; Chiers et al. 2002; Maes et al. 2001b). Serosurveys may be more effective than slaughter checks to detect subclinical infections that may affect some production parameters (Regula et al. 2000). Oral fluids (saliva and mucosal transudate) obtained from cotton ropes suspended in pens has been successfully used to detect agents and antibodies to some respiratory pathogens (Kittawornrat et al. 2010; Prickett et al. 2008; Strait et al. 2010). These minimally invasive techniques may be easy and efficient ways to monitor several respiratory diseases.

Slaughter checks: Examining the snout for atrophic rhinitis

Slaughter checks for atrophic rhinitis are usually performed by examining a transverse section of the snout (see Figures 21.1 and 21.4). Optimal results are obtained if the cut is placed between premolars 1 and 2 (Martineau-Doizé et al. 1990). Several methods of scoring atrophic rhinitis have been used (Bäckström et al. 1985; Straw et al. 1983). These methods are based on subjective and visual assessment of structures. Results from different slaughter checks should be compared with caution, as demonstrated by D'Allaire et al. (1988). Comparisons should be performed by the same experienced observer using the same scoring system. Morphometric techniques to quantify the degree of atrophic rhinitis have been developed (Gatlin et al. 1996). These yielded highly reproducible results but may be less practical for field use.

Slaughter checks: Examining thoracic viscera (plucks)

The progression and regression of pneumonia in growing pigs is highly dependent on the type and severity of pneumonia (Morrison et al. 1985; Noyes et al. 1988; Wallgren et al. 1994). Slaughter examinations of the respiratory tract may not detect subclinical diseases or diseases affecting young pigs that heal without scarring (Regula et al. 2000). Careful slaughter checks of thoracic viscera cannot normally be performed at the slaughter line. The material needs to be transferred to an appropriate location for thorough visual examination and palpation.

Slaughter checks supply useful information on the prevalence and severity of chronic lung lesions in a group, and the pattern of lesions provides insight into presumed underlying causes. Chronic consolidation and/or pleuritis of the cranioventral lung is presumed to represent enzootic pneumonia associated with *M. hyopneumoniae* infection and a secondary bacterial bronchopneumonia (Garcia-Morante et al. 2016). Fibrous

pleural adhesions in the dorsocaudal lung correlate with herd *A. pleuropneumoniae* seropositivity (Merialdi et al. 2012). Diffuse pleural adhesions identified in conjunction with epicardial/pericardial adhesions are indicative of a prior bacterial polyserositis due to agents such as *H. parasuis*, *S. suis*, and *M. hyorhinis*.

The percentage of pneumonia is usually based on the percentage of lung surface that is abnormally firm and discolored or has fibrous adhesions (Garcia-Morante et al. 2016) and can be given a numeric score (Christensen et al. 1999; Madec et al. 1982) or sketched onto a standard diagram of the lung followed by image analysis of the diagrams to determine the overall lung score. Some slaughter-check diagrams include more detailed information about the lung, such as the type of pneumonia, pleuritis, and adhesions, plus evaluations of the liver and heart (Christensen et al. 1999). Visual-only evaluations of digital images of lungs obtained at slaughter may be an effective alternative to drawings (Baysinger et al. 2010). In most situations slaughter checks should be performed on at least 30 pigs (with similar or known age if possible) to get a reliable picture of the herd problem (Straw et al. 1989). Chronic lung and pleural lesions present at the time of slaughter can provide valuable information regarding the health conditions on the farm and the effectiveness of intervention strategies.

References

Agostini PS, Fahey AG, Manzanilla EG, et al. 2014. *Animal* 8:1312–1318.

Alonso C, Raynor PC, Davies PR, et al. 2016. *Aerobiologia (Bologna)* 32:405–419.

Amass SF. 2005a. Biosecurity: Stopping the bugs from getting in. *Pig J* 55:104–114.

Amass SF. 2005b. Biosecurity: Reducing the spread. *Pig J* 56:78–87.

Amass SF, Clark LK, Van Alstine WG, et al. 1994. *J Am Vet Med Assoc* 204:102–107.

Anderson PL, Morrison RB, Molitor TW, et al. 1990. *J Am Vet Med Assoc* 196:877–880.

Andreasen M, Nielsen JP, Willeberg P, et al. 2000. *Prev Vet Med* 12:221–235.

Andreasen M, Mousing J, Krogsgaard Thomasen L. 2001. *Prev Vet Med* 52:147–161.

Andrews JJ, Schwartz KJ, Daniels GN, et al. 1986. *Vet Clin North Am Food Anim Pract* 2:159–172.

Bäckström L, Hoefling DC, Morkoc AC. 1985. *J Am Vet Med Assoc* 187:712–715.

Bak P, Havn KT, Bagger J, et al. 2008. The presence of respiratory pathogens in finisher farms impacts on performance. In Proceedings, International Pig Veterinary Society, p. 105.

Baskerville A. 1981. *N Z Vet J* 29:235–238.

Baysinger A, Polson D, Philips R, et al. 2010. Visual-only evaluation of lung lesions as an alternative to palpation at necropsy. In Proceedings, International Pig Veterinary Society, p. 659.

Bille N, Larsen JL, Svendsen J, et al. 1975. *Nord Vet Med* 27:482–495.

Bourry O, Fablet C, Simon G, et al. 2015. *Vet Microbiol* 180:230–236

Bradley R, Wrathall AE. 1977. *J Pathol* 122:145–151.

Brockmeier SL, Lager KM. 2002. *Vet Microbiol* 89:267–275.

Brockmeier SL, Halbur PG, Thacker EL. 2002. Porcine respiratory disease complex. In Brogden KA, Guthmiller JM, eds. *Polymicrobial Diseases*. Washington, DC: ASM Press, pp. 231–258.

Buddle JR, Mercy AR, Skirrow SZ, et al. 1997. *Aust Vet J* 75:274–281.

Bustamante-Marin XM, Ostrowski LE. 2017. Cilia and mucociliary clearance. *Cold Spring Harb Perspect Biol* 9(4):1–17.

Castryck F, Devriese LA, Hommez J, et al. 1990. Bacterial agents associated with respiratory disease in young feeder pigs. *Proc Int Pig Vet Soc* 11:112.

Chiers K, Donne E, Van Overbeke I, et al. 2002. *Vet Microbiol* 85:343–352.

Chiou MT, Jeng CR, Chueh LL, et al. 2000. *Vet Microbiol* 71:9–25.

Christensen LS, Mortensen S, Botner A, et al. 1993. *Vet Rec* 132:317–321.

Christensen G, Sørensen V, Mousing J. 1999. Diseases of the respiratory system. In Straw BE, D'Allaire S, Mengeling WL, et al., eds. *Diseases of Swine*, 8th ed. Ames, IA: Iowa State University Press, pp. 927–928.

Cleveland-Nielsen A, Nielsen EO, Ersbøll AK. 2002. *Prev Vet Med*. 55:121–135.

Curtis SE, Anderson CR, Simon J, et al. 1975. *J Anim Sci* 41:735–739.

D'Allaire S, Bigras-Poulin M, Paradis MA, et al. 1988. Evaluation of atrophic rhinitis: Are the results repeatable? *Proc Int Pig Vet Soc* 10:38.

Dee S, Otake S, Deen J. 2010a. *Virus Res*, 154: 177–184

Dee S, Otake S, Corzo C, et al. 2010b. Long distance airborne transport of viable PRRSV and MHP from a swine population infected with multiple viral variants. In Proceedings, International Pig Veterinary Society, p. 153.

Donham KJ. 1991. *Am J Vet Res* 52:1723–1730.

Donham KJ, Thorne PS, Breuer GM, et al. 2002. Exposure limits related to air quality and risk assessment. Iowa Concentrated Animal Feeding Operations Air Quality Study (Final Report).

Enoe C, Mousing J, Schirmer AL, et al. 2002. *Prev Vet Med* 54:337–349.

Fachinger V, Bischoff R, Jedidia SB, et al. 2008. *Vaccine* 26:1488–1499.

Flori J, Mousing J, Gardner IA, et al. 1995. *Prev Vet Med* 25:51–62.
Foged NT. 1992. Pasteurella multocida toxin. *APMIS Suppl* 25:1–56.
Fort M, Fernandes LT, Nofrarias M, et al. 2008. *Vet Immunol Immunopathol* 129:101–107.
Ganter M, Kipper S, Hensel A. 1990. Bronchoscopy and bronchoalveolar lavage of live anaesthetized pigs. *Proc Int Pig Vet Soc* 11:109.
Garcia-Morante B, Segalés J, Fraile L, et al. 2016. *J Comp Pathol* 154:125–134.
Gatlin CL, Jordan WH, Shryock TR, et al. 1996. *Can J Vet Res* 60:121–126.
Giesbrecht GG. 1995. *Aviat Space Environ Med* 66:890–902.
Gloster J, Blackall RM, Sellers RF, et al. 1981. *Vet Rec* 108:370–374.
Gloster J, Champion HJ, Sorensen JH, et al. 2003. *Vet Rec* 152:525–533. Erratum in: Vet Rec 152:628.
Gómez-Laguna J, Carrasco L, Guillermo R, et al. 2010. *J Vet Diagn Invest* 22: 123–127.
Gómez-Laguna J, Salguero FJ, Pallarés FJ, et al. 2013. *Vet J* 195:148–155.
Goodwin RFW. 1985. *Vet Rec* 116:690–694.
Gottschalk M.. 2012. Actinobacillosis. In *Diseases of Swine* 10th ed. Wiley-Blackwell, pp. 653–669.
Grandia J, Berges AC, Falceto MV. 2010. Swine respiratory diseases in Aragon (Spain): economic, pathology, and microbiological study during the year 2008. In Proceedings, International Pig Veterinary Society, p. 648.
Hamilton TD, Roe JM, Hayes CM, et al. 1998. *J Clin Microbiol* 36:1260–5.
Hansen MS, Pors SE, Jensen HE, et al. 2010. *J Comp Path* 143:120–131.
Henningsen D, Mousing J, Aalund O. 1988. *Dansk Vet Tidsskr* 71:1168–1177.
Hillen S, von Berg S, Köhler K, et al. 2014. *Prev Vet Med* 113:580–8.
Horiguchi Y. 2012. *Curr Top Microbiol Immunol* 361:113–129.
Jacobs AC, Hermann JR, Muñoz-Zanzi C, et al. 2010. *J Vet Diagn Invest* 22:257–260.
Jager HJ, McKinley TJ, Wood JL, et al. 2010. A tool to assess the economic impact of pleurisy in slaughter pigs. In Proceedings, International Pig Veterinary Society, p. 191.
Jeong J, Park C, Choi K, et al. 2016. *Vet Microbiol* 182:178–86
Jolie R, Olson L, Backstrom L. 2000. *J Vet Diagn Invest* 12:4
Jorsal SE, Thomsen BL. 1988. *Acta Vet Scand (Suppl)* 84:436–437.
Keenliside JM, Wilkinson C, Forgie S, et al. 2010. Pandemic H1N1 influenza virus infection in a swine herd. In Proceedings, International Pig Veterinary Society, p. 254.
Kim K, Ko H. 2007. *Environ Res* 103:352–357.
Kim D, Han K, Oh Y, et al. 2010. *Can J Vet Res* 74:314–6.
Kittawornrat A, Prickett JR, Main R, et al. 2010. Surveillance using oral fluid samples – PRRSv, SIV, PCV2, and TTV field data. In Proceedings, International Pig Veterinary Society, p. 661.
Knetter SM, Tuggle CK, Wannemuehler MJ, et al. 2014. *Vet Immunol Immunopathol* 157:20–30.
Kobisch M, Blanchard B, Le Potier MF. 1993. *Vet Res* 24:67–77.
Kristensen CS, Angen O, Andreasen M, et al. 2004a. *Vet Microbiol* 98:243–249.
Kristensen CS, Botner A, Takai H, et al. 2004b. *Vet Microbiol* 99:197–202.
Liljegren CH, Aalbaek B, Nielsen OL, et al. 2003. *APMIS* 111:531–538
López A. 2007. Respiratory system. In McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*, 4th ed. St. Louis, MO: Elsevier, pp. 463–558.
Loving CL, Brockmeier SL, Vincent AL, et al. 2010. *Microb Pathog* 49:237–245.
Lowe JF, Husmann R, Firkins LD, et al. 2005. *J Am Vet Med Assoc* 226:1707–1711.
Madec F, Gourreau JM, Kaizer C. 1982. *Epidemiol Sante Anim* 2:56–64.
Maes D. 2010. *Mycoplasma hyopneumoniae* infections in pigs: update on epidemiology and control. In Proceedings, International Pig Veterinary Society, pp. 30–35.
Maes D, Deluyker H, Verdonck M, et al. 2001a. *Vet Rec* 148:41–46.
Maes D, Chiers K, Haesebrouck F, et al. 2001b. *Vet Res* 32:409–419.
Martineau-Doizé B, Larochelle R, Boutin J, et al. 1990. Atrophic rhinitis caused by toxigenic *Pasteurella multocida* type D: morphometric analysis. *Proc Int Pig Vet Soc* 11:63.
McClendon CJ, Gerald CL, Waterman JT. 2015. *Curr Opin Allergy Clin Immunol* 15:137–144.
Merialdi G, Dottori M, Bonilauri P, et al. 2012. *Vet J* 193:234–9.
Meyns T, van Steelant J, Rolly E, et al. 2011. *Vet J* 187: 388–392.
Michiels A, Piepers S, Ulens T, et al. 2015. *Prev Vet Med* 121:99–107.
Mizgerd JP. 2002. *Semin Immunol* 14: 123–132.
Mohamed AM, El-Ella GA, Nasr EA. 2009. *J Vet Diagn Invest* 21: 48–52.
Møller K, Kilian M. 1990. *J Clin Microbiol* 28:2711–2716.
Morrison RB, Hilley HD, Leman AD. 1985. *Can Vet J* 26:381–384.
Mortensen S, Mousing J, Henriksen CA, et al. 1990. Evidence of long distance transmission of Aujeszky's disease virus. II: Epidemiological and meteorological investigations. *Proc Int Pig Vet Soc* 11:279.
Mortensen S, Stryhn H, Sogaard R, et al. 2002. *Prev Vet Med* 53(1–2):83–101.
Neumann R, Leonhardt W, Ballin A, et al. 1985. *Arch Exp Vet Med* 39:525–534.
Neumann R, Mehlhorn G, Buchholz I, et al. 1987. *J Vet Med B* 34:241–253.
Neumann EJ, Kliebenstein JB, Johnson CD, et al. 2005. *J Am Vet Med Assoc* 227:385–392.
Nicod, J 2005, Lung defences: An overview. *Eur Respir Rev* 14: 45–50.

Nielsen JP, Frederiksen W. 1990. Atrophic rhinitis in pigs caused by a human isolate of toxigenic *Pasteurella multocida*. *Proc Int Pig Vet Soc* 11:75.
Noyes EP, Feeney D, Pijoan C. 1988. A prospective radiographic study of swine pneumonia. *Proc Int Pig Vet Soc* 10:67.
Ohgami M, Doershuk CM, English D, et al. 1989. *J Appl Physiol* 66:1881–1885.
Ostanello F, Dottori M, Gusmara C, et al. 2007. *J Vet Med A Physiol Pathol Clin Med*. 54:70–75.
Palzer A, Ritzman M, Wolf G, et al. 2008. *Vet Rec* 162:267–271
Palzer A, Haedke K, Heinritzi K, et al. 2015. *Can Vet J* 56:285–287
Pattison IH. 1956. *Vet Rec* 68:490–494.
Pointon AM, McCloud P, Heap P. 1985. *Aust Vet J* 62:98–101.
Pol JM, van Leengoed LA, Stockhofe N, et al. 1997. *Vet Microbiol* 55:259–264.
Prickett J, Simer R, Christopher-Hennings J, et al. 2008. *J Vet Diagn Invest* 20:156–163.
Rademacher C, Bradley G, Pollmann S, et al. 2012. Electrostatic particle ionization (EPI) improves nursery pig performance and air quality. In Proceedings of the AASV, pp. 257–258.
Rafai P, Neumann R, Leonhardt W, et al. 1987. *Acta Vet Hung* 35:211–223.
Reams RY, Glickman LT, Harrington DD, et al. 1994. *J Vet Diagn Invest* 6:326–334.
Regula G, Lichtensteiger CA, Mateus-Pinilla NE, et al. 2000. *J Am Vet Med Assoc* 217:888–895.
Renukaradhya GJ, Alekseev K, Jung K, et al. 2010. *Viral Immunol* 23:457–466.
Sales TP, Soares P, Brito WM, et al. 2010. In Proceedings, Interenational Pig Veterinary Society, p. 620.
Scheepens CJM, Tielen MJM, Hessing MJC. 1988. Influence of climatic stress on health status of weaner pigs. In Ekesbo I, ed. Proceedings, 6th International Animal Hygiene Congress (Environment and Animal Health), Skara, Sweden. Vol. 2. pp. 543–547.
Schenker MB, Christiani D, Cormier Y. 1998. *Am J Resp Crit Care Med* 158:S1–S76.
Schurrer JA, Dee SA, Moon RD, et al. 2004. *Am J Vet Res* 65:1284–1292.
Segalés J, Valero O, Espinal A, et al. 2012. *Int J Biometeorol*, 56:1167–1171
Sørensen V, Ahrens P, Barfod K, et al. 1997. *Vet Microbiol* 54:23–34.
Stark KD, Keller H, Eggenberger E. 1992. *Vet Rec* 131:532–535.
Stärk KDC, Pfeiffer DU, Morris RS. 1998. *N Z Vet J* 46: 3–10.
Strait E, Roe C, Levy N, et al. 2010. Diagnosis of *Mycoplasma hyopneumoniae* in growing pigs. In Proceedings, International Pig Veterinary Society, p. 139.
Straw BE, Bürgi EJ, Hilley HP, et al. 1983. *J Am Vet Med Assoc* 182:607–611.
Straw BE, Tuovinen VK, Bigras-Poulin M. 1989. *J Am Vet Med Assoc* 195:1702–1706.
Swenson SL, Hill HT, Zimmermann JJ, et al. 1994. *J Am Vet Med Assoc* 204:1943–1948.
Thacker BJ, Strait EL, Kesl LD. 2010. Comparison of mycoplasmal lung lesion scoring methods. In Proceedings, International Pig Veterinary Society, p. 144.
Thanawongnuwech R, Thacker B, Halbur P, et al. 2004. *Clin Diagn Lab Immunol* 11:901–908.
Torremorell M, Pijoan C, Janni K, et al. 1997. *Am J Vet Res* 58:828–832.
Tsai YC, Jeng CR, Hsiao SH, et al. 2010. *Vet Res* 41:60.
Vahle JL, Haynes JS, Andrews JJ. 1995. *J Vet Diagn Invest* 7:476–80.
Van Alstine WG, Kanitz CL, Stevenson GW. 1993. *J Vet Diagn Invest* 5:621–622.
Vangroenweghe FA, Labarque GG, Piepers S, et al. 2015. *M Vet J* 205:93–97.
Vincent AL, Ciacci-Zanella JR, Lorusso A, et al. 2010. *Vaccine* 28:2782–2787.
Wallgren P, Beskow P, Fellström C, et al. 1994. *J Vet Med B* 41:441–452.

22

Urinary System

Richard Drolet

Anatomy

The kidneys of swine are bean shaped, smooth, brown, elongated, flattened dorsoventrally, and at least twice as long as wide. At the middle of the medial border of each kidney is an indentation, the hilus, where the vessels, nerves, and ureter communicate with the organ. The kidneys are located ventral to the psoas muscles at the level of the first four lumbar vertebrae. Contrary to most species, the left kidney of pigs usually is situated cranially to the right. In the adult, the ratio of the combined weight of the kidneys to that of the body is about 0.50–0.66% (Sisson 1975).

The kidneys are enveloped by a rather thin fibrous capsule that can be easily peeled off. In a kidney section, the relative surface occupied by the cortex and the medulla is readily apparent (Figure 22.1). Pigs have multipyramidal or multilobar kidneys but without the external lobation typically found in the bovine species. The medullary portion of each lobe is called a pyramid; some are simple, whereas others are compound, that is, formed by the fusion of two or more primitively separate pyramids. The pale apical portion of a pyramid, called the papilla, projects into the renal pelvis or its ramifications; the latter are referred to as calyces. Papillae of simple pyramids are generally narrow and conical, whereas those of compound pyramids, often located in the area of the renal poles, are broad and flattened. There are 8–12 papillae per kidney. Collecting ducts of the kidneys have their openings at the tips of the papillae.

The ureters, which are continuous with the renal pelvis, leave the kidneys in a sharp caudal curve and reach the dorsolateral sides of the bladder neck area. In newborn piglets, the length of the portion of the ureter running beneath the bladder mucosa is about 5 mm, whereas it reaches a mean length of about 35 mm in the adult (Carr et al. 1990). The intravesical portion of the ureters acts as a valve that prevents vesicoureteral reflux of urine.

The urinary bladder of the pig is large and has a long neck. When full, it lies well down into the abdominal cavity. The bladder is supported by one median (ventrally located) and two lateral ligaments. The urethra of the adult female is about 7–8 cm long, and its external ostium is located ventrally, at the junction of the vagina and vestibule; beneath it is a small depression, the suburethral diverticulum. In the male, a urethral recess is present near the ischial arch in the area where the ducts of the bulbourethral glands empty into the urethra (Garret 1987). These fold-like structures represent a significant barrier to urethral catheterization. The urethra finally opens into a slit-like structure at the tip of the penis.

Physiology

Histophysiology

The kidney is involved in many vital functions: the elimination of waste products from the body, the conservation of water, and the regulation of the acid–base balance and electrolyte composition. In addition, it has an endocrine function, producing a variety of hormones including erythropoietin, renin, prostaglandins, and vitamin D_3. The function units of the kidney are called nephrons, each consisting of a renal corpuscle, proximal tubule, loop of Henle, and distal tubule, which form the bulk of the renal parenchyma. The renal corpuscle comprises the glomerulus, a tuft of arterial capillaries, and Bowman's capsule. The kidney of a pig contains well over 1 million nephrons. Newborn piglets have immature kidneys, and nephrogenesis continues during the first 3 months of life (Friis 1980).

The first mechanism used to accomplish renal function is glomerular filtration, where the volume of plasma filtered depends on the renal perfusion, blood pressure, and integrity of the glomerulus itself. The glomerular filtrate is an ultrafiltrate of blood plasma that contains

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

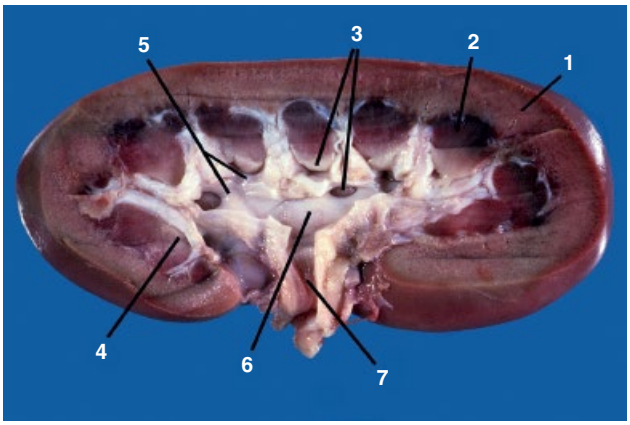


Figure 22.1 Section of the kidney from a feeder pig. (1) Cortex, (2) medulla, (3) papillae, (4) compound papilla, (5) calyces, (6) pelvis, and (7) ureter.

water, glucose, salts, ions, amino acids, and small amounts of protein of low molecular weight. The tubular components of the nephron modify glomerular filtrate as homeostasis processes dictate. The proximal tubules are lined by well-developed and metabolically active epithelial cells that can actively reabsorb 100% of the filtered glucose and many other substances, such as water, sodium, amino acids, albumin, and bicarbonate, in significant amounts (Banks 1993). Pigs reabsorb very few urates from glomerular filtrate compared with most other species. Tubular secretion complements glomerular filtration in clearance of certain waste products. The filtrate finally enters the collecting ducts, where it may be further concentrated. Urine expelled into the pelvis normally is not modified as it travels through the rest of the lower urinary tract, which is lined with transitional epithelium (urothelium).

Urine

The volume of urine produced daily depends on several variables, including diet, fluid intake, ambient temperature and humidity, and the size and weight of the animal. Accurate data on the normal ranges of the amount of urine excreted per day in pigs are limited. Salmon-Legagneur et al. (1973) reported a mean urinary output of 9 L and 5.3 L/day in gestating and lactating sows, respectively. Other factors, such as the water distribution system used, may also affect the production of urine if they influence drinking behavior.

The mean specific gravity of urine in adult swine is about 1.020 (plasma >1.010), one of the lowest found in domestic animals (Ruckebusch et al. 1991). Young animals have even lower values. Specific gravity of urine is usually inversely related to urine volume. Urinary pH is usually between 5.5 and 7.5. It is influenced by the metabolism and the composition of the feed; starvation or a high protein intake lowers urinary pH. Urinary infection with urea-splitting bacteria (e.g. *Actinobaculum suis*) may result in a significant alkalinization of urine.

A small amount of protein passes through the glomeruli and is usually reabsorbed by proximal tubules. The presence of proteinuria is of diagnostic significance but should be interpreted in conjunction with the specific gravity. Significant proteinuria occurs with glomerulonephritis (GN) (increased permeability to protein), tubular necrosis (decreased reabsorption of protein), pyelonephritis (inflammation), and lower urinary tract inflammation. The presence of protein in the urine is, however, not always pathologic because transient physiological or functional proteinuria also occurs in some instances. Proteinuria is expected in the first few days of life because glomeruli are permeable to colostral proteins found in high levels in the blood. Transient proteinuria can occur following excessive physical activity (e.g. transport) or intense stress or when excessive amounts of protein are ingested.

The color of urine is usually yellow to amber depending on the concentration of urochromes. Abnormal coloration of urine is observed with some underlying urinary tract diseases. Urine sediment examination can be informative, particularly for inflammatory diseases.

Impaired renal function

In some pathological situations, renal function is impaired so much that renal failure ensues. Renal failure may have a prerenal (e.g. any condition that reduces renal blood flow), postrenal (e.g. obstructive uropathy), or primary renal origin (e.g. extensive renal parenchymal disease).

Renal failure can cause metabolic acidosis, electrolyte imbalances, and intravascular accumulations of various metabolic waste products, including blood urea nitrogen (BUN) and creatinine. Determination of both BUN and serum creatinine concentration may be used to assess the renal function. The serum creatinine concentration is a more accurate index of the glomerular filtration rate than the BUN because it is less dependent on nonrenal factors. Concentrations of BUN may rise in animals with high dietary protein intake or in any conditions resulting in increased protein catabolism. Friendship et al. (1984) reported normal range values for BUN and serum creatinine in weaned and feeder pigs, gilts, and sows. In sows, for example, the mean BUN concentration has been reported as 5.3 mmol/L (n = 102) (Friendship et al. 1984) and 5.0 mmol/L (n = 120) (McLaughlin and McLaughlin 1987), whereas the mean serum creatinine concentrations reported in the same two studies are 160 and 186 µmol/L, respectively.

Developmental anomalies

Anomalies of development occur in all body systems, and the urinary tract is no exception. These anomalies may involve the kidneys as well as the lower urinary tract. Many of these conditions in swine are relatively rare and of little economic significance. Few of these malformations in pigs are common, and only rarely are they associated with clinical signs. In some instances, developmental anomalies have been shown to be inherited.

Malformations of the kidneys

Most of the well-characterized renal anomalies of development occurring in domestic animals have also been documented in pigs. Depressions of the external surface and partial persistence of fetal lobation of the kidneys have been reported to be relatively common in Norwegian slaughtered pigs (Jansen and Nordstoga 1992). Unilateral renal agenesis (aplasia) occurs sporadically in pigs and is relatively common compared with other domestic animals (von Höfliger 1971). Bilateral renal agenesis is incompatible with life and would be encountered in the fetus or stillborn piglet. Cases of bilateral renal agenesis and renal hypoplasia have been described in pigs and linked to a genetic cause (Cordes and Dodd 1965; Mason and Cooper 1985). Malposition of the kidneys (renal ectopia) is not rare and is often characterized by caudal displacement of one kidney, often the left, to the pelvic area (Sisson 1975). Duplication of one kidney has been observed on a number of occasions in pigs (Nieberle and Cohrs 1967). Horseshoe kidney, rarely observed in swine, is a condition in which the kidneys have fused at either the cranial or the caudal poles, resulting in a horseshoe-shaped organ (Nieberle and Cohrs 1967). Renal dysplasia, a disorganized development of renal parenchyma due to anomalous differentiation, also is relatively rare. Some cases have been attributed to hypovitaminosis A (Cianciolo and Mohr 2016).

Congenital renal cysts are frequent in various species but are more common in swine (Figure 22.2). The presence of one or a few cysts in the kidney is a common incidental finding at abattoirs, and the affected organs are generally condemned. The cystic cavities are filled with serous fluid and vary from a few millimeters in diameter to larger than the organ itself. They are commonly found in the cortex and often protrude from the surface of the organ, where they may appear translucent or opaque depending on the amount of fibrous connective tissue present in the cyst wall. Histologically, these cysts are lined with a layer of tubular epithelial cells surrounded by a fibrous capsule.

Polycystic kidney disease, another form of congenital cysts, occurs far less frequently. It is characterized by the presence of numerous smaller cysts that occupy a large portion of the renal parenchyma; cystic bile ducts may be found in the liver as well. Affected piglets usually die from renal failure during the neonatal period (Webster and Summers 1978). The distinction between simple renal cysts and polycystic kidney disease is not always clear. Wells et al. (1980) reported a prevalence of renal cysts of nearly 50% from a single herd that experienced an abnormally high rate of kidney condemnations at the abattoir where affected kidneys had extremes in variation of numbers and sizes of cysts. Further investigation showed that affected animals were the progeny of a Landrace boar and disease was found to be inherited as an autosomal dominant trait, the number of cysts being determined by polygenic inheritance (Wijeratne and Wells 1980).

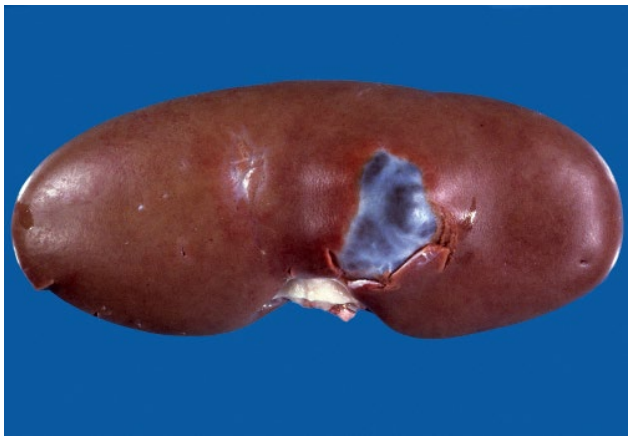


Figure 22.2 Congenital renal cyst in a slaughtered sow.

Malformations of the lower urinary tract

Developmental anomalies of the lower urinary tract appear to be rare in swine. Cases of duplication of the ureter (Benko 1969), persistent urachus (Weaver 1966), and congenital ureteral occlusion (Nieberle and Cohrs 1967) have been reported in pigs. A case of congenital urethral stenosis has also been described recently (Pouleur-Larrat et al. 2014).

Circulatory disturbances

Circulatory disturbances may occur in the urinary tract as well as in any other tissues of the body. Some of these disturbances of the circulation produce lesions that may be of diagnostic significance on postmortem inspection.

Hemorrhage

Hemorrhages, in the form of widespread petechiae or less commonly ecchymoses, may be found in any part of the kidney or lower urinary tract in various septicemic

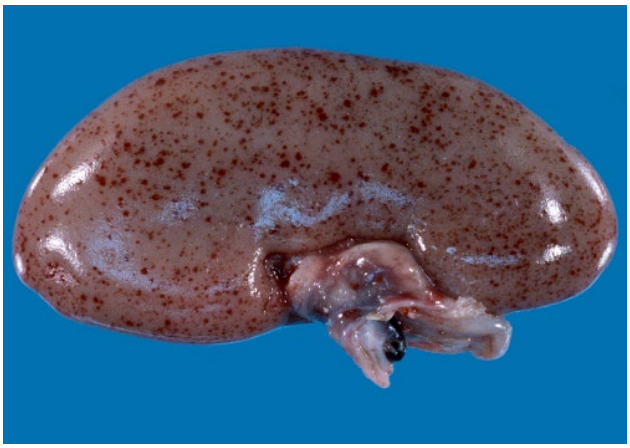


Figure 22.3 Widespread petechiae in the kidney of a piglet with *Streptococcus suis* septicemia.

illnesses (Figure 22.3). Bacterial infections commonly associated with these lesions include septicemia due to *Salmonella*, streptococci, *Erysipelothrix rhusiopathiae*, and *Actinobacillus* spp. These lesions are also seen in acute viral diseases such as classical swine fever, African swine fever, or systemic cytomegalovirus infection (Orr et al. 1988). Petechiation of the renal cortex is also observed occasionally in acute GN, in some acute intoxications, and in electrocuted animals. Neonates normally have dilated, congested glomerular tufts within an otherwise normal renal cortex that erroneously can be interpreted as petechiae.

Larger intrarenal or subcapsular hemorrhages are usually caused by trauma, necrosis, or clotting defect, including poisoning by anticoagulant rodenticides. They may also occur in some cases of disseminated intravascular coagulation. Widespread hemorrhages in almost all body systems, including the urinary tract, are a striking pathologic feature in suckling piglets with isoimmune thrombocytopenic purpura due to passively transferred antiplatelet antibody from the dam's colostrum (Andersen and Nielsen 1973; Dimmock et al. 1982). In this relatively common disease, affected piglets appear normal at birth but eventually die between 1 and 3 weeks of age from hemorrhagic diathesis.

Infarction

Renal infarcts, infrequently found in the kidneys of swine, are localized areas of ischemic coagulative necrosis produced by the occlusion of the renal artery or one of its tributaries. The localization and size of the infarct reflect the area vascularized normally by the involved blood vessel. The occlusion is usually due to thrombosis or aseptic emboli (the consequences of septic emboli are discussed under embolic nephritis). In some instances, infarction of the renal parenchyma is primarily associated with renal vasculitis (Jansen and Nordstoga 1992), including polyarteritis nodosa (Nieberle and Cohrs 1967). Bilateral renal cortical necrosis is seen on rare occasions in pigs and is considered to be the result of an infarct of a major part of the cortex of both kidneys (Häni and Indermühle 1980). The reaction is characterized by disseminated intravascular coagulation with a marked tropism for the small arterial blood vessels of the renal cortex. The etiopathogenesis of these lesions remains unclear, but the condition has been associated with septicemia, endotoxemia, and hemorrhagic shock due to bleeding gastric ulcers.

Glomerular diseases

Renal diseases that involve primarily the glomeruli include amyloidosis and GN. Renal amyloidosis has been rarely reported in pigs (Cianciolo and Mohr 2016; Jakob 1971). GN represents an important category of renal diseases in animals, and in recent years this condition has been recognized with increasing frequency in swine.

Glomerulonephritis

Inflammatory changes in renal glomeruli may take place via a number of mechanisms, including immunologic, thrombotic, toxic, and as yet uncharacterized mechanisms. Most cases of GN in humans and animals are thought to be immune mediated. The main types of glomerular immunologic injury recognized are trapping of circulating antigen–antibody complexes, *in situ* immune complex formation, activation of the alternate pathway of complement, and cell-mediated processes (Spargo and Taylor 1988). Because immunoglobulins and complement components are frequently found in inflamed glomeruli, antibody-mediated injury has received the most attention. In veterinary medicine, the various morphologic types of immune-mediated GN are commonly classified as membranous, proliferative, mesangioproliferative, membranoproliferative, and exudative.

Although the pathogenesis of GN is now reasonably well understood, knowledge of the etiology or triggering event is still unknown in most cases (idiopathic immune-mediated GN). Theoretically GN may be triggered by a variety of factors including drugs, chemicals, food allergens, endogenous antigens, and infectious agents (Drolet et al. 1999).

GN is not often diagnosed in swine but does occur occasionally as a sporadic event (Bourgault and Drolet 1995; Cianciolo and Mohr 2016; Nieberle and Cohrs 1967; Slauson and Lewis 1979). It has also been reported as a sequel to chronic infectious diseases such as classical swine fever, African swine fever (Choi and Chae 2003; Hervas et al. 1996; Martin-Fernandez et al. 1991),

systemic cytomegalovirus infection (Yoshikawa et al. 1988), and group A streptococcal abscesses (Morales and Guzman 1976). In these cases, the resulting GN appears to be caused by the presence of glomerular immune complexes in which the antigen is related to the agent responsible for the underlying disease (Slauson and Lewis 1979). Nutritionally induced GN has also been reported, the apparent result of ingestion of a protein-rich by-product (Elling 1979) and ingestion of "smut fungus" contained in the feedstuff (Müller 1977).

An inherited renal disease, classified morphologically as membranoproliferative GN type II, has been described in Yorkshire piglets from Norway (Jansen and Nordstoga 1994). It is caused by an autosomal recessive deficiency of the complement inhibitory protein "factor H" (Hegasy et al. 2002; Jansen et al. 1995). Deficiency of factor H ultimately provokes activation of the alternate pathway of complement, with subsequent massive deposition of complement in renal glomeruli, large intramembranous dense deposits, and mesangial hypercellularity (Jansen et al. 1998).

In many species, generalized or focal GN is also observed in some cases of systemic vasculitis (mainly immune-mediated vasculitis). In pigs, the best example of this certainly is porcine dermatitis and nephropathy syndrome (PDNS). The condition, first described in the United Kingdom in 1993 (Smith et al. 1993; White and Higgins 1993), has been subsequently observed in most parts of the world. The disease affects nursery and growing pigs and, less commonly, breeding animals (Drolet et al. 1999). The prevalence of the syndrome in affected herds is usually less than 1% although higher prevalences have been detected in the United Kingdom and other countries, with case mortality in affected herds ranging from 0.25 to over 20% (Segalés et al. 2005).

Affected animals present a systemic necrotizing vasculitis with marked tropism for the skin and kidneys (Hélie et al. 1995; Smith et al. 1993; Thibault et al. 1998). Vascular lesions in the skin provoke a conspicuous dermatopathy (Figure 22.4). Kidney lesions in acute cases include exudative and occasionally necrotizing GN with or without cellular crescent formation (crescentic GN). Interstitial nephritis and renal vasculitis are also commonly observed. Vascular lesions in other tissues vary considerably in frequency and distribution in individual pigs (Thomson et al. 2002). In some atypical cases, there may be cutaneous lesions without renal lesions and vice versa. An animal with GN without any other vascular lesions elsewhere should probably not be diagnosed as PDNS since other glomerular diseases not related to this syndrome are known to occur in pigs.

In PDNS, the glomerular and systemic vascular damages are thought to be immune mediated, possibly through a type III hypersensitivity reaction, which is characterized by deposition of antigen–antibody aggregates or immune complexes within these sites (Hélie et al. 1995; Sierra et al. 1997; Smith et al. 1993; Thibault et al. 1998; Thomson et al. 2002; Wellenberg et al. 2004). Over the years several bacterial and viral pathogens, including porcine reproductive and respiratory syndrome (PRRS) virus and porcine circovirus type 2 (PCV2), have been incriminated as possible etiologies for PDNS (Opriessnig et al. 2007). Observations from the last decade support an association between PCV2 and PDNS. The precise way by which this virus promotes directly or indirectly the development of PDNS remains unknown at this time. It has been shown that animals with PDNS often have relatively low PCV2 viral loads (Olvera et al. 2004) but very high PCV2 antibody titers (Wellenberg et al. 2004). However, PDNS has been observed recently in a pig challenged with PCV2 during a vaccination trial, and the animal had low IgG titers against PCV2, but the IgM response was not measured (Opriessnig et al. 2017). Other hypotheses on the role of PCVs in triggering PDNS include PCV3 infection and a misdirected excessive immune response toward a decoy epitope called CP (169–180) located in ORF2 of PCV2 (Opriessnig et al. 2017).

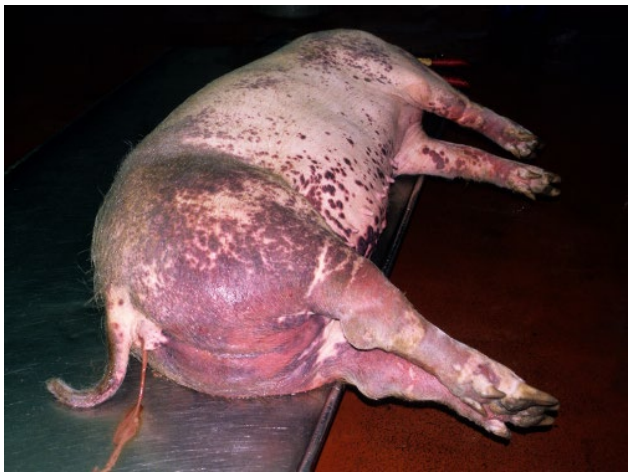


Figure 22.4 A white sow affected with PDNS. Note multifocal to coalescent erythematous macules and typical caudal distribution.

Other examples of glomerular disease/lesion in swine have been reported in the literature in recent years (Carrasco et al. 2003; Jansen and Nordstoga 1992; Pace et al. 1998; Shirota et al. 1984, 1995; Tamura et al. 1986; Vezzali et al. 2011; Yoshie 1991).

The clinical significance of GN is highly variable, with a spectrum ranging from a subclinical condition to a fulminating and rapidly fatal disease (Bourgault and Drolet 1995; Shirota et al. 1986). Mortalities are commonly recorded in pigs affected with PDNS (Hélie et al. 1995; Kavanagh 1994; Segalés et al. 1998; Smith et al. 1993; Thomson et al. 2002; White and Higgins 1993). In this latter condition, the survival of the affected pigs depends

on the extent and severity of the vascular lesions in the internal organs, particularly within the kidneys. The hereditary GN of Norwegian Yorkshire pigs appears to be invariably fatal; affected piglets die of renal failure within 11 weeks of birth (Jansen et al. 1995).

Unlike most other domestic animals affected with GN (with the exception of the familial glomerulopathies), pigs appear to be affected at a relatively young age. The condition has been described most commonly in weaned and feeder pigs (1.5–6 months old), occasionally in breeding animals, and rarely in nursing piglets. Clinical signs, when present, may include anorexia, lethargy, unwillingness to move, subcutaneous edema, rapid loss of condition, and death.

GN is rarely recognized clinically because most of the signs are nonspecific, and analysis of urine and blood from an individual is rarely considered of practical value in the herd medicine approach applied in our large units of production. Pigs affected with PDNS are often easier to detect clinically because of the presence of hemorrhagic and necrotizing skin lesions, mainly located on the hind limbs and perineal area (Segalés et al. 2003). Pigs with GN may show concomitant hypoproteinemia, hypoalbuminemia, and persistent proteinuria, which are highly suggestive of a protein-losing glomerulopathy. The urine protein/creatinine ratio may also be increased (Hélie et al. 1995). Proteinuria, hematuria, and pyuria, which are usually compatible with lower urinary tract lesions, may also occur in severe types of GN, namely, in acute exudative GN. Blood of affected animals may also reveal elevated urea and creatinine levels suggestive of renal failure (Drolet et al. 1999; Hélie et al. 1995; Jansen et al. 1995; Thomson et al. 2002; White and Higgins 1993).

The gross lesions of GN will depend largely on the severity of the glomerular lesions and the stage (acute vs. chronic) of the disease process. In acute GN, the kidneys may be slightly to markedly enlarged, pale, and edematous, often with cortical petechiation (Figure 22.5). The most important differential diagnoses to consider for such acutely affected kidneys are bacterial septicemia, acute viremia, and intoxications. With time, the surface of the kidneys may become finely granular, and in the chronic phase of the disease, the organs may appear shrunken and contracted due to progressive cortical fibrosis, which is difficult to distinguish from other chronic renal diseases. Perirenal and subcutaneous edema and serous effusions in body cavities may be observed in some cases of GN, and high prevalence of gastric ulcers has been reported with GN (Bourgault and Drolet 1995; Jansen 1993; Kavanagh 1994; White and Higgins 1993).

Treatment of GN is usually symptomatic since disease is not often diagnosed in live animals under normal farm-raising conditions. Pigs affected with PDNS have been treated with various antimicrobial agents, anti-inflammatory drugs, and multivitamin supplements with no significant effect (Segalés et al. 2003).

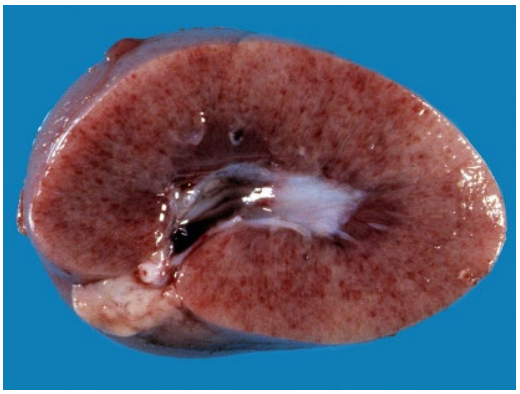


Figure 22.5 Acute glomerulonephritis in a grower pig. Note the edematous and finely petechiated cortical parenchyma. *Source:* Bourgault and Drolet 1995. Reproduced with permission of the American Association of Veterinary Laboratory Diagnosticians.

Tubular diseases

Renal diseases characterized primarily by degenerative changes of the tubular epithelium of the nephrons may occur under certain circumstances, where the epithelial lining cells of the tubules undergo degeneration, necrosis, and sloughing of the cells into the lumen. Acute tubular necrosis is termed nephrosis, which represents an important cause of acute renal failure in animals. The epithelial cells of the proximal tubules, because of their high metabolic activity, are especially susceptible to damage caused by prolonged ischemia or nephrotoxins, the two main causes of this type of nephropathy.

Ischemic tubular necrosis

Ischemic tubular necrosis is generally the result of a severe and prolonged period of hypotension associated with shock of endotoxic, hypovolemic, cardiogenic, or neurogenic origin (Cianciolo and Mohr 2016). These renal lesions are potentially life threatening, and the clinical signs of the resulting renal failure are often hidden by the marked systemic effects of the primary disease responsible for the state of shock.

Nephrotoxic tubular necrosis

Nephrotoxic tubular necrosis has been documented in domestic animals in association with a wide variety of exogenous natural and synthetic compounds. These toxic substances may affect tubular function and ultimately cause cellular damage by several mechanisms,

including metabolic alterations affecting cellular respiration, interference with the tubular transport system, and damage to specific organelles (Brown and Engelhardt 1987). Plants, mycotoxins, antimicrobial drugs, heavy metals, ethylene glycol, and some other industrial compounds are potential nephrotoxins in swine, covered elsewhere in this book.

Several species of pigweed, particularly redroot pigweed (*Amaranthus retroflexus*), may cause acute renal failure in pigs, clinically characterized by weakness, incoordination, posterior paresis, and death (Osweiler et al. 1969).

Fungi of some species of *Aspergillus* and *Penicillium* produce nephrotoxins that can contaminate grains used as feedstuff. Ochratoxin A and citrinin are the most common nephrotoxic mycotoxins. Monogastric animals, particularly pigs, may develop significant disease when moldy feed containing ochratoxin A is ingested. Acute clinical signs are relatively rare; a subacute to chronic wasting disease is more commonly associated with this poisoning (Osweiler 1996).

Antibiotic-associated nephropathies are well documented in domestic animals. Classes of antibiotics considered potentially nephrotoxic include aminoglycosides, tetracyclines (or their degradation products), and sulfonamides. Predisposing factors associated with the toxicity of these agents include the dosage and the route of administration, the duration of the treatment, the solubility of the products, and the general health status (dehydration, shock, preexisting renal disease) of the animal.

Ethylene glycol, found in high concentration in older antifreeze solutions, is ingested, absorbed from the gastrointestinal tract, enzymatically oxidized in the liver, and successively transformed into several nephrotoxic compounds and finally to oxalate. Poisoning occurs in swine with the ingestion of 4–5 mL of ethylene glycol/kg of body weight (Carson 2006).

Outbreaks of melamine- and cyanuric acid-associated nephrotoxicity have been reported in recent years in animals, mainly dogs and cats, and human infants. In these cases, feedstuffs were adulterated with melamine and its analogues, including cyanuric acid, to falsely elevate their protein concentrations. A syndrome characterized by progressive weight loss, pallor, and high mortality rate due to renal failure has been reported in pigs with this intoxication (Nilubol et al. 2009). Many metallic compounds are nephrotoxic, including inorganic mercury, arsenic, cadmium, lead, thallium, and bismuth. Cases of poisoning with these products are relatively rare in pigs.

Gross renal lesions observed in acute cases of nephrotoxic tubular necrosis are not always conspicuous, but the kidneys may appear slightly swollen, pale, and moist (Figure 22.6). In pigweed (A. retroflexus) poisoning, these renal lesions are often accompanied by a marked perirenal edema that may contain blood and possibly by serous effusions elsewhere in the body (Osweiler et al. 1969). In severe acute tubular necrosis, death from acute renal failure may ensue. Animals that survive the acute phase of the disease either recover or develop progressive fibrosis of the kidneys that may or may not lead to chronic renal failure. This chronic evolution appears relatively common in pigs with ochratoxin A toxicosis (Cook et al. 1986; Krogh 1977; Rutqvist et al. 1978).

Figure 22.6 Pale and swollen kidney associated with acute tubular necrosis in a sow dead of ethylene glycol poisoning. A leaking valve on the water heating system was responsible for the poisoning of several animals.

Acute tubular necrosis is characterized histologically by swelling and necrosis of the lining epithelial cells of the proximal and distal tubules, the presence of granular casts in the tubular lumen, dilated tubules, and mild interstitial edema. Abundant calcium oxalate crystals within tubules are a characteristic finding in ethylene glycol poisoning, whereas round, yellow-brown crystals with radiating striations within tubules and collecting ducts are the hallmark of melamine and analogue poisoning. Subacute or chronic changes are epithelial regeneration and eventually, at least in some cases, interstitial fibrosis accompanied by focal loss of nephrons and a mild interstitial inflammatory infiltrate.

Tubulointerstitial diseases

Tubulointerstitial diseases include a relatively large group of conditions characterized primarily by interstitial inflammation and tubular damage, namely, interstitial nephritis (such as that occurring from leptospirosis), embolic nephritis, and pyelonephritis (one of the most significant urinary tract diseases in swine). Immunologically mediated tubulointerstitial disease as occurs in humans has only rarely been documented in domestic animals.

Interstitial nephritis

Many serovars of *Leptospira*, especially those of the serogroups Pomona and Australis for which pigs act as maintenance hosts, cause reproductive disturbance in swine including infertility, abortion, and birth of weak or dead piglets. The pathogenesis of the disease involves preferential localization and persistence of the organisms at sites physically protected from antibodies, such as in the ocular vitreous humor, the cerebrospinal fluid, the genital tract, and the lumen of the renal proximal tubules (Cianciolo and Mohr 2016). The passage of the leptospires from the bloodstream to the interstitial tissue of the renal parenchyma and finally to the tubular lumen elicits multifocal lesions of interstitial nephritis (Cheville et al. 1980). The severity of the interstitial nephritis varies and ranges from grossly undetectable to extensive lesions, particularly when serovars of the Pomona serogroup are involved. The lesions are randomly distributed and appear as poorly circumscribed whitish foci of various shapes and sizes, becoming confluent in severe cases. Histologically these foci correspond to the infiltration of lymphocytes, plasma cells, and macrophages in the interstitial tissue, along with some degenerative changes of the surrounding nephrons. In chronic cases, interstitial fibrosis occurs. In most cases these lesions are not extensive enough to cause renal failure, so the generally asymptomatic animal may shed the leptospires in urine for a relatively long period of time (Mitchell et al. 1966).

The association between lesions of interstitial nephritis in pigs and the detection of leptospires within these kidneys are highly variable among studies (Baker et al. 1989; Boqvist et al. 2003; Hunter et al. 1987; Jones et al. 1987; McErlean 1973). Factors that may influence these results include the serovar of *Leptospira* spp. involved, the methods of leptospiral detection used, the phase of the infection, and also the prevalence of leptospirosis and of other infectious causes of interstitial nephritis in swine in a given geographical area. For instance, the prevalence of leptospirosis in fattening pigs and sows in southern Vietnam was found to be high (Boqvist et al. 2002, 2003), whereas similar studies conducted in slaughtered pigs in southwestern Quebec have shown a very low prevalence of this infection (Drolet et al. 2002; Ribotta et al. 1999).

Severe multisystemic disease from PCV2 infection can and often does cause lymphohistiocytic to granulomatous lesions involving multiple tissues, including kidney (Segalés and Domingo 2002). The interstitial nephritis is in some cases grossly detectable as patchy pallor or obvious white foci within renal parenchyma (Figure 22.7). Histologic examination of these affected kidneys by Sarli et al. (2008) revealed tubulointerstitial nephritis with lymphocytes, plasma cells, macrophages, and multinucleated giant cells in variable proportions.

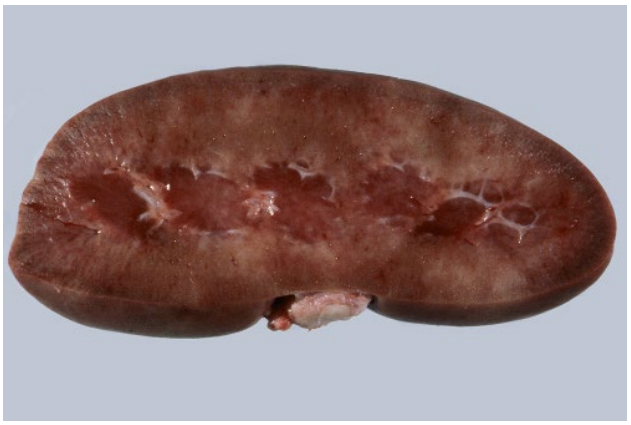


Figure 22.7 Interstitial nephritis in a pig naturally infected with porcine circovirus type 2.

Using an *in situ* hybridization technique, they also found that PCV2 nucleic acid detection was in general more frequent in tubular epithelial cells than in interstitial inflammatory cells. Renal lesions characterized by widespread necrosis and PCV2 infection of renal tubular epithelium with accompanying interstitial edema and hemorrhage (turkey-egg kidneys) have also been described (Imai et al. 2006).

Multifocal lesions of interstitial nephritis also occur in swine with other bacterial (see section "Embolic Nephritis") and viral hematogenous infections. Although lesions may not impair renal function, they are of diagnostic significance because they are suggestive of a systemic disease. Lesions of interstitial nephritis caused by systemic viral infections are often visible only by microscopy and are characterized by the presence of foci of nonsuppurative inflammation. Viral infections that may produce these lesions include cytomegalovirus (Kelly 1967), adenovirus (Nietfeld and Leslie-Steen 1993; Shadduck et al. 1967), PRRS virus, and possibly others. Multifocal lesions have been reproduced experimentally in PRRS virus-infected piglets examined 2–3 weeks post inoculation (Cooper et al. 1997; Rossow et al. 1995) and are also frequently found in naturally infected pigs.

Gross lesions of multifocal interstitial nephritis, often called white-spotted kidneys, represent a common cause of kidney condemnation at the slaughterhouse in some areas (Drolet et al. 2002). Lesions generally appear either as few randomly distributed or numerous widely disseminated whitish foci, 1–3 mm in diameter (Figure 22.8). Histologically these foci are composed of mononuclear inflammatory cells that often have a distinct lymphofollicular pattern (follicular nephritis) corresponding to the development of tertiary lymphoid structures within the kidneys. This type of interstitial reaction probably represents a nonspecific immunological response to prolonged local antigenic stimulation. In one study there

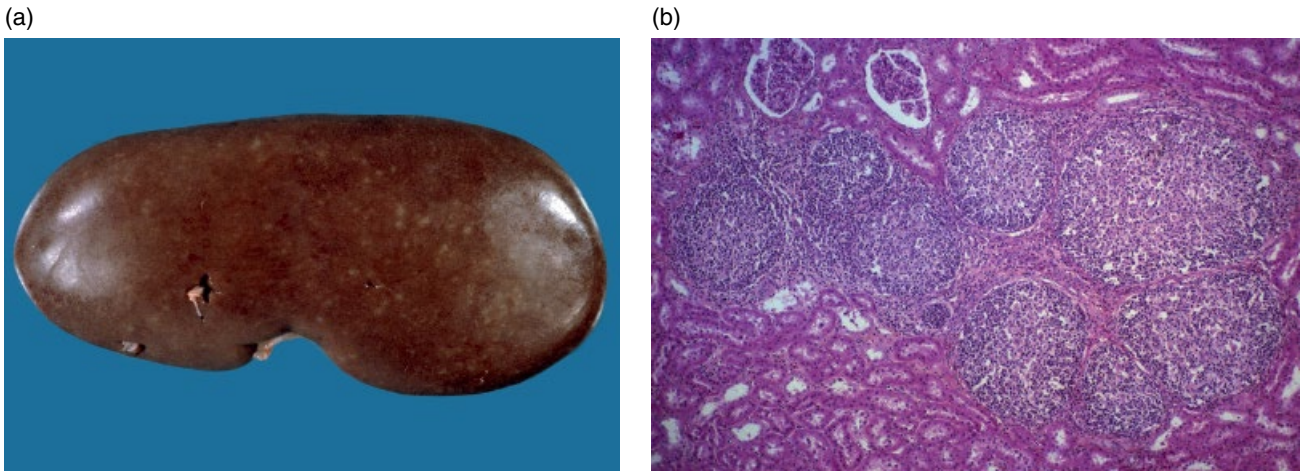


Figure 22.8 (a) Kidney from a slaughtered pig with multifocal interstitial nephritis. Note the relatively well-demarcated white dots randomly distributed throughout the cortex. (b) Well-demarcated focal areas of interstitial nephritis showing a distinct lymphofollicular pattern (follicular nephritis).

was a statistically significant association between the lesions and the presence of porcine parvovirus and PCV2, with a stronger association when both viruses were identified in the same kidney (Drolet et al. 2002). These renal lesions have also been observed in some cases of chronic leptospirosis (Pezzolato et al. 2012).

Embolic nephritis

Embolic nephritis may occur with bacteremia or septic thromboembolism when any of various species of bacteria is seeded within the vasculature of the kidneys. In bacteremia, small aggregates of microorganisms localizing in the renal microcirculation (particularly in the interstitial and glomerular capillaries) cause the formation of small suppurative foci. Early lesions appear grossly as small hemorrhagic foci bilaterally scattered throughout the renal cortex. They gradually form small (1–3 mm) whitish to yellowish abscesses that may be surrounded by a hyperemic rim (Figure 22.9). These lesions, although more numerous in the cortex, may also be found in the medulla. The finding of such renal lesions when performing a necropsy strongly suggests the possibility of a septicemia. In swine, infections with *Actinobacillus suis*, *Streptococcus* spp., *E. rhusiopathiae*, *Escherichia coli*, *Staphylococcus* spp., *Trueperella pyogenes*, and, more recently, *Actinobacillus equuli* (Benavente and Fuentealba 2012; Thompson et al. 2010) must be considered.

Septic thromboembolism occurs when fragments of a septic thrombus enter the bloodstream and occlude the arterial vasculature of the kidneys, resulting in necrosuppurative foci of variable sizes (Figure 22.10). Such renal lesions, if disclosed during a postmortem examination, should prompt a careful examination of the left cardiac valves (mitral and aortic) for the presence of vegetative

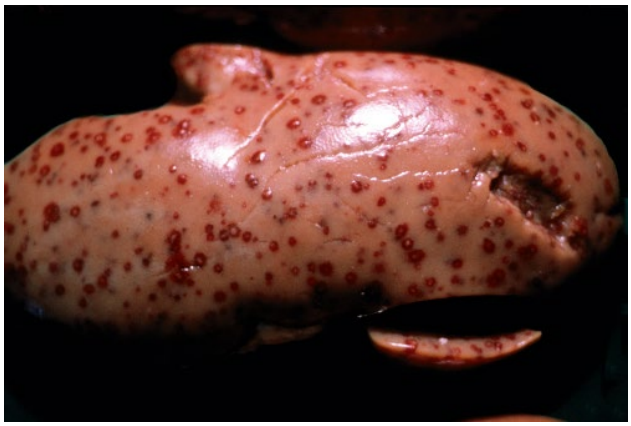


Figure 22.9 Embolic nephritis caused by *Actinobacillus suis*. Scattered suppurative foci are surrounded by a hemorrhagic rim.

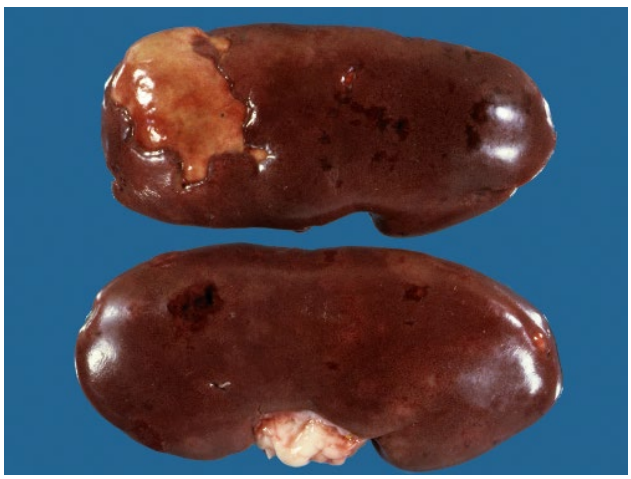


Figure 22.10 Septic thromboembolic nephritis secondary to *Streptococcus suis* endocarditis.

endocarditis. In these cases, bacteria most often involved include *Streptococcus* spp., *E. rhusiopathiae*, and *E. coli*.

Cystitis–pyelonephritis complex

Urine is formed by the kidneys and stored in the bladder by way of the ureters. Ascending infection from vagina and distal urethra to the sterile portions of the urinary tract may lead to cystitis and pyelonephritis. The cystitis–pyelonephritis complex has been documented as a leading cause of mortality in sows (D'Allaire and Drolet 2006). Porcine cystitis–pyelonephritis has been reported throughout the world, and the increased incidence appears to be correlated with changes in management, particularly the adoption of confinement housing for gestating sows.

A wide variety of bacteria have been isolated from cases of porcine cystitis and pyelonephritis, including *E. coli*, *T. pyogenes*, *Streptococcus* spp., and *Staphylococcus* spp. (Carr and Walton 1993). These endogenous and opportunistic organisms typically inhabit the lower urinary tract and are often referred to as being responsible for "nonspecific" urinary tract infections, which are reviewed in their respective chapters. *A. suis*, a specific urinary pathogen, is also an important cause of ascending infection in swine. Infection with *A. suis* frequently results in elevated sow mortality, and *A. suis* has been isolated, either alone or in combination with other bacteria, from nearly half of the reported cases of cystitis and pyelonephritis reviewed by Carr and Walton (1993).

The pathogenesis of the cystitis–pyelonephritis complex begins with the infection of the lower urinary tract. The normal complete voiding of urine is a major mechanism in maintaining sterility of the bladder; any factor that causes stasis of urine (decreased water intake, decreased urination, incomplete emptying of bladder, etc.) should be considered important in the development of this condition (Cianciolo and Mohr 2016). Environmental risk factors including the reduced availability of water, reduced consumption of poor quality water, high water pH, increased fecal contamination of the perineal area, excessive weight gain, or leg injuries all can result in a reduction in the frequency of urination and enhanced bacterial establishment in the urogenital tract. Advanced-parity sows are considered more susceptible to ascending infections possibly in part because older animals are more prone to obesity, limb injuries, and lack of exercise (D'Allaire and Drolet 2006). Once infection is established in the bladder (cystitis), deformation of the intravesical portion of the ureter and of ureteric orifices may facilitate vesicoureteral reflux, which is the retrograde flow of infected urine up the ureters and into the kidney (Cianciolo and Mohr 2016).

Clinical signs associated with infections of the urinary tract vary from asymptomatic, revealed only by urinalysis, to acute and severe cases of cystitis–pyelonephritis where affected animals may be found dead, probably from acute renal failure. Symptomatic animals are usually afebrile and may show anorexia, hematuria, and pyuria. The urine is often reddish brown in color with a strong odor. With *A. suis* infection, urinary pH may increase from normal values of 5.5–7.5 up to 8–9 due to the cleavage of urea into ammonia through the use of urease enzyme of the bacteria. Animals that survive the initial infection frequently experience weight loss and reduced productivity secondary to end-stage renal disease, resulting in premature removal from the breeding herd.

Grossly, the inflammatory reaction on the mucosal surface of the bladder may be catarrhal, hemorrhagic, purulent, or necrotic, and the bladder wall may be thickened. Struvites can also be found in the lumen. The ureters, often filled with exudate, may increase to as much as 2.5 cm in diameter.

Unilateral or bilateral pyelonephritis or pyelitis is the primary lesion detected in the kidneys. The pelvic region frequently distended with blood, pus, and foul-smelling urine often shows irregular ulceration and necrosis of the papillae. These suppurative lesions may eventually extend irregularly through the renal medulla and even into the cortex, causing exophytic and discolored deformations of the renal surface (Figure 22.11). These foci of cortical inflammation, when present, seem to occur more frequently at the renal poles (Isling et al. 2010). Compound papillae that are mainly located in these latter areas are considered more susceptible to intrarenal reflux of septic urine because of the inability of their papillary ducts to close under intrapelvic pressure (Carr et al. 1991; Carr and Walton 1993; Ransley and Risdon 1974). In long-standing cases of pyelonephritis, fibrosis ultimately replaces inflammation (Figure 22.11).

Microscopically, necrotizing ureteritis and pyelitis with accumulation of bacterial colonies can be seen (Woldemeskel et al. 2002). Renal tubules may contain protein casts, bacteria, and purulent exudate. The interstitium contains mononuclear inflammatory cells, neutrophils, and possibly some fibrosis. Examination of the ureteric valves may reveal inflammation, necrosis, and fibrosis.

Presumptive diagnosis of cystitis and pyelonephritis in live animals is best achieved when frequent micturition of bloodstained and cloudy urine can be observed. Examination of the urine sediments may also be very informative, because it may reveal the presence of inflammatory cells, erythrocytes, granular renal casts, bacteria, and crystals (Carr and Walton 1992). Blood concentrations of urea and creatinine may indicate renal failure. Due to the striking gross lesions, confirmatory diagnosis of the condition is usually not difficult.

Determination of urea concentration in ocular fluids can be a useful aid in diagnosing cystitis–pyelonephritis

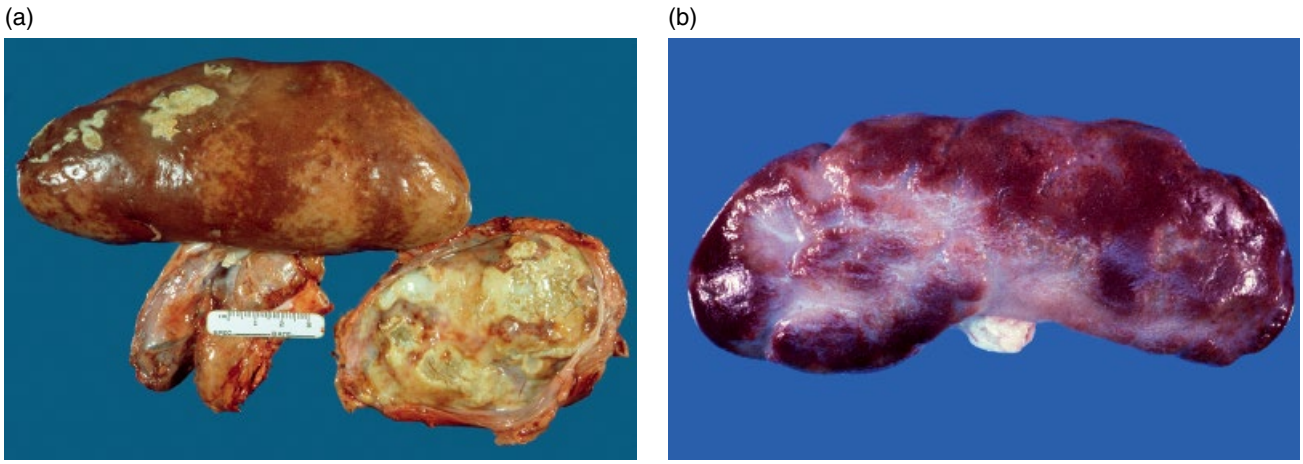


Figure 22.11 (a) Acute pyelonephritis due to *Actinobaculum suis* in a sow. Note the cortical foci of suppurative inflammation that have extended through the renal capsule (removed on the right). (b) Chronic pyelonephritis in a sow.

in dead animals, particularly when a complete necropsy is not possible or when it is difficult to ascertain that the lesions found in the urinary tract are responsible for death (Drolet et al. 1990). A significantly higher aqueous humor urea concentration was found in sows dead of cystitis–pyelonephritis (45–92 mmol/L) than in those dead of other causes (9–10 mmol/L) (Arauz and Perfumo 2000; Chagnon et al. 1991).

Treatment of urinary tract infections may be successful if the correct antibiotic is administered early in the course of the disease. Prevention of urinary tract infections should include the maintenance of a high degree of hygiene during breeding and parturition, as well as throughout the gestation period. Facilities need to be properly designed to reduce the spread of pathogens within the breeding herd and allow efficient removal of feces from the environment. It is recommended that free-choice water be available at all times to reduce the possibility of water deprivation and its sequels. Because a higher degree of urinary tract infection can be seen in older sows, proper culling procedures are important to ensure that an optimal parity distribution is maintained within the breeding herd.

Neoplasia

Neoplasms are infrequent in pigs because of the low average age of the population. However, those most often recorded have been from young animals (Nielsen and Moulton 1990). Tumors of the urinary tract in swine involve mainly the kidneys. Neoplasms of the lower urinary tract, although they have been reported (Nieberle and Cohrs 1967), are generally considered exceedingly rare.

Embryonal nephroma, also named nephroblastoma, is one of the most common neoplasms of swine and is certainly the most common primary renal tumor observed in this species, although its relative prevalence varies from one region to another. As its name implies, this neoplasm appears to originate from the embryonic renal blastema. The tumor arises from the kidney or, rarely, from the perirenal tissues (probably from remnants of embryonic renal tissues). Affected animals are typically young, and most of them reach market age without significant clinical signs, the tumor being discovered at postmortem inspection. Embryonal nephroma is most commonly found as a single mass involving one kidney, but it may be multiple or bilateral (Nielsen and Moulton 1990). The tumor, which can reach impressive size, often appears firm, pale, and nodular. Metastasis infrequently occurs in swine compared with other mammals affected with embryonal nephroma. Histologically, this tumor is very peculiar and resembles disorganized embryonic renal tissue. The primitive tissue from which it arises is pluripotent and accounts for the presence of neoplastic epithelial and mesenchymal elements simultaneously observed within the tumor. Hayashi et al. (1986) classified porcine nephroblastomas into four types according to their contents: nephroblastic, epithelial, mesenchymal, and miscellaneous. Only a few of the nephroblastic tumors described in their case series had metastasized.

Other primary renal tumors are believed to be uncommon in swine. Renal carcinomas have been occasionally reported (Anderson et al. 1969; Sandison and Anderson 1968). Neoplasms originating from the renal pelvis are very rare (Vitovec 1977).

Secondary renal involvement may occur with some multisystemic or generalized cancers such as the malignant lymphoma (lymphosarcoma). In pigs, this relatively common neoplasm occurs predominantly as multicentric and thymic forms. In advanced cases of multicentric

and thymic lymphomas, which involve primarily the lymph nodes and the thymus, respectively, infiltration of the liver, spleen, kidneys, and other organs may occur. Renal involvement is diffuse or more often nodular so that the organs appear either enlarged and pale or dotted with pale nodules often protruding from the cortical surface (Figure 22.12). During the disease, some animals may develop a leukemic phase. Renal lesions in some of these cases appear rather hemorrhagic (Marcato 1987; Stevenson and DeWitt 1973) and may be confused with some systemic infectious diseases (Figure 22.13). The precise pathogenesis of these latter lesions is uncertain but may involve either a coagulation defect or a phenomenon of acute infarction caused by the presence of intravascular neoplastic cells.

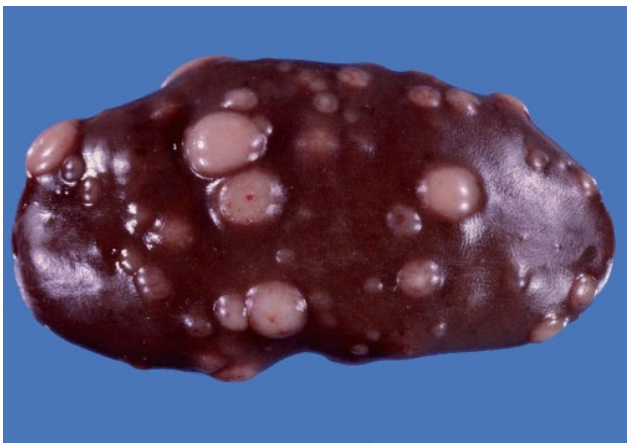


Figure 22.12 Lymphoma in a fattening pig. Multiple tumoral nodules in the kidney.

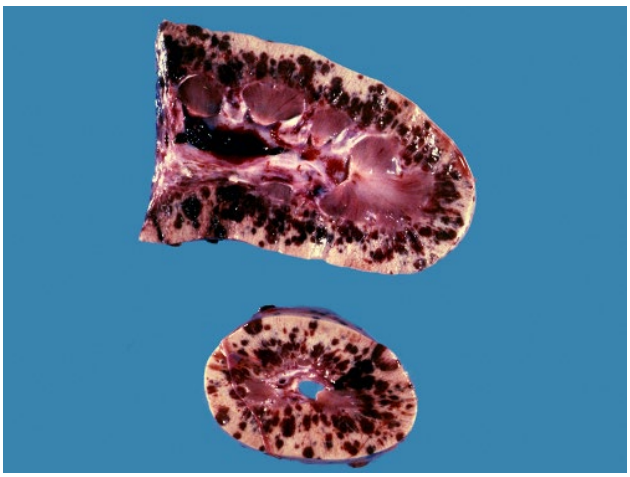


Figure 22.13 Leukemic lymphoma in a gilt showing multiple renal hemorrhagic foci. Histologically, these focal interstitial hemorrhages also contain neoplastic lymphoid cells.

Miscellaneous conditions

Urolithiasis

Urolithiasis is the presence of calculi, or uroliths, in the urinary passages. Uroliths are macroscopic mineral (polycrystalline) concretions that may contain small quantities of organic material; the term crystalluria is used for abnormal microscopic crystalloid precipitates in urine. Reports on the mineral composition of calculi found in pigs and their relative importance are relatively scarce (Osborne et al. 2009). Nevertheless, various types of calculi can be found, including calcium apatite (calcium phosphate), struvite (magnesium ammonium phosphate hexahydrate), calcium carbonate, and uric acid and urate. Factors known to predispose to the formation of uroliths include the diet, urinary pH, reduced water intake, urinary stasis, and preexisting urinary tract diseases.

Swine are rarely afflicted with urolithiasis in comparison with other domestic animals. The condition is sporadically found in pigs of all ages and is also occasionally observed as incidental findings in slaughtered pigs. Outbreaks of obstructive urolithiasis have been reported on some occasions (Inoue et al. 1977; Sim 1978; Smyth et al. 1986). In these outbreaks, which involved weaned and feeder pigs as well as breeding animals, the predisposing cause for the condition was not elucidated. Animals affected with obstructive urolithiasis may demonstrate decreased appetite, oliguria or anuria, abdominal distension and pain, and death from postrenal uremia. Ruptured bladder may also occur in some cases. Treatment of pigs with obstructive urolithiasis is theoretically feasible but is generally not considered cost effective.

The bladder of sows sometimes contains yellowish sediments that do not seem to be of clinical significance. On postmortem examination, such sediments, admixed with desquamated epithelial cells, may give the false impression of cystitis because of the turbidity of the urine. Infection-induced calculi are also observed occasionally in sows with cystitis and pyelonephritis (Figure 22.14).

Uric acid and urate uroliths are frequently found in the kidneys of newborn piglets (Figure 22.15). These often appear as fine orange precipitates in the medulla and pelvis. This peculiar form of urolithiasis is observed mainly in piglets that have no access to the sow's milk (which contains both fluids and nutrients) or are afflicted by a debilitating disease associated with anorexia and diarrhea (e.g. transmissible gastroenteritis), thus contributing to dehydration. Accelerated catabolism of tissue proteins and purines to supply energy needs and decreased kidney function related to dehydration are responsible for the high levels of blood urea and uric acid

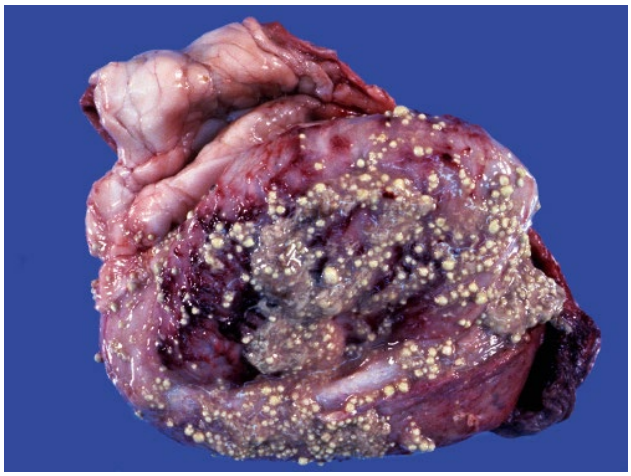


Figure 22.14 Infection-induced calculi in the bladder of a sow with a chronic suppurative cystitis.

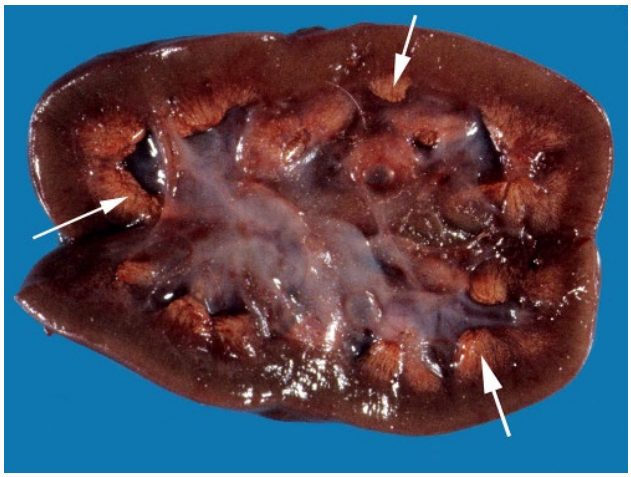


Figure 22.15 Urate calculi in the medulla (arrows) of a dehydrated suckling piglet.

found in these piglets. The excess solute, poorly reabsorbed from the glomerular filtrate, is ultimately deposited in the inner medulla and pelvis (Cianciolo and Mohr 2016).

Hydronephrosis

Distension of the renal pelvis and calyces with urine, associated with progressive atrophy of the kidney parenchyma, is the hallmark of hydronephrosis, which is uncommon and sporadic in swine. The pathogenesis of this condition always involves some form of obstructive impediment to the normal passage of urine within the lower urinary tract, anywhere from the pelvis to the distal urethra. The causes of the obstruction include urinary calculi, exudate within urinary passages, ureteral kinking, focal external compression (abscesses, tumors), and posttraumatic or postinflammatory strictures.

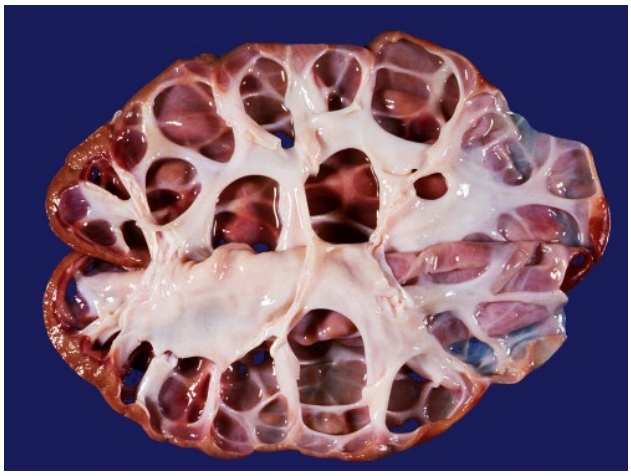


Figure 22.16 Chronic unilateral hydronephrosis.

Severe unilateral hydronephrosis (Figure 22.16) may develop unnoticed since the remaining kidney, if normal, may compensate adequately. In these cases, the affected kidney shows extensive dilation of the pelvis and calyces at the expense of the renal parenchyma, which may appear as a thin layer of cortical tissue. Depending on the location of the obstruction, hydroureter may also develop. In long-standing cases, the kidney may be virtually transformed into a large fluid-filled sac delimited by a severely distended renal capsule. These extreme lesions may take months to develop. Since stagnation of urine predisposes to infection, the urine may be transformed into a purulent exudate in some instances. In cases of bilateral hydronephrosis, affected animals usually die from uremia before renal lesions get fully developed.

Parasitic infections

The pig is the final or intermediate host of a number of parasitic helminths. Compared with some other body systems, the urinary tract is the niche of very few of these parasites. Renal infections with *Dioctophyma renale*, the giant kidney worm, and with larval stages of certain tapeworms may occur on rare occasions. The most significant helminth with tropism for the urinary system of swine is the nematode *Stephanurus dentatus*, the so-called kidney worm of swine.

Stephanurus dentatus is a widely distributed strongyloid worm and is most prevalent in warm climates, including the Southern United States. In endemic areas, this parasitic infection may have significant economic impact since it is associated with deaths, retarded growth, decreased feed efficiency, and condemnations at the abattoir (Batte et al. 1960). Larvae of this nematode need moisture and shade for optimal survival, so pigs raised on soil in this type of environment are the most prone to the disease. Infective larvae penetrate the skin

Table 22.1 Differential diagnosis of some renal diseases based on gross findings at necropsy.

Gross findings	Differential diagnosis
Normal size kidneys with widespread petechiae	• Bacterial septicemia (Figure 22.3) • Acute viremic diseases: classical swine fever (CSF), African swine fever (ASF), cytomegalovirus • Electrocution • Some intoxications
Enlarged and edematous kidneys with widespread petechiae	• Acute glomerulonephritis[a] (GN) including PDNS (Figure 22.5)
Enlarged, pale, and edematous kidneys	• Acute GN[a] • Acute tubular necrosis[a]: various toxic agents (Figure 22.6) Note: Occasionally with perirenal edema (pigweed, GN)
Multiple renal hemorrhages (larger than petechiae)	• Trauma • Necrosis • Clotting defects: anticoagulant poisoning, isoimmune thrombocytopenia
One or both kidneys with red or white foci of necrosis (often wedge shaped and cortical)	• Acute or subacute infarcts: thrombosis, vasculitis, emboli
Kidneys with patchy pallor or obvious white foci within renal parenchyma	• Interstitial nephritis: circovirus, leptospirosis, undetermined cause (Figure 22.7) Note: Kidneys may also be enlarged with circovirus
Kidneys with multiple microabscesses	• Suppurative embolic nephritis: *Erysipelothrix rhusiopathiae*, *Actinobacillus suis* (Figure 22.9)
One or both kidneys with exudate within dilated pelvis and possibly papillary necrosis and asymmetric foci of parenchymal suppurative inflammation	• Acute pyelonephritis[a]: various bacteria including *Actinobaculum suis* (Figure 22.11) Note: Often accompanied with ureteritis and cystitis
Kidneys diffusely pale, firm, and fibrotic with generalized fine granularity of the capsular surface	• Chronic GN[b] • Chronic tubular necrosis[b]: some toxic agents including ochratoxin A • Chronic generalized interstitial nephritis (uncommon in pigs)
One or both kidneys with large irregular fibrous bands with intervening areas of normal parenchyma; dilation of pelvis and deformities of papillae	• Chronic pyelonephritis (Figure 22.11)
One or both kidneys with large irregular fibrous bands with intervening areas of normal parenchyma; normal pelvis	• Chronic infarcts (septic or not) • Renal dysplasia (rare)
One or both kidneys with one to several fluid-filled cysts that do not communicate with the pelvis	• Congenital renal cysts (Figure 22.2)
One or both kidneys with dilation of the pelvis and calyces associated with atrophy and cystic enlargement of the kidney	• Hydronephrosis[b] (obstructive nephropathy) (Figure 22.16)
One or both kidneys with multiple exophytic nodules of various sizes	• Lymphoma (often bilateral) (Figures 22.12 and 22.13) • Embryonal nephroma (often unilateral) • Other neoplasms (rare)

[a] Acute renal failure possible.
[b] Chronic renal failure possible.

or are ingested by the pigs. Transplacental fetal infection is also possible (Batte et al. 1966).

After being introduced into the host, the larvae molt and migrate to the liver, where they remain for several months, causing severe hepatic damage and inflammation. The presence of the parasite within the hepatic parenchyma is often responsible for extensive liver condemnation in some herds (Hale and Marti 1983). Eventually some larvae escape from the liver and migrate to the abdominal cavity and potentially to various ectopic sites, eliciting a severe inflammatory reaction. To complete the cycle, some adults establish themselves in the

perirenal tissues or, more rarely, within the kidney. At that site, the worms, measuring about 3 cm in length, are found in cystic inflammatory nodules that communicate with either the pelvis or the ureter in order to shed their eggs successfully into urine. The prepatent period in most cases is at least 9 months, and adults may shed ova in urine for over 2 years (Batte et al. 1960, 1966). Preventive and curative measures for the control of parasites are addressed in detail elsewhere in this book.

Other conditions

Urethral polyps causing local obstruction have been reported on several occasions in male Vietnamese potbellied pigs (Helman et al. 1996). These inflammatory polyps have all occurred at the level of the urethral recess (see section "Anatomy"). The precise cause of the lesions could not be determined, but traumatic injuries secondary to urinary catheterization have been strongly suspected in some cases. Urethral catheterization should be performed with great care in male pet breeds of swine to avoid potential damage to the urethral recess.

Mineralization of the kidneys occurs in swine with acute vitamin D toxicosis. This poisoning is usually observed when excessive amounts of vitamin D_3 are inadvertently added to their feed (Kurtz and Stowe 1979; Long 1984). Affected pigs show lethargy, vomiting, diarrhea, respiratory distress, and death. Salient gross necropsy findings include hemorrhagic gastritis or gastroenteritis, myocardial necrosis, and pulmonary edema and congestion. Histologically, besides the gross lesions described above, there is widespread mineralization accompanied by degenerative changes of varying severity in the kidneys, myocardium, lungs, gastrointestinal tract, and blood vessels.

Chronic salt intoxication has been reported recently in growing pigs (Alonso et al. 2010). This poisoning was associated with a severe bilateral diffuse interstitial fibrosis in the middle and deep area of the renal cortex with loss of glomeruli and glomerulosclerosis.

Mucinous metaplasia of the epithelial cells lining the renal pelvis, ureter, and urinary bladder is occasionally observed in pigs. This rather nonspecific lesion, of uncertain pathogenesis, has been reported in pigs with various conditions, including exudative epidermitis, *E. coli* enteritis, classical swine fever, and suppurative arthritis (Brobst et al. 1971), as well as in urinary tract infections.

Ossification of the renal pelvis of unknown etiology has been reported in slaughtered pigs (Bundza 1990).

Differential diagnosis

Main differential diagnoses based on gross renal findings are presented in Table 22.1.

References

Alonso C, Llanes N, Segalés J. 2010. A case on chronic salt intoxication in growing pigs. *Proc Congr Int Pig Vet Soc* 21(1):98.

Andersen S, Nielsen R. 1973. *Nord Vet Med* 25:211–219.

Anderson LJ, Sandison AT, Jarrett WFH. 1969. *Vet Rec* 84:547–551.

Arauz SM, Perfumo CJ. 2000. *Rev Med Vet (Buenos Aires)* 81:342–344.

Baker TF, McEwen SA, Prescott JF, et al. 1989. *Can J Vet Res* 53:290–294.

Banks WJ. 1993. Urinary system. In Banks WJ, ed. *Applied Veterinary Histology*, 3rd ed. Baltimore, MD: Mosby-Year Book, pp. 374–389.

Batte EG, Harkema R, Osborne JC. 1960. *J Am Vet Med Assoc* 136:622–625.

Batte EG, Moncol DJ, Barber CW. 1966. *J Am Vet Med Assoc* 149:758–765.

Benavente CE, Fuentealba IC. 2012. *Arch Med Vet* 44:99–107.

Benko L. 1969. *Vet Rec* 84:139–140.

Boqvist S, Chau BL, Gunnarsson A, et al. 2002. *Prev Vet Med* 53:233–245.

Boqvist S, Montgomery JM, Hurst M, et al. 2003. *Vet Microbiol* 93:361–368.

Bourgault A, Drolet R. 1995. *J Vet Diagn Invest* 7:122–126.

Brobst DF, Cottrell R, Delez A. 1971. *Vet Pathol* 8:485–489.

Brown SA, Engelhardt JA. 1987. *Comp Cont Educ* 9:148–159.

Bundza A. 1990. *Can Vet J* 31:529.

Carr J, Walton JR. 1992. Characteristics of plasma and urine from normal adult swine and changes found in sows with either asymptomatic bacteriuria or cystitis and pyelonephritis. *Proc Congr Int Pig Vet Soc* 12:263.

Carr J, Walton JR. 1993. *Vet Rec* 132:575–577.

Carr J, Walton JR, Done SH. 1990. Observations on the intravesicular portion of the ureter from healthy pigs and those with urinary tract disease. *Proc Congr Int Pig Vet Soc* 11:286.

Carr J, Walton JR, Done SH. 1991. *Pig Vet J* 27:122–141.

Carrasco L, Madsen LW, Salguero FJ, et al. 2003. *J Comp Pathol* 128:25–32.

Carson TL. 2006. Toxic minerals, chemicals, plants, and gases. In Straw BE, Zimmerman JJ, D'Allaire S, et al. eds. *Diseases of Swine*, 9th ed. Ames, IA: Blackwell Publishing, pp. 971–984.

Chagnon M, D'Allaire S, Drolet R. 1991. *Can J Vet Res* 55:180–184.
Cheville NF, Huhn R, Cutlip RC. 1980. *Vet Pathol* 17:338–351.
Choi C, Chae C. 2003. *Vet Rec* 153:20–22.
Cianciolo RE, Mohr FC. 2016. Urinary system. In Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, 6th ed. Vol. 2. St. Louis, MO: Elsevier, pp. 376–464.
Cook WO, Osweiler GD, Anderson TD, et al. 1986. *J Am Vet Med Assoc* 188:1399–1402.
Cooper VL, Heese RA, Doster AR. 1997. *J Vet Diagn Invest* 9:198–201.
Cordes DO, Dodd DC. 1965. *Pathol Vet* 2:37–48.
D'Allaire S, Drolet R. 2006. Longevity in breeding animals. In Straw BE, Zimmerman JJ, D'Allaire S, et al. eds. *Diseases of Swine*, 9th ed. Ames, IA: Blackwell Publishing, pp. 1011–1025.
Dimmock CK, Webster WR, Shiels IA, et al. 1982. *Aust Vet J* 59:157–159.
Drolet R, D'Allaire S, Chagnon M. 1990. *J Vet Diagn Invest* 2:9–13.
Drolet R, Thibault S, D'Allaire S, et al. 1999. *Swine Health Prod* 7:283–285.
Drolet R, D'Allaire S, Larochelle R, et al. 2002. *Vet Rec* 150:139–143.
Elling F. 1979. *Acta Pathol Microbiol Scand* 87A: 387–392.
Friendship RM, Lumsden JH, McMillan I, et al. 1984. *Can J Comp Med* 48:390–393.
Friis C. 1980. *J Anat* 130:513–526.
Garret PD. 1987. *J Am Vet Med Assoc* 191:689–691.
Hale OM, Marti OG. 1983. *J Anim Sci* 56:616–620.
Häni H, Indermühle NA. 1980. *Vet Pathol* 17:234–237.
Hayashi M, Tsuda H, Okumura M, et al. 1986. *J Comp Pathol* 96:35–46.
Hegasy GA, Manuelian T, Hogasen K, et al. 2002. *Am J Pathol* 161:2027–2034.
Hélie P, Drolet R, Germain MC, et al. 1995. *Can Vet J* 36:150–154.
Helman RG, Hooper RN, Lawhorn DB, et al. 1996. *J Vet Diagn Invest* 8:137–140.
Hervas J, Gomezvillamandos JC, Mendez A, et al. 1996. *Vet Res Commun* 20:285–299.
von Höfliger H. 1971. *Schweizer Archiv Tierheilkund* 113:330–337.
Hunter P, van Der Vyver FH, Selmer-Olsen A, et al. 1987. *Onderstepoort J Vet Res* 54:59–62.
Imai DM, Cornish J, Nordhausen R, et al. 2006. *J Vet Diagn Invest* 18:496–499.
Inoue I, Baba K, Ogura Y, et al. 1977. *Nat Inst Anim Hlth Quart* 17:186.
Isling LK, Aalbaek B, Schroder M, et al. 2010. *Acta Vet Scand* 52:48.
Jakob W. 1971. *Vet Pathol* 8:292–306.
Jansen JH. 1993. *Acta Pathol Microbiol Immunol Scand* 101:281–289.
Jansen JH, Nordstoga K. 1992. *J Vet Med* A39:582–592.
Jansen JH, Nordstoga K. 1994. *J Vet Med* A41:91–101.
Jansen JH, Hogasen K, Grondahl AM. 1995. *Vet Rec* 137:240–244.
Jansen JH, Hogasen K, Harboe M, et al. 1998. *Kidney Int* 53:331–349.
Jones RT, Millar BD, Chappel RJ, et al. 1987. *Aust Vet J* 64:258–259.
Kavanagh NT. 1994. *Vet Rec* 134:311.
Kelly DF. 1967. *Res Vet Sci* 8:472–478.
Krogh P. 1977. *Nord Vet Med* 29:402–405.
Kurtz HJ, Stowe CM. 1979. Acute vitamin-D toxicosis in swine. *Proc Am Assoc Vet Lab Diagn* 22:61–68.
Long GG. 1984. *J Am Vet Med Assoc* 184:164–170.
Marcato PS. 1987. *Vet Res Commun* 11:325–337.
Martin-Fernandez J, Igual A, Rueda A, et al. 1991. *Histol Histopathol* 6:115–121.
Mason RW, Cooper R. 1985. *Aust Vet J* 62:413–414.
McErlean BA. 1973. *Vet J* 27:185–186.
McLaughlin PS, McLaughlin BG. 1987. *Am J Vet Res* 48:467–473.
Mitchell DA, Robertson A, Corner AH, et al. 1966. *Can J Comp Med* 30:211–217.
Morales GA, Guzman VH. 1976. Proliferative glomerulonephritis in young pigs. *Proc Congr Int Pig Vet Soc* 4:R1.
Müller E. 1977. *Dtsch Tierarztl Wochenschr* 48:43–45.
Nieberle K, Cohrs P. 1967. Urinary organs. In *Textbook of the Special Pathological Anatomy of Domestic Animals*. Toronto: Pergamon Press, pp. 659–720.
Nielsen SW, Moulton JE. 1990. Tumors of the urinary system. In Moulton JE, ed. *Tumors in Domestic Animals*, 3rd ed. Berkeley, CA: University of California Press, pp. 458–478.
Nietfeld JC, Leslie-Steen P. 1993. *J Vet Diagn Invest* 5:269–273.
Nilubol D, Pattanaseth T, Boonsri K, et al. 2009. *Vet Pathol* 46:1156–1159.
Olvera A, Sibila M, Calsamiglia M, et al. 2004. *J Virol Methods* 117:75–80.
Opriessnig T, Meng X-J, Halbur PG. 2007. *J Vet Diagn Invest* 19:591–615.
Opriessnig T, Xiao C-T, Halbur PG, et al. 2017. *Vaccine* 35:248–254.
Orr JP, Althouse E, Dulac GC, et al. 1988. *Can Vet J* 29:45–50.
Osborne CA, Albasan H, Lulich JP, et al. 2009. *Vet Clin North Am Small Anim Pract* 39:65–78.
Osweiler GD. 1996. Mycotoxins. In Osweiler GD, ed. *Toxicology*. Philadelphia, PA: Williams and Wilkins, pp. 409–436.
Osweiler GD, Buck WB, Bicknell EJ. 1969. *Am J Vet Res* 30:557–566.

Pace LW, Schreibman AE, Bouchard G, et al. 1998. Immune-complex mediated glomerulonephritis in miniature swine. *Proc Congr Int Pig Vet Soc* 15:394.
Pezzolato M, Maina E, Lonardi S, et al. 2012. *Vet Immunol Immunopathol* 145:546–550.
Pouleur-Larrat B, Maccolini E, Carmel EN, et al. 2014. *Can Vet J* 55:544–546.
Ransley PG, Risdon RA. 1974. *Lancet* 2:1114.
Ribotta M, Higgins R, Perron D. 1999. *Can Vet J* 40, 809–810.
Rossow KD, Collins JE, Goyal SM, et al. 1995. *Vet Pathol* 32:361–373.
Ruckebusch Y, Phaneuf LP, Dunlop R. 1991. The urinary collecting and voiding system. In Ruckebusch Y, Phaneuf L-P, Dunlop R, eds. *Physiology of Small and Large Animals*, 1st ed. Hamilton: B.C. Decker, pp. 184–188.
Rutqvist L, Bjorklund NE, Hult K, et al. 1978. *Appl Environ Microbiol* 36:920–925.
Salmon-Legagneur E, Gayral JP, Leveau JM, et al. 1973. *J Rech Porcine France* 5:285–291.
Sandison AT, Anderson LJ. 1968. *Cancer* 21:727–742.
Sarli G, Mandrioli L, Panarese S, et al. 2008. *Vet Pathol* 45:12–18.
Segalés J, Domingo M. 2002. *Vet Q* 24:109–124.
Segalés J, Piella J, Marco E, et al. 1998. *Vet Rec* 142:483–486.
Segalés J, Rosell C, Domingo M. 2003. Porcine dermatitis and nephropathy syndrome. In Morilla A, Yoon K-J, Zimmerman JJ, eds. *Trends in Emerging Viral Infections of Swine*, 1st ed. Ames, IA: Iowa State Press, pp. 313–318.
Segalés J, Allan G, Domingo M. 2005. *Anim Health Res Rev* 6:119–142.
Shadduck JA, Koestner A, Kasza L. 1967. *Pathol Vet* 4:537–552.
Shirota K, Nomura Y, Saito Y. 1984. *Vet Pathol* 21:158–163.
Shirota K, Koyama R, Nomura Y. 1986. *Jpn J Vet Sci* 48:15–22.
Shirota K, Masaki T, Kitada H, et al. 1995. *Vet Pathol* 32:236–241.
Sierra MA, de las Mulas JM, Molenbeek RF, et al. 1997. *Eur J Vet Pathol* 3:63–70.
Sim WW. 1978. Urinary obstruction in weaned piglets leading to increased mortality. *Proc Congr Pig Vet Soc* 4:57–59.
Sisson S. 1975. Porcine urogenital system. In Getty R, ed. *Sisson and Grossman's the Anatomy of Domestic Animals*, 5th ed. Philadelphia, PA: W. B. Saunders, pp. 1297–1303.
Slauson DO, Lewis RM. 1979. *Vet Pathol* 16:135–164.
Smith WJ, Thomson JR, Done S. 1993. *Vet Rec* 132:47.
Smyth JA, Rice DA, Kavanagh NT, et al. 1986. *Vet Rec* 119:158–159.
Spargo BH, Taylor JR. 1988. The kidney. In Rubin E, Farber JL, eds. *Pathology*, 1st ed. Philadelphia, PA: J. B. Lippincott, pp. 832–889.
Stevenson RG, DeWitt WF. 1973. *Can Vet J* 14:139–141.
Tamura T, Shirota K, Une Y, et al. 1986. *Jpn J Vet Sci* 48:1183–1189.
Thibault S, Drolet R, Germain MC, et al. 1998. *Vet Pathol* 35:108–116.
Thompson AB, Postey RC, Snider T, et al. 2010. *Can Vet J* 51:1223–1225.
Thomson JR, Higgins RJ, Smith WJ, et al. 2002. *J Vet Med* A49:430–437.
Vezzali E, Manno RA, Salerno D, et al. 2011. *Toxicol Pathol* 39:700–705.
Vitovec J. 1977. *J Comp Pathol* 87:129–134.
Weaver ME. 1966. *Anat Rec* 154:701–704.
Webster WR, Summers PM. 1978. *Aust Vet J* 54:451.
Wellenberg GJ, Stockhofe-Zurwieden N, de Jong MF, et al. 2004. *Vet Microbiol* 99:203–214.
Wells GAH, Hebert CN, Robins BC. 1980. *Vet Rec* 106:532–535.
White M, Higgins RJ. 1993. *Vet Rec* 132:199.
Wijeratne WVS, Wells GAH. 1980. *Vet Rec* 107:484–488.
Woldemeskel M, Drommer W, Wendt M. 2002. *J Vet Med A* 49:348–352.
Yoshie T. 1991. *Jpn J Nephrol* 33:179–189.
Yoshikawa T, Yoshikawa H, Oyamada T, et al. 1988. Immune-complex glomerulonephritis associated with porcine cytomegalovirus infection. *Proc Congr Int Pig Vet Soc* 10:245.

Section III

Viral Diseases

23

Overview of Viruses

Jianqiang Zhang, Kyoung-Jin Yoon, and Jeffrey J. Zimmerman

Characteritics of viruses

Historically, a virus was defined as a "filterable agent" (Hughes 1977) because filtration (<300 nm pore size) would separate viruses from most bacteria and other microorganisms. However, some mycoplasmas and chlamydia may be <300 nm in diameter (MacLachlan and Dubovi 2011), and "giant" viruses have been identified, such as mimiviruses (750 nm in diameter) and pandoraviruses (up to 1000 nm in diameter) (MacLachlan and Dubovi 2017) (Table 23.1).

Viruses are very distinct from other microorganisms. Viruses are obligate intracellular parasites. They cannot be cultivated on nonliving (i.e. artificial) medium because they only replicate within living cells. Indeed, they rely on the host cell to provide many of the resources required for their replication. Viruses also have other unique properties: they do not reproduce by binary fission, they contain DNA or RNA genomes (but not both), and they are not sensitive to antibiotics but to interferons (Table 23.1).

Compositions of virions

A virion is a complete virus particle consisting of the genomic RNA or DNA surrounded by a protein shell (sometimes with an external envelope as well) and is the infective form of a virus (Figure 23.1). The genome can be single stranded or double stranded, can be linear or circular in form, and can contain one or more segments (Table 23.2). If single stranded, the virion genome can be positive sense, negative sense, or ambisense (Table 23.2).

Genomic RNA or DNA encodes various viral proteins. Generally speaking, viral proteins produced in the process of virus life cycle, but not physically incorporated into the virion, are referred to as "nonstructural proteins." Viral proteins incorporated into the virion are "structural proteins." However, some virions include a small amount of nonstructural proteins (e.g. viral polymerase) used at the initiation of the next cycle of replication. Whether some viral polymerases are packaged into virions or not depends on virus replication/transcription strategies and subcellular sites (cytoplasm and/or nucleus) of viral replication (Table 23.2).

Within a virion, the viral genome is surrounded by a protein coat ("capsid") composed of viral protein subunits ("capsomere"). Together, the viral genome and capsid form the nucleocapsid. For some viruses, the nucleocapsid is "naked" (non-enveloped viruses). For some viruses, the nucleocapsid is covered by an outer structure ("envelope") that is acquired as the nucleocapsid passes ("buds") through a cell membrane, i.e. cytoplasmic, intracytoplasmic, or nuclear membrane. Due to the nature of the budding process, the major constituent (lipid bilayer) of the viral envelope is structurally identical to the cell membrane from which it was derived. An enveloped virion also has additional virus-encoded proteins on the surface of envelope. These proteins are attached to the matrix protein (or the tegument in the case of herpesviruses) located between the envelope and the nucleocapsid. These envelope proteins, sometimes called envelope glycoproteins or envelope-associated proteins, play a critical role in the process of virus life cycle. For example, they are involved in virus attachment (receptor binding), membrane fusion and uncoating, and release of progeny virus (receptor destruction).

Viral morphology

Viruses vary in size and morphology (Figure 23.2 and Table 23.2). Nucleocapsid symmetry and virion morphology are two important aspects of viral morphology (Condit 2013; MacLachlan and Dubovi 2011). Nucleocapsid symmetry is often described as icosahedral (isometric), helical, or complex. Descriptions of virion morphology are not standardized, and various descriptors may be seen in the literature (e.g. icosahedral, spherical, complex, pleomorphic, bullet shaped, and others).

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 23.1 Comparison of basic properties among unicellular microorganisms.

Property	Bacteria	Mycoplasma	Rickettsia	Chlamydia	Virus
Size >300 nm[a]	Yes	Yes	Yes	Yes	No
Grow on artificial media	Yes	Yes	No	No	No
Divide by binary fission	Yes	Yes	Yes	Yes	No
Contain both DNA and RNA	Yes	Yes	Yes	Yes	No
Sensitive to antibiotics	Yes	Yes	Yes	Yes	No

Source: Adapted from Murphy et al. (1999).
[a] Some mycoplasmas and chlamydia are <300 nm in diameter. Some viruses, e.g. mimiviruses and pandoraviruses, are >300 nm in diameter.

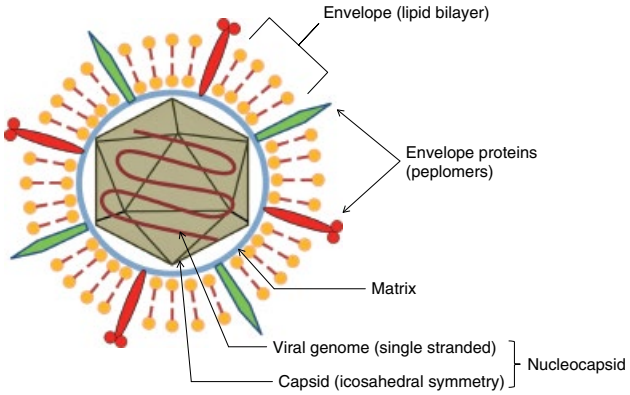


Figure 23.1 Schematic diagram of an enveloped virion with a single-stranded genome surrounded by an icosahedral capsid.

Virus taxonomy

Basis of taxonomy

Initially, viruses were named or categorized on the basis of (1) the disease or pathology associated with the virus (e.g. foot-and-mouth disease virus, hepatitis virus), (2) the geographic location where the virus was found (e.g. Rift Valley fever virus), (3) transmission vectors (e.g. arthropod-borne viruses or arboviruses), and (4) other overt characteristics. As more viruses were discovered and the scientific basis of characterizing viruses expanded, this system became inadequate. Therefore, the International Committee on Taxonomy of Viruses (ICTV) was established in 1966 with the objective of developing an internationally recognized taxonomy and nomenclature for viruses.

The current virus taxonomy system classifies viruses on the basis of specific characteristics: (1) genome characteristics (RNA vs. DNA, strandedness, segmentation, circular vs. linear, polarity, haploid vs. diploid), (2) virion structure (morphology, envelope, nucleocapsid symmetry, capsomere number), (3) genome organization and viral replication and transcription strategies, and (4) other features.

Hierarchy and nomenclature

Virus taxonomic classification is an ongoing process and evolves as new information is brought to light. *Diseases of Swine* (11th edition) relied on the information in the 10th Report of the ICTV (2017). Updates and new information on virus taxonomy may be found on the ICTV website https://ictv.global/report.

The ICTV has adopted a universal system to describe the hierarchical taxonomic levels of viruses: order, family, subfamily, genus, and species. Within this system, a virus species is defined as "a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche." A type species is defined for each genus, but the type species is not necessarily the most well-characterized virus in the genus. Rather, it is the virus that initially necessitated the creation of the genus. A genus is a group of virus species sharing some common characters, a subfamily is a group of genera sharing some common characters, a family is a group of genera or subfamilies sharing some common characters, and an order is a group of families sharing some common characters. However, not all families have a defined higher taxonomic level "order," and, in fact, only a few families have been grouped into orders. In addition, not all families include subfamilies; for most cases, species are grouped into genera and genera directly into families.

The ICTV has defined specific naming conventions for the taxa in this system. The names of orders end with the suffix "*-virales*," families with the suffix "*-viridae*," subfamilies with the suffix "*-virinae*," and genera with the suffix "*-virus*." In formal nomenclature, order, family, subfamily, and genus names are a single word in italics with the first letter capitalized, for example, the order *Herpesvirales*, the family *Herpesviridae*, the subfamily *Alphaherpesvirinae*, and the genus *Varicellovirus*. Taxonomic species name is written in italics as well, but there is no specific suffix for species. In addition, the species name can be more than one word. For a species name, the first word begins with a capital letter, and other words only begin with a capital letter if they are

Table 23.2 Viral families containing animal and human pathogens and their virion and genome properties.

		Virion			Genome					
Family[a]	Nucleocapsid symmetry[b]	Diameter (nm)	Envelope	Morphology[d]	Type	Structure[e]	Segment	Size (kb)	Virion Polymerase[f]	Virus replication and assembly[c]
Adenoviridae	I	80–110	–	I	DNA	Linear, ds	1	32–40	–	Nu
Anelloviridae	I	30–32	–	I	DNA	Circular, ss(–)	1	2–4	–	Nu
Asfarviridae	I	175–215	+[g]	I	DNA	Linear, ds	1	170–190	+	Cy
Circoviridae	I	17–24	–	I	DNA	Circular, ss(– or ±)	1	1.7–2.3	–	Nu
Hepadnaviridae	I	40–48	+	S	DNA	Circular, ds	1	3–3.3	+	Nu/Cy
Herpesviridae	I	150–200	+	S	DNA	Linear, ds	1	120–235	–	Nu
Iridoviridae	I	160–350	±	I	DNA	Linear, ds	1	150–300	–	Nu/Cy
Papillomaviridae	I	40–55	–	I	DNA	Circular, ds	1	5.3–8	–	Nu
Parvoviridae	I	18–26	–	I	DNA	Linear, ss(– or +)	1	5	–	Nu
Polyomaviridae	I	40–55	–	I	DNA	Circular, ds	1	5.3–8	–	Nu
Poxviridae	C	250–300 × 200	+	C	DNA	Linear, ds	1	130–375	+	Cy
Arenaviridae	H	100–300	+	S	RNA	Circular, ss(±)	2	10–14	+	Cy
Arteriviridae	I	50–70	+	S	RNA	Linear, ss(+)	1	13–15	–	Cy
Astroviridae	I	27–30	–	I	RNA	Linear, ss(+)	1	6.8–7.9	–	Cy
Birnaviridae	I	60	–	I	RNA	Linear, ds	2	7	+	Cy
Bornaviridae	I	50–60	+	S	RNA	Linear, ss(–)	1	8.9	+	Nu
Bunyaviridae	H	80–120	+	S	RNA	Linear, ss(– or ±)	3	11–22.7	+	Cy
Caliciviridae	I	35–39	–	I	RNA	Linear, ss(+)	1	7.4–8.3	–	Cy
Coronaviridae	H	80–160	+	S	RNA	Linear, ss(+)	1	20–33	–	Cy
Filoviridae	H	800–950 × 80	+	P	RNA	Linear, ss(–)	1	18.9–19.1	+	Cy
Flaviviridae	I	40–60	+	S	RNA	Linear, ss(+)	1	9.5–12.5	–	Cy
Hepeviridae	I	27–34	–	I	RNA	Linear, ss(+)	1	7.1–7.2	–	Cy
Orthomyxoviridae	H	80–120	+	P,S	RNA	Linear, ss(–)	6–8	10–14.6	+	Nu
Paramyxoviridae	H	150–600	+	P,S	RNA	Linear, ss(–)	1	15–18	+	Cy
Picornaviridae	I	22–30	–	I	RNA	Linear, ss(+)	1	7–8.5	–	Cy
Reoviridae	I	60–80	–	I	RNA	Linear, ds	10–12	16–27	+	Cy
Retroviridae	I	80–100	+	S	RNA	Linear, ss(+) dimer	1	7–11	+	Cy/Nu
Rhabdoviridae	H	180 × 75	+	Bullet shaped	RNA	Linear, ss(–)	1	13–16	+	Cy
Roniviridae	H	150–200 × 40–60	+	S	RNA	Linear, ss(+)	1	26.2	–	Cy
Togaviridae	I	65–70	+	S	RNA	Linear, ss(+)	1	9.7–11.8	–	Cy

[a] Families are listed in the alphabetical order of family names but with DNA viruses first followed by RNA viruses.
[b] Nucleocapsid symmetry abbreviation: I, icosahedral; C, complex; H, helical.
[c] Location where virus replication takes place: Cy, cytoplasm; Nu, nucleus.
[d] Virion morphology abbreviation: I, icosahedral; S, spherical; C, complex; P, pleomorphic.
[e] ds, double stranded; ss, single stranded; –, negative sense; +, positive sense; ±, ambisense.
[f] Viral polymerase packaged into virion: – No; + Yes.
[g] Intracellular virion is not enveloped.

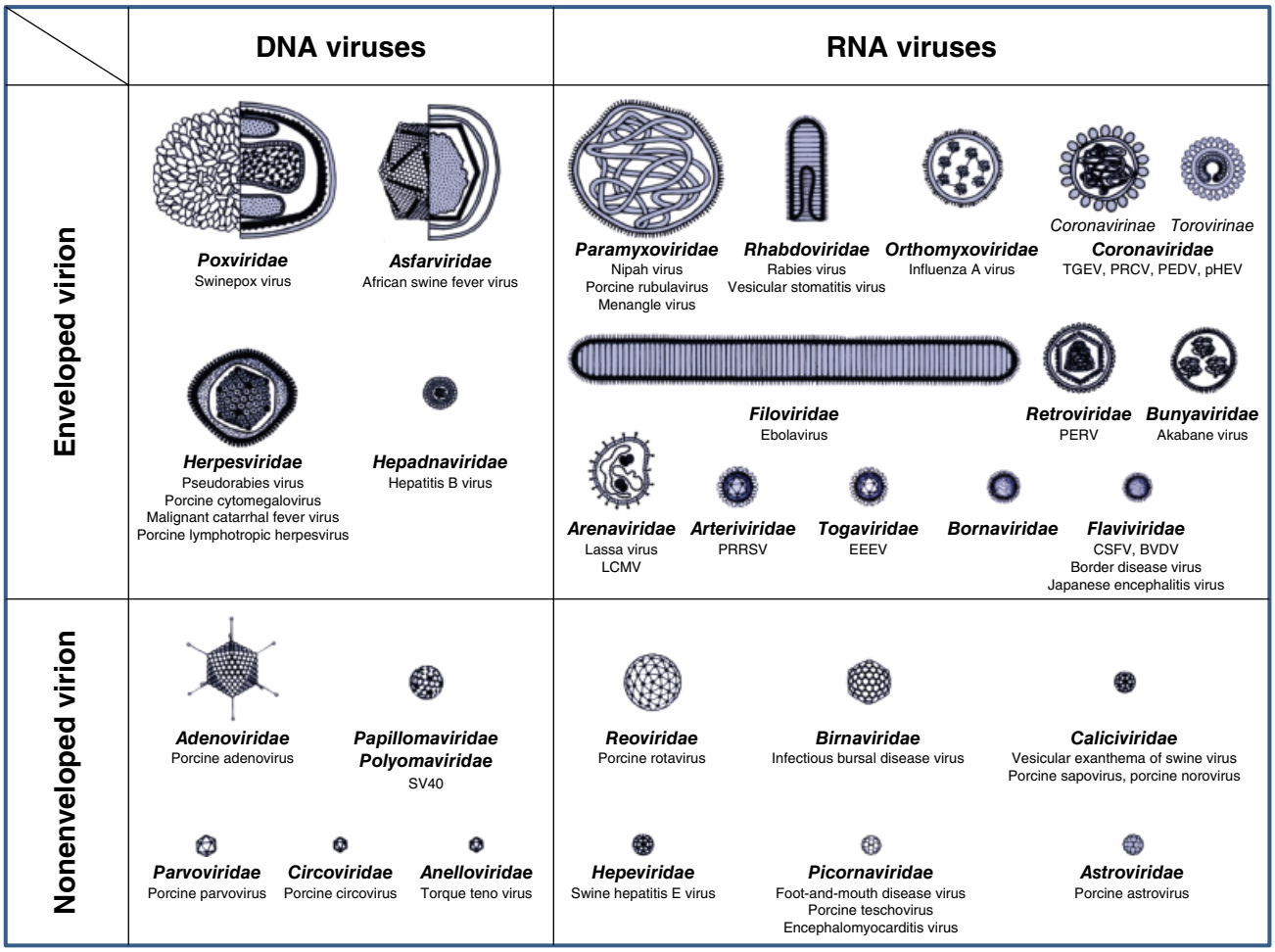


Figure 23.2 Grouping of animal viruses based on morphological characteristics and composition of nucleic acid. *Source:* Adapted with permission from Murphy et al. (1999). Reproduced with permission of Elsevier. LCMV, lymphocytic choriomeningitis virus; EEEV, Eastern equine encephalitis virus; CSFV, classical swine fever virus; BVDV, bovine viral diarrhea virus; PEDV, porcine epidemic diarrhea virus; PERV, porcine endogenous retrovirus; pHEV, porcine hemagglutinating encephalomyelitis virus; PRCV, porcine respiratory coronavirus; PRRSV, porcine reproductive and respiratory syndrome virus; TGEV, transmissible gastroenteritis virus.

proper nouns or alphabetical identifiers, for example, the species *Suid alphaherpesvirus 1*, the species *Vesicular exanthema of swine virus*, the species *West Nile virus*, and the species *Teschovirus A*. A species name should not be abbreviated.

Taxonomic levels lower than species are not officially specified by the ICTV. For that reason, there are no formal rules of naming members of a virus species, although recommendations are provided on the ICTV website. A virus name should not be italicized and should be written in lowercase. Words in a virus name, including the first word, should only begin with a capital letter when these words are proper nouns or when a sentence starts with a virus name. Virus names may be abbreviated. Some examples are given below:

- "Porcine epidemic diarrhea virus (PEDV) was detected in the fecal samples."
- "Fecal samples tested positive by a porcine epidemic diarrhea virus (PEDV)-specific real-time RT-PCR."
- "Detection of West Nile virus (WNV) can be achieved using a virus-specific RT-PCR."

Sometimes the common virus name is the same as the species name, e.g. the virus name is "porcine epidemic diarrhea virus" and the species name is "*Porcine epidemic diarrhea virus*." Sometimes the common virus name is different from the species name, e.g. the virus is named "Seneca Valley virus," and the species is named "*Senecavirus A*."

A species may consist of just one virus or a species can include multiple virus members. For example, the species *Alphacoronavirus 1* includes virus members, such as transmissible gastroenteritis virus, porcine respiratory coronavirus, canine coronavirus, feline infectious peritonitis virus, and feline enteric coronavirus.

Occasionally a collective name is used to describe a group of viruses belonging to a genus, a family, or an order. Under such a circumstance, the collective name is neither italicized nor capitalized. For example, the

collective name "nidoviruses" is used to describe viruses in the order *Nidovirales*. However, collective names must be used carefully to avoid miscommunication. For example, does "flaviviruses" represent viruses in the genus *Flavivirus* or all viruses in the family *Flaviviridae*? Does the collective name "picornaviruses" represent all viruses in the family *Picornaviridae* or all viruses in the order *Picornavirales*?

Utility of taxonomy

Virus classification provides useful information. For example, DNA viruses tend to be more genetically stable (lower rate of genetic change) than RNA viruses during the process of genome replication. Enveloped viruses are more susceptible to environmental stresses than nonenveloped viruses and lose their infectivity easily when exposed to lipid solvents or detergents. Most importantly, virus taxonomy provides a standard framework for properly classifying viruses and comparing viruses between different groups. When a newly identified virus is classified into a given family, some basic information on its properties and certain viral characteristics can be inferred.

Detection of viruses

The technology for virus detection continues to evolve, but current methods for detecting viruses fit into five categories (MacLachlan and Dubovi 2017; Storch and Wang 2013): (1) detection of infectious virus (virus cultivation), (2) electron microscopy (EM), (3) viral antigen-based detection methods, (4) viral nucleic acid-based detection methods, and (5) detection of virus antibodies. These detection methods can be agent dependent, agent independent, or both.

Virus cultivation

Initially, propagation of animal viruses was limited to live animals, either their natural animal hosts or laboratory animals. In the 1930s, some avian and animal viruses were successfully propagated using embryonated chicken eggs (Goodpasture et al. 1932) and embryonated chicken eggs are still widely used for the isolation and propagation of influenza viruses and many avian viruses. In 1948, the growth and maintenance of mammalian cells *in vitro* became possible (MacLachlan and Dubovi 2017). This led to the development of *in vitro* cell culture systems, including organ cultures, primary explant cultures, primary cell cultures, and cell lines. In particular, continuous cell lines are routinely used for virus cultivation. Although virus cultivation in animal hosts has largely been replaced by cell culture and embryonated chicken eggs, not all viruses can be cultivated *in vitro*, and inoculation of animals (bioassays) is sometimes necessary to assess the viability of some viruses in samples.

Electron microscopy

EM enables direct visualization of virus particles. The virus of interest is then assigned to the appropriate family on the basis of the morphology and size of the viral particles. In disease outbreak investigations, EM is particularly useful in identifying previously unrecognized viruses or uncultivable viruses. In addition to the need for expensive equipment and a highly skilled microscopist, another limitation of EM is its low analytical sensitivity; that is, the agent needs to be present in abundance. However, immunoelectron microscopy can enhance sensitivity to some extent.

Viral antigen-based detection methods

Several methods can be used for the direct detection of viral antigens in clinical specimens or virus-infected cells. These include fluorescent antibody (FA) staining, immunohistochemical (immunoperoxidase) staining, solid-phase enzyme immunoassays (e.g. antigen-capture enzyme-linked immunosorbent assay [ELISA]), and lateral flow immunochromatographic assays (e.g. strip test). These assays detect viral antigens in clinical specimens by reacting with virus-specific antibody. Thus, these viral antigen-based detection methods are agent specific. FA staining and immunohistochemistry (IHC) are extremely useful in establishing a diagnosis because they are able to identify viral antigens in a tissue specimen in the context of microscopic lesions. Solid-phase enzyme assays or lateral flow chromatographic assays are useful for a liquid specimen (e.g. feces).

Viral nucleic acid-based detection methods

Nucleic acid-based methods for virus detection and discovery include sequence-dependent approaches, sequence-independent metagenomics approaches, and next-generation sequencing (NGS).

Nucleic acid sequence-dependent approaches

Polymerase chain reaction (PCR) assays are generally agent specific and require virus-specific sequence information to design primers and/or probes. Development of consensus, degenerate, or pan-family PCRs partially overcomes this limitation, but degenerate PCRs lack analytical sensitivity and are still highly dependent on prior sequence information on the virus genera/families being investigated.

Microarrays are another nucleic acid sequence-dependent approach for virus detection. Microchips consist of hundreds to thousands of oligonucleotide probes bound to a solid matrix. Virus detection and identification is based on hybridization of viral nucleic acids to specific probes. Microarrays have the potential to simultaneously detect multiple viruses in one sample. However, the current microarrays are not as sensitive as real-time PCRs and are not routinely used for virus detection in diagnostic laboratories (MacLachlan and Dubovi 2017).

In situ hybridization (ISH) utilizes a virus-specific probe to directly detect a virus present in clinical samples, especially tissues. Similar to FA or IHC, ISH is able to correlate the presence of virus with lesions observed in tissues. ISH is particularly useful for those viruses against which a validated antibody reagent is not available to conduct FA staining or IHC but whose nucleotide sequences are known.

Nucleic acid sequence-independent approaches

Nucleic acid sequence-dependent approaches efficiently detect known viruses but have limitations in discovering previously unrecognized viruses. Metagenomics is a technique that detects the totality of genetic material present in one sample (Handelsman 2004). A number of viral metagenomics approaches based on random amplification techniques have been utilized to amplify viral genetic materials for cloning and sequencing, including sequence-independent single-primer amplification, virus discovery cDNA-AFLP, and rolling circle amplification (Bexfield and Kellam 2011; Delwart 2007; Thurber et al. 2009). These approaches have allowed for the discovery of new viruses, but they have been mainly replaced by NGS-based metagenomics approaches.

NGS technologies became commercially available in 2005 and many different platforms of NGS have been developed since then. One common feature of NGS platforms is "massive parallel sequencing," an approach that generates millions of sequence reads in a single run, thereby producing sequences at a lower cost and faster speed. In addition, the hypothesis-free basis of NGS provides an unbiased approach for detecting multiple agents in a sample and for discovering new microorganisms (Datta et al. 2015; Temmam et al. 2014).

Detection of virus antibodies

Unless the antibody is maternally acquired, detection of virus-specific antibodies in a bodily fluid provides evidence that an animal has previously been exposed. This may be particularly useful in the case of viruses that are rapidly cleared by the animal. Classically, antibody-based detection of a viral infection was based on testing paired samples (2–4-week interval) from the same animal(s), with significant increases in antibody levels indicative of active viral infection. Likewise, detection of virus-specific IgM antibody in a single acute-phase serum sample can also indicate active viral infection. In contemporary production systems, collection and testing of serum, oral fluid, or other clinical specimens at 2- to 4-week intervals either for antibody or viral nucleic acid is used to detect infections prior to the appearance of clinical signs or disease outbreaks.

Depending on the clinical specimen and the viral pathogen, a variety of assays are available for detecting virus antibodies, including ELISA, virus neutralization, indirect FA, immunodiffusion, hemagglutination inhibition, and fluorescent microsphere immunoassay (FMIA). In particular, the differentiation of infected and vaccinated animals (DIVA) using vaccines and antibody ELISAs specifically designed for this purpose has improved the success of disease control and elimination programs, as in the case of Aujeszky's disease (pseudorabies) virus.

Characterization of viruses

Characterization of virion structure

EM is the most direct way to determine the morphology and size of a virus particle. Conventional transmission EM is generally used for detecting and characterizing virus particles in fluid matrices (negative stain EM) and cells/tissues (thin-section positive stain EM). Only the surface topography of virus particles can be imaged by transmission EM.

Some advanced techniques can be used to further characterize virion structures (Mateu 2013) although they are not used in diagnostic medicine. For example, cryo-electron microscopy (cryo-EM) together with three-dimensional reconstruction techniques can determine the three-dimensional structural model of virus particles by averaging the images of many individual virus particles. Cryo-EM is best applied to symmetric viruses and is not suitable for pleomorphic viruses because the procedures involve image averaging. Cryo-electron tomography (cryo-ET) can image an individual particle from various angels of view and, thus, can determine the three-dimensional structure of a single virus particle including symmetric and pleomorphic viruses. X-ray crystallography can be used to determine the crystal structures of virus particles or individual viral proteins, but crystals must be obtained first.

Ultrafiltration can be used to estimate the virion's size. In this procedure, a concentrated purified virus suspension is passed through a series of membrane filters with different pore sizes (10–300 nm). The presence and quantity of virus are then established for each filtrate.

Table 23.3 Taxonomy of the main viruses infecting pigs (based on ICTV Virus Taxonomy released in 2017).

Order	Family	Subfamily	Genus	Species	Virus common name
Bunyavirales	*Peribunyaviridae*		*Orthobunyavirus*	*Akabane orthobunyavirus*	Akabane virus
				?	Oya virus, Lumbo virus, Tahyna virus
			Herbevirus	*Herbert herbevirus*	Herbert virus
	Phenuiviridae		*Goukovirus*	*Gouleako goukovirus*	Gouleako virus
Herpesvirales	*Herpesviridae*	*Alphaherpesvirinae*	*Varicellovirus*	*Suid alphaherpesvirus 1*	Pseudorabies virus (PRV) or Aujeszky's disease virus
		Betaherpesvirinae	?	*Suid betaherpesvirus 2*	Porcine cytomegalovirus (PCMV)
		Gammaherpesvirinae	*Macavirus*	*Suid gammaherpesvirus 3*	Porcine lymphotropic herpesvirus 1 (PLHV-1)
				Suid gammaherpesvirus 4	PLHV-2
				Suid gammaherpesvirus 5	PLHV-3
				Ovine gammaherpesvirus 2	Ovine herpesvirus 2 (OvHV-2)
Mononegavirales	*Filoviridae*		*Ebolavirus*	*Reston ebolavirus*	Reston virus (RESTV)
				Zaire ebolavirus	Ebolavirus (EBOV)
	Paramyxoviridae		*Rubulavirus*	*Menangle rubulavirus*	Menangle virus
				Porcine rubulavirus	Blue eye paramyxovirus (BEPV) or La Piedad-Michoacan virus (LPMV)
			Henipavirus	*Nipah henipavirus*	Nipah virus (NiV)
				Hendra henipavirus	Hendra virus (HeV)
			Respirovirus	*Porcine respirovirus 1*	Porcine parainfluenza virus 1 (PPIV-1)
	Rhabdoviridae		*Vesiculovirus*	*Indiana vesiculovirus*	Vesicular stomatitis Indiana virus (VSIV)
				New Jersey vesiculovirus	Vesicular stomatitis New Jersey virus (VSNJV)
			Lyssavirus	*Rabies lyssavirus*	Rabies virus
Nidovirales	*Arteriviridae*		*Porartevirus*	*Porcine reproductive and respiratory syndrome virus 1*	Porcine reproductive and respiratory syndrome virus 1 (PRRSV-1)
				Porcine reproductive and respiratory syndrome virus 2	Porcine reproductive and respiratory syndrome virus 2 (PRRSV-2)
	Coronaviridae	*Coronavirinae*	*Alphacoronavirus*	*Alphacoronavirus 1*	Transmissible gastroenteritis virus (TGEV); porcine respiratory coronavirus (PRCV)
				Porcine epidemic diarrhea virus	Porcine epidemic diarrhea virus (PEDV)
			Betacoronavirus	*Betacoronavirus 1*	Porcine hemagglutinating encephalomyelitis virus (pHEV)
			Deltacoronavirus	*Coronavirus HKU15*	Porcine deltacoronavirus (PDCoV)
		Torovirinae	*Torovirus*	*Porcine torovirus*	Porcine torovirus (PToV)
Ortervirales	*Retroviridae*	*Orthoretrovirinae*	*Gammaretrovirus*		Porcine endogenous retrovirus (PERV)

Picornavirales	*Picornaviridae*	*Aphthovirus*	*Foot-and-mouth disease virus*	Foot-and-mouth disease virus (FMDV)
		Cardiovirus	*Cardiovirus A*	Encephalomyocarditis virus (EMCV)
		Enterovirus	*Enterovirus B*	Coxsackievirus B4 (including swine vesicular disease virus 2 [SVDV-2]); coxsackievirus B5 (including SVDV-1)
			Enterovirus G	Enterovirus (EV) G1 (old name PEV-9); EV-G2 (PEV-10); EV-G3 (PEV-14); EV-G4 (PEV-15); EV-G6 (PEV-16), EV-G8 to EV-G19
		Kobuvirus	*Aichivirus C*	Porcine kobuvirus (PKV)
		Pasivirus	*Pasivirus A*	Swine pasivirus A1 (PaV-A1); PaV-A2; PaV-A3
		Sapelovirus	*Sapelovirus A*	Porcine sapelovirus (PSV)
		Senecavirus	*Senecavirus A*	Seneca Valley virus (SVV)
		Teschovirus	*Teschovirus A*	Porcine teschovirus (PTV) 1–13
		Unassigned	Unassigned	Porcine picornavirus Japan
	Adenoviridae	*Mastadenovirus*	*Porcine mastadenovirus A*	Porcine adenovirus 1, 2, 3 (PAdV-1, 2, 3)
			Porcine mastadenovirus B	Porcine adenovirus 4 (PAdV-4)
			Porcine mastadenovirus C	Porcine adenovirus 5 (PAdV-5)
	Anelloviridae	*Iotatorquevirus*	*Torque teno sus virus 1a*	Torque teno sus virus 1a (TTSuV1a)
			Torque teno sus virus 1b	Torque teno sus virus 1b (TTSuV1b)
		Kappatorquevirus	*Torque teno sus virus k2a*	Torque teno sus virus k2a (TTSuVk2a)
			Torque teno sus virus k2b	Torque teno sus virus k2b (TTSuVk2b)
	Asfarviridae	*Asfivirus*	*African swine fever virus*	African swine fever virus (ASFV)
	Astroviridae	*Mamastrovirus*	*Mamastrovirus 3*	Porcine astrovirus 1 (PAstV-1)
			?	Porcine astrovirus 2, 3, 4, 5 (PAstV-2, 3, 4, 5)
	Caliciviridae	*Vesivirus*	*Vesicular exanthema of swine virus*	Vesicular exanthema of swine virus (VESV)
		Norovirus	*Norwalk virus*	Porcine norovirus
		Sapovirus	*Sapporo virus*	Porcine sapovirus (historically porcine enteric calicivirus)
		Unassigned	?	St-Valérien virus
	Circoviridae	*Circovirus*	*Porcine circovirus 1*	Porcine circovirus 1 (PCV1)
			Porcine circovirus 2	Porcine circovirus 2 (PCV2)
			?	Porcine circovirus 3 (PCV3)
	Flaviviridae	*Flavivirus*	*Japanese encephalitis virus*	Japanese encephalitis virus (JEV)
			West Nile virus	West Nile virus (WNV)
			Murray Valley encephalitis virus	Murray Valley encephalitis virus (MVEV)
		Pestivirus	*Classical swine fever virus*	Classical swine fever virus (CSFV)
			Bovine viral diarrhea virus 1	Bovine viral diarrhea virus 1 (BVDV-1)

(*Continued*)

Table 23.3 (Continued)

Order	Family	Subfamily	Genus	Species	Virus common name
				Bovine viral diarrhea virus 2	Bovine viral diarrhea virus 2 (BVDV-2)
				Border disease virus	Border disease virus (BDV)
				?	Bungowannah virus
				?	Atypical porcine pestivirus (APPV)
	Hepeviridae		*Orthohepevirus*	*Orthohepevirus A*	Hepatitis E virus (HEV)
	Orthomyxoviridae		*Influenzavirus A*	*Influenza A virus*	Swine influenza virus (SIV) or influenza A virus in swine (IAV-S)
			Influenzavirus B	*Influenza B virus*	Influenza B virus
			Influenzavirus C	*Influenza C virus*	Influenza C virus
			Influenzavirus D	*Influenza D virus*	Influenza D virus
	Parvoviridae	*Parvovirinae*	*Protoparvovirus*	*Ungulate protoparvovirus 1*	Porcine parvovirus 1 (PPV1)
			Tetraparvovirus	*Ungulate tetraparvovirus 3*	Porcine parvovirus 2 (PPV2)
				Ungulate tetraparvovirus 2	Porcine parvovirus 3 (PPV3), or porcine hokovirus, or PARV4-like
			Copiparvovirus	*Ungulate copiparvovirus 2*	Porcine parvovirus 4 (PPV4)
				?	Porcine parvovirus 5 (PPV5)
			?	?	Porcine parvovirus 6 (PPV6)
			?	?	Porcine parvovirus 7 (PPV7)
			Bocaparvovirus	*Ungulate bocaparvovirus 2, 3, 4, 5*	Porcine bocaviruses
	Poxviridae	*Chordopoxvirinae*	*Suipoxvirus*	*Swinepox virus*	Swinepox virus
	Reoviridae	*Sedoreovirinae*	*Rotavirus*	*Rotavirus A*	Rotavirus A (RVA)
				Rotavirus B	Rotavirus B (RVB)
				Rotavirus C	Rotavirus C (RVC)
				Rotavirus E	Rotavirus E (RVE)
				Rotavirus H	Rotavirus H (RVH)
		Spinareovirinae	*Orthoreovirus*	*Mammalian orthoreovirus*	Porcine reovirus
	Togaviridae		*Alphavirus*	*Eastern equine encephalitis virus*	Eastern equine encephalitis virus (EEEV)
				Getah virus	Getah virus (GETV)
				?	Sagiyama virus (SAGV)

Virion size is based on the pore sizes of two filters – one allowing virus to pass and the other not allowing virus to pass or substantially reducing the amount of viruses passing. While EM estimates the size of the dehydrated virion, ultrafiltration gives an estimate of the hydrated virion.

The presence of a viral envelope can usually be determined by exposing the virus to a lipid solvent (ether, chloroform) or detergent (sodium deoxycholate, Triton® X). The infectivity of enveloped virions is easily destroyed by these reagents, whereas non-enveloped viruses retain their infectivity.

Characterization of viral genome

Compositions of viral genome

Either enzymatic or chemical methods can be used to determine the type of viral nucleic acid in a virus. In the enzymatic method, virus material is digested with RNase or DNase and then subjected to gel electrophoresis to determine which enzyme digested the genetic material. Chemical determination uses the diphenylamine reaction for DNA or the orcinol reaction for RNA to characterize the makeup of the genome.

Acridine orange staining can be used to differentiate double-stranded DNA or RNA viruses from single-stranded DNA or RNA viruses in infected cells. Double-stranded DNA or RNA is visualized as a yellow-green fluorescence, whereas single-stranded RNA or DNA is orange-red. In combination with the location of fluorescence in cells (nucleus vs. cytoplasm), both the nucleic acid composition (DNA vs. RNA) and the strandedness of the virus can be determined.

Electrophoretic techniques can be used to determine if a virus has more than one molecule of double-stranded RNA or DNA. For certain viruses (e.g. rotaviruses), electrophoretic typing based on migration pattern of RNA segments has been used to determine groups. The polarity (sense) of viral nucleic acid can be determined through transfection or *in vitro* translation studies with a extracted viral genome. Appropriate cells transfected with positive-sense viral genome will produce virions because the genomic material serves as mRNA and is translated into proteins. Under specific conditions, an *in vitro* translation study can demonstrate the same effect.

Virus genetic diversity and relatedness

Viruses can sometimes be divided into different types, subtypes, serotypes, or genotypes. For example, influenza viruses can be classified into different types (A, B, C, and D); influenza A virus (IAV) can be classified into different subtypes (e.g. H1N1, H1N2, H3N2) based on the hemagglutinin (HA or H) and neuraminidase (NA or N); rotaviruses can be classified into different serogroups (based on the VP6 protein) or G genotypes/serotypes (based on the VP7 protein) and P genotypes (based on the VP4 protein). Determination of subtypes and/or genotypes of viruses can be achieved using subtyping PCRs or genotyping PCRs. However, genomic sequences can more definitely determine the subtypes and genotypes. In addition, sequences can be analyzed in depth to fulfill other purposes.

From the 1970s to the early 2000s, Sanger sequencing was the primary method for DNA/cDNA sequencing. This method is still used by many diagnostic and research laboratories, but is being replaced by NGS technologies. Virus sequences can be used in comparative sequence analyses (sequence homology), phylogenetic analyses, and recombination analyses to determine the genetic diversity and genetic relatedness of viruses. If collection dates of the samples containing the virus are available, molecular clock phylogenetic analyses can be conducted to establish the evolutionary relationship of viruses. For those viruses in which genetic sequences have been associated with certain phenotypes, determination of virus sequences can predict the corresponding phenotypes. For example, sequencing the HA gene of avian influenza viruses can predict whether the virus will be highly pathogenic.

Quantitation of viruses

The concentration (titer) of infectious virus in a sample can be determined using a dose–response curve, with the results expressed as infectious dose 50 (ID_{50}) (i.e. the dilution at which 50% of the population becomes infected). For viruses that can be cultivated in cell culture or embryonated chicken eggs, wells of susceptible cells or individual eggs are inoculated with sequential dilutions of the virus. Data (positive or negative for virus replication) are then analyzed using one of several possible statistical procedures (Finney 1978) to estimate the 50% infectious dose (e.g. tissue culture infectious dose [$TCID_{50}$] or egg infectious dose [EID_{50}]). For cytopathic viruses that produce clear plaques in cell culture, the concentration of infectious virus can also be estimated in terms of plaque-forming units (PFU). For viruses with hemagglutinating activity, a hemagglutination (HA) test can be performed to determine HA titers.

Real-time PCR has the capability to quantitate genomic copies of a virus in a sample (quantitative real-time PCR), but unlike the infectivity assays described above, PCR detects both infectious and noninfectious virus. In order to accurately quantitate virus in a sample, two criteria should be met: (1) control samples with known genomic copies of the virus should be included in the quantitative PCR to generate a standard curve, and (2) the suspension of test samples should be treated with nucleases to degrade all non-virion nucleic acids. However, in a diagnostic laboratory, it is impractical to treat every clinical

sample with nucleases before testing by a quantitative real-time PCR; thus, under diagnostic setting, quantitative real-time PCR just estimates the genomic copies of a virus in a sample based on standards.

Characterization of specific viral phenotypes

Some viruses cause cytopathic effect (CPE), inclusion bodies, and hemadsorption. For cytopathic viruses, CPE is characterized by morphological changes of cells infected with viruses. Inclusion bodies are alterations to the intracellular architecture induced by virus infection and can be observed after infected tissues/cells are fixed and treated with cytological stains. For example, paramyxoviruses, reoviruses, rabies virus, and poxviruses can induce cytoplasmic inclusion bodies, whereas herpesviruses, adenoviruses, and parvoviruses can induce intranuclear inclusion bodies. However, not all viruses produce obvious inclusion bodies. Hemadsorption refers to adsorption of erythrocytes to the surface of virus-infected cells. Examples of hemadsorbing viruses include some parainfluenza viruses and coronaviruses.

Viral susceptibility to temperature, pH, disinfectants, and other factors can be assessed by comparing the infectious titers of untreated with treated viruses.

Virulence phenotypes, pathogenicity, pathogenesis, and immunity of a virus can be determined by inoculating test animals followed by assessment of appropriate parameters such as clinical signs (including morbidity and mortality), viral shedding, gross lesions, microscopic lesions, immune responses, and so on. For novel or previously uncharacterized viruses, detection of the virus in a sample (especially by molecular methods) does not necessarily mean the virus is the causative agent of a disease. In order to establish causality between a previously unrecognized virus and disease, animal inoculation studies may be required to determine whether Koch's postulates can be fulfilled.

Viral phenotypes and their genetic determinants

Even if well-characterized viral phenotypic and genetic data are available, it is often difficult to identify the genetic determinants of some phenotypes. However, advancements in recombinant DNA and reverse genetics technologies have revolutionized virus studies. Construction and genetic manipulation of full-length cDNA clones (RNA viruses) and DNA clones (DNA viruses) have become important tools in the study of the biology, pathogenesis, and virulence determinants of viruses. After introducing the defined nucleotide changes into the gene(s) of interest in a cDNA/DNA clone, phenotypes of the recombinant progeny virus can be compared with those of the parental viruses. Thus, reverse genetics systems make it possible to achieve functional characterization of genes of interest and their association with viral phenotypes and genetic determinants.

Swine viruses

The taxonomy of the principal viruses infecting swine is summarized in Table 23.3. The detailed descriptions of each virus and its consequences of infecting swine are provided in Chapters 24–46.

References

Bexfield N, Kellam P. 2011. *Vet J* 190:191–198.

Condit RC. 2013. Principles of virology. In Knipe DM, Howley PM, eds. *Fields of Virology*, 6th ed. Philadelphia: Lippincott Williams & Wilkins, pp. 21–51.

Datta S, Budhauliya R, Das B, et al. 2015. *World J Virol* 4:265–276.

Delwart EL. 2007. *Rev Med Virol* 17:115–131.

Finney DJ. 1978. *Statistical Method in Biological Assay*, 3rd ed. New York: MacMillan Publishing Company, Inc.

Goodpasture EW, Woodruff AM, Buddingh GJ. 1932. *Am J Pathol* 8:271–281.

Handelsman J. 2004. *Microbiol Mol Biol Rev* 68:669–685.

Hughes SS. 1977. *The Virus: A History of the Concept*. London: Heinemann Education Books.

MacLachlan NJ, Dubovi EJ, eds. 2011. *Fenner's Veterinary Virology*, 4th ed. San Diego: Academic Press.

MacLachlan NJ, Dubovi EJ, eds. 2017. *Fenner's Veterinary Virology*, 5th ed. San Diego: Academic Press.

Mateu MG, ed. 2013. *Structure and Physics of Viruses*. Dordrecht, Heidelberg, New York and London: Springer.

Murphy FA, Gibbs EPJ, Horzinek MG, et al., eds. 1999. *Veterinary Virology*, 3rd ed. San Diego: Academic Press.

Storch GA, Wang D. 2013. Diagnostic virology. In Knipe DM, Howley PM, eds. *Fields of Virology*, 6th ed. Philadelphia: Lippincott Williams & Wilkins, pp. 414–451.

Temmam S, Davoust B, Berenger JM, et al. 2014. *Int J Mol Sci* 15:10377–10397.

Thurber RV, Haynes M, Breitbart M, et al. 2009. *Nat Protoc* 4:470–483.

24

Adenoviruses

David A. Benfield and Richard A. Hesse

Relevance

Adenoviruses have been isolated from pigs with diarrhea (Coussement et al. 1981; Haig et al. 1964; McAdaragh et al. 1980), encephalitis (Kasza 1966), nephritis (Nietfeld and Leslie-Steen 1993), respiratory disease (Hirahara et al. 1990), aborted fetuses (Dee 1995), and pigs with no clinical signs (Clarke et al. 1967; Sharpe and Jessett 1967). In general, porcine adenoviruses (PAdVs) produce subclinical infections and rarely cause disease of economic significance in swine herds.

Etiology

PAdVs are classified in the genus *Mastadenovirus* in the family *Adenoviridae*. There are three species (*Porcine mastadenovirus A*, *Porcine mastadenovirus B*, and *Porcine mastadenovirus C*) and five serotypes identified by virus neutralization assays (Clarke et al. 1967; Haig et al. 1964; Hirahara et al. 1990; Kadoi et al. 1995; Kasza 1966). The species *Porcine mastadenovirus A* includes PAdV serotypes 1, 2, and 3; the species *Porcine mastadenovirus B* includes serotype 4, the most commonly isolated serotype; and the species *Porcine mastadenovirus C* includes serotype 5 (Büchen-Osmond 2003).

Serotype 1 was isolated from a rectal swab from a pig with diarrhea (Haig et al. 1964), types 2 and 3 were isolated from pigs with normal feces (Sharpe and Jessett 1967), type 4 was isolated from the brain of a pig with neurological signs and enteritis (Kasza 1966), and type 5 was isolated from nasal secretions of pigs with respiratory disease (Hirahara et al. 1990) and the brain of a newborn piglet (Kadoi et al. 1995). In a study of 24 pools of swine fecal samples, 17/24 pools from pigs 3 to 18 months of age were positive for PAdV with serotype 3 most commonly detected (Maluquer de Motes et al. 2004). Recently, a new serotype of PAdV (WI) was isolated from the wash water in a swine facility (Sibley et al. 2011), and another novel serotype (SVN1) from urinary bladder urothelial cell cultures prepared from the bladders of normal pigs (Jerman et al. 2014). Infections of porcine urinary bladder cells with PAdV had not been previously reported, although bladder tissue did not show visible signs of infection (Jerman et al. 2014).

The morphology, structure, and physical properties of PAdVs are similar to other adenoviruses. Virions are nonenveloped icosahedra 80–90 nm in diameter with a capsid consisting of 240 hexon capsomeres, and 12 penton capsomeres (pentamers) that occupy the 12 corners of the virion. A fiber protein 20–50 nm in length with a terminal knob projects from each pentamer. The adenovirus genome is double-stranded linear DNA approximately 32–34 kilobases in length (Kleiboeker et al. 1993; Nagy et al. 2001; Reddy et al. 1998). The genome contains sufficient genetic information to code for 10 proteins, but actually encodes up to 40 proteins due to a complex RNA splicing mechanism (Kleiboeker 2005). Viral replication of PAdV is similar to other adenoviruses, with early protein expression needed for viral replication and late proteins consisting of structural components of the virion. Adenoviral virions are very stable, resisting heat inactivation at room temperature for up to 10 days, but readily inactivated by bleach, formaldehyde, alcohol, and phenolic compounds (Derbyshire and Arkell 1971).

PAdVs can be isolated and amplified in primary porcine kidney, continuous porcine kidney cells (PK-15), primary thyroid (Dea and El Azhary 1984), and primary testicular cell cultures (Hirahara et al. 1990). Replication in cell cultures produces a cytopathic effect (CPE) 2–5 days after inoculation. CPE is characterized by rounding and swelling of infected cells into aggregates of "grape-like" clusters that eventually detach from the substrate (Derbyshire et al. 1968). The most notable morphologic feature of adenovirus replication is the presence of Cowdry type A intranuclear inclusions that represent crystalline arrays of viral proteins in the cell nucleus (Koestner et al. 1968). These inclusions have been

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

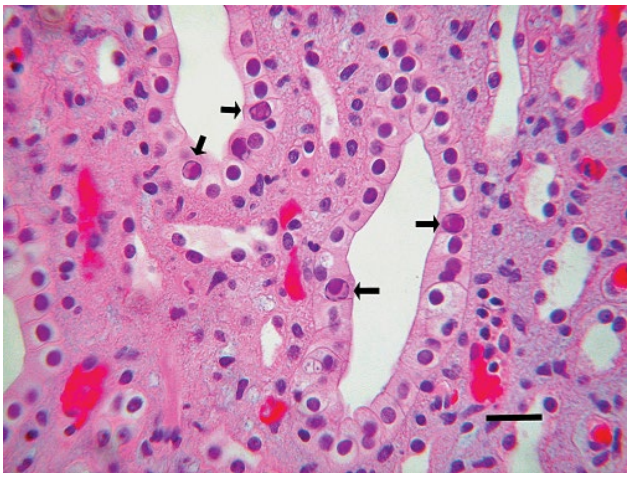


Figure 24.1 Intranuclear adenovirus inclusion bodies (arrows) in tubular epithelial cells in the papilla of a pig kidney (hematoxylin and eosin stain). Bar = 30 µm. *Source:* Courtesy of Jerome Nietfeld.

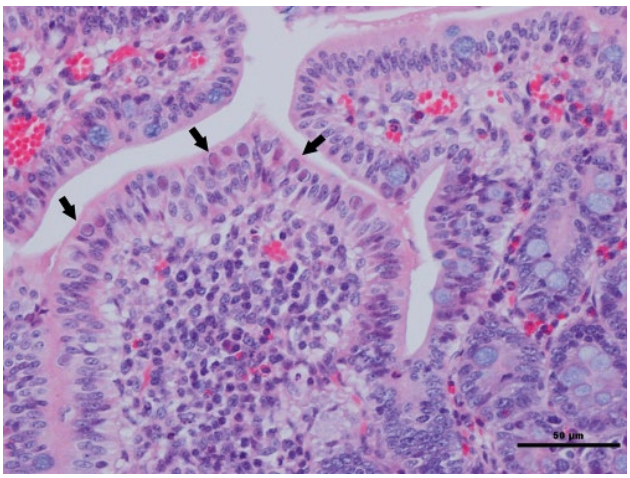


Figure 24.2 Intranuclear porcine adenovirus inclusions (arrows) in the follicle-associated epithelium of a small intestinal dome of a naturally infected pig (hematoxylin and eosin stain). Bar = 50 µm. *Source:* Courtesy of Jerome Nietfeld.

observed in cell cultures (Derbyshire et al. 1968) and in several tissues, most notably the kidney (Figure 24.1) and intestinal epithelium in the distal jejunum and ileum (Figure 24.2) of naturally and experimentally infected pigs (Coussement et al. 1981; Ducatelle et al. 1982; Sanford and Hoover 1983).

Public health

PAdVs are not known to have public health significance.

Epidemiology

Adenoviruses tend to be host specific and swine are the only known species susceptible to PAdV. While PAdV does not infect humans, swine can be experimentally infected with human adenoviruses (Betts et al. 1962). Cross-species infection between human adenoviruses and swine has been useful in that replication-defective human adenovirus type 5 vectored vaccines have been successfully developed and used to immunize swine against swine influenza (Braucher et al. 2012; Wesley and Lager 2005, 2006; Wesley et al. 2004).

Serological surveys indicate that most adult animals have antibodies to adenoviruses, but in the context of the low incidence of clinical disease, the data suggest that most infections are subclinical. The present incidence of PAdV in swine populations is unknown because most of the serological surveys were done in the 1960s–1970s. These studies indicated 26–53% of the swine population in southwest England had adenovirus group-specific antibodies (Darbyshire 1967; Darbyshire and Pereira 1964). Other reports from England indicated that 50–60% of adult swine had antibodies to adenovirus, as determined by virus neutralization and immunodiffusion tests (Darbyshire 1967; Kasza et al. 1969). The prevalence of adenovirus type 4 in Quebec was found to be considerably lower, with 83/540 (15.2%) of swine seropositive in one study (Dea and El Azhary 1984) and 64/350 (18.3%) in another (Elazhary et al. 1985).

Most PAdVs are transmitted via fecal–oral route or possibly by aerosol exposure (Benfield 1990). Vectors are not known to be involved, but the stability of PAdVs in the environment makes transmission possible via fomites, e.g. boots, clothing, bedding, transport vehicles, and feed utensils.

Most outbreaks of adenoviral diarrhea occur in pigs 1–4 weeks of age (Abid et al. 1984; Coussement et al. 1981; Sanford and Hoover 1983). PAdVs are most frequently isolated from rectal swabs of weaned pigs and rarely from adult animals (Derbyshire et al. 1966). The presence of high antibody titers in adults may prevent active replication of virus. Shedding of PAdV in feces has been reported up to 14 weeks post weaning (Derbyshire et al. 1966), and PAdV has been isolated from brain, nasal tissue, pharynx, lungs, and intestines up to 48 days after experimental inoculation (Kasza 1966). Viral antigen has also been observed in enterocytes by fluorescent antibody (FA) staining up to 45 days after infection, suggesting that long-term fecal shedding may occur (Kleiboeker 2005).

Pathogenesis

PAdVs are most commonly associated with gastrointestinal disease in swine (Abid et al. 1984; Coussement et al. 1981; Derbyshire et al. 1966, 1975; Ducatelle et al. 1982; Haig et al. 1964; Sanford and Hoover 1983). Respiratory (Hirahara et al. 1990), reproductive (Dee 1995), and neurological (Edington et al. 1972; Kasza 1966; Shadduck

et al. 1967) signs have been associated with PAdV infection, but rarely reported as the principal pathogen in pigs with these clinical presentations.

Infection of swine with PAdV occurs via ingestion and/or inhalation. Primary replication occurs in the tonsil and villous enterocytes and lymphoid tissue in the distal small intestine (Coussement et al. 1981; Ducatelle et al. 1982; Sanford and Hoover 1983; Shadduck et al. 1967; Sharpe and Jessett 1967). In all experimental studies, independent of the route of inoculation, virus replication is always demonstrated in the short blunt villi overlying lymphoid tissue or Peyer's patches in the ileum (Figure 24.2).

Piglets inoculated orally with PAdV-4 developed a watery diarrhea 3–4 days post inoculation (DPI) that lasted for 3–6 days. Adenoviral particles were demonstrated in intestinal contents up to 9 DPI. Antigen was detected in villous epithelial cells of the distal jejunum and the ileum as early as 24 hours after inoculation and for at least 15 DPI and, in one pig, up to 45 DPI (Ducatelle et al. 1982). In another study, diarrhea was induced in 8-day-old gnotobiotic piglets within 48 hours post inoculation, and adenovirus inclusions and antigens were demonstrated by light microscopy and immunofluorescence in the short blunted villi overlying lymphoid aggregates or Peyer's patches in the ileum (McAdaragh et al. 1980).

Infection of colostrum-deprived pigs causes pneumonia and lesions in the kidney, thyroid, and lymph nodes (Shadduck et al. 1967). Inoculation of pregnant swine can result in abortion, with virus replication demonstrated in fetal tissues (Dee 1995). Adenoviruses may also play a role in coinfections. Serotype 4 adenovirus in combination with *Mycoplasma hyopneumoniae* produced a more severe pneumonia (Kasza et al. 1969), and adenovirus inclusion bodies are more commonly observed in the kidneys of pigs with porcine circovirus-associated disease (J. Nietfeld, 2010, personal communication).

Common histological lesions include villous blunting and the presence of intranuclear basophilic inclusion bodies in enterocytes of the distal jejunum and ileum (Coussement et al. 1981; Ducatelle et al. 1982). In naturally infected pigs, affected enterocytes are usually present along the sides and tips of villi or on the apical border of the short blunt villi over Peyer's patches. These enterocytes appear to be desquamating and the nuclei contain large eosinophilic-to-amphophilic inclusion bodies (Sanford and Hoover 1983) (Figure 24.2). Intranuclear inclusion bodies are also commonly observed in the lung, kidney (Figure 24.1), and occasionally brain following experimental inoculation (Shadduck et al. 1967).

Shadduck et al. (1967) induced encephalitis only when pigs were inoculated via the intracerebral route; intranasal/oral inoculation produced a nonsuppurative pneumonitis. Intranuclear inclusions were observed in a variety of organs including the lung, kidney, and brain. Virus was isolated from these tissues for several weeks after inoculation.

Interstitial nephritis has also been described in a pig naturally infected with adenovirus. Kidney lesions included inflammation and intranuclear inclusion bodies in cells lining the tubules of the medulla. These cells were confirmed to be adenovirus-infected by direct FA staining, electron microscopy, and virus isolation from kidneys (Nietfeld and Leslie-Steen 1993). An unusual disseminated adenovirus infection in a nursing pig with cutaneous and visceral hemorrhages has also been described (Tang et al. 1995).

Clinical signs

Watery to pasty diarrhea is the most consistent clinical sign observed in pigs naturally or experimentally inoculated with PAdV. Diarrhea is typically observed 3–4 days after oronasal inoculation of Cesarean-derived colostrum-deprived piglets and persists for 3–6 days. Pigs may present with mild dehydration and reduced weight gain, but mortalities are rare (Coussement et al. 1981; Derbyshire et al. 1969, 1975; Ducatelle et al. 1982; Sanford and Hoover 1983). Respiratory signs and abortion are also rare clinical presentations.

Diagnosis

Adenovirus infection should be considered in the differential diagnosis of gastrointestinal and possibly respiratory diseases of pigs. PAdV can be detected by negative stain electron microscopy of feces or intestinal contents, detection of viral antigens in tissue by FA, or immunohistochemistry (IHC) staining of infected cells. FA staining using frozen sections is rapid, and results may be available the day of sample submission. IHC also offers rapid and specific identification of adenoviruses, but usually requires 1–2 days processing time. Although intranuclear inclusion bodies may be detected by light microscopy in histological samples of tissues, definitive diagnosis of PAdV should be confirmed by FA, IHC, or virus isolation.

PAdV can be readily isolated in cell culture from fecal samples or homogenates of lung or kidney. PAdV replicates and induces CPE in primary swine kidney cells, PK-15 cells, primary pig thyroid, and pig testicular cells. PAdV is frequently isolated from the kidneys, spleen, or testes harvested for the production of primary cell cultures (Hirahara et al. 1990). Thus, interpretation of virus isolation results can be complicated by the ubiquitous presence of adenoviruses in swine herds. This becomes an adventitious agent "problem" when primary pig kidney cells are used as vaccine substrate or in xenotransplantation procedures where pig tissues are used in

humans. Once isolated, the serotype of adenoviruses can be determined by virus neutralization assays with type-specific antisera, but this is rarely done because such reagents are not readily available.

PAdV infection can be serologically diagnosed by demonstrating a rising antibody titer in the presence of clinical disease. Serological diagnosis is done by either viral neutralization assays or an indirect FA test with adenovirus-infected cells as substrate to detect antibodies (Dea and El Azhary 1984).

Polymerase chain reaction (PCR) and quantitative polymerase chain reaction (qPCR) assays have been developed for the detection of PAdV serotype 3 in feces (Hundesa et al. 2006; Maluquer de Motes et al. 2004). To date, PCR has not been evaluated as a diagnostic tool for the detection of PAdV in clinical samples.

Immunity

Since most PAdV infections are either asymptomatic or cause moderate disease, there is little information on immunity. Most adult swine are seropositive to adenovirus. Colostrum-deprived and conventional newborn piglets are free of adenovirus antibodies prior to nursing. After nursing, antibody titers peak within a few days and persist for 8–9 weeks after weaning. Presumably, antibodies are protective, or at least moderate adenovirus infection, because the frequency of adenovirus shedding increases after weaning as colostral antibody titers decline (Derbyshire et al. 1966).

Prevention and control

PAdVs are stable for up to 10 days in the environment (Dee 1995) and are reported to remain viable for 18 days downstream of the site of a manure spill (Haack et al. 2015). Control and prevention is dependent on practicing sanitation procedures that reduce environmental fecal contamination. Due to the mild nature of adenovirus infections, there is little incentive for the production of vaccines. PAdVs have proven to be potential vectors for veterinary vaccines incorporating antigens from transmissible gastroenteritis and African swine fever viruses (Bangari and Mittal 2004; Ferreira et al. 2005).

References

Abid HN, Holscher MA, Byerly CS. 1984. *Vet Med Small Anim Clin* 79:105–107.

Bangari DS, Mittal SK. 2004. *Virus Res* 105:127–136.

Benfield DA. 1990. Enteric adenoviruses of animals. In Saif LJ, Theil KW, eds. *Viral Diarrheas of Man and Animals*. Boca Raton, FL: CRC Press, Inc., pp. 115–136.

Betts AO, Jennings AR, Lamont PH, et al. 1962. *Nature (London)* 193:45–46.

Braucher DR, Henningson JN, Loving CL, et al. 2012. *Clin Vaccine Immunol* 9:1722–1729.

Büchen-Osmond C. 2003. Mastadenovirus. In ICTVdB – The universal virus database, version e. ICTVdB management, The Earth Institute, Biosphere 2 Center, Columbia University, Oracle AZ USA.

Clarke MC, Sharpe HBA, Derbyshire JB. 1967. *Arch Gesamte Virusforsch* 21:91–97.

Coussement W, Ducatelle R, Charlier G, et al. 1981. *Am J Vet Res* 42:1905–1911.

Darbyshire JH. 1967. *Vet Res* 81:118–121.

Darbyshire JH, Pereira HG. 1964. *Nature (London)* 201:895–897.

Dea S, El Azhary MA. 1984. *Am J Vet Res* 45(10):2109–2112.

Dee SA. 1995. *Comp Cont Educ Pract Vet* 17(7):962–972.

Derbyshire JB, Arkell S. 1971. *Br Vet J* 127:137–141.

Derbyshire JB, Clarke MC, Jessett DM. 1966. *Vet Rec* 79:595–599.

Derbyshire JB, Chandler RL, Smith K. 1968. *Res Vet Sci* 9:300–303.

Derbyshire JB, Clarke MC, Jessett DM. 1969. *J Comp Pathol* 79:97–100.

Derbyshire JB, Clarke MC, Collins AP. 1975. *J Comp Pathol* 85:437–443.

Ducatelle R, Coussement W, Hooren J. 1982. *Vet Pathol* 19:179–189.

Edington N, Kasza L, Christofinis GJ. 1972. *Res Vet Sci* 13:289–291.

Elazhary MASY, Dea S, Mittal KR, et al. 1985. *Can Vet J* 26:190–192.

Ferreira TB, Alves PM, Aunins JG, et al. 2005. *Gene Ther* 12:S73–S83.

Haack SK, Duris JW, Kolpin DW, et al. 2015. *Appl Environ Microbiol* 81:3430–3441.

Haig DA, Clarke MC, Pereira MS. 1964. *J Comp Pathol* 74:81–84.

Hirahara T, Yasuhara H, Matsui O, et al. 1990. *Jpn J Vet Sci* 52:1089–1091.

Hundesa A, DeMotes CM, Bofill-Mas S, et al. 2006. *Appl Environ Microbiol* 72:7886–7893.

Jerman UD, Kolene M, Steyer A, et al. 2014. *Viruses* 6:2505–2518.

Kadoi K, Inoue Y, Ikeda T, et al. 1995. *J Basic Microbiol* 35:195–204.

Kasza L. 1966. *Am J Vet Res* 27:751–758.

Kasza L, Hodges RT, Retts AO, et al. 1969. *Vet Rec* 84:262–267.

Kleiboeker S. 2005. Chapter 12: Porcine reproductive and respiratory syndrome. In Straw BE, Sylvie D'Allaire, and

David J. Taylor, eds. *Diseases of Swine*, 9th ed. Ames, IA: Iowa State University Press, pp. 287–290.
Kleiboeker SB, Seal BS, Mengeling WL. 1993. *Arch Virol* 133: 357–368.
Koestner A, Kasza L, Kindig O, et al. 1968. *Am J Pathol* 53:651–665.
Maluquer de Motes C, Clemente-Casares P, Hundesa A, et al. 2004. *Appl Environ Microbiol* 70:1448–1454.
McAdaragh JP, Eustis S, Benfield DA. 1980. Adenovirus associated with diarrhea in pigs. Abstract. In Conference of Research Workers in Animal Diseases, Chicago, IL, 10–11 November.
Nagy M, Nagy E, Tuboly T. 2001. *J Gen Virol* 82:525–529.
Nietfeld J, Leslie-Steen P. 1993. *J Vet Diagn Invest* 5:269–273.
Reddy PS, Idamakanti N, Song JY, et al. 1998. *Virology* 251:414–426.
Sanford SE, Hoover DM. 1983. *Can J Comp Med* 47:396–400.
Shadduck JA, Koestner A, Kasza L. 1967. *Pathol Vet* 4:537–547.
Sharpe HD, Jessett DM. 1967. *J Comp Pathol* 77:45–50.
Sibley SD, Goldberg TL, Pederson JA. 2011. *Appl Environ Microbiol* 77:5001–5008.
Tang KN, Baldwin CA, Manseli JL, et al. 1995. *Vet Pathol* 32:433–437.
Wesley RD, Lager KM. 2005. *Am J Vet Res* 66:1943–1947.
Wesley RD, Lager KM. 2006. *Vet Microbiol* 118:67–75.
Wesley RD, Tang M, Lager KM. 2004. *Vaccine* 22:3427–3434.

25

African Swine Fever Virus

José Manuel Sánchez-Vizcaíno, Alberto Laddomada, and Marisa L. Arias

Relevance

Historical overview

African swine fever (ASF) was first described in 1921 in Kenya when the virus (ASFV) was transmitted from wild African suids to domestic pigs, causing a disease with 100% mortality (Montgomery 1921). Since this discovery, ASFV has left Africa on three occasions. The first incursion occurred in 1957 from Angola to Lisbon (Manso-Ribeiro et al. 1958). The second (1960) was from Africa to Lisbon and then to Spain and other European countries, including France (1964), Italy (1967, 1969), the island of Sardinia (1978), Malta (1978), Belgium (1985), and the Netherlands (1986). From Europe, ASFV spread to Latin America, including Cuba (1971, 1980), Brazil (1978), the Dominican Republic (1978), and Haiti (1979). Subsequently ASFV was eradicated from all of these countries, except Portugal and Spain, where it remained endemic until 1995, and Sardinia, where it remains endemic today. A third ASFV incursion from Africa occurred in 2007, when it entered the Caucasian region and spread to the Russian Federation (2007), Ukraine (2012), Belarus (2013), reaching the Baltic regions and Poland in 2014, and spreading westward to Central and Western Europe in 2017 and 2018. In August 2018 ASF was reported for the first time in China. All of these countries remain infected with ASFV at present.

The current ASFV epidemiological situation poses a serious threat to animal health, pig production, and therefore the economies of affected and neighboring countries. ASFV remains endemic in many sub-Saharan countries on the African continent.

Public health

ASFV is not infectious to humans and does not directly affect public health (EFSA 2009). However, ASFV has a serious social and economic impact on the trade of swine, pig by-products, and food security, particularly in countries in which pigs are an important source of protein.

Etiology

ASFV is a large, icosahedral, linear double-stranded DNA virus and the only member of the family *Asfarviridae*, genus *Asfivirus* (Dixon et al. 2005). The virion is composed of four concentric layers and an external hexagonal membrane (Figure 25.1) acquired by budding through the cell plasma membrane (Salas and Andrés 2013). ASFV replication occurs mainly in the cytoplasm of infected macrophages, though an early stage of replication has also been described in the nucleus.

The viral genome varies in length from 170 to 193 kb and contains 150–167 open reading frames. The genome consists of a conserved central region of approximately 125 kb and two variable ends containing five multigene families (MGFs) (Yañez et al. 1995). Deletions and insertions of copied regions as long as 20 kb occur within the MGF genes, suggesting that these regions may help generate antigenic variability and therefore help ASFV evade the host immune system.

The virus is genotyped and subtyped on the basis of small variations in a central variable region (CVR) within the conserved central region. Complete genome sequences are available for 15 African and European ASFV isolates to date, coming from different regions and hosts (domestic pigs, warthogs, and ticks). These isolates display different levels of virulence and significant genomic diversity (De Villiers et al. 2010). Sequence variation within MGF regions has been associated with the degree of virulence in macrophages and with tick host range (Burrage et al. 2004; Zsak et al. 2001).

ASFV particles are extremely complex: two-dimensional electrophoresis indicates at least 28 structural proteins

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

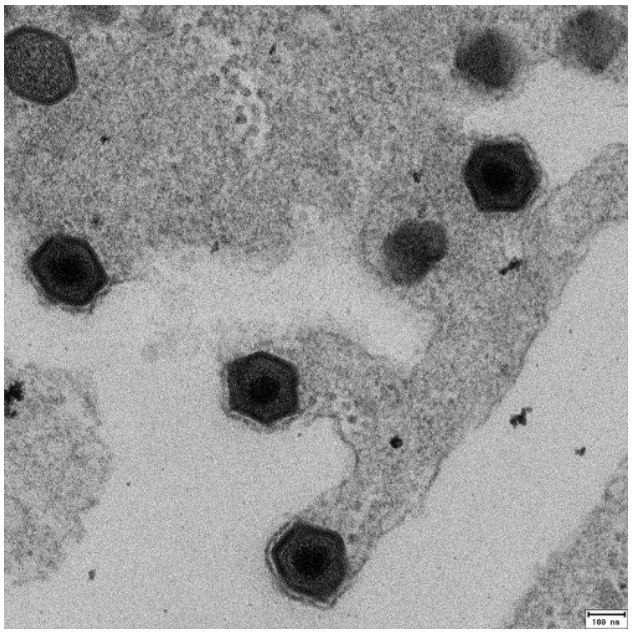


Figure 25.1 Electron micrograph of an ASFV particle. *Source:* Courtesy of CBM-CSIC-UAM.

in particles within the cell and 54 in purified extracellular particles. More than 100 virus-induced proteins have been described in infected porcine macrophages (Esteves et al. 1986). Attachment proteins p12 and p24 can be found in the external membrane of extracellular particles, while proteins p150, p37, p34, and p14 localize to the virus core. The external envelope also contains the hemagglutinin (HA) protein (virus homologue of cellular CD2), the only known glycoprotein in the viral particle.

Several ASFV proteins are highly antigenic, including the major structural component of the viral capsid (p72) as well as the membrane proteins p54, p30, and p12. More than 50 viral proteins induce an antibody response in infected or recovered pigs. They are useful as antigens in serological diagnosis, although their role in inducing protective immunity is unclear (Neilan et al. 2004).

ASFV does not induce a full neutralizing antibody immune response, preventing the development of a serotyping classification scheme. Instead, a genotyping strategy has been used to define 24 ASFV genotypes based on partial nucleotide sequencing of the p72 gene (Achenbach et al. 2017; Quembo et al. 2017). Subtyping is performed based on sequence analysis of tandem repeat sequences in a CVR within the B602L gene (Nix et al. 2006) as well as in the region between the I73R and I329L genes on the right end of the genome (Gallardo et al. 2014). Genome regions encoding p54, p30, or HA have also proven useful for virus tracking.

All 24 known ASFV genotypes have been identified in sub-Saharan Africa (Achenbach et al. 2017; Quembo et al. 2017). ASFV isolates detected in Europe and the Western Hemisphere before 2006 were limited to genotype I from West Africa. In 2007, a new ASFV isolate with genotype II from southeast Africa was detected throughout the Caucasian region in Europe (EFSA 2010).

ASFV particles remain stable in serum-free culture medium at pH 4–10, but they are inactivated in minutes at pH below 4 or higher than 11.5 (EFSA 2010). Particles in serum can remain infectious for 6 years at 5 °C (41 °F) and can remain infectious for days at pH 13.4 in medium containing 25% serum. ASFV is inactivated by heating at 60 °C (140 °F) for 30 minutes (Plowright and Parker 1967) or 56 °C (133 °F) for 70 minutes (Mebus 1988). Many organic solvents can inactivate the virus by disrupting the lipid envelope, but ASFV is resistant to proteases and nucleases (Plowright and Parker 1967).

ASFV field isolates must be recovered in porcine monocytes and macrophages because they do not replicate in conventional cell culture. For research purposes, several ASFV isolates have been adapted to grow in African green monkey kidney stable cell lines such as VERO, MS, and CV-1 cells. More recently, several porcine cell lines of monocyte origin have been developed for research purposes (Chitko-McKown et al. 2013; Hurtado et al. 2010). For example, the COS-1 cell line is used for detection, growth, and titration of field isolates, as well as for generation of laboratory-engineered ASFV. Despite this progress, a suitable cell line has yet to be identified or developed in which the virus isolates or potential vaccine candidates can replicate without undergoing genomic changes that alter its immunogenicity. This remains a significant obstacle to ASFV research and vaccine development.

Epidemiology

ASF has been described in 25 countries in sub-Saharan Africa, where different epidemiological patterns and scenarios are present. In Europe, ASF is endemic in Sardinia (Italy) and in some areas of Eastern Europe (Gogin et al. 2013). In June 2007, ASF was recognized in the Caucasian region following an outbreak in Georgia. Subsequently the outbreak spread to Armenia, Azerbaijan, and the Russian Federation, reaching the border with Ukraine and northwest Russia near the Baltic Sea and Barents Sea. Since 2011, ASF has spread to the northwest, reaching new territories in the Russian Federation (around Moscow), Ukraine (2012), Belarus (2013), Estonia (2014), Latvia (2014), Lithuania (2014), Poland (2014), Moldova (2016), Czech Republic (2017), Romania (2017), Hungary (2017), Bulgary (2018), Belgium (2018). From August 2018, ASF has rapidly spread to many provinces and municipalities in China. The Eastern European outbreaks resulted from a single introduction of a southeast African genotype II ASFV isolate into Europe in 2007. Two genetic variants have been identified so far

among isolates from the various Eastern European countries affected (Gallardo et al. 2014). The mortality associated with these outbreaks has decreased over time, while the rates of surviving animals positive for antibodies or with unspecific clinical signs have increased. This fact could be related at least in part to the presence of moderate virulent viruses that have been recently identified in certain regions of Europe (Gallardo et al, 2018).

The natural hosts of ASFV are wild and domestic suids. European wild boars are susceptible to ASFV infection and present clinical signs and mortality rates similar to those observed in domestic pigs (McVicar et al. 1981; Sánchez-Botija 1982). In contrast, ASFV usually produces subclinical infection in the three African wild suid species that serve as reservoirs: warthogs (*Phacochoerus aethiopicus*), giant forest hogs (*Hylochoerus meinertzhageni*), and bushpigs (*Potamochoerus porcus*) (De Tray 1957).

ASFV is maintained in Africa via a complex transmission cycle involving African wild suid species, soft ticks, and domestic pigs. In eastern and southern regions, the virus follows an ancient sylvatic cycle involving soft ticks and asymptomatic infected warthogs and bushpigs. Two additional cycles have been described in endemic areas: a domestic pig/tick cycle without warthog involvement and a domestic pig/pig cycle.

ASFV is transmitted within Europe most often directly via contact between sick and healthy animals, including domestic pigs and wild boar. ASFV spreads among domestic pigs via oral and nasal routes (Colgrove et al. 1969). Pigs can also be infected by other routes, including tick bite (Plowright et al. 1969), cutaneous scarification, and injection (intramuscular, subcutaneous, intraperitoneal, or intravenous) (McVicar 1984).

Natural infection in the European wild boar has been described in the Iberian Peninsula, Sardinia, and Eastern Europe. For reasons that remain unclear, the wild boar currently plays a significant role in ASFV spread and persistence in Eastern Europe (EFSA 2015). In contrast, in Spain and Portugal, the wild boar was not a major ASFV reservoir and did not pose a major obstacle to eradication. The difference may be that the wild boar population density is much higher now than in the past (Arias and Sánchez-Vizcaíno 2002a). Evidence in Sardinia suggests that ASFV naturally disappears from wild boar populations if the disease is eradicated from domestic pigs in the same area (Laddomada et al. 1994).

Several species of soft ticks are ASFV reservoirs and vectors, including *Ornithodoros moubata* in Africa (Plowright et al. 1969) and *Ornithodoros erraticus* on the Iberian Peninsula (Sánchez-Botija 1963). Indirect transmission by biological vectors such as *O. erraticus* occurs on the Iberian Peninsula, especially in outdoor pig production. The role of these vectors in Eastern Europe is unknown. In Africa, both transovarial and transstadial transmissions of ASFV have been described involving *O. moubata* (Plowright et al. 1970), while in Europe, only transstadial transmission involving *O. erraticus* has been observed. *O. savignyi*, present in Africa, can transmit ASFV to domestic pigs in the laboratory (Mellor and Wilkinson 1985), although this has not been described in the field. Several other tick species widely distributed in North and South America are capable of harboring and transmitting ASFV (Groocock et al. 1980). All *Ornithodoros* spp. tested to date are susceptible to ASFV infection (EFSA 2010).

The ASFV incubation period varies from 4 to 19 days, depending on the ASFV isolate and route of exposure. Domestic pigs infected with virulent isolates begin shedding virus during the incubation period, prior to the observation of clinical signs. After the onset of clinical signs, ASFV is shed at high levels in all secretions and excretions, including nasal secretions, saliva, feces, urine, conjunctival exudate, genital discharges, and bleeding wounds. Surviving pigs show high antibody titers and long-term viremia; virus may be recovered from tissues for weeks or months. Consequently, once ASFV is established in domestic pigs, carrier pigs become an important ASFV reservoir and therefore must be a focal point of ASF eradication strategies.

Infection in wild African suids typically results in low virus titers in tissues and low or undetectable viremia (Plowright 1981). Genetic factors and immunologic responses in the host that may be associated with low viral load remain unclear. These levels of virus are sufficient for transmission to domestic pigs through tick vectors, but usually not through direct contact between animals. This transmission cycle makes ASFV eradication in Africa extremely difficult.

ASFV is very stable in the environment and can remain infectious for more than 3 days in contaminated pens and for up to several weeks in pig feces. After storage at room temperature, ASFV can be isolated from sera or blood after 18 months and from putrefied blood after 15 weeks (EFSA 2009). ASFV persists for weeks to months in frozen or uncooked meat. In cured or processed products, such as Parma ham, infectious virus was not found after 300 days of processing and curing (McKercher et al. 1987). Spanish cured pig meat products, such as Serrano hams and shoulders, were free of viable ASFV by day 140 and Iberian loins by day 112 (Mebus et al. 1993). No infectious ASFV was found in cooked or canned hams heated to 70 °C (158 °F). Infectious ASFV was undetectable by 110 days in chilled deboned meat, bone-in meat, or ground pork and by 30 days in smoked deboned meat (Adkin et al. 2004).

ASFV is inactivated by organic solvents, detergents, oxidizing agents such as hypochlorite and phenol, and commercial disinfectants as a function of exposure time and temperature. For example, ASFV is inactivated in 30 minutes by exposure to 2.3% chlorine, 3% orthophenylphenol, or iodine-containing compounds. Other effective virucidal treatments include formalin, sodium

hydroxide, beta-propiolactone, glyceraldehydes, or acetyl-ethyleneimine (EFSA 2010). In general, soaps, detergents, and alkalis are effective for disinfecting animal housing, machinery, clothing, vehicles, and areas of human habitation. Disinfectants such as Virkon® are recommended for use in aircraft. Virus-contaminated feed, effluents, and manure may be buried or burned. ASFV-contaminated pig slurry can be treated at 4 °C (39 °F) with 1% sodium or calcium hydroxide for 3 minutes or with 0.5% sodium or calcium hydroxide for 30 minutes (Turner and Williams 1999). Insecticides (organophosphates and synthetic pyrethroids) are recommended for tick eradication.

Pathogenesis

The sites of primary ASFV replication are the monocytes and macrophages of the lymph nodes nearest the point of virus entry. In the case of oral exposure, the monocytes and macrophages of the tonsils and mandibular lymph nodes are the first involved. Subsequently the virus spreads via the blood and/or lymphatic system to sites of secondary replication: lymph nodes, bone marrow, spleen, lung, liver, and kidney. Viremia usually begins 4–8 days postentry and, because of the absence of fully neutralizing antibodies, persists for weeks or months.

While ASFV replicates mostly in monocytes and macrophages (Mínguez et al. 1988), it also replicates in endothelial cells (Wilkinson and Wardley 1978), hepatocytes, renal tubular epithelial cells (Gómez-Villamandos et al. 1995), and neutrophils (Carrasco et al. 1996). No infection has been observed in T or B lymphocytes (Mínguez et al. 1988). The virus particle enters susceptible cells by receptor-mediated endocytosis (Alcamí et al. 1989) and replicates in distinct areas of the cytoplasm close to the nucleus. Studies on the interaction of ASFV with monocytes and derived macrophage subsets showed that virulent isolates have evolved mechanisms to counteract activated macrophage responses, thereby promoting viral survival, dissemination in the host, and ASF pathogenesis (Franzoni et al. 2017).

Most ASFV isolates bind to red blood cell membranes (Quintero et al. 1986) and platelets (Gómez-Villamandos et al. 1996) and cause hemadsorption in affected pigs. Some isolates, however, do not induce hemadsorption.

The hemorrhaging observed in the final stages of the ASF acute form is believed to reflect phagocytosis of the endothelial cells in which the virus is replicating. In contrast, hemorrhaging in ASF subacute form reflects mainly an increase in vascular permeability (Gómez-Villamandos et al. 1995). Lymphopenia in ASF acute form is associated with lymphocyte apoptosis, mainly in the T area of lymphoid organs (Carrasco et al. 1996). This apoptosis does not appear to involve viral replication, since no evidence of replication in T or B cells has been reported; instead, other processes are likely to be involved, perhaps triggered by cytokines or apoptotic mediators released by ASFV-infected macrophages (Oura et al. 1998).

ASF subacute form is characterized by transitory thrombocytopenia (Gómez-Villamandos et al. 1996). The alveolar edema observed in the final stages of acute and subacute forms of ASF is the primary cause of ASF-related death. This edema results from the activation of pulmonary intravascular macrophages (Sierra et al. 1990).

Clinical signs

Wild African pigs are extremely resistant to clinical disease and do not generally present lesions. Domestic pigs and European wild boars exhibit a wide range of clinical signs from acute to chronic. ASF may resemble several other pig diseases, especially classical swine fever (hog cholera) and erysipelas.

The incubation period ranges from 4 to 19 days in natural infections but only 2–5 days in experimental infections, depending on the virus dose and inoculation route (Mebus et al. 1983). Clinical manifestations of ASF depend on the virulence of the isolate, exposure dose, and route of infection. Highly virulent ASFV isolates are mainly involved in the peracute and acute forms of the disease. Moderately virulent isolates can generate a wide range of clinical forms: acute, subacute, and chronic or inapparent. Low virulent isolates may induce subacute, chronic, or inapparent disease.

Morbidity ranges from 40 to 85%, depending on whether the virus isolate causes acute or subacute disease, the virulence of the isolate, the route of exposure, and the presence or absence of bleeding (epistasis or hemorrhagic diarrhea). Highly virulent isolates may cause 90–100% mortality, moderately virulent isolates cause 20–40% mortality in adult animals and 70–80% in young animals, and lowly virulent isolates produce 10–30% mortality.

Peracute form

The peracute form of ASF is characterized by loss of appetite, body temperature >41 °C, depression, and cutaneous hyperemia. Death usually follows 1–4 days after clinical signs appear. The peracute form is usually reported in ASF-naïve areas.

Acute form

The acute form of disease is characterized by loss of appetite, elevated body temperature (40–42 °C, 104–108 °F), inactivity, early leukopenia, pulmonary edema,

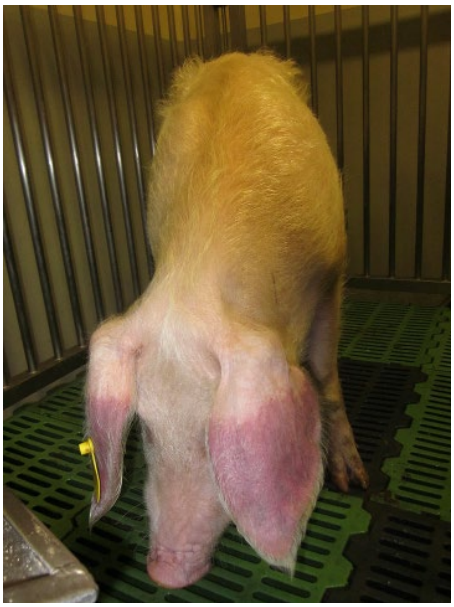


Figure 25.2 Purple marks on the ears of a pig with acute ASF because of intense hyperemia.

extensive necrosis and hemorrhage of lymphoid tissue, hemorrhages in skin (especially the skin of ears and flanks), splenomegaly, and high mortality (Mebus et al. 1983). In the final stages, rapid, labored breathing may be observed, as well as serous or seromucous nasal secretions caused by pulmonary edema.

In some cases there may be nasal hemorrhaging, constipation, vomiting, and, to a lesser extent, diarrhea. Hemorrhagic discharge from the anus (melena) is sometimes observed. Obvious signs of disease are exanthemas, in which the skin turns pinkish and nearly purple because of intense hyperemia, and/or cyanotic foci, which appear as irregular purple marks on the skin of the extremities, ears, chest, abdomen, and perineum (Figure 25.2). Hematomas and necrotic areas may be observed, although these lesions are more intense in pigs infected with moderately virulent isolates. Abortion frequently occurs in gestating females and is sometimes the first clinical sign of an outbreak. Mortality rates range from 90 to 100% at 7 days after the appearance of clinical signs. This clinical form is observed mainly in ASF-naïve areas.

Subacute form

Subacute disease involves similar, but less severe, clinical signs than the acute form. The subacute form is characterized by transitory thrombocytopenia, leukopenia, and numerous hemorrhagic lesions (Gómez-Villamandos et al. 1997). Other clinical signs include moderate to high fever, ascites, hydropericardium, edema in several organs (gallbladder or kidneys), abortion, or splenomegaly. Mortality rates range from 30 to 70%, and animals may recover after 3–4 weeks. This clinical form can be observed in endemic scenarios.

In Sardinia, surviving animals have been described with anti-ASFV antibodies, intermittent viremia, or subacute disease, as well as survivors with no clinical signs or unspecific signs (Mur et al. 2016a). Similar observations have been made under field conditions in the Russian Federation (Mur et al. 2016b) and under experimental conditions in animals inoculated with Eastern European isolates (Gallardo et al. 2016).

Chronic form

Chronic disease has been reported mainly on the Iberian Peninsula (Portugal and Spain) and countries infected with isolates from the Iberian Peninsula. Recently, chronic forms have been described in experimental animal inoculated with Eastern European isolates (Gallardo et al. 2018; 2016).

Lesions

A variety of lesions may be observed in animals infected with ASFV, depending on the virulence of the virus isolate. Acute and subacute ASF is characterized by extensive hemorrhaging and lymphoid tissue destruction. Conversely, lesions may be minimal or absent in subclinical or chronic disease (Mebus et al. 1983).

The principal gross lesions are observed in the spleen, lymph nodes, kidneys, and heart (Sánchez-Botija 1982). The spleen may be darkened, enlarged, infarcted, and friable (Figure 25.3). Lesions may be large infarcts with

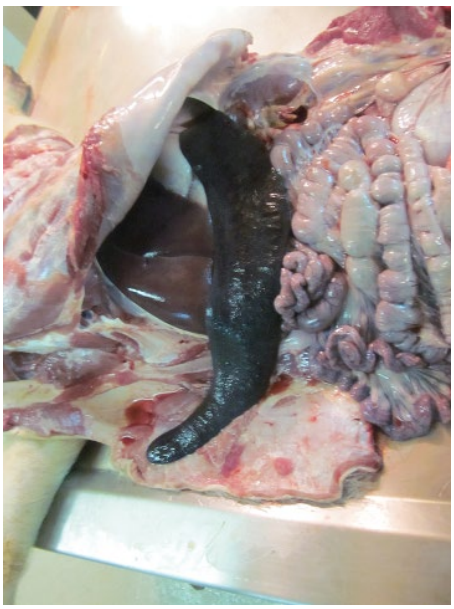


Figure 25.3 Enlarged and darkened spleen from ASF acute form.

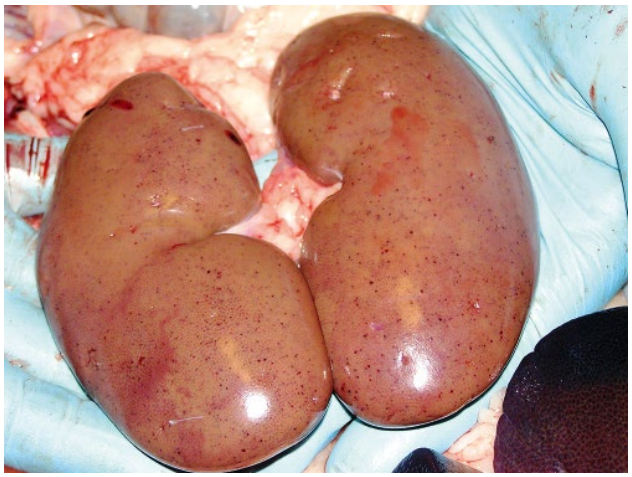


Figure 25.4 Kidneys from a pig infected with ASFV showing numerous petechiae on cortical surfaces.

subcapsular hemorrhages. Lymph nodes, especially the gastrohepatic and renal lymph nodes, are hemorrhagic, edematous, and friable and often resemble dark red hematomas. Because of congestion and subcapsular hemorrhage, cut sections of affected lymph nodes sometimes have a marbled appearance. Kidneys usually have petechial hemorrhages on cortical surfaces (Figure 25.4) and cut surfaces as well as in the renal pelvis. Intense hydropericardium with serohemorrhagic fluid is present in some cases. Petechial and ecchymotic hemorrhages may be observed in the epicardium and endocardium. Other lesions can also be observed in acute ASF, such as serohemorrhagic fluid in the abdominal cavity, with edema and hemorrhages throughout the gastrointestinal tract. Congestion of the liver and gallbladder may be observed, as well as petechial hemorrhages in the mucosa of the urinary bladder. Hydrothorax and petechial hemorrhages of the pleura are frequently found in the thoracic cavity, and lungs are usually edematous. Intense congestion is observed in the meninges, choroid plexus, and encephalon (Mebus et al. 1983).

Lesions in subacute ASF are similar to, but milder than, those in acute disease. Subacute disease is characterized by large hemorrhages in the lymph nodes and kidney. The spleen is enlarged and hemorrhagic. Congestion and edema can be observed in lungs, and, in some cases, interstitial pneumonia may be found.

In acute ASF, histopathological lesions are present in blood vessels and lymphoid organs. These lesions are characterized by hemorrhages, microthrombosis, and damage to endothelial cells, with accumulations of dead cells in the subendothelium (Gómez-Villamandos et al. 1995). The hemorrhagic splenomegaly characteristic of acute and subacute disease arises when viral replication leads to necrosis of splenic macrophages, destroying the splenic architecture. Lymphoid tissue destruction in acute ASF is observed mainly in the T area of lymphoid organs, but no evidence of virus replication in lymphocytes has been reported (Carrasco et al. 1996; Mínguez et al. 1988).

Chronic ASF is characterized by alterations in the respiratory tract, but lesions in the chronic form may be minimal or absent (Gómez-Villamandos et al. 1995; Mebus et al. 1983). Lesions include fibrinous pleuritis, pleural adhesions, caseous pneumonia, and hyperplasia of the lymphoreticular tissues. Fibrinous pericarditis and necrotic skin lesions are also common.

Diagnosis

Laboratory tests are essential for definitive diagnosis of ASFV because ASF clinical signs and lesions resemble those of other hemorrhagic diseases of pigs, such as classical swine fever (hog cholera), erysipelas, and septicemic salmonellosis. Thus, ASF cannot be diagnosed on the basis of clinical signs or gross lesions. In ASF-affected areas, it is also crucial to perform diagnostic evaluations of surviving animals that have recovered from infection, as well as animals showing only unspecific clinical signs.

Various laboratory tests are available for ASFV diagnosis (Arias and Sánchez-Vizcaíno 2002b). Samples recommended for laboratory analyses include the lymph nodes, kidney, spleen, lung, blood, and serum. When testing wild boar, bone marrow from leg is an excellent sample (Gallardo et al. 2015). Tissue samples are useful for virus isolation or detection; tissue exudates and serum samples may also be used for virus detection, though they serve primarily for antibody detection.

Identification of ASFV

The most convenient, safe, and frequently used techniques to detect and identify ASFV are the polymerase chain reaction (PCR) (Agüero et al. 2003; Fernández-Pinero et al. 2013; King et al. 2003) and the hemadsorption test (HAT) (Malmquist and Hay 1960). HAT is the reference standard test for ASF.

Several PCR-based methods have been assessed as consistent, analytically specific, and highly analytically sensitive for the detection of ASFV genotypes currently circulating, as well as for the detection of non-hemadsorbing and low virulence virus isolates. Primer sets and probes target sequences within a highly conserved region in the VP72 region of the genome. Some of them, when used in a real-time PCR system (Fernández-Pinero et al. 2013), provided the highest sensitivity for ASFV detection in chronically infected animals.

The diagnostic sensitivity and specificity of the HAT test makes it useful under a broad range of conditions. It should be used to evaluate suspected outbreaks,

especially when other tests are negative. HAT is based on the attachment of erythrocytes to the external (cytoplasmic) membrane of ASFV-infected porcine macrophages cultured *in vitro*. Typically, the erythrocytes form a rosette around the infected macrophages before the appearance of ASFV-induced cytopathic effects (Malmquist and Hay 1960). A few ASFV field strains have been isolated that induce cytopathic effects in macrophages without inducing hemadsorption (Sánchez-Botija 1982). These strains can be identified using PCR or direct immunofluorescence.

Serological tests

Several techniques have been adapted to the detection of anti-ASFV antibodies. ELISA (Sánchez-Vizcaíno 1986; Sánchez-Vizcaíno et al. 1979) is recommended for large-scale screening, while the indirect immunoperoxidase test, indirect immunofluorescence, and immunoblotting tests are recommended as confirmatory serological tests for positive or doubtful results (Gallardo et al. 2013, 2015; Pastor et al. 1987).

ELISA is well suited for control and eradication programs (Arias and Sánchez-Vizcaíno 2002a; Gallardo et al. 2015). Two indirect ELISAs described in the World Organization for Animal Health manual (OIE 2012) and several commercial competitive and indirect ELISAs have been validated for use under different epidemiological situations. These ELISAs may show lower diagnostic sensitivity when samples have been poorly preserved or when blood samples are used instead of serum, at least in the case of samples from wild boar (Arias et al. 1993; Gallardo et al. 2015). This limitation does not apply to "in-house" and commercial ELISAs based on new recombinant proteins (Gallardo et al. 2006, 2015).

The indirect immunoperoxidase and indirect immunofluorescence tests are highly diagnostically sensitive and specific as confirmatory assays following ELISA testing, as long as animals have been infected for at least 1 week (Arias and Sánchez-Vizcaíno 2002a). The immunoblotting test is recommended for confirming a clear positive for the presence of antibodies, which usually occurs at least 2 weeks post infection, and for confirming results when sera are suspected of being poorly preserved (Arias et al. 1993).

The serological diagnosis of ASF plays an important role in the surveillance program due to the fact that no vaccine is currently available against ASFV and, therefore, the presence of anti-ASFV antibodies in an animal indicates infection. This is particularly important for the detection of pigs that have recovered from subacute and inapparent ASF infection. These animals usually show high levels of ASFV-specific antibodies: IgM can be detected by 4 days post infection, and IgG by 6–8 days post infection. Antibody can circulate concurrently with virus for up to 6 months after infection (Arias and Sánchez-Vizcaíno 2002b; Wilkinson 1984) and remains detectable for years after the first exposure. The early appearance and subsequent persistence of anti-ASFV antibodies make them useful for detecting subacute and inapparent disease.

Immunity

Despite substantial progress toward an effective ASFV vaccine, no commercial vaccine is currently available. Pigs that survive ASFV infection develop efficient protective immunity against homologous viruses and, in certain cases, cross-protection against heterologous viruses (Boinas et al. 2004; Burmakina et al. 2016; King et al. 2011; Ruiz-Gonzalvo et al. 1981). The immune mechanisms involved in protection against ASFV are poorly understood, although both cellular and humoral immunities appear to be required (Takamatsu et al. 2013). The primary obstacles to protective immunity appear to be the absence of fully neutralizing antibodies and the great variability among virus isolates. Passive transfer of humoral immunity by administration of immune serum from ASFV-infected pigs or other infected animals can provide partial protection against ASFV, for example, a delay in the onset of clinical signs or mitigation of these signs, reduced levels of viremia, and higher survival rates (Onisk et al. 1994; Schlafer et al. 1984a,b; Wardley et al. 1985).

In vivo and *in vitro* studies have suggested a possible role for antibodies in complement-mediated cell lysis and antibody-dependent cell-mediated cytotoxicity (Rock 2017; Takamatsu et al. 2013). The innate immune response such as elevated activity of natural killer cells also plays an important role (Leitão et al. 2001) as well as the cytotoxic activity by a specific subset of CD8+ T lymphocytes (Martins and Leitão 1994; Oura et al. 2005).

Prevention and control

When ASF is suspected for any reason, pig movements should be restricted, and diagnostic testing performed immediately. It is important to remember that low virulence ASFV strains do not cause evident signs or lesions.

No treatment or effective vaccine against ASFV is available. Many attempts have been made to develop an efficacious ASF vaccine. The first trial with a live attenuated vaccine in 1963 in Portugal was unsuccessful (Manso-Ribeiro et al. 1963). Since then, some candidate vaccines based on attenuated ASFV have shown promising results (King et al. 2011; Leitão et al. 2001; O'Donnell

et al. 2016; Reis et al. 2016). However, there are still substantial shortcomings in regard to safety and efficacy.

Given the lack of an effective vaccine and the high costs associated with ASF, it is critical to protect ASF-free areas from the introduction of ASFV. Epidemiological studies have shown that contaminated garbage from international airports and ports is an important source of the virus (EFSA 2010). Therefore, all food leftovers from airplanes and ships should be incinerated.

In endemic European areas where mild or inapparent infections have been recognized, such as Sardinia, prevention measures include control of pig movements and pig products in combination with extensive serological surveys to detect carriers. In endemic areas of South and Est Africa, the most important prevention measure is the control of natural reservoirs, i.e. soft ticks (*O. moubata*) and wild African suids, with the objective of preventing their contact with domestic pigs. In Eastern Europe, it will be necessary to understand the role of biological vectors in the cycle and to control the movements of domestic pigs, wild boars, and pig by-products.

Methods to control ASF and eliminate ASFV from herds may differ depending on the region and continent, the specific epidemiological situation and circumstances, economic resources, and the situation in neighboring regions. Between 1985 and 1995, Spain carried out an extensive coordinated program to eradicate ASF, with the support of the European Community (Arias and Sánchez-Vizcaíno 2002a; Bech-Nielsen et al. 1995). Eradication of ASFV can be achieved without vaccination by the application of effective contingency programs. Every country should have an ASF contingency plan prepared and ready to implement in case of an ASF emergency.

References

Achenbach JE, Gallardo C, Nieto-Pelegrín E, et al. 2017. *Transbound Emerg Dis* 64:1393–1404.

Adkin A, Coburn H, England T, et al. 2004. *Risk assessment for the illegal import of contaminated meat and meat products into Great Britain and the subsequent exposure of GB livestock (IIRA): Foot and mouth disease (FMD), classical swine fever (CSF), African swine fever (ASF), swine vesicular disease (SVD)*. New Haw: Veterinary Laboratories Agency.

Agüero M, Fernández J, Romero L, et al. 2003. *J Clin Microbiol* 41:4431–4434.

Alcamí A, Carrascosa AL, Viñuela E. 1989. *Virology* 171:68–75.

Arias M, Sánchez-Vizcaíno JM. 2002a. African swine fever eradication: The Spanish model. In Morilla A, Yoon KJ, Zimmerman JJ, eds. *Trends in Emerging Viral Infections of Swine*. Ames, IA: Iowa State Press, pp. 133–139.

Arias M, Sánchez-Vizcaíno JM. 2002b. African swine fever. In Morilla A, Yoon KJ, Zimmerman JJ, eds. *Trends in Emerging Viral Infections of Swine*. Ames, IA: Iowa State Press, pp. 119–124.

Arias M, Escribano JM, Sánchez-Vizcaíno, JM. 1993. *Vet Rec* 133:189–190.

Bech-Nielsen S, Fernández J, Martínez-Pereda F, et al. 1995. *Br Vet J* 151:203–214.

Boinas FS, Hutchings GH, Dixon LK, et al. 2004. *J Gen Virol* 85:2177–2187.

Burmakina G, Magolovkin A, Tulman ER, et al. 2016. *J Gen Virol* 97:1670–1675.

Burrage TG, Lu Z, Neilan JG, et al. 2004. *J Virol* 78:2445–2453.

Carrasco L, de Lara FC, de las Mulas JM, et al. 1996. *Vet Res* 27:305–312.

Chitko-McKown CG, Chapes SK, Miller LC, et al. 2013. *Res Immunol* 3:26–32.

Colgrove G, Haelterman EO, Coggins L. 1969. *Am J Vet Res* 30:1343–1359.

De Tray DE. 1957. *J Am Vet Med Assoc* 130: 537–540.

De Villiers EP, Gallardo C, Arias M, et al. 2010. *Virology* 400:128–136.

Dixon LK, Escribano JM, Martins C, et al. 2005. The Asfarviridae. In Fauquet CM, Mayo MA, Maniloff J, et al., eds. *Virus Taxonomy: VIII Report of the International Committee for the Taxonomy of Viruses*. London: Elsevier/ Academic Press, pp. 135–143.

Esteves A, Marques MI, Costa JV. 1986. *Virology* 152:192–206.

European Food Safety Authority (EFSA). 2009. Scientific review on African swine fever. *EFSA J* 6:1–141.

European Food Safety Authority (EFSA). 2010. Scientific opinion on African swine fever. *EFSA J* 8:1–149.

European Food Safety Authority (EFSA). 2015. Scientific opinion on African swine fever. *EFSA J* 13:1–92.

Fernández-Pinero J, Gallardo C, Elizalde M, et al. 2013. *Transbound Emerg Dis* 60:48–58.

Franzoni G, Graham SP, Giudici SD, et al. 2017. *Vet Microbiol* 198:88–98.

Gallardo C, Blanco E, Rodríguez MJ, et al. 2006. *J Clin Microbiol* 44:1489–1495.

Gallardo C, Soler A, Nieto R, et al. 2013. *Vet Microbiol* 162:32–43.

Gallardo C, Fernández-Pinero J, Pelayo V, et al. 2014. *Emerg Infect Dis* 20:1544–1547.

Gallardo C, Nieto R, Soler A, et al. 2015. *J Clin Microbiol* 53:2555–2565.

Gallardo C, Nurmoja I, Soler A, et al. 2018. *Vet. Microbiol.* 219:70–79.

Gogin A, Gerasimov V, Malogolovkin A, et al. 2013. *Virus Res* 173:198–203.

Gómez-Villamandos JC, Hervás J, Méndez A, et al. 1995. *J Gen Virol* 76:2399–2405.
Gómez-Villamandos JC, Bautista MJ, Hervás J, et al. 1996. *J Comp Pathol* 59:146–151.
Gómez-Villamandos JC, Bautista MJ, Carrasco L, et al. 1997. *Vet Pathol* 34:97–107.
Groocock CM, Hess WR, Gladney WJ. 1980. *Am J Vet Res* 41:591–594.
Hurtado C, Bustos MJ, Carrascosa AL. 2010. *J Virol Methods* 164:131–134.
King DP, Reid SM, Hutchings GH, et al. 2003. *J Virol Methods* 107:53–61.
King K, Chapman D, Arguilaguet JM, et al. 2011. *Vaccine* 29:4593–4600.
Laddomada A, Patta C, Oggiano A, et al. 1994. *Vet Rec* 134:183–187.
Leitão A, Cartaxeiro C, Coelho R, et al. 2001. *J Gen Virol* 82:513–523.
Malmquist WA, Hay D. 1960. *Am J Vet Res* 21:104–108.
Manso-Ribeiro J, Azevedo R, Teixeira J, et al. 1958. Peste porcine provoquée par une souche différente (Souche L) de la souche classique. *Bull Off Int Epizoot* 50:516–534.
Manso-Ribeiro, J, Petisca NJ, Lopes-Frazao F, et al. 1963. Vaccination contra la peste porcina Africana. *Bull Off Int Epizoot* 60:921–937.
Martins CL, Leitão AC. 1994. *Vet Immunol Immunopathol* 43:99–106.
McKercher PD, Yedloutschnig RJ, Callis JJ, et al. 1987. *Can Inst Food Sci Technol J* 20:267–272.
McVicar JW. 1984. *Am J Vet Res* 45:1535–1541.
McVicar JW, Mebus CA, Becker HN, et al. 1981. *J Am Vet Med Assoc* 179:441–446.
Mebus CA. 1988. *Adv Virus Res* 35:251–269.
Mebus CA, McVicar JW, Dardiri AH. 1983. Comparison of the pathology of high and low virulence African swine fever infections. In Wilkinson PJ, ed. African Swine Fever. Proceedings CEC/FAO Research Seminar, Sardinia, September 1981, EUR 8466 EN, pp. 183–194.
Mebus CA, House C, Ruiz F, et al. 1993. *Food Microbiol* 10:133–143.
Mellor PS, Wilkinson PJ. 1985. *Res Vet Sci* 39:353–356.
Mínguez I, Rueda A, Domínguez J, et al. 1988. *Vet Pathol* 25:193–198.
Montgomery RE. 1921. On a form of swine fever occurring in British East Africa (Kenya Colony) *J Comp Pathol* 34:159–191.
Mur L, Atzeni M, Martínez-López B, et al. 2016a. *Transbound Emerg Dis* 63:e165–e177.
Mur L, Igolkin A, Varentsova A, et al. 2016b. *Transbound Emerg Dis* 63:e436–e440.
Neilan JC, Zsak L, Lu Z, et al. 2004. *Virology* 319:337–342.
Nix RJ, Gallardo C, Hutchings G, et al. 2006. *Arch Virol* 151:2475–2494.
O'Donnell V, Risatti GR, Holinka LG, et al. 2016. *J Virol* 91:e1760-16.
Onisk D, Borca M, Kutish G, et al. 1994. *Virology* 198:350–354.
Oura CAL, Powell PP, Parkhouse RM. 1998. *J Gen Virol* 79:1427–1438.
Oura CA, Denyer MS, Takamatsu H, et al. 2005. *J Gen Virol* 86:2445–2450.
Pastor MJ, Laviada MD, Sánchez-Vizcaíno JM, et al. 1987. *Can J Vet Res* 53:105–107.
Plowright W. 1981. African swine fever. In Davis JW, Karstand LH, Trainer DO, eds. *Infectious Diseases of Wild Mammals*, 2nd ed. Ames, IA: Iowa State University Press, pp. 178–190.
Plowright W, Parker J. 1967. *Arch Gesamte Virusforsch* 21:383–402.
Plowright W, Parker J, Peirce MA. 1969. *Vet Rec* 85:668–674.
Plowright W, Perry CT, Peirce MA. 1970. *Arch Gesamte Virusforsch* 31:33–50.
Quembo CJ, Jori F, Vosloo W, et al. 2017. Genetic characterization of African swine fever virus isolates from soft ticks at the wildlife/domestic interface in Mozambique and identification of a novel genotype. *Transbound Emerg Dis*. doi: https://doi.org/10.1111/tbed.12700
Quintero J, Wesley RD, Whyard TC, et al. 1986. *Am J Vet Res* 47:1125–1131.
Reis AL, Abrams CC, Goatley LC, et al. 2016. *Vaccine* 34:4698–4705.
Rock DL. 2017. *Vet Microbiol* 206:52–58.
Ruiz-Gonzalvo F, Carnero ME, Bruyel V. 1981. Immunological responses of pigs to partially attenuated ASF and their resistance to virulent homologous and heterologous viruses. In Wilkinson PJ, ed. Rome, FAO/CEC Expert Consultation in ASF Research, pp. 206–216.
Salas ML, Andrés G. 2013. *Virus Res* 173:29–41.
Sánchez-Botija C. 1963. Reservorios del virus de la Peste Porcina Africana. Investigación del virus de la PPA en los artropodos mediante la prueba de la hemoadsorción. *Bull Off Int Epizoot* 60:895–899.
Sánchez-Botija C. 1982. African swine fever: New developments. *Rev Sci Technol Off Int Epizoot* 1:1065–1094.
Sánchez-Vizcaíno JM. 1986. Africa swine fever diagnosis. In Becker J, ed. *African Swine Fever*. Boston: Martinus Nijhoff Publishers, pp. 63–71.
Sánchez-Vizcaíno JM, Martín L, Ordás A. 1979. Adaptación y evaluación del Enzimoinmunoensayo para la detección de anticuepos para la Peste porcina africana. *Laboratorio* 67:311–319.
Schlafer DH, Mebus CA, McVicar JW. 1984a. *Am J Vet Res* 45:1367–1372.
Schlafer DH, McVicar JW, Mebus CA. 1984b. *Am J Vet Res* 45:1361–1366.
Sierra MA, Carrasco L, Gómez J, et al. 1990. *J Comp Pathol* 102:323–334.
Takamatsu HH, Denyer MS, Lacasta A, et al. 2013. *Virus Res* 173:110–121.
Turner C, Williams SM. 1999. *J Appl Microbiol* 87:148–157.
Wardley RC, Norley SG, Wilkinson PJ, et al. 1985. *Vet Immunol Immunopathol* 9:201–212.

Wilkinson PJ. 1984. *Prev Vet Med* 2:71–82.
Wilkinson PJ, Wardley RC. 1978. *Br Vet J* 134:280–282.
World Organisation for Animal Health (OIE). 2012. Chapter 2.08.01: African swine fever. In Oura C, Arias M, eds. *Manual Diagnostic Tests Vaccines for Terrestrial Animals,* Vol. 2, pp. 1067–1081.
Yañez RJ, Rodríguez JM, Nogal ML, et al. 1995. *Virology* 208:249–278.
Zsak L, Lu Z, Burrage TG, et al. 2001. *J Virol* 75:3066–3076.

26

Anelloviruses

Joaquim Segalés and Tuija Kekarainen

Relevance

In 1997, a previously unrecognized virus was detected in a Japanese patient with posttransfusion non-A-G hepatitis (Nishizawa et al. 1997). The virus was initially designated "TT virus" (TTV), referring to the initials of the patient. It was later renamed *Torque teno virus* from the Latin "torques" (necklace) and "tenuis" (thin) in reference to the organization of TTV DNA genome (Todd et al. 2005).

Human TTVs have been associated with liver and respiratory diseases, hematological disorders, and cancer. However, a causal role for TTV has not been established in any species, and therefore, these viruses are considered nonpathogenic commensal inhabitants of vertebrates (Simmonds et al. 1999).

Torque teno sus viruses (TTSuVs) are the TTV species infecting swine. Research on TTSuV is very recent, with the first epidemiological studies published in mid-2000s (Bigarré et al. 2005; McKeown et al. 2004). The link between TTSuVs and disease in swine has not been clearly established (Kekarainen and Segalés 2012).

Etiology

Increasing numbers of TTVs have been described and the nomenclature is changing rapidly. Among the 12 genera currently in the family *Anelloviridae*, 2 genera, *Iotatorquevirus* and *Kappatorquevirus*, include TTSuVs. Genus *Iotatorquevirus* includes two genetically distinct viral species, *Torque teno sus virus 1a* (TTSuV1a) (formerly *Torque teno sus virus 1* [TTSuV1]) and *Torque teno sus virus 1b* (TTSuV1b) (formerly *Torque teno sus virus 2* [TTSuV2]). Genus *Kappatorquevirus* also includes two species, namely, *Torque teno sus virus k2a* (TTSuVk2a) (formerly *Torque teno sus virus k2* [TTSuVk2]) and *Torque teno sus virus k2b* (TTSuVk2b) (Cornelissen-Keijsers et al. 2012; Kekarainen and Segalés 2012). The classification of TTSuVs is based on several tentative pairwise identity (PI) thresholds: variants (>95% PI), subtypes (85–95% PI), types (67–85% PI), species (55–67%), and genus (36–55%) (Huang et al. 2010). For simplicity, viruses in the genus *Iotatorquevirus* will be referred to as TTSuV1, and viruses in the genus *Kappatorquevirus* will be referred to as TTSuV2 in this chapter.

TTV virions are icosahedral and non-enveloped with a diameter of 30–32 nm. The buoyant density in cesium chloride is 1.31–1.33 g/cm^3 for TTV in serum and 1.33–1.35 g/cm^3 for TTV in feces (Okamoto et al. 1998).

The TTV genome is a single-stranded, negative-sense, circular DNA molecule of approximately 2.1–3.8 kb, depending on the host species (2.8 kb for TTSuVs) (Okamoto et al. 2002). The genomic organization and predicted transcriptional profile of TTSuVs resemble those of TTVs of humans, although the sequence similarity is less than 50% (Niel et al. 2005; Okamoto et al. 2002). The TTSuV genome contains untranslated region (UTR), occupying 24% of the viral genome (Okamoto et al. 2002), and three major ORFs: ORF1, ORF2, and ORF3. By analogy with circoviruses, ORF1 (635 amino acids) encodes the putative coat protein and presents sequence motifs that are characteristic of replication-associated proteins of circular ssDNA viruses. ORF2 (73 amino acids) contains sequences characteristic of tyrosine phosphatases (Kekarainen and Segalés 2012). The function of ORF3 (224 amino acids) is currently unknown. Splicing could be involved in generation of TTSuV mRNAs. For example, ORF3 mRNA is generated by splicing. Also, alternative splicing of TTSuV ORF1 could result in several mRNAs and different protein isoforms (Huang et al. 2012b; Martínez-Guinó et al. 2011).

The wide distribution of TTSuV in tissues (Aramouni et al. 2010) suggests that the virus can replicate in distinct cells, but no *in vitro* cell culture system has been identified (Kekarainen and Segalés 2012).

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Public health

There is no documented transmission of swine anelloviruses to humans. TTSuV DNA has been found in human drugs containing components of swine origin, but the biological significance is undetermined (Kekarainen et al. 2009). Also, TTSuV genome has been detected in pork meat and liver (Jiménez-Melsió et al. 2013; Leblanc et al. 2014; Monini et al. 2016), as well as in human feces (Jiménez-Melsió et al. 2013). The latter finding probably represents viral DNA coming from ingested contaminated pork products.

Epidemiology

TTVs have been found in humans, nonhuman primates, livestock species (pigs, sheep, cattle, camels, and poultry), companion animals (cats and dogs), wild boars, badgers, pine martens, tupaias, rodents, bats, sea turtles, and sea lions (Manzin et al. 2015). Each host has its own species-specific, genetically distinct TTV. However, human TTV can infect chimpanzees under experimental conditions (Luo et al. 2000).

No comprehensive study on geographic distribution has been done, but TTSuVs appear to be ubiquitous worldwide (Kekarainen and Segalés 2012). It is considered that the same TTSuV strains circulate among wild boars and domestic pigs. A retrospective study showed that TTSuVs were present as early as 1985 in Spain (Segalés et al. 2009).

TTSuVs can be detected in tissues, blood, semen, colostrum, nasal, and fecal samples (Aramouni et al. 2010; Kekarainen et al. 2007; Nieto et al. 2013; Sibila et al. 2009), indicating the potential for both horizontal and vertical transmission. As observed in human TTV, the rate of TTSuV detection in serum and nasal excretions increases with age (Sibila et al. 2009). Increasing prevalence and viral load in tissues with age (Xiao et al. 2012) suggests that transmission is very efficient, probably by the oral–nasal exposure.

The current inability to propagate virus *in vitro* precludes studies on the virus persistence in the environment or susceptibility to disinfectants. However, the physicochemical properties of TTSuVs are thought to be similar to those of other ssDNA viruses like members of the family *Circoviridae*.

Pathogenesis

The primary site of virus replication is unknown. In fetuses, the highest concentration of TTSuV is found in the lung, heart, spleen, and kidney, suggesting that these tissues contain a significant number of cells supporting viral replication (Aramouni et al. 2010). In older animals, high concentrations of virus are found in all tissues (Nieto et al. 2013). Whether this reflects actively replicating virus within these tissues or virus accumulated over time is uncertain. Recently, by using an *in situ* hybridization method, lymphoid tissues, including superficial inguinal, mesenteric, and hilar lymph nodes, tonsil, intestinal lamina propria of mucosa, and Peyer's patches, and sometimes spleen, were found to contain significant levels of positive signals and were suggested as target sites for TTSuV (Lee et al. 2014). T lymphocytes were speculated to be the major target cells for TTSuV (Lee et al. 2014).

TTSuVs may be found in a high proportion of apparently healthy animals (Kekarainen and Segalés 2012), which suggests that infection by itself does not produce clinical signs. However, TTSuVs have been associated with economically important diseases like porcine circovirus type 2 systemic disease (PCV2-SD) (classically known as post weaning multisystemic wasting syndrome) (Ellis et al. 2008; Kekarainen et al. 2006). Also, TTSuVs have been linked with a porcine dermatitis and nephropathy syndrome (PDNS)-like condition (Krakowka et al. 2008), weight gain reduction (de Menezes Cruz et al. 2016), inflammatory lung lesions (Aramouni et al. 2013b), porcine respiratory disease complex (PRDC) (Rammohan et al. 2012) and found in coinfection with many pathogens, including African (Luka et al. 2016) and classical (Aramouni et al. 2013a) swine fever. The association of TTSuV with disease is controversial, and it has been suggested that TTSuV species could differ genetically and in their role in clinical disease (Cornelissen-Keijsers et al. 2012; Rogers et al. 2017).

Clinical signs and lesions

At present, no clinical signs are specifically associated with TTSuV infection, and no clinicopathological experimental studies involving conventional pigs have been done due to the lack of readily available isolates. Inoculation of gnotobiotic pigs with TTSuV1-containing tissue homogenate caused mild interstitial pneumonia, transient thymic atrophy, membranous glomerulonephropathy, and modest lymphocytic-to-histiocytic liver infiltrates (Krakowka and Ellis 2008). One-half of gnotobiotic pigs inoculated intraperitoneally with TTSuV1 and 7 days later oronasally with PCV2 developed acute fatal PCV2-SD (Ellis et al. 2008). No PCV2-SD was observed when TTSuV or PCV2 was the only inoculum or if PCV2 preceded the inoculation with TTSuV inoculation. However, there are also contrary reports concerning the association of TTSuV infection with PCV2-SD

(Lee et al. 2010; McMenamy et al. 2013; Teixeira et al. 2015). Thus, TTSuV infection as a cofactor in the development of PCV2-SD is undetermined.

Diagnosis

No protocols for virus isolation have been described for TTSuVs. Therefore, diagnosis of TTSuV infection is based on the detection of viral DNA and/or antibodies against the virus. To date, the developed methods have been used for research purposes, and no commercial methods are available. Several PCR-based assays have been developed (Segalés et al. 2009), including a quantitative PCR (qPCR) for all TTSuV1 and TTSuV2 species in serum (Cornelissen-Keijsers et al. 2012; Gallei et al. 2009; Lee et al. 2010; Nieto et al. 2011) and a nonspecific qPCR that detects both TTSuV genera and bovine TTV (Brassard et al. 2010). A number of serological methods have been used in research, all of them based on ELISA (Huang et al. 2012a; Li et al. 2015; Nieto et al. 2015), with the exception of one report of a fluorescent microbead-based immunoassay (FMIA) (Giménez-Lirola et al. 2014).

Immunity

Few studies on immune responses against TTSuV have been reported. Both sows and piglets develop antibodies against TTSuV1 and TTSuV2 (Giménez-Lirola et al. 2014; Huang et al. 2012a; Nieto et al. 2015), although the kinetics are different for both viral genera (high levels in sows and pigs at early ages for TTSuV1, but low levels for TTSuV2) (Nieto et al. 2015). The high percentage of viremic, but antibody-positive, pigs reflects an inefficient anti-TTSuV immune response. Further, *in utero* infection prior to the gestational age at which the fetus becomes immunologically responsive and evidence of lifelong infection in pigs raise the possibility of immunotolerance (Aramouni et al. 2010). Further studies are needed to understand these observations.

The immunopathogenesis of swine anelloviruses is unknown. An *in vitro* study suggested that TTSuV1 does not have a significant immunosuppressive effect on antiviral immunity (Singh and Ramamoorthy 2016). In contrast, another study suggested that TTSuV1 natural infection has an adverse effect on the development of host immune responses, suppresses immunization by a porcine reproductive and respiratory syndrome (PRRS) attenuated vaccine, and exacerbates PRRS to a certain extent (Zhang et al. 2012). These preliminary results await confirmation.

Prevention and control

The impact of TTSuV infection and the consequences for herd health have not yet been established. Since a significantly higher TTSuVk2a load in serum has been described in PCV2-SD (Aramouni et al. 2011), measures to decrease the viral load might have an impact on disease outcome. One study reported that TTSuV viremia was controlled by a combined DNA and protein immunization (Jiménez-Melsió et al. 2015). PCV2 vaccination has also been tested as a potential measure to avoid TTSuV upregulation in the context of PCV2-SD. However, PCV2 vaccination did not alter TTSuV loads (Lee et al. 2012; Nieto et al. 2012).

References

Aramouni M, Segalés J, Cortey M, et al. 2010. *Vet Microbiol* 146:350–353.

Aramouni M, Segalés J, Sibila M, et al. 2011. *Vet Microbiol* 153:377–381.

Aramouni M, Kekarainen T, Ganges L, et al. 2013a. *Virus Res* 172:81–84.

Aramouni M, Martínez J, Nieto D, et al. 2013b. *Vet Microbiol* 162:338–344.

Bigarré L, Beven V, de Boisseson C, et al. 2005. *J Gen Virol* 86:631–635.

Brassard J, Gagne MJ, Houde A, et al. 2010. *J Appl Microbiol* 108:2191–2198

Cornelissen-Keijsers V, Jiménez-Melsió A, Sonnemans D, et al. 2012. *J Gen Virol* 93:2682–2691.

Ellis JA, Allan G, Krakowka S. 2008. *Am J Vet Res* 69:1608–1614.

Gallei A, Pesch S, Esking WS, et al. 2009. *Vet Microbiol* 43:202–212.

Giménez-Lirola LG, Gerber PF, Rowland RR, et al. 2014. *Res Vet Sci* 96:543–550.

Huang YW, Ni YY, Dryman BA, et al. 2010. *Virology* 396:289–297.

Huang YW, Harrall KK, Dryman BA, et al. 2012a. *J Virol* 86:10628–10639.

Huang YW, Patterson AR, Opriessnig T, et al. 2012b. *J Virol* 86:6042–6054.

Jiménez-Melsió A, Parés S, Segalés J, et al. 2013. *Virus Res* 178:522–524.

Jiménez-Melsió A, Rodriguez F, Darji A, et al. 2015. *Vaccine* 33:3497–3503.

Kekarainen T, Segalés J. 2012. *Transbound Emerg Dis* 59(Suppl 1):103–108.

Kekarainen T, Sibila M, Segalés J. 2006. *J Gen Virol* 87:833–837.
Kekarainen T, López-Soria S, Segalés J. 2007. *Theriogenology* 68:966–971.
Kekarainen T, Martinez-Guino L, Segales J. 2009. *J Gen Virol* 90:648–653.
Krakowka S, Ellis JA. 2008. *Am J Vet Res* 69:1623–1629.
Krakowka S, Hartunian C, Hamberg A, et al. 2008. *Am J Vet Res* 69:1615–1622.
Leblanc D, Houde A, Gagné MJ, et al. 2014. *Int J Food Microbiol* 178:60–64.
Lee SS, Sunyoung S, Jung H, et al. 2010. *J Vet Diagn Invest* 22:261–264.
Lee S, Shin J, Kim C, et al. 2012. *Res Vet Sci* 93:1039–1041.
Lee Y, Lin CM, Jeng CR, et al. 2014. *Vet Microbiol* 172:390–399.
Li Z, Qiao J, He Y, et al. 2015. *Virol J* 12:125.
Luka PD, Erume J, Yakubu B, et al. 2016. *Adv Virol* 2016:6341015.
Luo K, Liang W, He H, et al. 2000. *J Med Virol* 61:159–164.
Manzin A, Mallus F, Macera L, et al. 2015. *J Infect Dev Ctries* 9:562–570.
Martínez-Guinó L, Ballester M, Segalés J, et al. 2011. *J Gen Virol* 92:2446–2457.
McKeown NE, Fenaux M, Halbur PG, et al. 2004. *Vet Microbiol* 104:113–117.
McMenamy MJ, McKillen J, McNair I, et al. 2013. *Vet Microbiol* 164:293–298.
de Menezes Cruz AC, Silveira RL, Baez CF, et al. 2016. *Vet Microbiol* 195:154–157.
Monini M, Vignolo E, Ianiro G, et al. 2016. *Food Environ Virol* 8:283–288.
Niel C, Diniz-Mendes L, Devalle S. 2005. *J Gen Virol* 86:1343–1347.
Nieto D, Aramouni M, Grau-Roma L, et al. 2011. *Vet Microbiol* 152:284–290.
Nieto D, Aramouni M, Sibila M, et al. 2012. *Vet Microbiol* 157:8–12.
Nieto D, Kekarainen T, Aramouni M, et al. 2013. *Vet Microbiol* 163:364–367.
Nieto D, Martínez-Guinó L, Jiménez-Melsió A, et al. 2015. *Vet Microbiol* 180:22–27.
Nishizawa T, Okamoto H, Konishi K, et al. 1997. *Biochem Biophys Res Commun* 241:92–97.
Okamoto H, Akahane Y, Ukita M, et al. 1998. *J Med Virol* 56:128–132.
Okamoto H, Takahashi M, Nishizawa T, et al. 2002. *J Gen Virol* 83:1291–1297.
Rammohan L, Xue L, Wang C, et al. 2012. *Vet Microbiol* 157:61–68.
Rogers AJ, Huang YW, Heffron CL, et al. 2017. *Transbound Emerg Dis* 64:1877–1883.
Segalés J, Martínez-Guinó L, Cortey M, et al. 2009. *Vet Microbiol* 134:199–207.
Sibila M, Martínez-Guinó L, Huerta E, et al. 2009. *Vet Microbiol* 139:213–218.
Simmonds P, Prescott LE, Logue C, et al. 1999. *J Infect Dis* 180:1748–1750.
Singh P, Ramamoorthy S. 2016. *Virology* 495:63–70.
Teixeira TF, Cibulski SP, dos Santos HF, et al. 2015. *Res Vet Sci* 101:38–41.
Todd D, Bendinelli M, Biagini P, et al. 2005. *Virus Taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses*. Cambridge: Academic Press.
Xiao CT, Giménez-Lirola L, Huang YW, et al. 2012. *J Virol Methods* 183:40–44.
Zhang Z, Wang Y, Fan H, et al. 2012. *Arch Virol* 157:927–933.

27

Astroviruses

Gábor Reuter and Nick J. Knowles

Relevance

Astroviruses (AstV) have been identified in many mammalian and avian host species (Boujon et al. 2017). In some species, AstV have been linked to hepatic (e.g. duck), nephritic (e.g. chicken), gastroenteric (e.g. human, turkey, sheep), and/or neurologic (e.g. human, mink, cattle, sheep and pig) diseases (Arruda et al. 2017; Boros et al. 2017; De Benedictis et al. 2011; Mendez and Arias 2013; Reuter et al. 2018). AstV from different animal species are antigenically distinct (Mendez and Arias 2013). Porcine astroviruses (PAstVs) were first recognized by electron microscopy of diarrheic feces (Bridger 1980; Geyer et al. 1994; Shimizu et al. 1990; Shirai et al. 1985) and postulated to cause a mild, self-limiting diarrhea. Recently, PAstV-associated neurological disease was reported in pigs in Hungary and the United States (Arruda et al. 2017; Boros et al. 2017). But their causal role in enteric or other diseases in pigs still remains obscure.

Etiology

PAstVs are members of the family *Astroviridae*, which is divided into two genera, *Mamastrovirus* (AstV of mammals) and *Avastrovirus* (AstV of birds). Approximately 30 nm diameter and non-enveloped AstV particles are distinctive in appearance, with some particles showing a 5- or 6-pointed star surface pattern when viewed by negative stain electron microscopy (Figure 27.1; Bridger 1980; Shimizu et al. 1990). Not all particles show this distinctive appearance, and care is needed so as not to confuse them with the indistinct appearance of noroviruses, particularly in the presence of antibody.

The AstV genome is composed of positive-sense, single-stranded RNA 6.4–7.9 kb in length (excluding the poly[A] tail at the 3′ end) and consisting of three open reading frames: ORF1a, ORF1b, and ORF2 (Mendez and Arias 2013). The list of animal species susceptible to AstV infection has expanded in the last decade, and additional species will probably be identified. Studies of evolutionary genomic relationships, mainly based on ORF2 (capsid protein), showed that human and animal AstV belonged to phylogenetically distinct genomic clusters (Figure 27.2; Jonassen et al. 2001; Kapoor et al. 2009; Lukashov and Goudsmit 2002; Reuter et al. 2011). Multiple lineages (and serotypes) of AstV have been identified within the same host species, for example, humans (classical HAstV1-8, HAstV-MLB1, and HMOAstVs), bats, turkeys, and pigs. Thus, AstV are genetically highly diverse, with each lineage potentially representing an independent origin. The International Committee on Taxonomy of Viruses has officially classified 19 genotype species of mamastroviruses (*Mamastrovirus 1* through *Mamastrovirus 19*), but a number of AstV have not yet been classified. For example, up to five PAstV lineages (PAstV1 to PAstV5) have been identified (Laurin et al. 2011), but only PAstV1 has been classified under the species *Mamastrovirus 3*, and the taxonomic species of PAstV2–PAstV5 have not been officially classified.

The physicochemical and biological properties of PAstVs have not been fully characterized. Cytopathic PAstV1 from diarrheic pigs was successfully isolated on porcine kidney cell lines by incorporating trypsin into the medium (Indik et al. 2006; Shimizu et al. 1990). Immunofluorescent cells and AstV particles were also detected. A virus with a buoyant density of 1.35 g/ml was cloned, and a serum virus neutralization test was developed. The PAstV1 isolate was stable to treatment with lipid solvents and resisted heating at 56 °C (133 °F) for 30 minutes but showed some susceptibility to acid treatment at pH 3.0 for 3 hours. In most AstV, virion protein composition has not been established, but is generally thought to consist of three capsids ranging between 24 and 39 kD.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

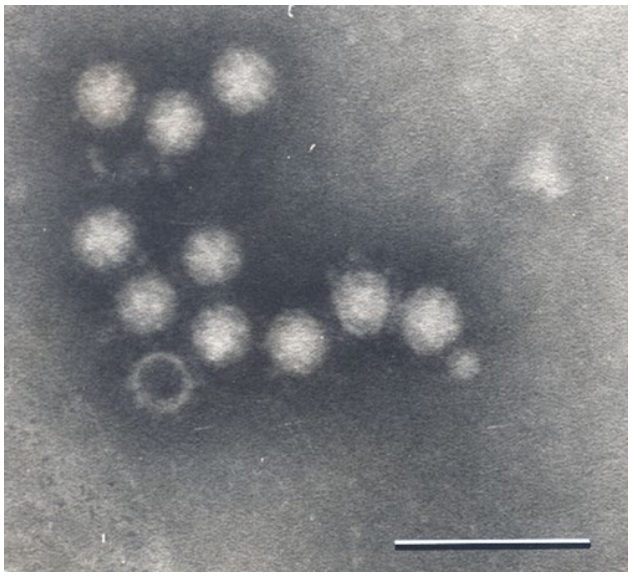


Figure 27.1 The 30 nm diameter, non-enveloped astrovirus particles are distinctive in appearance, with some particles showing a 5- or 6-pointed star pattern on their surface when viewed by negative stain electron microscopy. *Source:* Bridger (1980). Reproduced with permission of British Veterinary Association.

Public health

The zoonotic potential of AstV is unclear. The possibility of zoonotic transmission is suspected, but additional data is needed.

Epidemiology

PAstVs have been identified in pig feces in the United Kingdom (Bridger 1980), Japan (Shimizu et al. 1990), South Africa (Geyer et al. 1994), and Czech Republic (Indik et al. 2006) and recently in 12 more countries, indicating a worldwide distribution. The prevalence of PAstVs can be high in both diarrheic and clinically healthy swine. For example, PAstVs (PAstV1 to PAstV5), but predominantly PAstV4, were detected in 64% (326/509) of US porcine fecal samples (Xiao et al. 2013). Coinfections of a single pig with two or more PAstVs were observed (Xiao et al. 2013). One or more PAstVs were identified in 31.6% (25/79) of the suckling pigs sampled, 75.1% (136/181) of the nursery pigs, 72.3% (107/148) of the grow–finish pigs, and 28.6% (2/7) of mature pigs. PAstV prevalence was significantly higher in nursery pigs compared with suckling and mature pigs (Xiao et al. 2013). In a previous serological survey in Japan, 39% of 128 pigs in 8 herds had serum neutralizing antibodies to porcine astrovirus PAstV1 (Shimizu et al. 1990). All but one herd was antibody positive, and the in-herd prevalence ranged from 7 to 83%. PAstVs (PAstV4 and PAstV5) were also detected in wild boars (Cai et al. 2016; Reuter et al. 2012).

Transmission is presumed to be fecal–oral, but PAstV was also detected in nasal swab (Padmanabhan and Hause 2016) and blood samples (Brnic et al. 2013). Discoveries of novel AstV, in combination with genetic and evolutionary studies, support the hypothesis that cross-species transmission and natural recombination among AstV of humans, pigs, and other animal species, possibly through unknown intermediate hosts, may have occurred in the past. Given the diversity of AstV, interspecies transmission could be underestimated because no common molecular probes, antibodies, or oligonucleotide primers are available to identify AstV strains across different species (Mendez and Arias 2013).

Pathogenesis, clinical signs, lesions, and immunity

In most mammalian species, AstV infections are associated with gastroenteritis. In humans, AstV were found to be the third to fourth most common cause of viral diarrhea in young children worldwide. PAstVs have been associated with severe diarrhea in natural infections, but only in the presence of other known enteric pathogens (Bridger 1980; Shimizu et al. 1990). Mild diarrhea developed in 4-day-old pigs after oral inoculation with PAstV1 grown in cell culture (Shimizu et al. 1990). Diarrhea commenced 1 day after inoculation and continued for 5–6 days. Cytopathic AstV was recovered from feces and pigs seroconverted. Similarly, PAstV-induced mild diarrhea was observed in 7-day-old gnotobiotic piglets in a per os experimental inoculation study in the Czech Republic (Indik et al. 2006).

Recent studies also demonstrated that extraintestinal consequences of PAstV infections may also be possible. PAstVs capable of causing viremia (RNAemia) (Brnic et al. 2013) were detected in nasal swabs collected from young pigs in unexplained acute respiratory disease (Padmanabhan and Hause 2016) and in brain tissues of piglets with congenital tremor (Blomström et al. 2014). More recently, neuroinvasive PAstV3-associated neurological disease (meningoencephalomyelitis, weakness, and paralysis) has been reported on swine farms in Hungary and the United States (Arruda et al. 2017; Boros et al. 2017). These recent results suggest that PAstV infections have a more complex pathogenesis and more serious disease outcome than previously thought (Reuter et al. 2018).

Nothing is known about antigenic differences between PAstVs and the immunity induced by infection with these viruses.

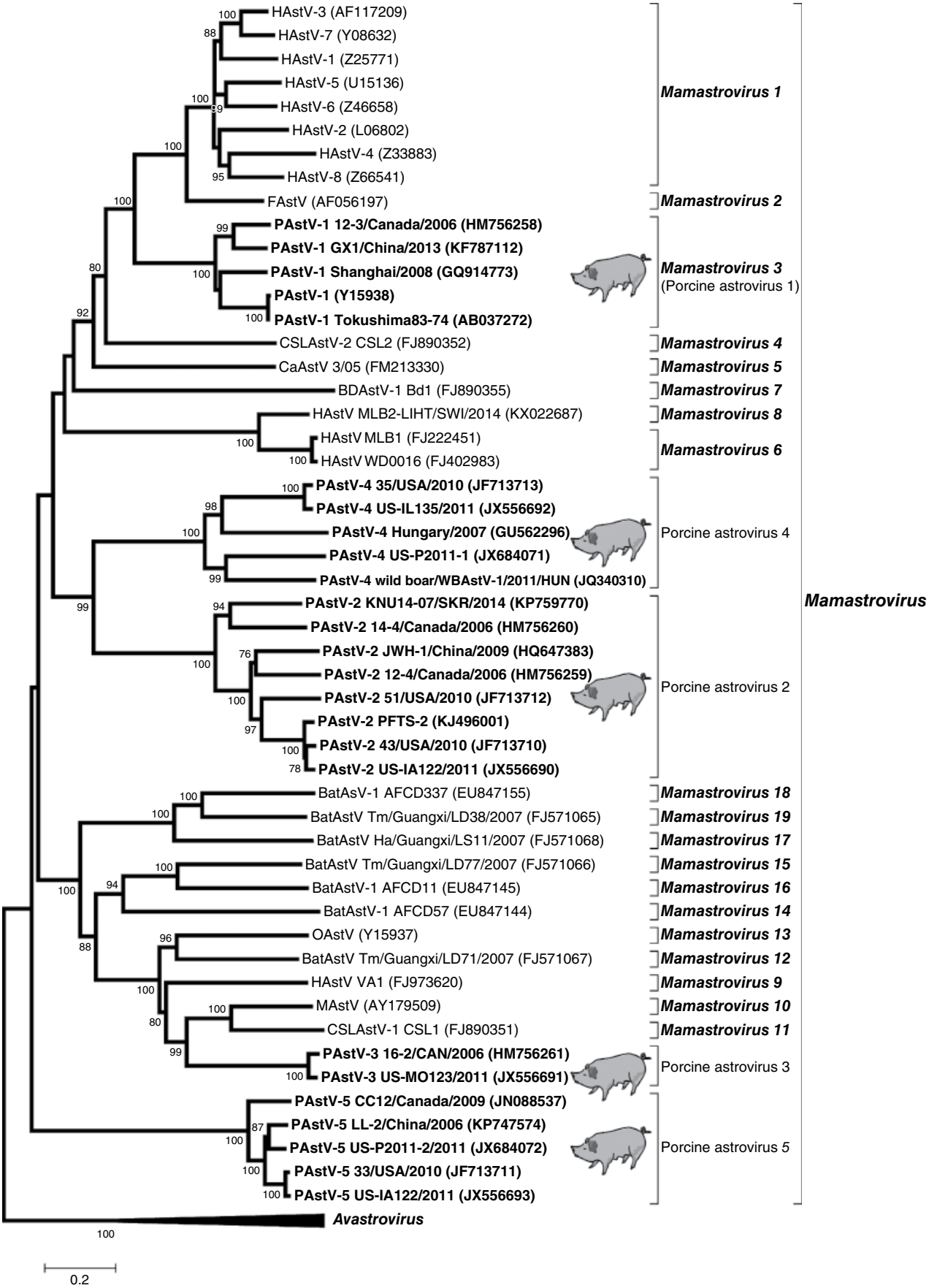


Figure 27.2 Midpoint-rooted neighbor-joining tree showing the relationships between the capsid proteins of porcine astroviruses and other representative members of the family in genera *Mamastrovirus* and *Avastrovirus*. Porcine astroviruses (PAstV1 to PAstV5) are in bold letters. BatAstV, bat astrovirus; BDAstV, bottlenose dolphin astrovirus; CaAstV, canine astrovirus; CSLAstV, California sea lion astrovirus; FAstV, feline astrovirus; HAstV, human astrovirus; MAstV, mink astrovirus; OAstV, ovine astrovirus.

Diagnosis

Assays have not been developed for routine diagnosis, but methods such as electron microscopy, isolation on cell culture, identification with immunofluorescence staining, detection of nucleic acids by reverse transcription polymerase chain reaction (RT-PCR) (Arruda et al. 2017; Boros et al. 2017), and next-generation sequencing methods using random primer-based amplification (Arruda et al. 2017; Shan et al. 2011) can be used to identify PAstV infection. Virus isolation for PAstVs has proven difficult. PAstV1 has been isolated on porcine kidney cells with the addition of trypsin (Indik et al. 2006; Shimizu et al. 1990), but there have been no reports of successful isolation of PAstV2–PAstV5. Virus neutralization and immunofluorescent antibody assays can be used to demonstrate PAstV1 seroconversion (Shimizu et al. 1990), but the diagnostic performance of these methods has not been established.

Prevention and control

PAstVs may be just one of several viruses that contribute to pre- and post weaning diarrhea and, perhaps, encephalomyelitis. Elimination of AstV from infected farms would be difficult because they are apparently widely distributed in swine populations and stable in the environment. Efforts to eliminate AstV would also be difficult to justify on the basis of their clinical effects. Assuming that lesions caused by infection with AstV are confined to the intestinal tract, oral rehydration would be expected to be effective in affected pigs. There are no commercially available vaccines.

References

Arruda B, Arruda P, Hensch M, et al. 2017. *Emerg Infect Dis* 23:2097–2100.

Blomström AL, Ley C, Jacobson M. 2014. *Acta Vet Scand* 56:82.

Boros A, Albert M, Pankovics P, et al. 2017. *Emerg Infect Dis* 23:1999–2010.

Boujon C, Koch M, Seuberlich T. 2017. *Adv Virus Res* 99:109–137.

Bridger JC. 1980. *Vet Rec* 107:532–533.

Brnic D, Prpic J, Keros T, et al. 2013. *Infect Genet Evol* 14:258–264.

Cai Y, Yin W, Zhou Y, et al. 2016. *Virol J* 13:6.

De Benedictis P, Schultz-Cherry S, Burnham A, et al. 2011. *Infect Genet Evol* 11:1529–1544.

Geyer A, Steele AD, Peenze I, et al. 1994. *J S Afr Vet Assoc* 65:164–166.

Indik S, Valicek L, Smid B, et al. 2006. *Vet Microbiol* 117:276–283.

Jonassen CM, Jonassen TO, Saif YM, et al. 2001. *J Gen Virol* 82:1061–1067.

Kapoor A, Li L, Victoria J, et al. 2009. *J Gen Virol* 90:2965–2972.

Laurin MA, Dastor M, L'Homme Y. 2011. *Arch Virol* 156:2095–2099.

Lukashov VV, Goudsmit J. 2002. *J Gen Virol* 83:1397–1405.

Mendez E, Arias CF. 2013. Astroviruses. In Knipe DM, Howley PM, eds. *Fields Virology*, 6th ed. Philadelphia: Lippincott Williams & Wilkins, pp. 609–628.

Padmanabhan A, Hause BM. 2016. *Arch Virol* 161:2575–2579.

Reuter G, Pankovics P, Boros Á. 2011. *Arch Virol* 156:125–128.

Reuter G, Nemes C, Boros Á, et al. 2012. *Arch Virol* 157:1143–1147.

Reuter G, Pankovics P, Boros Á 2018. *Clin Microbiol Rev* 31:e00040–18.

Shan T, Li L, Simmonds P, et al. 2011. *J Virol* 85:11697–11708.

Shimizu M, Shirai J, Narita M, et al. 1990. *J Clin Microbiol* 28:201–206.

Shirai J, Shimizu M, Fukusho A. 1985. Coronavirus-, calicivirus, and astrovirus-like particles associated with acute porcine gastroenteritis. *Nippon Juigaku Zasshi* 47:1023–1026.

Xiao CT, Giménez-Lirola LG, Gerber PF, et al. 2013. *J Gen Virol* 94:570–582.

28

Bunyaviruses

Chih-Cheng Chang

Relevance

Although more important in humans and ruminants, swine are nonetheless susceptible to several bunyaviruses, as supported by studies on Akabane, Oya, Lumbo, and Tahyna viruses (Arunagiri et al. 1991; Huang et al. 2003; Hubálek et al. 1993; Kono et al. 2002; Lim et al. 2007; Yadav et al. 2016). Bunyaviruses constitute a large and highly diverse group. Thus, it is possible that swine are susceptible to additional bunyaviruses. However, a consensus has not been achieved on the susceptibility of pigs to Gouléako virus and Herbert virus (Chung et al. 2014; Junglen et al. 2015). Similarly, Poskin et al. (2014) reported seroconversion, but no detection of viral replication in pigs inoculated with Schmallenberg virus.

Etiology

Bunyaviruses are single-stranded, negative-sense, enveloped viruses 80–100 nm in diameter. The genome is composed of three segments of negative-sense RNA that differ in size: large (L), medium (M), and small (S) (Elliott 1985, 1990). The L segment encodes a large polypeptide (L protein) with replicase and transcriptase activities (Jin and Elliott 1991, 1992). The M segment encodes two structural glycoproteins (G1 and G2) and a nonstructural protein (NSm) (Elliott 1985; Fazakerley et al. 1988; Fuller and Bishop 1982; Gentsch and Bishop 1979; Gerbaud et al. 1992). These glycoproteins, inserted into the membrane of the virion and projected onto the surface, are critical in viral virulence, attachment, cell fusion, and hemagglutination activity (Schmaljohn 1996). The nucleocapsid (N) and a nonstructural protein (NSs) are encoded by overlapping reading frames in the S segment (Elliott 1990).

Bunyavirus taxonomy underwent significant changes in 2016. Prior to 2016, the family *Bunyaviridae* was organized in five genera: *Hantavirus*, *Nairovirus*, *Orthobunyavirus*, *Phlebovirus*, and *Tospovirus*. In 2016, the International Committee on Taxonomy of Viruses created a new order, *Bunyavirales*, with nine families. Bunyaviruses known to be infectious for pigs are in the family *Peribunyaviridae*, genus *Orthobunyavirus* (Akabane, Oya, Lumbo, and Tahyna viruses). Traditionally, viruses in the genus *Orthobunyavirus* are divided into five serogroups: Bunyamera, Bwamba, California, Guama, and Simbu (Calisher 1996). Bunyaviruses of swine fall into the Simbu serogroup (Akabane, Oya, and Schmallenberg viruses) and California serogroup (Lumbo and Tahyna viruses) (Calisher 1996; Elliott 1997).

Public health

Humans are not susceptible to Akabane or Oya viruses (Bryant et al. 2005). Tahyna virus and the closely related Lumbo virus are widespread in some human populations, occasionally with clinical consequences (Gould et al. 2006; Vapalahti et al. 1996). Overall, the role of pigs in the complex ecology of bunyaviruses is largely undefined, but likely to be minor.

Epidemiology

Given the extensive global movement of people, animals, and goods, the geographical distribution of specific bunyaviruses is plastic and reflects each virus' ability to exploit available vector(s) and host(s). Akabane and Oya viruses are found worldwide, except Europe, and are transmitted to vertebrates by culicoid midges and mosquitoes (Calisher 1996). Lumbo (Africa) and Tahyna viruses (Asia, Africa, and Europe) are mosquito-borne viruses (Elliott 1997).

Akabane virus (Simbu serogroup) was first isolated from mosquitoes in Japan in 1968. Midges and mosquitoes are vectors of Akabane virus (Jennings and Mellor 1989; Kurogi et al. 1987; Oya et al. 1961), and herbivores, including cattle, sheep, giraffe, horses, and goats, are the natural vertebrate hosts (Al-Busaidy et al. 1988; Cybinski et al. 1978). Some work has shown that Akabane virus

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

may be prevalent in pig populations in Asia (Huang et al. 2003; Lim et al. 2007; Yanase et al. 2018), with biting midges (*Culicoides* spp.) serving as the primary vectors (Hsu et al. 1997; Huang et al. 2003). Akabane virus transmission in pigs may not be completely arthropod dependent. That is, infection occurred following experimental oronasal exposure (Huang et al. 2003).

In Korea, a survey found that 37% of pigs were seropositive for Akabane virus, with positive pigs on all of the 15 farms surveyed (Lim et al. 2007). In Taiwan, a national survey (Huang et al. 2003) found that approximately 75% of pigs were serum antibody positive for Akabane virus, with seropositivity rates in sow breeding herds reaching 99%. A high percentage of positive testing results in young pigs (98%) suggested the presence of maternal antibodies. Consistent with this interpretation, seropositivity declined to 17% in 20-week-old pigs and then returned to high levels (71%) in late finishers. Seropositivity rates for Akabane virus in cattle in Taiwan were also high (96%).

Sequential analysis of Taiwanese Akabane virus isolates from swine (NT-14) and cattle (PT-17) showed a high degree of homology (99.6%) for the small (S) RNA segment. Akabane virus was also identified in piglets with neurological disorders or congenital malformations in 2011 and 2013 in Japan, and the virus detected in piglets shared high nucleotide identity (99.02–99.83%) to the viruses detected in cattle at the same year (Yanase et al. 2018).

Serosurveys for other bunyaviruses have also been done in swine populations. A study of Oya virus (Simbu serogroup) in Malaysia found that 93% of 360 pigs sampled on 24 farms in 6 states were serum antibody positive (Kono et al. 2002). Hubálek et al. (1993) reported that 38% of wild boars (*Sus scrofa*) in the Czech Republic were seropositive for Tahyna virus (California serogroup). A serosurvey in China showed IgM and IgG antibodies against Tahyna virus in swine by indirect fluorescent antibody (IFA) assay (Li et al. 2010). Seroconversion of pigs to Lumbo virus, another member of the California serogroup, was reported in Sri Lanka (Arunagiri et al. 1991).

Pathogenesis

Little research on the pathogenesis of bunyaviruses in pigs has been reported. Huang et al. (2003) infected 4-week-old pigs with Akabane virus (isolate NT-14) under experimental conditions by oronasal exposure. Viremia occurred 1–6 days post inoculation (DPI). During this period, the virus was isolated from a variety of tissues (spleen, lungs, brain, small intestine, lymph node, thymus, and salivary gland) and persisted in the tonsil for 14 DPI. The virus was also isolated from oronasal discharge, but not feces. Naive pigs in direct contact with the inoculated pigs did not become infected.

Clinical signs and lesions

Akabane virus infection is inapparent in adult swine (Huang et al. 2003), but whether Akabane virus induces lesions in pregnant sows or their fetuses is not known. Under experimental conditions, Akabane virus (isolate NT-14) did not produce gross lesions, but mild lymphocytic encephalitis and cerebral vasculitis were observed (Huang et al. 2003).

Clinical signs and lesions have not been described for Tahyna and/or Lumbo viruses. Lesions caused by Oya virus in pigs have not been described, but Kono et al. (2002) suggested that Oya virus could cause clinical signs similar to Nipah virus infection.

Diagnosis

Bunyaviruses may be recovered in suckling mice or on susceptible cell culture systems, including hamster lung (HmLu-1), baby hamster kidney (BHK-21) cells, monkey kidney cells (Vero, MA-104, MARC-145), and mosquito cell lines (Bryant et al. 2005; Gerdes 2008; Huang et al. 2003; Kono et al. 2002). Techniques for bunyavirus detection and identification include transmission electron microscopy, serology (Huang et al. 2003), immunohistochemistry, reverse transcription–polymerase chain reaction (Bryant et al. 2005; Huang et al. 2003), and sequencing (Saeed et al. 2001).

Prevention and control

There are no effective treatments or vaccines available for bunyavirus infection in pigs. However, these viruses are inactivated by most common disinfectants, for example, hypochlorite, detergents, chlorhexidine, and phenols.

References

Al-Busaidy SM, Mellor PS, Taylor WP. 1988. *Vet Microbiol* 17:141–149.

Arunagiri CK, Perera LP, Abeykoon SB, et al. 1991. *Am J Trop Med Hyg* 45:377–382.

Bryant JE, Crabtree MB, Nam VS, et al. 2005. *Am J Trop Med Hyg* 73:470–473.

Calisher CH. 1996. History, classification, and taxonomy of viruses in the family Bunyaviridae. In Elliott RM, ed. *The Bunyaviridae*. New York: Plenum Press, pp. 1–17.

Chung HC, Nguyen VG, Goede D, et al. 2014. *Emerg Infect Dis* 20:2072–2075.

Cybinski DH, George TDS, Paull NI. 1978. *Aust Vet J* 54:1–3.

Elliott RM. 1985. *Virology* 143:119–126.
Elliott RM. 1990. *J Gen Virol* 71:501–522.
Elliott RM. 1997. *Mol Med* 3:572–577.
Fazakerley JK, Gonzales-Scarano F, Strickler J, et al. 1988. *Virology* 167:422–432.
Fuller F, Bishop DHL. 1982. *J Virol* 41:643–648.
Gentsch JR, Bishop DHL. 1979. *J Virol* 30:767–770.
Gerbaud S, Pardigon N, Vialat P, et al. 1992. *J Gen Virol* 73:2245–2254.
Gerdes GH. 2008. Bunyaviral diseases of animals (excluding Rift Valley fever). In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Vol. 2, 6th ed. Paris: World Organisation for Animal Health (OIE), pp. 1165–1176.
Gould EA, Higgs S, Buckley A, et al. 2006. *Emerg Infect Dis* 12:549–554.
Hsu HS, Liao YK, Hung HH. 1997. *J Chin Soc Vet Sci* 23:303–310.
Huang CC, Huang TS, Deng MC, et al. 2003. *Vet Microbiol* 94:1–11.
Hubálek Z, Juricova Z, Svobodov I, et al. 1993. *J Wildl Dis* 29:604–607.
Jennings M, Mellor PS. 1989. *Vet Microbiol* 21:125–131.
Jin H, Elliott RM. 1991. *J Virol* 65:4182–4189.
Jin H, Elliott RM. 1992. *J Gen Virol* 73:2235–2244.
Junglen S, Marklewitz M, Zirkel F, et al. 2015. *Emerg Infect Dis* 21:2190–2193.
Kono Y, Yusnita Y, Ali AM, et al. 2002. *Arch Virol* 147:1623–1630.
Kurogi H, Akiba K, Inaba Y, et al. 1987. *Vet Microbiol* 15:243–248.
Li WJ, Wang JL, Li MH, et al. 2010. *Am J Trop Med Hyg* 82:705–711.
Lim SI, Kweon CH, Tark DS, et al. 2007. *J Vet Sci* 8:45–49.
Oya A, Okuno T, Ogata T, et al. 1961. *Jpn J Med Sci Biol* 14: 101–108.
Poskin A, Van Campe W, Mostin L, et al. 2014. *Vet Microbiol* 170:398–402.
Saeed MF, Li L, Wang H, et al. 2001. *J Gen Virol* 82:2173–2181.
Schmaljohn CS. 1996. Bunyaviridae: The viruses and their replication. In Fields BN, Knipe DM, Howley PM, eds. *Fields Virology*, 3rd ed. New York: Raven Press, pp. 1447–1471.
Vapalahti O, Plyusnin A, Cheng Y, et al. 1996. *J Gen Virol* 77:1769–1774.
Yadav P, Shete A, Bondre V, et al. 2016. *Infect Genet Evol* 44:122–126.
Yanase T, Kato T, Hayama Y, et al. 2018. *Transbound Emerg Dis* 65:e434–e443.

29

Caliciviruses

Nick J. Knowles and Gábor Reuter

Overview

The family *Caliciviridae* consists of five genera: *Lagovirus*, *Vesivirus*, *Norovirus*, *Sapovirus*, and *Nebovirus* (Clarke et al. 2012). The *Lagovirus* genus is composed of two species, *Rabbit hemorrhagic disease virus* and *European brown hare syndrome virus*, while the *Vesivirus* genus is composed of two species, *Vesicular exanthema of swine virus* and *Feline calicivirus*, plus a number of unassigned viruses. The *Norovirus*, *Sapovirus*, and *Nebovirus* genera each consist of a single species, *Norwalk virus*, *Sapporo virus*, and *Newbury-1 virus*, respectively. Some recently discovered caliciviruses are phylogenetically distinct from the existing genera and currently remain unclassified (although six novel genera have been proposed): Tulane virus detected from rhesus macaques (Farkas et al. 2008), St-Valérien virus detected from pigs (L'Homme et al. 2009b), chicken calicivirus (Wolf et al. 2011), turkey/chicken/goose calicivirus (Liao et al. 2014; Wolf et al. 2012), Atlantic salmon calicivirus (Mikalsen et al. 2014), and bat calicivirus (Kemenesi et al. 2016). Caliciviruses infecting pigs include VESV, Norwalk virus, Sapporo virus, and the unclassified St-Valérien virus.

Calicivirus virions are non-enveloped with icosahedral symmetry. They are 27–40 nm in diameter by negative stain electron microscopy (Wawrzkiewicz et al. 1968) and 35–40 nm by cryo-electron microscopy and X-ray crystallography (Prasad et al. 1999). The capsid is composed of 90 dimers of the major structural protein VP1 arranged on a $T = 3$ icosahedral lattice. In noroviruses, the VP1 forms a subunit composed of a shell and two protruding domains. A characteristic feature of calicivirus capsid architecture is the 32 cup-shaped depressions at each of the icosahedral fivefold and threefold axes. In negative stain virus preparations, some cup-shaped depressions appear distinct and well defined, while in others, these depressions are less prominent.

Caliciviruses have single-stranded, positive-sense genomic RNA organized into either two or three major open reading frames (ORFs). The nonstructural proteins are encoded in the 5′ part of the genome and the structural proteins in the 3′ part. Replication occurs in the cytoplasm, and two major positive-strand RNA species are found in infected cells: (1) the genome-sized, positive-strand RNA serves as the template for the translation of a large polyprotein that undergoes cleavage by a virus-encoded protease to form the mature nonstructural proteins, and (2) a subgenomic-sized, positive-strand RNA coterminal with the 3′ terminus of the genome is the template for the translation of the VP1 as well as the 3′ terminal ORF product VP2. A double-stranded RNA (dsRNA) corresponding in size to full-length genomic RNA has been identified in cells infected with feline calicivirus and San Miguel sea lion virus (SMSV), indicating that replication occurs via a negative-strand intermediate.

Virion molecular mass (Mr) is about 15×10^6. Virion buoyant density is 1.33–1.41 g/cm^3 in CsCl and 1.29 g /cm^3 in glycerol–potassium tartrate gradients. Virion S_{20w} is 160–187S (Wawrzkiewicz et al. 1968). Physicochemical properties have been established for some members of the family. Generally, caliciviruses are stable in the environment, and many strains are resistant to inactivation by heat and certain chemicals (ether, chloroform, and mild detergents) (Wawrzkiewicz et al. 1968). Enteric caliciviruses are acid stable, while vesiviruses are labile below pH 4.5–5.0 (Wawrzkiewicz et al. 1968).

Vesicular exanthema of swine virus

Relevance

Vesicular exanthema of swine (VES) is an acute, highly infectious disease characterized by fever and formation of blisters on the snout, oral mucosa, soles of the feet, the coronary band, and between the toes. In pigs, the clinical disease is indistinguishable from foot-and-mouth disease,

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

vesicular stomatitis, swine vesicular disease, and vesicular disease caused by Seneca Valley virus.

Originally confined to California, VES became widespread in the United States during the 1950s, but a vigorous campaign to eradicate the disease was successful. In 1959, the United States was declared free of VES, and the disease was designated a foreign animal disease; it has never been reported as a natural infection of pigs in any other parts of the world.

Since 1972, a virus indistinguishable from vesicular exanthema of swine virus (VESV), designated as SMSV, has been isolated from throat and rectal swabs from premature and 4-month-old California sea lion pups, dead and weanling northern fur seal pups, and nursing northern elephant seal pups. It has also been isolated from vesicular lesions on marine mammals, commercial seal meat produced in Alaska, and perch-like fish collected from tidal pools off the southern California coast. SMSV isolated from both fish and marine mammals is capable of producing vesicular exanthema in pigs and humans (Smith et al. 1998b). In addition, caliciviruses isolated from throat and rectal swabs from dairy calves cause clinical vesicular exanthema in exposed pigs.

Etiology

The genus *Vesivirus* is composed of two species: *Vesicular exanthema of swine virus* and *Feline calicivirus*. A number of other vesiviruses, isolated from dogs and mink and as cell culture contaminants, are distinct from the two species and remain unclassified. Viruses in the species *Vesicular exanthema of swine virus* are classified into more than 40 serotypes (although not all have been serologically compared) (Neill et al. 1995). Thirteen are known as VESV (VESV-B34, VESV-101-43, VESV-A48, VESV-B51, VESV-C52, VESV-D53, VESV-E54, VESV-F55, VESV-G55, VESV-H54, VESV-I55, VESV-J56, VESV-K54), 17 as SMSV (SMSV-1, SMSV-2, SMSV-4 to SMSV-7, SMSV-9 to SMSV-11, SMSV-13 to SMSV-17, SMSV-FADDL 7005, SMSV-693 M, SMSV-3709), and the remainder named after the hosts from which they were first isolated, that is, California sea lion virus 02012181, Steller sea lion virus (SSLV) V810, SSLV-V1415, bovine calicivirus (BCV) Bos-1, BCV Bos-2, cetacean calicivirus Tur-1, primate calicivirus Pan-1, reptile calicivirus Cro-1, walrus calicivirus, skunk calicivirus, rabbit calicivirus, and human calicivirus. Seven VESV serotypes isolated during the 1930s and 1940s have since been lost or destroyed and have not been compared with later serotypes. SMSV-3 was found to be a mixture of SMSV-1 and SMSV-2. Complete genome sequencing of SMSV-8 and SMSV-12 shows them to be distinct from other members of the species *Vesicular exanthema of swine virus* (Neill 2014; N.J. Knowles et al., unpublished data). Members of the species *Vesicular exanthema of swine virus* all replicate in mammalian cell cultures (e.g. monkey kidney or porcine kidney) usually causing a rapid and destructive CPE.

Public health

These viruses are not thought to be of public health significance; however, human infection by two different marine mammal serotypes has been reported. SMSV-5 was recovered from vesicular lesions on the palms and soles of a researcher working with the virus (Smith et al. 1998b). In a separate incident, an individual who handled diseased Steller sea lions (*Eumetopias jubatus*) in the Bering Sea developed blistering of the hard palate, upper lip, and facial area. A novel vesivirus (strain McAll) was isolated from throat washings sampled 30 days post onset (Smith et al. 1998b).

Epidemiology

VESV/SMSV and related viruses have been found along the Pacific Coast of North America as far north as the Bering Sea. Outbreaks of VES occurring between 1932 and 1951 in pigs in the United States were limited to California. However, in 1951, one serotype (B51) spread to 41 states and the District of Columbia. Two further serotypes appeared in New Jersey in 1954 and 1956.

Natural infections have been found in pigs, pinnipeds, cetaceans, cattle, horses, skunk, primates (including humans), reptiles, and fish (Smith et al. 1998a). SMSV-7 and SMSV-17 have been isolated from a sea lion liver fluke (*Zalophatrema* sp.) and a mussel (*Mytilus californianus*), respectively (Smith et al. 1998a). Experimentally, at least six of the SMSV serotypes have been shown to cause vesicular disease in pigs indistinguishable from that caused by VESV (Berry et al. 1990; Bresse and Dardiri 1977; Gelberg and Lewis 1982; Smith et al. 1974, 1980; Van Bonn et al. 2000).

The principal form of transmission during outbreaks of vesicular exanthema in the United States was thought to be by feeding untreated contaminated garbage. However, spread by direct contact of infected animals occurs readily (Madin 1975).

Clinical signs and lesions

Following an elevated temperature, vesicles appear at one or more of the following sites: snout, lips, tongue, mucosa of the oral cavity, and on the sole, interdigital space, and coronary band of the foot (Madin 1975). Lesions may also appear on the teats, particularly in nursing sows.

Diagnosis

Presumptive diagnosis in pigs is based on fever and the presence of typical vesicles, which normally rupture within 24–48 hours to form erosions. Laboratory

differentiation of VESV from other vesicular disease-causing viruses, such as foot-and-mouth disease virus (*Aphthovirus*, *Picornaviridae*), swine vesicular disease virus (*Enterovirus*, *Picornaviridae*), Seneca Valley virus (*Senecavirus*, *Picornaviridae*), and vesicular stomatitis virus (*Vesiculovirus*, *Rhabdoviridae*), is essential.

Virus may be detected by a variety of serologically based laboratory tests, including complement fixation (Bankowski et al. 1953), virus neutralization (Bankowski et al. 1953; Holbrook et al. 1959), and enzyme-linked immunosorbent assay (ELISA) (Ferris and Oxtoby 1994). However, the serologically based tests rely on specific antisera for each serotype.

Electron microscopy may be performed on epithelial tissue suspensions or after passage in swine tissue cultures. Various reverse transcription polymerase chain reactions (RT-PCRs) have been developed for the identification of vesiviruses (McClenahan et al. 2009; Reid et al. 1999, 2007).

VES viruses may be isolated in cell cultures (usually pig or monkey kidney cell lines). Once the serotype involved in an outbreak has been confirmed, serological assays can be used for the detection/diagnosis of infected herds.

Immunity

Animals that have recovered from VESV develop a strong immune response and are protected against the same serotype for at least 6 months (Madin 1975). Neutralizing antibody may be detected 10–12 days post infection and peak at 21–28 days post infection.

Prevention and control

No specific treatment is available for VESV infection. Vaccines are not available, and the number of serotypes would probably preclude their development. Suspected cases of vesicular exanthema should be reported immediately to the proper authorities. Garbage and fish should be cooked before being fed to pigs. Despite VES having not appeared since its eradication in the 1950s, VES-like viruses are widespread in the Pacific Ocean and periodically appear in domesticated and captive wildlife along the Western United States. The potential for vesicular disease to appear in pigs remains an ever-present threat.

Porcine caliciviruses (noroviruses and sapoviruses)

Relevance

Porcine caliciviruses (noroviruses and sapoviruses) were first recognized when diarrheic feces from post weaning and nursing pigs in the United Kingdom and the United States were examined by electron microscopy (Bridger 1980; Saif et al. 1980). Porcine caliciviruses have not been widely studied, and much is unknown concerning their role in naturally occurring swine disease. This is in contrast to the recognized role of caliciviruses in sporadic and epidemic acute gastroenteritis in humans.

Human caliciviruses belong to two genera, *Norovirus* (formerly Norwalk-like viruses) and *Sapovirus* (formerly Sapporo-like viruses), in the family *Caliciviridae* (Mayo 2002). Viruses in both genera (porcine noroviruses and porcine sapoviruses) are now accepted as common infectious agents on pig farms, and it seems possible that caliciviruses may have a significant role in porcine enteric disease. Public health concerns over potential cross-species transmission and animal reservoirs for caliciviruses have been raised (Mattison et al. 2007; Reuter et al. 2010; Wang et al. 2005a). However, the question is still open. Based on limited data, there is no clear evidence that known porcine caliciviruses pose a threat to human health.

Porcine noroviruses

Etiology

Noroviruses, possessing a 27–32 nm non-enveloped capsid and an indistinct morphology, were reported in swine in 1980 (Bridger 1980) (Figure 29.1). The genome is composed of positive-sense, single-stranded RNA of 7.3–7.7 kb in length, excluding the poly(A) tail at the 3′ end. It is composed of three ORFs. ORF1 encodes a polyprotein that undergoes protease processing to produce several nonstructural proteins, including an RNA-dependent RNA polymerase (RdRp). ORF2 and ORF3 encode a major capsid protein (VP1) and a minor capsid protein (VP2), respectively (Green 2007).

Noroviruses are genetically highly diverse. Phylogenetic analysis of the virus capsid has been used to designate seven genogroups (G) named GI–GVII (Vinjé 2015).

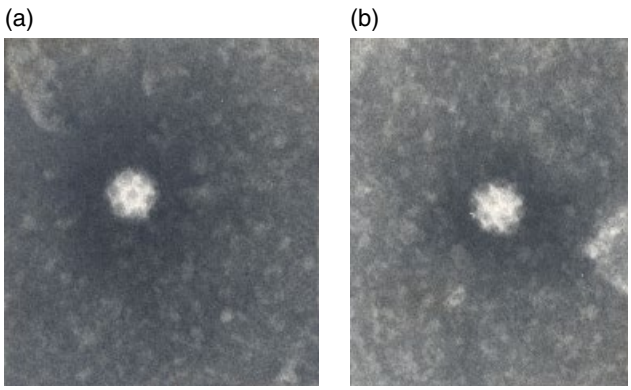


Figure 29.1 Negative stain electron microscopy. Panels (a) and (b): The sapovirus virion has typical calicivirus morphology with clear cup-shaped depressions and measures approximately 35 nm in diameter.

Viruses within each genogroup have been grouped into genotypes: 1–9 in GI, 1–22 in GII, 1–3 in GIII, 1 in GVII, and 2 each in GIV, GV, and GVI (Vinjé 2015; Wang et al. 2005a; Zheng et al. 2006). Due to the high sequence diversity between these genogroups (up to 60% amino acid difference), it has been suggested that they may represent distinct virus species (Zheng et al. 2006). GI has been found in humans and possibly pigs (Sisay et al. 2016a,b), GII in humans (genotypes 1–10 and 12–22) and pigs (genotypes 11, 18–19), GIII in cattle and sheep, GIV in humans and lion, GV in rats and mice, and GVI and GVII in dogs (Figure 29.2). Porcine norovirus sequences, including the prototype porcine norovirus strain Sw918/1997/JP (AB074893), grouped with the GII human noroviruses but formed three separate genotypes (11, 18, and 19) distinct from human noroviruses (Sugieda et al. 1998). Homologous recombination probably also occurs between porcine norovirus genotypes (Wang et al. 2005a).

Porcine norovirus genomes have been identified in pigs from Japan, the Netherlands, the United States, Hungary, Belgium, Canada, China, Korea, Slovenia, and Brazil (Cunha et al. 2010; Keum et al. 2009; L'Homme et al. 2009a; Mauroy et al. 2008; Mijovski et al. 2010; Reuter et al. 2007; Shen et al. 2009; Sugieda et al. 1998; Sugieda and Nakajima 2002; van der Poel et al. 2000; Wang et al. 2005a). Interestingly, virus-like particles (VLPs) of Sw918/JP (genotype 11) and QW101/2003/US (genotype 18) cross-react with antibodies against human GII noroviruses (Farkas et al. 2005; Wang et al. 2005a). In addition, human recombinant norovirus VLP binds specifically to histo-blood group antigens (HBGAs) in epithelial cells of pig gastrointestinal tissue (Tian et al. 2007). More data are required to determine whether human and porcine noroviruses are antigenically distinct or related and whether humans and pigs might share noroviruses.

Porcine noroviruses have not been cultured *in vitro*, and nothing is known about their physicochemical and other biological properties. In general, noroviruses are characterized by stability in the environment and relative resistance to inactivation.

Public health

The close genetic and antigenic relationships between human and porcine noroviruses raise public health concerns regarding their potential for cross-species transmission and as reservoirs for noroviruses. However, there is no evidence for the direct threat of porcine noroviruses to human health.

Epidemiology

Noroviruses have been identified in humans, swine, cattle, sheep, mice, dogs, and lion. It is assumed that natural norovirus transmission is fecal–oral.

Norovirus RNA was detected relatively infrequently (0.4–6%) in pigs by RT-PCR in 4 of 1017 normal slaughtered pigs collected in 1997 from 26 Japanese farms (Sugieda et al. 1998), 2 of 100 pooled fecal samples from fattening pigs (3–9 months of age) on 100 farms in the Netherlands (van der Poel et al. 2000), 6 of 275 fecal samples from normal adult swine in the United States, and 1 of 17 fecal samples from normal swine under the age of 2 years in Hungary (Reuter et al. 2007). These figures are likely to be an underestimate of porcine norovirus prevalence as PCR primers designed for human noroviruses were used, and pre- and post weaning diarrheic piglets were not examined.

The geographical distribution indicates the worldwide occurrence of porcine noroviruses among pigs in farms. Little is known about the association of porcine noroviruses with natural disease. It is not established if porcine noroviruses are species specific, but it has been postulated that the close genetic similarity of porcine noroviruses and human noroviruses may mean that porcine noroviruses are a reservoir of human infection (Sugieda and Nakajima 2002; van der Poel et al. 2000).

Pathogenesis, clinical signs, lesions, and immunity

All porcine noroviruses were detected from pigs with no clinical signs. Subclinically infected pigs may be natural reservoirs for noroviruses. The isolate QW101/2003/US (genotype 18) replicated in gnotobiotic pigs with fecal shedding coincident with mild diarrhea (Wang et al. 2005a).

In an experiment using GII.4 human norovirus, 48 (74%) of 65 gnotobiotic piglets developed mild diarrhea (Cheetham et al. 2006). Histopathological examination showed mild lesions in the proximal small intestine of only one of the seven pigs. Evidence was found for replication of the virus in intestinal enterocytes in 18 of 31 cases. Seroconversion after 21 days post inoculation was detected by antibody ELISA in 13 of 22 virus-inoculated pigs, indicating human norovirus replication in pigs.

Seroprevalence of GII norovirus in swine was 97% in the United States and 36% in Japan (Farkas et al. 2005). Immune responses against porcine noroviruses, protective immunity, and/or the role of maternal antibodies have not been assessed. It may be assumed that protective immune mechanisms are similar to those for other enteric virus pathogens. Porcine norovirus infections in pigs may potentially provide useful insights into protective immunity of the equivalent viruses of humans.

Diagnosis

No diagnostic tests for porcine noroviruses have been developed for use outside the research laboratory. Porcine noroviruses have been detected using electron

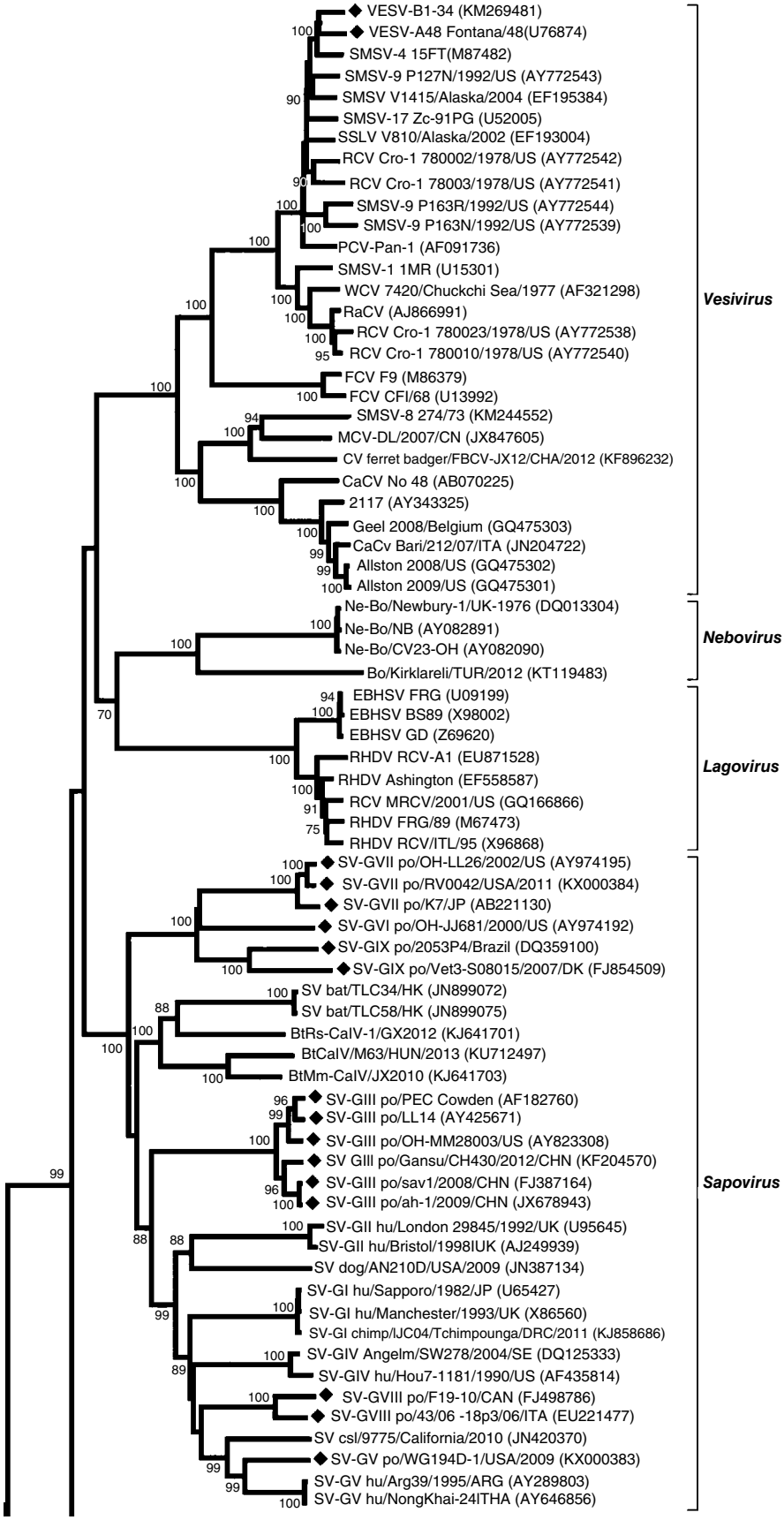


Figure 29.2 Unrooted neighbor-joining tree showing the relationships between the capsid proteins of caliciviruses in swine and other members of the family. Swine caliciviruses are indicated by ◆ (black diamond). Members of the five accepted calicivirus genera (*Lagovirus*, *Nebovirus*, *Norovirus*, *Sapovirus*, and *Vesivirus*) and candidate genera (names in quotation marks) are represented.

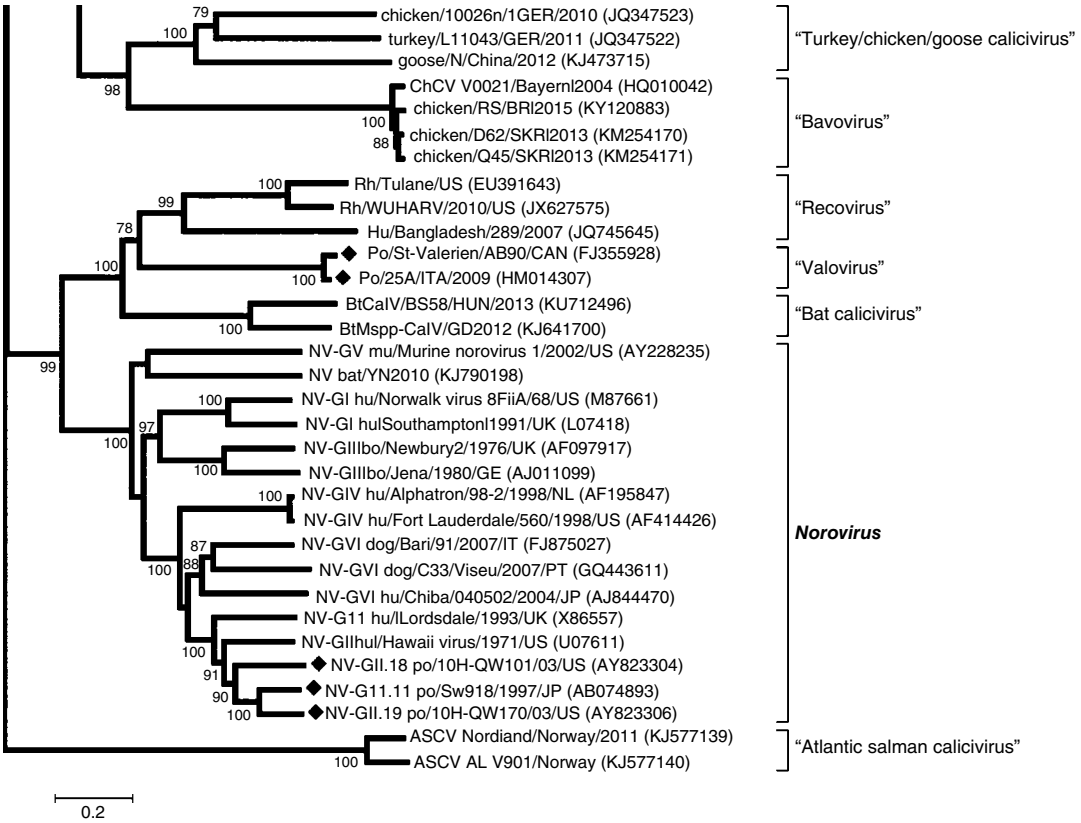


Figure 29.2 (Cont'd)

microscopy, RT-PCR, and real-time RT-PCR (Reuter et al. 2007; Sugieda et al. 1998; Sugieda and Nakajima 2002; van der Poel et al. 2000; Wang et al. 2005a). However, the sensitivity of molecular tests for laboratory diagnosis of porcine norovirus infections has not been evaluated.

Prevention and control

Assuming that porcine norovirus epidemiology and immunology are similar to porcine rotaviruses, it is likely that these viruses persist in the environment, and it may be impossible to eliminate the infection from pig herds and prevent natural infection of piglets. However, the persistence of porcine enteric viruses in animal waste depends on the waste treatment technology (Costantini et al. 2007). It is also likely that sows pass on maternal antibody in colostrum and milk and thus limit infection and damage in the gut of nursing piglets. Treatment with oral rehydration fluids is likely to be successful.

Porcine sapoviruses

Etiology

Sapoviruses, possessing a 30–35 nm non-enveloped capsid and typical calicivirus morphology with clear cup-shaped depressions, were reported in swine in the United States in 1980 (Saif et al. 1980) and genetically characterized as sapovirus in 1999 (Guo et al. 1999). The genome is composed of positive-sense, single-stranded RNA of 7.3–7.5 kb in length, excluding the poly(A) tail at the 3′ end. It is composed of two ORFs. ORF1 encodes a polyprotein that undergoes protease processing to produce several nonstructural proteins including an RdRp, and a major capsid protein (VP1). ORF2 is predicted to encode the minor capsid protein (VP2) (Green 2007).

Sapoviruses are genetically highly diverse. Phylogenetic analysis of the major virus capsid protein has been used to designate five official genogroups (G) named GI–GV (Green 2007). However, further novel sapovirus genogroups (GVI–GX) identified in swine were also proposed (Reuter et al. 2010; Wang et al. 2005b). Within each genogroup, viruses have been grouped into genotypes: 1–3 in GI, 1–3 in GII, and 1 each in GIII–GX (Green 2007; Reuter et al. 2010). Due to the extremely high sequence diversity between these genogroups, it has been suggested that they may represent distinct virus species. Human sapoviruses belong to genogroups GI, GII, GIV, and GV. Porcine sapovirus belongs to genogroups GIII, GVI, GV, GVII, GVIII, GIX, and GX (Figure 29.2). GIII strains were detected the most frequently in pigs.

Double infections with different porcine sapoviruses are common among pigs (Reuter et al. 2010). Recombination

probably also occurs between porcine sapovirus genotypes (Hansman et al. 2005; Wang et al. 2005b). By phylogenetic analysis, a higher degree of genetic diversity was seen among porcine sapoviruses than among the known human sapoviruses, indicating that the coevolution of sapoviruses in swine was longer (Reuter et al. 2010). On the other hand, GVIII is genetically more closely related to human sapoviruses (especially those of GV and GI) than to other sapovirus genogroups in swine (Martella et al. 2008; Reuter et al. 2010; Wang et al. 2005b).

Porcine sapoviruses (historically called porcine enteric calicivirus [PEC]) have been studied more extensively than porcine norovirus. PEC/Cowden, a prototype porcine sapovirus (GIII), has been studied in detail (Flynn and Saif 1988; Flynn et al. 1988; Guo et al. 1999, 2001a; Parwani et al. 1990; Saif et al. 1980). The PEC/Cowden sapovirus was cultured in primary porcine kidney cells and the continuous porcine kidney cell line (LLC-PK) but only by inclusion of intestinal contents in the culture medium (Flynn and Saif 1988; Parwani et al. 1991). Interestingly, bile acids were identified as the factor that allowed cultivation by affecting the protein kinase A cell signaling pathway (Chang et al. 2002, 2004).

Public health

Public health concerns over potential cross-species transmission and animal reservoir for sapovirus have been raised. However, there is no evidence for the direct threat of porcine sapoviruses to human health.

Epidemiology

Porcine sapovirus RNA has been identified frequently in pigs (8–67%) and pig farms (7–88%) by RT-PCR in several countries, including Belgium (Mauroy et al. 2008), Brazil (Barry et al. 2008), Canada (L'Homme et al. 2009a), China (Shen et al. 2009), Ethiopia (Sisay et al. 2016b), Hungary (Nagy et al. 1996; Reuter et al. 2007), Italy (Martella et al. 2008), Japan (Shirai et al. 1985; Sugieda et al. 1998; Sugieda and Nakajima 2002), the Netherlands (van der Poel et al. 2000), South Korea (Kim et al. 2006), the United Kingdom (Bridger 1980), the United States (Guo et al. 1999; Saif et al. 1980), and Venezuela (Martinez et al. 2006), and in six European countries (Reuter et al. 2010). The geographical distribution indicates the worldwide occurrence and endemic circulation of porcine sapoviruses among pigs and in pig farms. Pigs are infected with porcine sapovirus early in life (Reuter et al. 2010). The one study conducted to examine the prevalence of porcine sapoviruses related to PEC/Cowden showed that at least 83% of 30 sow serum samples from Ohio pig herds with PEC-associated post weaning diarrhea had antibodies reactive to PEC/Cowden (Guo et al. 2001b).

It has not been established whether porcine sapoviruses are species specific. At present, sapoviruses have been identified only in humans, swine, and mink. It is assumed that the main natural transmission mode is fecal–oral. In general, sapoviruses are characterized by stability in the environment and relative resistance to inactivation.

Pathogenesis

Experimental infections with the porcine sapovirus PEC/Cowden produced enteric lesions and disease (Flynn et al. 1988; Guo et al. 2001a). Unusual for viral enteric pathogens, disease and small intestinal lesions in the duodenum and jejunum resulted from intravenous inoculation of PEC/Cowden, as well as by oral exposure. Viral replication in enterocytes was demonstrated by immunofluorescence with anti-PEC/Cowden antiserum. Sapovirus particles were demonstrated in intestinal contents and in the bloodstream, the first time viremia has been associated with an enteric calicivirus. The mechanism by which the virus reached the small intestine and villous enterocytes from the bloodstream was not determined. When infected by the oral route, fecal shedding of the sapovirus PEC/Cowden occurred for up to 9 days. When infected by the intravenous route, fecal shedding was observed for at least 8 days.

Clinical signs

Porcine sapoviruses are one of the viral agents that cause diarrhea in swine. With PEC/Cowden, the incubation period ranged from 2 to 4 days after oral inoculation, and clinical signs of anorexia and diarrhea persisted for 3–7 days (Flynn et al. 1988; Guo et al. 2001a). All inoculated pigs became infected and developed clinical signs ranging from mild to severe diarrhea. Control pigs and pigs infected with the tissue culture-adapted PEC failed to develop clinical signs, although intestinal lesions were observed in the exposed pigs.

Lesions

Infection with PEC/Cowden produced lesions indistinguishable from those produced by other enteric viral pathogens (Flynn et al. 1988; Guo et al. 2001a). Lesions included shortening, blunting, fusion or absence of duodenal and jejunal villi, and, by scanning electron microscopy, an irregular microvillus coat on enterocytes. Crypt cell hyperplasia and a reduction of villus/crypt ratios occurred with cytoplasmic vacuolation and infiltration of polymorphonuclear and mononuclear cells into the lamina propria.

Diagnosis

No diagnostic tests for porcine sapoviruses have been developed for use outside the research laboratory. Porcine sapoviruses have been detected using electron

microscopy, RT-PCR, and real-time RT-PCR (Reuter et al. 2010; Sugieda et al. 1998; Sugieda and Nakajima 2002; van der Poel et al. 2000; Wang et al. 2005a). However, the sensitivity of molecular tests for laboratory diagnosis of porcine sapovirus infections has not been evaluated. An antigen and antibody ELISA was developed and used to study PEC/Cowden (Guo et al. 2001b).

Immunity

Immune responses, protective immunity induced by porcine sapoviruses, and the role of maternal antibodies have not been assessed. It may be assumed that protective immune mechanisms are similar to those for other enteric virus pathogens. However, the finding of an extraintestinal phase to the pathogenesis of a porcine sapovirus may mean that other immune strategies might be used for their control.

Prevention and control

Assuming that porcine sapovirus epidemiology and immunology are similar to porcine rotaviruses, it is likely that these viruses persist in the environment, and it may be impossible to eliminate the infection from pig herds and prevent natural infection of piglets. However, the persistence of porcine enteric viruses in animal waste depends on the waste treatment technology (Costantini et al. 2007). It is also likely that sows will pass on maternal antibody in colostrum and milk and thus limit infection and damage in the gut of nursing piglets. Treatment with oral rehydration fluids is likely to be successful.

St-Valérien virus

In 2009, a report described a novel calicivirus from pigs in Quebec, Canada (L'Homme et al. 2009b). Genomic analysis revealed a positive-sense RNA genome of 6409 nucleotides encoding two major ORFs. Phylogenetic analysis showed that these viruses form a unique cluster with a common root with the noroviruses and the Tulane virus isolated from captive juvenile rhesus macaques. The genus name *Valovirus* has been suggested with the St-Valérien virus as the prototype (Di Martino et al. 2011). The virus has also been found in the United States (Wang et al. 2011), Italy (Di Martino et al. 2011), and Japan (Sato et al. 2014). In a study in Italy, 10.3% of 614 pigs had antibodies to St-Valérien virus (Di Martino et al. 2012). The role of St-Valérien virus in swine disease is unknown.

References

Bankowski RA, Wichmann R, Kummer M. 1953. *Am J Vet Res* 14:145–149.

Barry AF, Alfieri AF, Alfieri AA. 2008. *Vet Microbiol* 131:185–191.

Berry ES, Skilling DE, Barlough JE, et al. 1990. *Am J Vet Res* 51:1184–1187.

Bresse SS Jr., Dardiri AH. 1977. *J Gen Virol* 36:221–225.

Bridger JC. 1980. *Vet Rec* 107:532–533.

Chang KO, Kim Y, Green KY, et al. 2002. *Virology* 304:302–310.

Chang KO, Sosnovtsev SV, Belliot G, et al. 2004. *Proc Natl Acad Sci U S A* 101:8733–8738.

Cheetham S, Souza M, Meulia T, et al. 2006. *J Virol* 80:10372–10381.

Clarke IN, Estes MK, Green KY, et al. 2012. *Caliciviridae*. In King AMQ, Adams MJ, Carstens EB, et al., eds. *Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press, pp. 977–986.

Costantini VP, Azevedo AC, Li X, et al. 2007. *Appl Environ Microbiol* 73:5284–5291.

Cunha JB, de Mendonca MC, Miagostovich MP, et al. 2010. *Res Vet Sci* 89:126–129.

Di Martino B, Martella V, Di Profio F, et al. 2011. *Vet Microbiol* 149:221–224.

Di Martino B, Di Profio F, Ceci C, et al. 2012. *J Gen Virol* 93:102–105.

Farkas T, Nakajima S, Sugieda M, et al. 2005. *J Clin Microbiol* 43:657–661.

Farkas T, Sestak K, Wei C, et al. 2008. *J Virol* 82:5408–5416.

Ferris NP, Oxtoby JM. 1994. *Vet Microbiol* 42:229–238.

Flynn WT, Saif LJ. 1988. *J Clin Microbiol* 26:206–212.

Flynn WT, Saif LJ, Moorhead PD. 1988. *Am J Vet Res* 49:819–825.

Gelberg HB, Lewis RM. 1982. *Vet Pathol* 19:424–443.

Green KY. 2007. *Caliciviridae*: The noroviruses. In Knipe D, Howley P, eds. *Fields Virology*. Philadelphia: Lippincott Williams and Wilkins, pp. 949–979.

Guo M, Chang KO, Hardy ME, et al. 1999. *J Virol* 73:9625–9631.

Guo M, Hayes J, Cho KO, et al. 2001a. *J Virol* 75:9239–9251.

Guo M, Qian Y, Chang KO, et al. 2001b. *J Clin Microbiol* 39:1487–1493.

Hansman GS, Takeda N, Oka T, et al. 2005. *Emerg Infect Dis* 11:1916–1920.

Holbrook AA, Geleta JN, Hopkins SR. 1959. Two new immunological types of vesicular exanthema virus. *Proc Meet US Livest Saint Assoc* 63:332–339.

Kemenesi G, Gellért Á, Dallos B, et al. 2016. *Infect Genet Evol* 41:227–232.

Keum HO, Moon HJ, Park SJ, et al. 2009. *Arch Virol* 154:1765–1774.

Kim HJ, Cho HS, Cho O, et al. 2006. *J Vet Med B Infect Dis Vet Public Health* 53:155–159.
L'Homme Y, Sansregret R, Plante-Fortier E, et al. 2009a. *Arch Virol* 154:581–593.
L'Homme Y, Sansregret R, Plante-Fortier E, et al. 2009b. *Virus Genes* 39:66–75.
Liao Q, Wang X, Wang D, et al. 2014. *Arch Virol* 159:2529–2531.
Madin SH. 1975. Vesicular exanthema. In Dunne HW, Leman AD, eds. *Diseases of Swine*, 4th ed. Ames, IA: Iowa State University Press, pp. 285–307.
Martella V, Lorusso E, Banyai K, et al. 2008. *J Clin Microbiol* 46:1907–1913.
Martinez MA, Alcala AC, Carruyo G, et al. 2006. *Vet Microbiol* 116:77–84.
Mattison K, Shukla A, Cook A, et al. 2007. *Emerg Infect Dis* 13:1184–1188.
Mauroy A, Scipioni A, Mathijs E, et al. 2008. *Arch Virol* 153:1927–1931.
Mayo MA. 2002. *Arch Virol* 147:1655–1663.
McClenahan SD, Bok K, Neill JD, et al. 2009. *J Virol Methods* 161:12–18.
Mijovski JZ, Poljsak-Prijatelj M, Steyer A, et al. 2010. *Infect Genet Evol* 10:413–420.
Mikalsen AB, Nilsen P, Frøystad-Saugen M, et al. 2014. *PLoS One* 9:e107132.
Nagy B, Nagy G, Meder M, et al. 1996. *Acta Vet Hung* 44:9–19.
Neill JD. 2014. *Genome Announc* 2 pii: e01286-14.
Neill JD, Meyer RF, Seal BS. 1995. *J Virol* 69:4484–4488.
Parwani AV, Saif LJ, Kang SY. 1990. *Arch Virol* 112:41–53.
Parwani AV, Flynn WT, Gadfield KL, et al. 1991. *Arch Virol* 120:115–122.
van der Poel WH, Vinje J, van Der Heide R, et al. 2000. *Emerg Infect Dis* 6:36–41.
Prasad BV, Hardy ME, Dokland T, et al. 1999. *Science* 286:287–290.
Reid SM, Ansell DM, Ferris NP, et al. 1999. *J Virol Methods* 82:99–107.
Reid SM, King DP, Shaw AE, et al. 2007. *J Virol Methods* 140:166–173.
Reuter G, Bíró H, Szűcs G. 2007. *Arch Virol* 152:611–614.
Reuter G, Zimsek-Mijovski J, Poljsak-Prijatelj M, et al. 2010. *J Clin Microbiol* 48:363–368.
Saif LJ, Bohl EH, Theil KW, et al. 1980. *J Clin Microbiol* 12:105–111.
Sato G, Ido H, Kiuchi M, et al. 2014. *J Vet Med Sci* 76:1045–1050.
Shen Q, Zhang VV, Yang S, et al. 2009. *Arch Virol* 154:1625–1630.
Shirai J, Shimizu M, Fukusho A. 1985. *Nippon Juigaku Zasshi* 47:1023–1026.
Sisay Z, Djikeng A, Berhe N, et al. 2016a. *Arch Virol* 161:2169–2182.
Sisay Z, Djikeng A, Berhe N, et al. 2016b. *Arch Virol* 161:2739–2747.
Smith AW, Prato CM, Gilmartin WG, et al. 1974. *J Wildl Dis* 10:54–59.
Smith AW, Skilling DE, Dardiri AH, et al. 1980. *Science* 209:940–941.
Smith AW, Skilling DE, Cherry N, et al. 1998a. *Emerg Infect Dis* 4:13–20.
Smith AW, Berry ES, Skilling DE, et al. 1998b. *Clin Infect Dis* 26:434–439.
Sugieda M, Nakajima S. 2002. *Virus Res* 87:165–172.
Sugieda M, Nagaoka H, Kakishima Y, et al. 1998. *Arch Virol* 143:1215–1221.
Tian P, Jiang X, Zhong VV, et al. 2007. *Res Vet Sci* 83:410–418.
Van Bonn W, Jensen ED, House C, et al. 2000. *J Wildl Dis* 36:500–507.
Vinjé J. 2015. *J Clin Microbiol* 53:373–381.
Wang QH, Han MG, Funk JA, et al. 2005a. *Emerg Infect Dis* 12:1874–1881.
Wang QH, Han MG, Funk JA, et al. 2005b. *J Clin Microbiol* 43:5963–5972.
Wang Q, Scheuer K, Ahang Z, et al. 2011. *Emerg Infect Dis* 17:1103–1106.
Wawrzkiewicz J, Smale CJ, Brown F. 1968. *Arch Gesamte Virusforsch* 25:337–351.
Wolf S, Reetz J, Otto P. 2011. *Arch Virol* 156:1143–1150.
Wolf S, Reetz J, Hoffmann K, et al. 2012. *Arch Virol* 157:1499–1507.
Zheng DP, Ando T, Fankhauser RL, et al. 2006. *Virology* 346:312–323.

30

Circoviruses

Joaquim Segalés, Gordon M. Allan, and Mariano Domingo

Relevance

In the late 1990s, a porcine circovirus (PCV) distinct from a known PCV contaminant of PK-15 cell cultures (Tischer et al. 1974, 1982) was detected in North America and Europe (Allan et al. 1998; Ellis et al. 1998). The original cell culture contaminant was designated porcine circovirus type 1 (PCV1), and the new pathogenic virus was named porcine circovirus type 2 (PCV2) (Allan et al. 1999b). Subsequently, the term "porcine circovirus diseases" (PCVDs) was proposed to group diseases or conditions linked to PCV2 (Allan et al. 2002b). In North America, the term "porcine circovirus-associated diseases" (PCVAD) is used (Opriessnig et al. 2007). PCVD can have different clinical manifestations: PCV2 systemic disease (PCV2-SD), which was historically known as post weaning multisystemic wasting syndrome (PMWS) (Clark 1996; Harding 1996); PCV2 reproductive disease (PCV2-RD) (West et al. 1999); porcine dermatitis and nephropathy syndrome (PDNS) (Rosell et al. 2000b); and subclinical infection. PCV2 has also been linked to respiratory (Kim et al. 2003) and enteric (Kim et al. 2004a) diseases, but both have shown to be part of PCV2-SD (Baró et al. 2015; Ticó et al. 2013). Among PCVDs, PCV2-SD and the subclinical infection have a worldwide impact on swine production. Commercial PCV2 vaccines have been available since 2006, and economic losses attributed to PCVDs, including subclinical PCV2 infections, have been markedly reduced (Segalés 2015). PCV2 is also an important coinfecting agent, and vaccination against PCV2 can assist in the control of clinical disease associated with polymicrobial infections (Chae 2016). Porcine circovirus type 3 (PCV3) has recently been described and is thought to be linked to PCVD-like scenarios (Palinski et al. 2016; Phan et al. 2016), although no experimental evidence is currently available to support this association. Also, a recent limited study does not support disease association with PCV3 (Franzo et al. 2018a). Further studies will be warranted to establish the potential pathogenicity of PCV3.

Etiology

PCVs belong to the genus *Circovirus* in the family *Circoviridae* (Rosario et al. 2017). Three PCV types have been described: PCV1, PCV2, and PCV3. It should be noted that PCV3 has not been yet officially listed in the genus *Circovirus*. PCV1 was first described as a PK-15 cell contaminant (Tischer et al. 1974, 1982) and was not pathogenic for swine (Allan and Ellis, 2000). PCV2 has been the most commonly studied species because of its link to PCVDs (Segalés 2012). PCV3 has recently been identified (Palinski et al. 2016; Phan et al. 2016), but little is known about PCV3 beyond its genomic sequence.

PCVs are non-enveloped and 12–23 nm in diameter (Figure 30.1) (Tischer et al. 1982). Based on studies of PCV2, the nucleocapsids exhibit icosahedral symmetry, and three-dimensional studies have shown a polygonal outline containing 60 capsid (Cap) protein elements arranged in 12 slightly protruding pentameric units, giving an overall diameter of about 20.5 nm (Crowther et al. 2003). The circular, covalently closed single-stranded DNA (ssDNA) genome contains 1759 (PCV1), 1767–1768 (PCV2), and 2000 (PCV3) nucleotides (Hamel et al. 1998; Meehan et al. 1997, 1998; Palinski et al. 2016; Phan et al. 2016). PCV1 and PCV2 may have a common evolutionary origin, but a common ancestor has not been identified (Olvera et al. 2007).

After PCV infects a cell, the ssDNA is converted to a double-stranded DNA (dsDNA) intermediate known as the "replicative form" (RF) (Mankertz et al. 2004). The RF is ambisense, with genes encoded by both the viral (positive) and the complementary (negative) strand. PCV2 genes are arranged in 11 putative open reading frames (ORFs) (Hamel et al. 1998), but protein expression

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

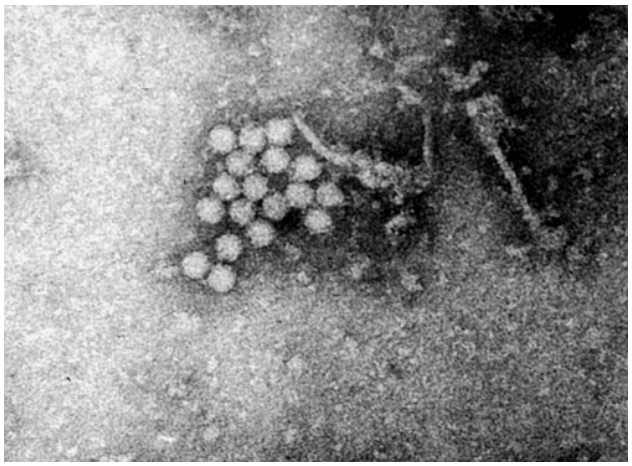


Figure 30.1 PCV2-SD-affected pig. Electron microscopy of PCV2 particles isolated from an affected pig. Virion particles are 15–20 nm in diameter. Transmission electron photomicrograph of negatively stained virions.

has been described for only four. ORF1 (Rep gene) is located on the positive strand in clockwise orientation. It codes for the nonstructural replicase proteins Rep and Rep', 314 and 178 amino acids (aa) in length, respectively (Cheung 2003; Mankertz et al. 1998). ORF2 (capsid gene) is on the complementary strand and oriented counterclockwise. It encodes for the capsid, the only structural protein (233–234 aa) (Mankertz et al. 2004; Nawagitgul et al. 2000). ORF3 is located on the complementary strand, oriented counterclockwise, and completely overlaps the ORF1 gene. ORF3 codes for a nonstructural protein 105 aa in length. *In vitro*, the ORF3 protein induces apoptosis in PK-15 cells (Liu et al. 2005), and an ORF3-defficient PCV2 mutant was shown to be less virulent in pigs compared with wild type PCV2 (Karuppannan et al. 2009). An ORF4, located also on the complementary strand and oriented counterclockwise, encodes a protein with a role in suppressing caspase activity and regulation of $CD4^+$ and $CD8^+$ T lymphocytes (He et al. 2013).

PCV2 genotypes

Analyses have shown close phylogenetic relationships and nucleotide sequence identities >93% among PCV2 viruses from around the world (Franzo et al. 2016; Xiao et al. 2015). PCV2 genotype definitions based on pairwise sequence comparisons (PASC) was proposed in 2008 (Segalés et al. 2008), and five genotypes (PCV2a, PCV2b, PCV2c, PCV2d, and PCV2e) have since been identified. However, the growing number of the PCV2 sequences reported since 2008 have made it apparent that the 2008 PASC thresholds for PCV2 genotyping do not apply to all PCV2 strains (Franzo et al. 2015a) and a new PCV2 genotyping method is needed. A PCV2 genotyping method based on marker positions was proposed (Franzo et al. 2015a), but has not been widely used.

PCV2a was the most prevalent genotype in clinically affected pigs from 1996 to the early 2000s, after which PCV2b predominated ("genotype shift"). The emergence of PCV2b in North America and Europe was associated with the appearance of a more severe clinical disease (Carman et al. 2006; Cortey et al. 2011; Timmusk et al. 2008; Wiederkehr et al. 2009). PCV2d (also called mutant PCV2b by some research groups) was first reported in China (Guo et al. 2010), and a second "genotype shift" (from PCV2b to PCV2d) may be currently occurring globally (Franzo et al. 2016; Xiao et al. 2016), perhaps driven by the worldwide use of PCV2 vaccines. PCV2c has been detected in archival material in Denmark (Dupont et al. 2008), in wild pigs from Brazil (Franzo et al. 2015b), and in domestic swine in China (Liu et al. 2016). PCV2e has been identified in swine samples collected in the United States and Mexico (Davies et al. 2016; Harmon et al. 2015). The clinical significances of PCV2c and PCV2e are unknown.

PCV2 strains of the same or different genotype may coinfect the same pig (Cheung et al. 2007; de Boisseson et al. 2004; Gagnon et al. 2007; Grau-Roma et al. 2008; Hesse et al. 2008). *In vivo* and *in vitro* studies provided evidence of viral recombination (Cheung 2009; Hesse et al. 2008; Lefebvre et al. 2009; Olvera et al. 2007). Thus, the emergence of new genotypes could have been the result of recombination between strains infecting the same animal. At least three circulating recombinant forms (CRF) have been found in pigs in different parts of the world (Franzo et al. 2016). A recombinant virus containing PCV1 ORF1 and PCV2a ORF2 was identified in Quebec (Canada), apparently derived from a chimeric killed vaccine strain that was not effectively inactivated (Gagnon et al. 2010).

Antigenic variability

Initially, similarities in reactivity to monoclonal and polyclonal antibodies led to the conclusion that no major antigenic differences existed among PCV2 strains (Allan et al. 1999b; McNeilly et al. 2001). Subsequent work revealed antigenic variability among PCV2 genotypes (Guo et al. 2010; Lefebvre et al. 2008a; Saha et al. 2012; Shang et al. 2009). Despite genotypic and antigenic differences, it is considered that PCV2 exists as a single serotype.

Physicochemical and biological properties

PCV1 has a buoyant density of 1.37 g/mL in CsCl, does not hemagglutinate erythrocytes from a wide range of species, is resistant to inactivation at pH 3 and by chloroform, and is stable at 70 °C (158 °F) for 15 minutes (Allan et al. 1994b). The biological and physicochemical prop-

erties of PCV2 are not well characterized. Infectivity decreases somewhat in an acid buffer, but PCV2 remains viable even at pH < 2. Infectivity is markedly decreased at pH 11–12 (Kim et al. 2009). PCV2 resists inactivation at high temperatures 56 °C (133 °F) for 1 hour and 75 °C (167 °F) for 15 minutes, which suggests that the virus is able to remain infectious in the environment at high ambient temperatures (e.g. summer) (Kim et al. 2009; O'Dea et al. 2008). Exposure of PCV2 (10 minutes to 24 hours) at room temperature to a number of commercial disinfectants (chlorhexidine, formaldehyde, iodine, oxidizing agents, and alcohols) leads to a significant reduction in virus titers (Kim et al. 2009; Martin et al. 2008; Royer et al. 2001).

Laboratory cultivation

Replication of PCVs *in vitro* is best achieved by inoculation of semi-confluent monolayers of PCV-free PK-15 cells. Since PCVs do not produce cytopathic effect, viral replication is monitored by immunofluorescence or immunoperoxidase staining (Allan and Ellis 2000). PCV1 has been shown to replicate in other porcine-derived cell lines and Vero cells (Allan et al. 1994a; Tischer et al. 1982). PCV2 has also been shown to replicate in IPEC-J2 (Yan et al. 2014) and lymphoblastoid L35 (Rodríguez-Cariño et al. 2011) cell lines. PCV DNA replication seems to be dependent on cellular enzymes expressed during the S phase of growth (Tischer et al. 1987) or perhaps cell repair (Sánchez et al. 2003) and has been reported to be dependent on the porcine cyclin A (CycA) as an important regulator of the PCV2 life cycle (Tang et al. 2013). Viral yields may be enhanced by cell treatment with glucosamine, but care must be taken because glucosamine is cytotoxic (Allan and Ellis 2000). Glutamine starvation of PK-15 cultures has been shown to increase PCV2 replication by promoting p38 MAPK activation, which was associated with the downregulation of intracellular glutathione levels (Chen et al. 2015). There have been no reports on successful PCV3 isolation in cell culture yet.

Public health

PCVs are not zoonotic. PCV DNA has been detected in vaccines produced for use in humans and swine (Kuehn 2010; Quintana et al. 2006; Victoria et al. 2010). These findings reflect quality control issues in vaccine production.

Epidemiology

PCV1 and PCV2 are ubiquitous in both domestic and feral swine (Allan and Ellis 2000; Calsamiglia et al. 2002; Segalés and Domingo 2002; Vicente et al. 2004). Nucleotide sequence analysis grouped PCV1 detected in wild boar with PCV1 from domestic swine (Csagola et al. 2008). Likewise, the nucleotide identity of PCV2 isolates from wild boars was almost identical to isolates from domestic swine and included both PCV2a and PCV2b genotypes (Ellis et al. 2003; Schulze et al. 2004; Sofia et al. 2008). PCV3 DNA has been detected in domestic swine in the United States, China, Italy, Poland, South Korea, Brazil, the United Kingdom, Denmark, Spain, and Germany (Collins et al. 2017; Franzo et al., 2018b; Fux et al. 2018; Ku et al. 2017; Li and Tian 2017). Interestingly, it was reported that PCV3 DNA was detected in archived clinical samples as far back as in 2002 in the United Kingdom and in 1996 in Spain (Collins et al. 2017; Klaumann et al. 2018a). Currently the transmission, duration of virus shedding, and other epidemiological data of PCV3 mainly remain unknown.

With the exception of mice and rats, non-porcine species are not susceptible to PCV2 infection (Allan et al. 2000b; Ellis et al. 2000, 2001; Rodríguez-Arrioja et al. 2003). PCV2 can replicate in and transmit between mice to a limited degree (Kiupel et al. 2001; Opriessnig et al. 2009a). PCV2 has been found in mice and rats from pig farms, suggesting a possible role as alternate hosts or mechanical vectors, but not in rodents collected outside swine herds (Lorincz et al. 2010) or houseflies (Blunt et al. 2011). PCV2 sequences were also found in buffalo meat in China (Zhai et al. 2014), although their origin was unclear.

Oronasal exposure is the primary route of transmission, but PCV2 has been found in nasal, tonsillar, bronchial, and ocular secretions, feces, saliva, urine, colostrum, milk, and semen (Rose et al. 2012). Pigs can become infected by eating raw tissues from viremic animals (Opriessnig et al. 2009c). Transplacental infection occurred in pregnant sows intranasally exposed to PCV2 3 weeks prior to farrowing (Ha et al. 2008, 2009; Park et al. 2005). Naïve sows inseminated with semen contaminated with PCV2 exhibited reproductive failure, and their fetuses became infected (Madson et al. 2009a; Sarli et al. 2012). However, it is believed that the quantity of PCV2 naturally shed in semen is not sufficient to infect sows or their fetuses (Madson et al. 2009a).

Transmission of PCV2 among pigs can occur by mixing susceptible with infected animals (Albina et al. 2001; Bolin et al. 2001). Direct contact is more efficient than transmission between animals in separate pens (Andraud et al. 2008). Longitudinal studies quantifying PCV2 in serum, nasal excretions, and feces found that most pigs became infected at 4–11 weeks of age, depending on the farm (Carasova et al. 2007; Grau-Roma et al. 2009). A variable percentage of sows and piglets may be viremic during lactation (Grau-Roma et al. 2009; Shen et al. 2010), suggesting the possibility of transmission from sows to nursing piglets through respiratory secretions, colostrum, and milk (Rose et al. 2012).

PCV2 persistence in individuals or groups of pigs has not been studied extensively, but Bolin et al. (2001) isolated virus or detected viral DNA in tissues from experimentally inoculated pigs for up to 125 days post inoculation (DPI). In the field, viral DNA has been detected in serum from pigs for up to 22 weeks (Rodríguez-Arrioja et al. 2002). PCV2 DNA was repeatedly found in sera from pigs 7–70 days of age (Grau-Roma et al. 2009), providing further evidence that some animals remain persistently infected despite the presence of high levels of PCV2-specific antibodies (McIntosh et al. 2006; Rodríguez-Arrioja et al. 2002).

Pathogenesis, clinical signs, lesions, and diagnosis

PCV1 is nonpathogenic to swine. PCV3 has been reported to be found in cases of PDNS, reproductive failure, and cardiac and multisystemic inflammation (Palinski et al. 2016; Phan et al. 2016), but its pathogenesis and clinical significance are still under debate (Franzo et al. 2018a; Klaumann et al. 2018b). The remainder of this section focuses on pathogenesis, clinical signs, lesions, and diagnosis of PCV2-SD, PCV2-RD, and PDNS.

Porcine circovirus type 2 systemic disease

Pathogenesis

PCV2-SD is characterized by severe impairment of the immune system, and clinical signs and lesions are often the result of the additive effect of co-infectious pathogens in different organs. Thus, PCV2-SD is a multifactorial disease in which PCV2 infection is the necessary cause. Genotypes PCV2a, PCV2b, and PCV2d are all considered pathogenic, that is, able to cause PCV2-SD (Allan et al. 1999a; Lager et al. 2007; Opriessnig et al. 2017). Differences in virulence among genotypes or strains has been postulated, but not adequately addressed (Segalés et al. 2013).

PCV2 viremia is first detected around 7 DPI and peaks at 14–21 DPI (Allan et al. 1999a; Opriessnig et al. 2008; Rovira et al. 2002). Under field conditions, seroconversion usually occurs at 7–12 weeks of age, and antibodies last until at least 28 weeks of age (Rodríguez-Arrioja et al. 2002).

There is little information regarding the target cells for initial viral replication, the early events of PCV2 infection, or the cell type(s) that supports PCV2 replication *in vivo*. PCV2 does not code for its own DNA polymerases, and cells in the S phase are presumed necessary for the virus to complete its infectious cycle (Tischer et al. 1987). If so, cells with a high mitotic rate should be the most efficient for viral replication. Lymphoid tissues contain the highest concentration of PCV2 (Quintana et al. 2001; Rosell et al. 1999), but virus may also be detected in epithelial cells from the kidney and respiratory tracts, endothelial cells, lymphocytes, enterocytes, hepatocytes (including Kupffer's cells in the liver), smooth muscle cells, and pancreatic acinar and ductal cells (Opriessnig and Langohr 2013).

The role of monocytes and lymphocytes in sustaining PCV2 replication is still unresolved (Meng 2013). Initial work suggested that macrophages and lymphocytes did not play a significant role in PCV2 replication (Gilpin et al. 2003; Vincent et al. 2003), but later studies found that they (mainly macrophages) may support replication (Hamberg et al. 2007; Pérez-Martín et al. 2007; Rodríguez-Cariño et al. 2010; Yu et al. 2007). Characterization of PCV2-infected leukocyte subpopulations from peripheral blood mononuclear cells (PBMCs) showed that circulating T lymphocytes ($CD4^+$ and $CD8^+$), and to a lesser extent B lymphocytes, supported PCV2 replication, but not PBMC-derived monocytes (Lefebvre et al. 2008b; Lin et al. 2008; Yu et al. 2007). In PCV2-SD-affected pigs, the highest concentration of virus is found in the cytoplasm of monocyte–macrophage lineage cells (Rosell et al. 1999; Sánchez et al. 2004). *In vitro* studies have shown that PCV2 infects these cells, after which they become persistently infected with little or no virus replication (Gilpin et al. 2003; Vincent et al. 2003). For this reason, it has been suggested that monocytic cells facilitate dissemination of PCV2 within the host, rather than being a primary target for PCV2 replication (Vincent et al. 2003).

Although PCV2-SD has been reproduced under experimental conditions, a definitive, consistently repeatable disease model is lacking. Horizontal transmission of PCV2-SD by commingling affected and healthy pigs and between affected and naïve pigs in adjacent pens has been reported (Kristensen et al. 2009), but both infectious and noninfectious factors are believed to play a role in PCV2-SD (Rose et al. 2012).

Reasons for inconsistency in the experimental reproduction of PCV2-SD are not known, but may be related to the origin of pigs, age of the animals at inoculation, immunological status, genetic predisposition, PCV2 strain, type of inoculum, infectious dose, and/or route of administration. Meta-analysis suggested that the experimental reproduction of PCV2-SD was most probable in colostrum-deprived pigs <3 weeks of age inoculated with high doses ($>1 \times 10^5$ $TCID_{50}$ per pig) of PCV2b in combination with another pathogen (Tomás et al. 2008).

Field observations suggested that certain genetic lines of pigs might be more susceptible to PCV2-SD. This observation was supported by experimental studies in which Landrace were shown to be more likely to develop PCV2-SD than Duroc, Large White, and Pietrain pigs (Opriessnig et al. 2006, 2009b). However, field studies have reported contradictory results, with the Pietrain

boar line showing no effect in one study (Rose et al. 2005) and lower general post weaning and PCV2-SD-associated mortalities in another (López-Soria et al. 2011).

The most repeatable PCV2-SD models are based on PCV2 inoculation in combination with other infectious and/or noninfectious cofactors (Allan et al. 2004). Noninfectious risk factors for PCV2-SD are summarized in Table 30.1. Infectious agents considered potential PCV2-SD triggers include infection with porcine parvovirus (PPV), porcine reproductive and respiratory syndrome (PRRS) virus, and/or *Mycoplasma hyopneumoniae* (Opriessnig and Halbur 2012). Stimulation and/or activation of the immune system of PCV2-infected pigs by other viral infections or noninfectious factors upregulates PCV2 replication and increases viral loads in tissues and serum (Allan et al. 1999a, 2000a; Harms et al. 2001; Kennedy et al. 2000; Krakowka et al. 2001; Rovira et al. 2002). This suggests that PCV2 infection and immunostimulation may be pivotal events in the development of PCV2-SD, although the mechanism by which coinfection or immunostimulation triggers the development of PCV2-SD in PCV2-infected pigs is unknown (Allan et al. 2012).

Conversely, regular features of PCV2-SD in severely affected pigs are suggestive of immunosuppression, such as microscopic lymphoid lesions (Clark 1997; Rosell et al. 1999), the association of PCV2-SD with opportunistic pathogens (Grau-Roma et al. 2011), and changes in immune cell subpopulations in lymphoid tissues and PBMCs (Darwich and Mateu 2012). The most striking evidence of immunosuppression are the extensive lesions in lymphoid tissues of PCV2-SD-affected pigs (i.e. depletion of B and T lymphocytes), an increase in the number of macrophages, and loss or redistribution of interfollicular dendritic cells (Chianini et al. 2003). In lymphoid tissues, depletion of T lymphocytes primarily involved $CD4^+$ cells and, to a lesser extent, $CD8^+$ cells (Sarli et al. 2001).

Another feature suggestive of immunosuppression in PCV2-SD-affected pigs is the alteration of PBMC subsets, mainly lymphopenia (Darwich et al. 2002; Nielsen et al. 2003). Depletion of B and T lymphocytes was experimentally observed only in PCV2-inoculated pigs that later developed PCV2-SD. Changes in T-cell subsets involved mainly $CD4^+CD8^+$ memory T cells. In PCV2-inoculated pigs that did not show clinical signs, the number of cytotoxic ($CD4^-CD8^+$) and γδ ($CD4^-CD8^-$) T lymphocytes was increased in comparison with that of control pigs, suggesting an active response to PCV2 infection.

Although lymphoid depletion and lymphopenia are consistent features of PCV2-SD-affected pigs, it is not known whether the loss of lymphocytes is a direct or an indirect effect of PCV2 infection. PCV2-infected PBMCs

Table 30.1 Noninfectious risk factors for post weaning multisystemic wasting syndrome (PMWS).

	Factors increasing the risk of PMWS	Factors decreasing the risk of PMWS
Animals	• Gender (male) • Litter of origin • Low birth weight • Low weaning weight • Low weight at the beginning of fattening period	• Gender (female)
Facilities	• Large number of sows • Large pens at nursery and growing ages • Proximity to other pig farms	• Separate pit for adjacent fattening rooms • Shower facilities
Management practices	• High level of cross-fostering • Short empty periods at weaning and fattening • Large range in age and weight entering the nursery • Continuous flow nursery • Purchase of replacement gilts • Sows with neck injuries due to poor injection technique • Early weaning (<21 days of age)	• Sorting pigs by sex at nursery stage • Greater minimum weight at weaning • Group housing sows during pregnancy • Visitors with no pig contact for several days before visiting farm • Use of semen from an insemination center
Vaccination/treatment/nutrition	• Vaccination of gilts against PRRSV • Vaccination of sows against *Escherichia coli* • Use of separate vaccines against erysipelas and porcine parvovirus on gilts	Vaccination of sows against atrophic rhinitis Regular treatment for ectoparasites Use of oxytocin during farrowing Use of spray-dried plasma in initial nursery ration

Source: Grau-Roma et al. (2011). Reproduced with permission of Elsevier.
PRRSV, porcine reproductive and respiratory syndrome virus.

undergo morphological changes typical of cellular degeneration (Lefebvre et al. 2008b), and B and T lymphocytes, albeit a low proportion, support PCV2 replication (Pérez-Martín et al. 2007; Yu et al. 2007). Alternatively, lymphoid depletion might be the result of virus-induced apoptosis, but this is controversial (Ren et al. 2016).

Clinical signs

PCV2 is ubiquitous and most PCV2 infections are subclinical. In the field, the proportion of PCV2-infected pigs and their viral load increases gradually from lactation onward, coincident with the decline in maternal immunity. Only a variable proportion of PCV2-infected pigs develop PCV2-SD, typically at 2–4 months of age (Segalés 2012). At that time, clinically affected pigs have a higher concentration of virus in serum, shed higher levels of virus, and demonstrate a weaker antibody response compared with subclinically infected pigs (Fort et al. 2007; Grau-Roma et al. 2009). Morbidity in affected farms is commonly 4–30% (occasionally 50–60%), and mortality 4–20% (Segalés 2012). PCV2-SD is characterized clinically by wasting (Figure 30.2), pallor of the skin, respiratory distress, diarrhea, and, occasionally, icterus (Harding and Clark 1997). Enlarged subcutaneous lymph nodes are a common finding in the early clinical phases of PCV2-SD.

Experimental infections comparing PCV2-inoculated groups with negative controls rarely detected significant differences in production parameters (Fernandes et al. 2007). However, the evaluation of production records on farms without clinical disease showed that the use of PCV2 vaccines reduced mortality and increased average daily gain (ADG) (Segalés 2015).

Figure 30.2 PCV2-SD-affected pig (left) compared with an age-matched healthy pig (right). Note the severe growth retardation and the marked spinal cord of the affected animal.

Lesions

PCV2-SD lesions are primarily found in lymphoid tissues, and enlargement of lymph nodes is the most prominent feature of the early clinical phase of PCV2-SD (Clark 1997; Rosell et al. 1999). Normal sized, or even atrophied, lymph nodes are usually seen in more advanced phases of PCV2-SD (Segalés et al. 2004), and the thymus is frequently atrophied in diseased pigs (Darwich et al. 2003a). Characteristic histopathological lymphoid lesions in PCV2-SD-affected pigs include lymphocyte depletion with infiltration by large histiocytic cells and giant multinucleate cells (Figure 30.3) (Clark 1997; Rosell et al. 1999). In the thymus, cortical atrophy is a prominent finding (Darwich et al. 2003a). Cytoplasmic viral inclusions may be found in histiocytes or dendritic cells (Figure 30.4).

Lungs may be enlarged, non-collapsed, and rubbery in consistency, in a diffuse or patchy distribution, with fluid-distended interlobular septa. These findings correspond microscopically to interstitial pneumonia, with thickening of alveolar septa, pneumocyte type II hyperplasia, and the presence of macrophages and few neutrophils in alveolar spaces. Peribronchial fibrosis and fibrinous bronchiolitis occur in advanced cases (Clark 1997; Segalés et al. 2004).

In a few cases of PCV2-SD, the liver is enlarged or atrophied, pale, and firm, with a fine granular surface that corresponds microscopically to widespread cytopathic changes and inflammation (Clark 1997; Segalés et al. 2004). Pigs may show generalized icterus at this latter stage. Microscopic lesions in the liver may vary from mild lymphohistiocytic hepatitis to massive inflammation,

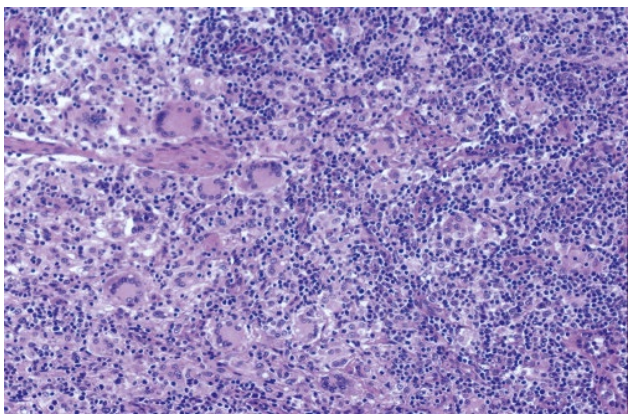


Figure 30.3 Histological appearance of a mesenteric lymph node from a PCV2-SD-affected pig. Note the lack of lymphoid follicles together with a change in cell subpopulations, which are dominated by macrophages and multinucleate giant cells with a marked loss of lymphoid cells. Hematoxylin and eosin stain.

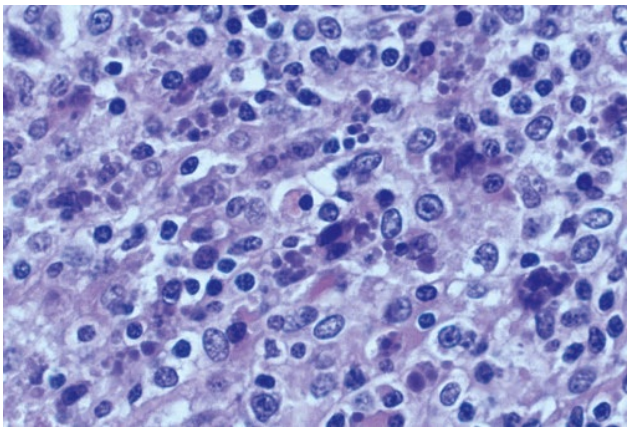


Figure 30.4 Inguinal superficial lymph node. Presence of various intracytoplasmic rounded inclusion bodies characteristics of porcine circovirus type 2 infection in a PCV2-SD-affected pig. Hematoxylin and eosin stain.

with apoptotic bodies, disorganization of hepatic plates, and perilobular fibrosis. Some hepatocytes may show large prominent nucleus and apoptosis (Resendes et al. 2011; Rosell et al. 2000a). PCV2 antigen may be found in periportal inflammatory infiltrates, in Kupffer's and endothelial sinusoidal cells, and in the nucleus and cytoplasm of hepatocytes.

Some pigs show white spots in the kidney cortex that correspond to mononuclear interstitial nephritis. PCV2 antigen may be found in renal epithelial tubular cells and infiltrating mononuclear cells. Foci of lymphohistiocytic inflammatory infiltrates may be seen in many tissues of pigs affected by PCV2-SD (Segalés et al. 2004). Brain lesions, consisting primarily of vasculitis, have occasionally been described (Correa et al. 2007; Seeliger et al. 2007). Vasculitis and lymphangitis have been found as relatively regular findings in PCV2-SD-affected pigs (Resendes and Segalés 2015).

Diagnosis

A diagnosis of PCV2-SD in a pig or group of pigs is warranted if the following criteria are fulfilled (Sorden 2000):

1) Growth retardation and wasting, frequently with dyspnea and enlargement of inguinal lymph nodes and occasionally with jaundice.
2) Moderate to severe characteristic histopathological lesions in lymphoid tissues.
3) Moderate to high amounts of PCV2 within the lesions in lymphoid and other tissues of affected pigs.

This case definition does not exclude the concomitant presence of other diseases together with PCV2-SD. Neither clinical signs nor gross lesions observed in suspected PCV2-SD-affected pigs are sufficient to diagnose the disease. In particular, the respiratory form of PRRS virus infection and all diseases and conditions that cause wasting must be differentiated from PCV2-SD (Harding and Clark 1997).

A herd diagnosis of PCV2-SD is based on the occurrence of a clinical process characterized mainly by wasting and mortality in excess of the expected and/or historical level for the farm and the individual diagnosis of PCV2-SD, as described above, in a number of pigs (Grau-Roma et al. 2012). This case definition is very useful in an epidemic situation, but the evolution of PCV2-SD to a more chronic, milder form with lower mortality makes more difficult establishing a diagnosis in farms suspected of PCV2-SD that subsequently responded to PCV2 vaccination.

Several methods have been developed to detect PCV2 in tissues. *In situ* hybridization (ISH) and immunohistochemistry (IHC) are the most widely used tests for the diagnosis of PCV2-SD (McNeilly et al. 1999; Rosell et al. 1999). PCV2 nucleic acid or antigen is usually found in the cytoplasm of histiocytes, multinucleate giant cells, and other monocyte–macrophage lineage cells, as well as in other cell types (Segalés et al. 2004).

A strong correlation has been observed between the quantity of PCV2 seen in tissues and the severity of microscopic lymphoid lesions in PCV2-SD (Figure 30.5) (Rosell et al. 1999). Since the amount of PCV2 in damaged tissues is the primary difference between PCV2-SD-affected and PCV2 subclinically infected pigs, techniques that allow PCV2 quantification in tissues and/or serum could potentially be used to diagnose PCV2-SD (Brunborg et al. 2004; McNeilly et al. 2002; Olvera et al. 2004). However, while quantitative polymerase chain reaction (PCR) shows potential for the diagnosis of PCV2-SD on a population basis, histopathology in combination with the detection of PCV2 in tissues is required for the diagnosis of PCV2-SD in

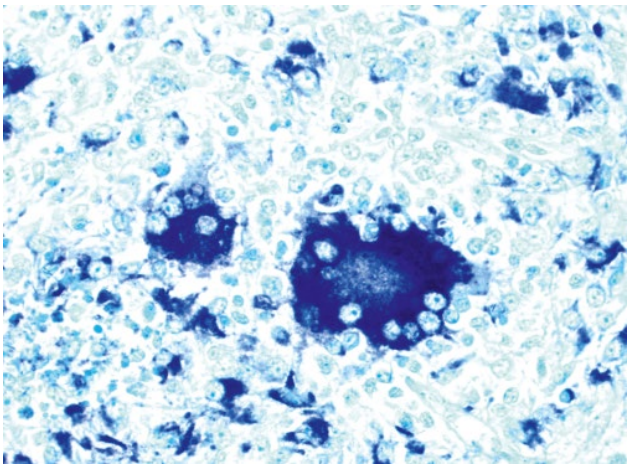


Figure 30.5 Mesenteric lymph node. Marked presence of PCV2 nucleic acid in the cytoplasm of multinucleate giant cells and macrophages (blue-stained cells) in a PCV2-SD-affected pig. *In situ* hybridization to detect PCV2; fast green counterstain.

individual pigs (Grau-Roma et al. 2009). Qualitative PCR techniques should not be used to diagnose PCV2-SD because the virus is ubiquitous and positive results in the absence of clinical disease are common.

Serologic assays for the detection of antibodies to PCV2 have been developed (Segalés and Domingo 2002), but diagnosis of PCV2-SD using serological techniques is problematic because PCV2 is ubiquitous and seroconversion patterns are relatively similar in affected and non-affected farms.

Porcine circovirus type 2 reproductive disease

Pathogenesis

Porcine embryos are susceptible to PCV2 infection, and their susceptibility increases with developmental stage (Mateusen et al. 2004). PCV2 replicates in *in vivo* produced zona-pellucida-free morulae and blastocysts (Mateusen et al. 2004, 2007), which might lead to embryonic death and eventually return to estrus. However, the relevance of this finding to naturally occurring reproductive disease is unknown.

Direct intra-fetal inoculation with PCV2 at 57, 75, and 92 days of gestation showed that PCV2 replicated in all inoculated fetuses, with significantly higher replication in fetuses inoculated at 57 days of gestation (Sánchez et al. 2001). PCV2-susceptible cells in fetuses include myocardiocytes, hepatocytes, and cells of the monocyte–macrophage lineage (Sánchez et al. 2003). Myocarditis-like lesions were observed in the heart, and cardiac tissues contained the highest virus titer and the highest proportion of infected cells.

Pensaert et al. (2004) reported mummification in fetuses inoculated at 57 days of gestation, with transmission of PCV2 to neighboring non-inoculated fetuses. At farrowing, fetuses inoculated at 75 days of gestation were stillborn, whereas fetuses inoculated at 92 days of gestation showed no lesions. In both cases, cardiac tissues were virus positive, and pigs were PCV2 antibody positive at birth.

Intranasal inoculation of pregnant sows with PCV2 or artificial insemination with PCV2-contaminated semen produced infection in fetuses/newborn piglets and/or reproductive failure (Madson et al. 2009a; Park et al. 2005; Sarli et al. 2012). Park et al. (2005) intranasally inoculated six sows with PCV2 3 weeks before the expected farrowing date. Subsequently, three sows aborted and three farrowed prematurely. PCV2 antigen and nucleic acid were detected in lymphoid and non-lymphoid tissues from stillborn and live-born piglets, but no PCV2-SD-like or cardiac lesions were observed. Madson et al. (2009a) artificially inseminated three sows with PCV2a-contaminated semen and three with PCV2b-contaminated semen. Sows that received semen with PCV2b became pregnant and maintained pregnancy to term, but most of the fetuses were mummified with myocardial lesions associated with PCV2 antigen. Sows inseminated with PCV2a-contaminated semen failed to become pregnant. Whether this was due to early embryonic death caused by PCV2a or another factor was undetermined.

Clinical signs

PCV2 has been linked to late-term abortions and stillbirths (West et al. 1999), but the contribution of PCV2 infection to reproductive failure in the field is unclear. Some reports suggest that its occurrence is rare (Ladekjaer-Mikkelsen et al. 2001; Maldonado et al. 2005; Pensaert et al. 2004; Sharma and Saikumar 2010), while others suggest that 13–46% of aborted fetuses and/or stillborns are infected with PCV2 (Kim et al. 2004b; Lyoo et al. 2001). Mummification is also an outcome of late reproductive problems caused by PCV2 infection (Madson et al. 2009a).

Based on experimental data, it is feasible to conclude that return to estrus would be the outcome of PCV2 infection at early gestation, since the virus is able to replicate and cause death of zona-pellucida-free embryos (Mateusen et al. 2007). However, the real impact of early gestation infection is currently unknown.

PCV2 infections can be found in clinically normal live-born piglets, indicating intrauterine infection (Eddicks et al. 2016; Shen et al. 2010). The viability and performance of these animals may not be overtly affected, although a subclinical impact of transplacental infection cannot be ruled out.

Lesions

Stillborn and nonviable neonatal piglets show chronic passive hepatic congestion and cardiac hypertrophy with multifocal areas of myocardial discoloration (West et al. 1999). The primary microscopic lesion corresponds to a nonsuppurative, fibrotic, and/or necrotic myocarditis (Mikami et al. 2005; West et al. 1999).

Diagnosis

The diagnosis of PCV2-RD at late gestation includes three criteria (Segalés 2012):

1) Late-term abortions and stillbirths, sometimes with evident hypertrophy of the fetal heart.
2) The presence of heart lesions characterized by extensive fibrosing and/or necrotizing myocarditis.
3) The presence of high amounts of PCV2 in myocardial lesions and other fetal tissues.

In aborted and mummified fetuses, PCV2 is mainly found in the myocardiocyte (Madson et al. 2009a; West et al. 1999). Recent studies suggested that this case definition is useful for the diagnosis of acute reproductive failure, whereas quantitative PCR is an appropriate diagnostic method across a wider time span (Hansen et al.

2010). Serology is not useful for the detection of intrauterine infection with PCV2 (Hansen et al. 2010). This is reinforced by the recent evidence that small amounts of PCV2 antibodies are able to cross the placenta in some fetuses coming from sows with very high serological titers (Saha et al. 2014), probably related to small lesions in the placental barrier during gestation. PCV2-RD at late gestation is clinically indistinguishable from other swine diseases that cause late-term abortions and stillbirths. In addition, the presence of mummies of different sizes must be differentiated from the infection by PPV.

The putative diagnosis of PCV2-RD at early gestation has been suggested based on two criteria (Segalés 2012):

1) Regular return to estrus.
2) PCV2 seroconversion following the return to estrus and/or PCV2 PCR positivity around return-to-estrus occurrence.

PCV2-RD at early gestation should be clinically differentiated from other infectious and noninfectious conditions causing return to estrus in sows.

Porcine dermatitis and nephropathy syndrome (PDNS)

Pathogenesis

PDNS is classified as an immune-mediated disease, although the specific antigen triggering this condition is unknown. In principle, the nature of such an antigen could be diverse, but the clinical and epidemiological data supports PCV2 as the most likely suspect. It has been suggested that excessive PCV2 serum antibody titers may somehow be involved with triggering the condition (Wellenberg et al. 2004); however, this hypothesis awaits experimental confirmation.

Clinical signs

PDNS affects nursery, growing, and adult pigs (Drolet et al. 1999). The prevalence of PDNS is usually <1% (Segalés et al. 1998), although higher frequency has been described (Gresham et al. 2000). Mortality approaches 100% in pigs older than 3 months versus approximately 50% in younger pigs. Severe, acutely affected pigs die within a few days after the onset of clinical signs. Surviving pigs tend to recover and gain weight 7–10 days after the beginning of the syndrome (Segalés et al. 1998).

PDNS-affected pigs are anorexic and depressed, with little or no pyrexia (Drolet et al. 1999). They may be prostrate, reluctant to move, and/or stiff-gaited. The most obvious sign of PDNS is the presence of irregular, red to purple macules and papules in the skin, primarily on the hind limbs and perineal area (Figure 30.6), but sometimes more generally distributed. With time, the lesions become covered by dark crusts. The lesions gradually fade, sometimes leaving scars (Drolet et al. 1999). Serum BUN and creatinine are markedly elevated, indicating acute renal failure.

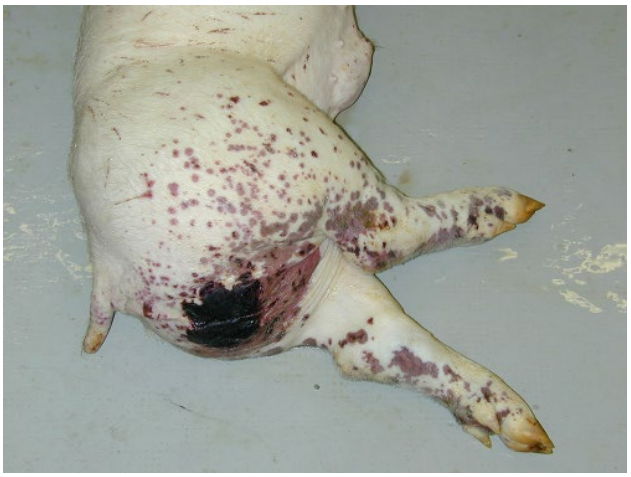


Figure 30.6 PDNS-affected pig. Presence of necrotizing cutaneous lesions of generalized distribution, which tend to be more numerous and coalescent on the hind limbs.

Lesions

Macules and papules from the skin are seen microscopically as necrotic and hemorrhagic tissue associated with necrotizing vasculitis (Segalés et al. 1998). Necrotizing vasculitis is a systemic feature. Studies on tissue sections from pigs with PDNS have failed to consistently demonstrate PCV2 antigen or nucleic acid associated with PDNS vascular lesions.

Pigs that die acutely with PDNS have bilaterally enlarged kidneys with a fine granular cortical surface, small reddish pinpoint cortical lesions, and edema of the renal pelvis (Segalés et al. 2004). These lesions correspond to a fibrinonecrotizing glomerulitis with fibrin and neutrophils filling and occluding Bowman's spaces and a non-purulent interstitial nephritis. Pigs with prolonged disease may show chronic glomerulonephritis (Segalés et al. 1998). Normally, both skin and renal lesions are present in PDNS, but in few occasions, skin or renal lesions may occur alone. Lymph nodes may be enlarged and red in color. Splenic infarcts may be also present (Segalés et al. 1998). Microscopically, lymphoid lesions like those of PCV2-SD but milder are frequently observed in PDNS-affected pigs (Rosell et al. 2000b).

Diagnosis

Since the triggering antigen for PDNS is unknown, detection of PCV2 is not a diagnostic requirement. Instead, the diagnosis of PDNS is based on two criteria (Segalés 2012):

1) The presence of hemorrhagic and necrotizing skin lesions, primarily on the hind limbs and perineal area, and/or swollen and pale kidneys with generalized cortical petechiae.
2) The presence of systemic necrotizing vasculitis and necrotizing fibrinous glomerulonephritis.

PCV2 nucleic acid or antigen is usually found in PDNS-affected pigs, mainly in the cytoplasm of histiocytes, multinucleate giant cells, and other monocyte–macrophage lineage cells, as well as in some epithelial cell types (Segalés et al. 2004).

Differential diagnoses for PDNS include any condition that causes red to dark discoloration of the skin, as well as conditions that cause petechial hemorrhages in the kidneys (Segalés 2012). Special note should be made of the similarity of gross lesions between PDNS, classical swine fever (CSF), and African swine fever.

Immunity

In vitro studies demonstrated that PCV2 modulates the immune system. The addition of PCV2 to cultured alveolar macrophages altered the production of certain cytokines and/or chemokines (Chang et al. 2006). The altered functionality of PCV2-infected alveolar macrophages may favor the spread of PCV2, as well as render pigs more susceptible to opportunistic and secondary pulmonary infections.

PCV2 infection of myeloid dendritic cells did not interfere with dendritic cell maturation or their ability to process and present antigen to T lymphocytes (Vincent et al. 2003, 2005), but PCV2 interaction with plasmacytoid dendritic cells (natural interferon-producing cells) impaired their responsiveness to danger signals (Vincent et al. 2007). PCV2-induced impairment of dendritic cell function does not require viral replication, but is mediated by viral DNA in a dose-dependent manner (i.e. a minimum concentration of dsDNA (RF) is necessary to mediate inhibition) (Vincent et al. 2007).

The addition of PCV2 to PBMCs from either healthy or clinically affected pigs altered their cytokine profiles (Darwich et al. 2003a) and seemingly modulated the specific immune responses to other pathogens as well (i.e. PCV2 downregulated the cytokine recall antigen response) (Kekarainen et al. 2008). Inhibition of some of cytokine responses was mediated by the release of PCV2-induced interleukin-10 (IL-10) by monocytic cells. *Ex vivo*, increased serum levels of this cytokine in PCV2-infected pigs were associated with the development of PCV2-SD (Darwich et al. 2003b; Stevenson et al. 2006). It has been suggested that IL-10-producing cells are of myeloid and lymphoid origin, but they are rarely, if ever, infected with PCV2 (Crisci et al. 2010; Doster et al. 2010).

Most current knowledge on PCV2 immunity was generated under PCV2-SD and PCV2 vaccination scenarios in piglets.

Under field conditions, colostral antibodies decline during the lactating and nursery periods, followed by active seroconversion during the growing–finishing period (Carasova et al. 2007; Grau-Roma et al. 2009; Larochelle et al. 2003). PCV2-SD is very rarely observed in pigs younger than 4 weeks of age (Segalés and Domingo 2002), suggesting that certain levels of maternal immunity protect against the development of PCV2-SD (Allan et al. 2002a; Calsamiglia et al. 2007).

Specific immune responses to PCV2 develop 2–3 weeks after inoculation. In the field, seroconversion occurs in both subclinically infected and PCV2-SD-affected pigs (Grau-Roma et al. 2009). Some field studies found no difference in total anti-PCV2 antibody titer levels between non-PCV2-SD- and PCV2-SD-affected pigs (Larochelle et al. 2003; Sibila et al. 2004), but others have reported weaker humoral immune responses in PCV2-SD-affected pigs (Grau-Roma et al. 2009; Meerts et al. 2006).

An insufficient humoral response (Bolin et al. 2001; Okuda et al. 2003; Rovira et al. 2002) and, in particular, a poor neutralizing antibody response (Fort et al. 2007; Meerts et al. 2006) is associated with increased viral replication, severe lymphoid lesions, and the eventual development of PCV2-SD. During infection, antibodies are produced especially against the capsid protein, but also against nonstructural replication-associated proteins (Pérez-Martín et al. 2010; Pogranichnyy et al. 2000). All current commercial PCV2 vaccines elicit anti-capsid antibodies only, as they are all based either on capsid protein or on non-replicative viruses (Segalés 2015).

Pigs with subclinical PCV2 infections develop specific humoral and T-cell responses, although relatively slowly (Fort et al. 2009a; Steiner et al. 2009). The kinetics of the helper and cytotoxic T-cell responses, as measured by the number of IFN-γ secreting cells (SC), are dependent on the individual animal and the time post inoculation at which the cells are assayed.

PCV2-SD-affected pigs show B- and T-cell lymphopenia induced by PCV2 infection. Most notably, B and $CD3^+CD4^+CD8^+$ memory/activated T lymphocytes are depleted in PCV2-SD-affected animals (Nielsen et al. 2003). Both vaccination and infection elicit memory/activated T cells. These cells persist long term post infection/vaccination and are able to expand rapidly after recall antigen recognition (Ferrari et al. 2014). More specifically, it has been suggested that PCV2-specific IFN-γ/TNF-α-co-producing CD4+ cells, produced upon vaccination/infection, play a pivotal role in controlling and clearing PCV2 infection (Koinig et al. 2015).

Overall, current data on the adaptive immune response against PCV2 infection/vaccination suggest that the cell-mediated response, as measured by IFN-γ-SC, together with a significant neutralizing antibody response, is responsible for viral clearance in infected animals and protection (Fort et al. 2009a; Kekarainen and Segalés 2015). It has been hypothesized that a failure in one or

both responses could result in PCV2-SD development (Kekarainen et al. 2010).

Prevention and control

PCV2-SD is a multifactorial disease that can be controlled by the use of PCV2 vaccines, but prior to the advent of vaccines, prevention and control focused on eliminating environmental and infectious cofactors and triggers believed to trigger PCV2-SD (e.g. "Madec's 20-point plan") (Ellis et al. 2004; Madec et al. 2000; Segalés et al. 2005). These issues are still pertinent.

Most current vaccines worldwide are based on PCV2a strains (Segalés 2015). Although vaccines are effective in reducing clinical signs of PCVDs, their efficacy against mixed infection of PCV2a and PCV2b and the recently emerged PCV2d is less clear (Jeong et al. 2015; Opriessnig et al. 2014; Rose et al. 2016; Shen et al. 2010) and requires further study (Afghah et al. 2017).

Vaccination of both sows and piglets has been shown to be beneficial in the continuous control of PCVD (Segalés 2015). In this protocol it is important to take into account the possible interference of maternally derived immunity upon PCV2 vaccine efficacy in piglets.

Vaccination of sows has two potential objectives: (1) to prevent PCVDs in offspring or (2) to protect against PCV2-RD. To prevent PCVDs in offspring, vaccination should take place during late gestation. PCV2 infection and/or low serological titers to PCV2 in sows at farrowing increased the overall mortality of their offspring to PCV2-SD (Allan et al. 2002a; Calsamiglia et al. 2007). Measures that increase maternal immunity and decrease sow viremia at farrowing should diminish piglet PCV2-SD mortality in problem herds (Pejsak et al. 2010).

If the objective is to prevent PCV2-RD, vaccination may be done during the acclimatization of gilts, prior to mating, during lactation, or at weaning. Sow vaccination has been shown to increase fertility, farrowing rate, number of piglets born alive, birth weight of piglets, and number of piglets weaned per a litter (Pejsak et al. 2012). However, one study using a PCV2 commercial subunit vaccine (Madson et al. 2009b) found that vertical transmission of PCV2 could occur in PCV2-vaccinated dams exposed at 56 days of gestation, although clinical protection was achieved. To date, minimal data exist regarding the benefits of the continuous vaccination of the breeding stock and its effects on PCV2-RD.

Piglet vaccination to control PCV2-SD in affected farms is faster than sow vaccination because vaccine administered to piglets elicits protective immunity in the animal at risk of the disease. Low antibody responses, or even lack of detectable antibody after vaccination, do not necessarily indicate lack of protection. High levels of maternally derived antibodies interfere with active seroconversion following vaccination (Fort et al. 2009b; Fraile et al. 2012; Opriessnig et al. 2010), although vaccine efficacy under field conditions seems not to be jeopardized in spite of moderate to high antibody titers (Fachinger et al. 2008; Feng et al. 2016). Cell-mediated immunity is also assumed to be important for protection (Fenaux et al. 2004; Pérez-Martín et al. 2010). Development of cell-mediated immunity was demonstrated by production of PCV2-specific IFN-γ-SC in piglets vaccinated with a PCV2 subunit vaccine (Fort et al. 2009b).

References

Afghah Z, Webb B, Meng XJ, et al. 2017. *Vet Microbiol* 206:21–28.

Albina E, Truong C, Hutet E, et al. 2001. *J Comp Pathol* 125:292–303.

Allan GM, Ellis JA. 2000. *J Vet Diagn Invest* 12:3–14.

Allan GM, Mackie DP, McNair J, et al. 1994a. *Vet Immunol Immunopathol* 43:357–371.

Allan GM, Phenix KV, Todd D, et al. 1994b. *Zentralbl Veterinarmed B* 41:17–26.

Allan GM, McNeilly F, Kennedy S, et al. 1998. *J Vet Diagn Invest* 10:3–10.

Allan GM, Kennedy S, McNeilly F, et al. 1999a. *J Comp Pathol* 121:1–11.

Allan GM, Mc Neilly F, Meehan BM, et al. 1999b. *Vet Microbiol* 66:115–123.

Allan GM, McNeilly F, Ellis J, et al. 2000a. *Arch Virol* 145:2421–2429.

Allan GM, McNeilly F, McNair I, et al. 2000b. *Arch Virol* 145:853–857.

Allan G, McNeilly F, McNair I, et al. 2002a. *Pig J* 50:59–67.

Allan GM, Krakowka S, Ellis JA. 2002b. *Pig Progress* 18:2.

Allan GM, McNeilly F, Ellis J, et al. 2004. *Vet Microbiol* 98:165–168.

Allan G, Krakowka S, Ellis J, et al. 2012. *Virus Res* 164:4–9.

Andraud M, Grasland B, Durand B, et al. 2008. *Vet Res* 39:43.

Baró J, Segalés J, Martínez J. 2015. *Vet Microbiol* 176:83–87.

Blunt R, McOrist S, McKillen J, et al. 2011. *Vet Microbiol* 149:452–455.

de Boisseson C, Beven V, Bigarre L, et al. 2004. *J Gen Virol* 85:293–304.

Bolin SR, Stoffregen WC, Nayar GP, et al. 2001. *J Vet Diagn Invest* 13:185–194.

Brunborg IM, Moldal T, Jonassen CM. 2004. *J Virol Methods* 122:171–178.

Calsamiglia M, Segalés J, Quintana J, et al. 2002. *J Clin Microbiol* 40:1848–1850.

Calsamiglia M, Fraile L, Espinal A, et al. 2007. *Res Vet Sci* 82:299–304.

Carasova P, Celer V, Takacova K, et al. 2007. *Res Vet Sci* 83:274–278.
Carman S, McEwen B, DeLay J, et al. 2006. *Can Vet J* 47:761–762.
Chae C. 2016. *Vet J* 212:1–6.
Chang HW, Jeng CR, Lin TL, et al. 2006. *Vet Immunol Immunopathol* 110:207–219.
Chen X, Shi X, Gan F, et al. 2015. *Vet Res* 46:32.
Cheung AK. 2003. *Virology* 313:452–459.
Cheung AK. 2009. *Arch Virol* 154:531–534.
Cheung AK, Lager KM, Kohutyuk OI, et al. 2007. *Arch Virol* 152:1035–1044.
Chianini F, Majo N, Segalés J, et al. 2003. *Vet Immunol Immunopathol* 94:63–75.
Clark EG. 1996. Post-weaning multisystemic wasting syndrome. *Proc West Can Assoc Swine Pract* 19–20.
Clark EG. 1997. Post-weaning multisystemic wasting syndrome. *Proc Annu Meet Am Assoc Swine Pract* 28:499–501.
Collins PJ, McKillen J, Allan G. 2017. *Vet Rec* 181:599.
Correa AM, Zlotowski P, de Barcellos DE, et al. 2007. *J Vet Diagn Invest* 19:109–112.
Cortey M, Pileri E, Sibila M, et al. 2011. *Vet J* 187:363–368.
Crisci E, Ballester M, Domínguez J, et al. 2010. *Vet Immunol Immunopathol* 136:305–310.
Crowther RA, Berriman JA, Curran WL, et al. 2003. *J Virol* 77:13036–13041.
Csagola A, Kiss I, Tuboly T. 2008. *Acta Vet Hung* 56:139–144.
Darwich L, Mateu E. 2012. *Virus Res* 164:61–67.
Darwich L, Segalés J, Domingo M, et al. 2002. *Clin Diagn Lab Immunol* 9:236–242.
Darwich L, Balasch M, Plana-Duran J, et al. 2003a. *J Gen Virol* 84:3453–3457.
Darwich L, Pie S, Rovira A, et al. 2003b. *J Gen Virol* 84:2117–2125.
Davies B, Wang X, Dvorak CM, et al. 2016. *Virus Res* 217:32–37.
Doster AR, Subramaniam S, Yhee JY, et al. 2010. *J Vet Sci* 11:177–183.
Drolet R, Thibault S, D'Allaire S, et al. 1999. *J Swine Health Prod* 7:283–285.
Dupont K, Nielsen EO, Baekbo P, et al. 2008. *Vet Microbiol* 128:56–64.
Eddicks M, Koeppen M, Willi S, et al. 2016. *Vet Microbiol* 187:70–74.
Ellis J, Hassard L, Clark E, et al. 1998. *Can Vet J* 39:44–51.
Ellis JA, Wiseman BM, Allan G, et al. 2000. *J Am Vet Med Assoc* 217:1645–1646.
Ellis JA, Konoby C, West KH, et al. 2001. *Can Vet J* 42:461–464.
Ellis J, Spinato M, Yong C, et al. 2003. *J Vet Diagn Invest* 15:364–368.
Ellis J, Clark E, Haines D, et al. 2004. *Vet Microbiol* 98:159–163.
Fachinger V, Bischoff R, Jedidia SB, et al. 2008. *Vaccine* 26:1488–1499.
Fenaux M, Opriessnig T, Halbur PG, et al. 2004. *J Virol* 78:6297–6303.
Feng H, Segalés J, Fraile L, et al. 2016. *Vaccine* 34:3044–3050.
Fernandes LT, Mateu E, Sibila M, et al. 2007. *Viral Immunol* 20:541–552.
Ferrari L, Borghetti P, De Angelis E, et al. 2014. *Vet Res* 45:44.
Fort M, Olvera A, Sibila M, et al. 2007. *Vet Microbiol* 125:244–255.
Fort M, Fernandes LT, Nofrarías M, et al. 2009a. *Vet Immunol Immunopathol* 129:101–107.
Fort M, Sibila M, Pérez-Martín E, et al. 2009b. *Vaccine* 27:4031–4037.
Fraile L, Grau-Roma L, Sarasola P, et al. 2012. *Vaccine* 30:1986–1992.
Franzo G, Cortey M, Olvera A, et al. 2015a. *Virol J*: 12:131.
Franzo G, Cortey M, de Castro AM, et al. 2015b. *Vet Microbiol* 178:158–162.
Franzo G, Cortey M, Segalés J, et al. 2016. *Mol Phylogenet Evol* 100:269–280.
Franzo G, Legnardi M, Tucciarone CM, et al. 2018a. *Vet Rec* 182:83.
Franzo G, Legnardi M, Hjulsager CK, et al. 2018b. *Transbound Emerg Dis*, 65(3):602–606.
Fux R, Söckler C, Link EK, et al. 2018. *Virol J* 15:25.
Gagnon CA, Tremblay D, Tijssen P, et al. 2007. *Can Vet J* 48:811–819.
Gagnon CA, Music N, Fontaine G, et al. 2010. *Vet Microbiol* 144:18–23.
Gilpin DF, McCullough K, Meehan BM, et al. 2003. *Vet Immunol Immunopathol* 94:149–161.
Grau-Roma L, Crisci E, Sibila M, et al. 2008. *Vet Microbiol* 128:23–35.
Grau-Roma L, Hjulsager CK, Sibila M, et al. 2009. *Vet Microbiol* 135:272–282.
Grau-Roma L, Fraile L, Segalés J. 2011. *Vet J* 187:23–32.
Grau-Roma L, Baekbo P, Rose N, et al. 2012. *J Swine Health Prod* 20:129–136.
Gresham A, Giles N, Weaver J. 2000. *Vet Rec* 147:115.
Guo LJ, Lu YH, Wei YW, et al. 2010. *Virol J* 7:273.
Ha Y, Lee YH, Ahn KK, et al. 2008. *Vet Pathol* 45:842–848.
Ha Y, Ahn KK, Kim B, et al. 2009. *Res Vet Sci* 86:108–110.
Hamberg A, Ringler S, Krakowka S. 2007. *J Vet Diagn Invest* 19:135–141.
Hamel AL, Lin LL, Nayar GP. 1998. *J Virol* 72:5262–5267.
Hansen MS, Hjulsager CK, Bille-Hansen V, et al. 2010. *Vet Microbiol* 144:203–209.
Harding JC. 1996. Postweaning multisystemic wasting syndrome: Preliminary epidemiology and clinical findings. *Proc West Can Assoc Swine Pract* 21.
Harding JC, Clark EG. 1997. *J Swine Health Prod* 5: 201–203.
Harmon KM, Gauger PC, Zhang J, et al. 2015. *Genome Announc* 3:e01315–15.
Harms PA, Sorden SD, Halbur PG, et al. 2001. *Vet Pathol* 38:528–539.
He J, Cao J, Zhou N, et al. 2013. *J Virol* 87:1420–1429.

Hesse R, Kerrigan M, Rowland RR. 2008. *Virus Res* 132:201–207.
Jeong J, Park C, Choi K, et al. 2015. *Vet Microbiol* 177:43–52.
Karuppannan AK, Jong MH, Lee SH, et al. 2009. *Virology* 383:338–347.
Kekarainen T, Segalés J. 2015. *Porcine Health Manag* 1:17.
Kekarainen T, Montoya M, Mateu E, et al. 2008. *J Gen Virol* 89:760–765.
Kekarainen T, McCullough K, Fort M, et al. 2010. *Vet Immunol Immunopathol* 136:185–193.
Kennedy S, Moffett D, McNeilly F, et al. 2000. *J Comp Pathol* 122:9–24.
Kim J, Chung HK, Chae C. 2003. *Vet J* 166:251–256.
Kim J, Ha Y, Jung K, et al. 2004a. *Can J Vet Res* 68:218–221.
Kim J, Jung K, Chae C. 2004b. *Vet Rec* 155:489–492.
Kim HB, Lyoo KS, Joo HS. 2009. *Vet Rec* 164:599–600.
Kiupel M, Stevenson GW, Choi J, et al. 2001. *Vet Pathol* 38:74–82.
Klaumann F, Franzo G, Sohrmann M, et al. 2018a. *Transbound Emerg Dis* 65:1290–1296.
Klaumann F, Correa-Fiz F, Franzo G, et al. 2018b. *Front Vet Sci* 5:5–315.
Koinig HC, Talker SC, Stadler M, et al. 2015. *Vet Res* 46:20.
Krakowka S, Ellis JA, McNeilly F, et al. 2001. *Vet Pathol* 38:31–42.
Kristensen CS, Baekbo P, Bille-Hansen V, et al. 2009. *Vet Microbiol* 138:244–250.
Ku X, Chen F, Li P, et al. 2017. *Transbound Emerg Dis* 64:703–708.
Kuehn BM. 2010. *JAMA* 304:30–31.
Ladekjaer-Mikkelsen AS, Nielsen J, Storgaard T, et al. 2001. *Vet Rec* 148:759–760.
Lager KM, Gauger PC, Vincent AL, et al. 2007. *Vet Rec* 161:428–429.
Larochelle R, Magar R, D'Allaire S. 2003. *Can J Vet Res* 67:114–120.
Lefebvre DJ, Costers S, Van Doorsselaere J, et al. 2008a. *J Gen Virol* 89:177–187.
Lefebvre DJ, Meerts P, Costers S, et al. 2008b. *Vet Microbiol* 132:74–86.
Lefebvre DJ, Van Doorsselaere J, Delputte PL, et al. 2009. *Arch Virol* 154:875–879.
Li X, Tian K. 2017. *Vet Rec* 181:659–660.
Lin CM, Jeng CR, Chang HW, et al. 2008. *Vet Immunol Immunopathol* 124:355–366.
Liu J, Chen I, Kwang J. 2005. *J Virol* 79:8262–8274.
Liu X, Wang FX, Zhu HW, et al. 2016. *Arch Virol* 161:1591–1599.
López-Soria S, Nofrarías M, Calsamiglia M, et al. 2011. *Vet Microbiol* 149:352–357.
Lorincz M, Csagola A, Biksi I, et al. 2010. *Acta Vet Hung* 58:265–268.
Lyoo KS, Park YH, Park BK. 2001. *J Vet Sci* 2:201–207.
Madec F, Eveno E, Morvan P, et al. 2000. *Livest Prod Sci* 63:223–233.
Madson DM, Patterson AR, Ramamoorthy S, et al. 2009a. *Vet Pathol* 46:707–716.
Madson DM, Patterson AR, Ramamoorthy S, et al. 2009b. *Theriogenology* 72:747–754.
Maldonado J, Segalés J, Martínez-Puig D, et al. 2005. *Vet J* 169:454–456.
Mankertz A, Mankertz J, Wolf K, et al. 1998. *J Gen Virol* 79:381–384.
Mankertz A, Caliskan R, Hattermann K, et al. 2004. *Vet Microbiol* 98:81–88.
Martin H, Le Potier MF, Maris P. 2008. *Vet J* 177:388–393.
Mateusen B, Sanchez RE, Van Soom A, et al. 2004. *Theriogenology* 61:91–101.
Mateusen B, Maes DG, Van Soom A, et al. 2007. *Theriogenology* 68:896–901.
McIntosh KA, Harding JC, Ellis JA, et al. 2006. *Can J Vet Res* 70:58–61.
McNeilly F, Kennedy S, Moffett D, et al. 1999. *J Virol Methods* 80:123–128.
McNeilly F, McNair I, Mackie DP, et al. 2001. *Arch Virol* 146:909–922.
McNeilly F, McNair I, O'Connor M, et al. 2002. *J Vet Diagn Invest* 14:106–112.
Meehan BM, Creelan JL, McNulty MS, et al. 1997. *J Gen Virol* 78:221–227.
Meehan BM, McNeilly F, Todd D, et al. 1998. *J Gen Virol* 79:2171–2179.
Meerts P, Misinzo G, Lefebvre D, et al. 2006. *BMC Vet Res* 2:6.
Meng XJ. 2013. *Annu Rev Anim Biosci* 1:43–64.
Mikami O, Nakajima H, Kawashima K, et al. 2005. *J Vet Med Sci* 67:735–738.
Nawagitgul P, Morozov I, Bolin SR, et al. 2000. *J Gen Virol* 81:2281–2287.
Nielsen J, Vincent IE, Botner A, et al. 2003. *Vet Immunol Immunopathol* 92:97–111.
O'Dea MA, Hughes AP, Davies LJ, et al. 2008. *J Virol Methods* 147:61–66.
Okuda Y, Ono M, Yazawa S, et al. 2003. *J Vet Diagn Invest* 15:107–114.
Olvera A, Sibila M, Calsamiglia M, et al. 2004. *J Virol Methods* 117:75–80.
Olvera A, Cortey M, Segalés J. 2007. *Virology* 357:175–185.
Opriessnig T, Halbur PG. 2012. *Virus Res* 164:20–32.
Opriessnig T, Langohr I. 2013. *Vet Pathol* 50:23–38.
Opriessnig T, Fenaux M, Thomas P, et al. 2006. *Vet Pathol* 43:281–293.
Opriessnig T, Meng XJ, Halbur PG. 2007. *J Vet Diagn Invest* 19:591–615.
Opriessnig T, Patterson AR, Elsener J, et al. 2008. *Clin Vaccine Immunol* 15:397–401.
Opriessnig T, Patterson AR, Jones DE, et al. 2009a. *Can J Vet Res* 73:81–86.
Opriessnig T, Patterson AR, Madson DM, et al. 2009b. *J Anim Sci* 87:1582–1590.
Opriessnig T, Patterson AR, Meng XJ, et al. 2009c. *Vet Microbiol* 133:54–64.
Opriessnig T, Patterson AR, Madson DM, et al. 2010. *Vet Microbiol* 142:177–183.

Opriessnig T, Gerber PF, Xiao CT, et al. 2014. *Vaccine* 32:4342–4348.
Opriessnig T, Xiao CT, Halbur PG, et al. 2017. *Vaccine* 35:248–254.
Palinski R, Piñeyro P, Shang P, et al. 2016. *J Virol* 91 pii: e01879-16.
Park JS, Kim J, Ha Y, et al. 2005. *J Comp Pathol* 132:139–144.
Pejsak Z, Podgorska K, Truszczynski M, et al. 2010. *Comp Immunol Microbiol Infect Dis* 33:e1–5.
Pejsak Z, Kusior G, Pomorska-Mól M, et al. 2012. *Pol J Vet Sci* 15:37–42.
Pensaert MB, Sanchez RE, Jr., Ladekjaer-Mikkelsen AS, et al. 2004. *Vet Microbiol* 98:175–183.
Pérez-Martín E, Rovira A, Calsamiglia M, et al. 2007. *J Virol Methods* 146:86–95.
Pérez-Martín E, Gómez-Sebastián S, Argilaguet JM, et al. 2010. *Vaccine* 28:2340–2349.
Phan TG, Giannitti F, Rossow S, et al. 2016. *Virol J* 13:184.
Pogranichnyy RM, Yoon KJ, Harms PA, et al. 2000. *Viral Immunol* 13:143–153.
Quintana J, Segalés J, Rosell C, et al. 2001. *Vet Rec* 149:357–361.
Quintana J, Segalés J, Calsamiglia M, et al. 2006. *Vet J* 171:570–573.
Ren L, Chen X, Ouyang H. 2016. *Virus Genes* 52:437–444.
Resendes AR, Segalés J. 2015. *Vet Pathol* 52:497–504.
Resendes AR, Majó N, van den Ingh TS, et al. 2011. *Vet J* 189:72–76.
Rodríguez-Arrioja GM, Segalés J, Calsamiglia M, et al. 2002. *Am J Vet Res* 63:354–357.
Rodríguez-Arrioja GM, Segalés J, Domingo M, et al. 2003. *Vet Rec* 153:371–372.
Rodríguez-Cariño C, Sánchez-Chardi A, Segalés J. 2010. *J Comp Pathol* 142:291–299.
Rodríguez-Cariño C, Duffy C, Sánchez-Chardi A, et al. 2011. *J Comp Pathol* 144:91–102.
Rosario K, Breitbart M, Harrach B, et al. 2017. *Arch Virol* 162:1447–1463.
Rose N, Abhervé-Guéguen A, Le Diguerher G, et al. 2005. *Livest Prod Sci* 95:177–186.
Rose N, Opriessnig T, Grasland B, et al. 2012. *Virus Res* 164:78–89.
Rose N, Andraud M, Bigault L, et al. 2016. *Vaccine* 34:3738–3745.
Rosell C, Segalés J, Plana-Duran J, et al. 1999. *J Comp Pathol* 120:59–78.
Rosell C, Segalés J, Domingo M. 2000a. *Vet Pathol* 37:687–692.
Rosell C, Segalés J, Ramos-Vara JA, et al. 2000b. *Vet Rec* 146:40–43.
Rovira A, Balasch M, Segalés J, et al. 2002. *J Virol* 76:3232–3239.
Royer RL, Nawagitgul P, Halbur PG, et al. 2001. *J Swine Health Prod* 9:4.
Saha D, Huang L, Bussalleu E, et al. 2012. *Vet Microbiol* 157:13–22.
Saha D, Del Pozo Sacristán R, Van Renne N, et al. 2014. *Virol Sin* 29:136–138.
Sánchez RE Jr, Nauwynck HJ, McNeilly F, et al. 2001. *Vet Microbiol* 83:169–176.
Sánchez RE Jr, Meerts P, Nauwynck HJ, et al. 2003. *Vet Microbiol* 95:15–25.
Sánchez RE Jr, Meerts P, Nauwynck HJ, et al. 2004. *J Vet Diagn Invest* 16:175–185.
Sarli G, Mandrioli L, Laurenti M, et al. 2001. *Vet Immunol Immunopathol* 83:53–67.
Sarli G, Morandi F, Panarese S, et al. 2012. *Acta Vet Scand* 31:54:51.
Schulze C, Segalés J, Neumann G, et al. 2004. *Vet Rec* 154:694–696.
Seeliger FA, Brugmann ML, Kruger L, et al. 2007. *Vet Pathol* 44:621–634.
Segalés J. 2012. *Virus Res* 164:10–19.
Segalés J. 2015. *Expert Rev Vaccines* 14:473–487.
Segalés J, Domingo M. 2002. *Vet Q* 24:109–124.
Segalés J, Piella J, Marco E, et al. 1998. *Vet Rec* 142:483–486.
Segalés J, Rosell C, Domingo M. 2004. *Vet Microbiol* 98:137–149.
Segalés J, Allan GM, Domingo M. 2005. *Anim Health Res Rev* 6:119–142.
Segalés J, Olvera A, Grau-Roma L, et al. 2008. *Vet Rec* 162:867–868.
Segalés J, Kekarainen T, Cortey M. 2013. *Vet Microbiol* 165:13–20.
Shang SB, Jin YL, Jiang XT, et al. 2009. *Mol Immunol* 46:327–334.
Sharma R, Saikumar G. 2010. *Tropl Anim Health Prod* 42:515–522.
Shen H, Wang C, Madson DM, et al. 2010. *Prev Vet Med* 97:228–236.
Sibila M, Calsamiglia M, Segalés J, et al. 2004. *Am J Vet Res* 65:88–92.
Sofia M, Billinis C, Psychas V, et al. 2008. *J Wildl Dis* 44:864–870.
Sorden SD. 2000. *J Swine Health Prod* 8:133–136.
Steiner E, Balmelli C, Gerber H, et al. 2009. *BMC Vet Res* 5:45.
Stevenson LS, McCullough K, Vincent I, et al. 2006. *Viral Immunol* 19:189–195.
Tang Q, Li S, Zhang H, et al. 2013. *Arch Virol* 158:2553–2560.
Ticó G, Segalés J, Martínez J. 2013. *Vet Microbiol* 163:242–247.
Timmusk S, Wallgren P, Brunborg IM, et al. 2008. *Virus Genes* 36:509–520.
Tischer I, Rasch R, Tochtermann G. 1974. *Zentralbl Bakteriol Orig A* 226:153–167.
Tischer I, Gelderblom H, Vettermann W, et al. 1982. *Nature* 295:64–66.
Tischer I, Peters D, Rasch R, et al. 1987. *Arch Virol* 96:39–57.
Tomás A, Fernandes LT, Valero O, et al. 2008. *Vet Microbiol* 132:260–273.
Vicente J, Segalés J, Hofle U, et al. 2004. *Vet Res* 35:243–253.

Victoria JG, Wang C, Jones MS, et al. 2010. *J Virol* 84:6033–6040.
Vincent IE, Carrasco CP, Herrmann B, et al. 2003. *J Virol* 77:13288–13300.
Vincent IE, Carrasco CP, Guzylack-Piriou L, et al. 2005. *Immunology* 115:388–398.
Vincent IE, Balmelli C, Meehan B, et al. 2007. *Immunology* 120:47–56.
Wellenberg GJ, Stockhofe-Zurwieden N, de Jong MF, et al. 2004. *Vet Microbiol* 99:203–214.
West KH, Bystrom JM, Wojnarowicz C, et al. 1999. *J Vet Diagn Invest* 11:530–532.
Wiederkehr DD, Sydler T, Buergi E, et al. 2009. *Vet Microbiol* 136:27–35.
Xiao CT, Halbur PG, Opriessnig T. 2015. *J Gen Virol* 96:1830–1841.
Xiao CT, Harmon KM, Halbur PG, et al. 2016. *Vet Microbiol* 197:72–77
Yan M, Zhu L, Yang Q. 2014. *Virol J* 11:193
Yu S, Opriessnig T, Kitikoon P, et al. 2007. *Vet Immunol Immunopathol* 115:261–272.
Zhai SL, He DS, Qi WB, et al. 2014. *Infect Genet Evol* 28:278–282.

31

Coronaviruses

Linda J. Saif, Qiuhong Wang, Anastasia N. Vlasova, Kwonil Jung, and Shaobo Xiao

Overview

The family *Coronaviridae* within the order *Nidovirales* consists of two subfamilies: (1) *Coronavirinae* comprising the genera *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus* and (2) *Torovirinae* comprising the genera *Torovirus* and *Bafinivirus* and an unassigned genus.

Five swine coronaviruses (CoVs) have been identified: transmissible gastroenteritis virus (TGEV) first described in 1946; porcine respiratory coronavirus (PRCV), a spike (S) gene deletion mutant of TGEV isolated in 1984; porcine epidemic diarrhea virus (PEDV) isolated in 1977; porcine hemagglutinating encephalomyelitis virus (pHEV) isolated in 1962; and porcine deltacoronavirus (PDCoV) detected in 2012. In addition, a TGEV/PEDV recombinant virus (TGEV backbone but with PEDV spike gene) has been identified in swine in Europe (Akimkin et al. 2016; Belsham et al. 2016; Boniotti et al. 2016), and a bat-HKU2-like alphacoronavirus has been identified in swine in China (Gong et al. 2017; Pan et al. 2017; Zhou et al. 2018). In pigs, CoVs and toroviruses (ToVs) affect a variety of organs, including the gastrointestinal and respiratory tracts, the peripheral and central nervous systems (CNS), and the mammary glands. Most ToVs and PRCV induce mainly subclinical infections in pigs, whereas TGEV, PEDV, PDCoV, and pHEV infections can result in fatal enteric or nervous diseases.

Swine CoVs comprise three distinct genera – *Alphacoronavirus*, *Betacoronavirus*, and *Deltacoronavirus* (Figure 31.1) – and share replication strategies common to *Coronaviridae*. TGEV and PRCV belong to the *Alphacoronavirus 1* species that also contains closely related CoVs of domestic cats and dogs. PEDV and two human CoVs (229E and NL63) are separate species in the same genus *Alphacoronavirus*. The newly identified bat-HKU2-like swine enteric alphacoronavirus also belongs to the genus *Alphacoronavirus*, but its taxonomic name has not been defined. pHEV and PDCoV are genetically distinct, and they belong to the *Betacoronavirus and Deltacoronavirus* genera, respectively. Together with bovine, human OC43, equine, and canine respiratory CoVs, pHEV is a member of the *Betacoronavirus 1* species. PDCoV is most closely related to other mammalian deltacoronaviruses from Asian leopard cats and Chinese ferret badgers (Ma et al. 2015). For each swine CoV, only a single serotype is recognized.

CoVs are enveloped and pleomorphic, with an overall diameter of 60–160 nm as viewed by negative staining electron microscopy (EM) (Figure 31.2). Most have a single layer of club-shaped spikes (S protein) 12–25 nm in length, but pHEV and some other betacoronaviruses have a second shorter layer of surface spikes, the hemagglutinin-esterase (HE) protein.

Genomic organization and gene expression: TGEV prototype

Swine CoVs contain one large, polyadenylated, single-stranded, genomic RNA (25–30 kb) of positive-sense polarity. The genome organization, replication strategy, and expression of viral proteins are similar to those of other human and animal CoVs (Enjuanes and Van der Zeijst 1995; Gonzalez et al. 2003; Laude et al. 1993). The complete genomes of the Purdue and Miller strains of TGEV are 28,546–28,580 nucleotide (nt) long and share 96% overall identity (Penzes et al. 2001; Zhang et al. 2007). Most CoVs have buoyant densities in sucrose of 1.18–1.20 g/mL. The phospholipids and glycolipids incorporated into the virus envelope are derived from the host cell, and thus, the envelope composition is host cell dependent (Enjuanes and Van der Zeijst 1995).

Most CoVs contain four structural proteins: a large surface glycoprotein (spike or S protein visible as the corona; Figure 31.2), a small membrane protein (E), an integral membrane glycoprotein (M), and a nucleocapsid protein (N). However, pHEV also contains an HE protein (de Groot et al. 2008).

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

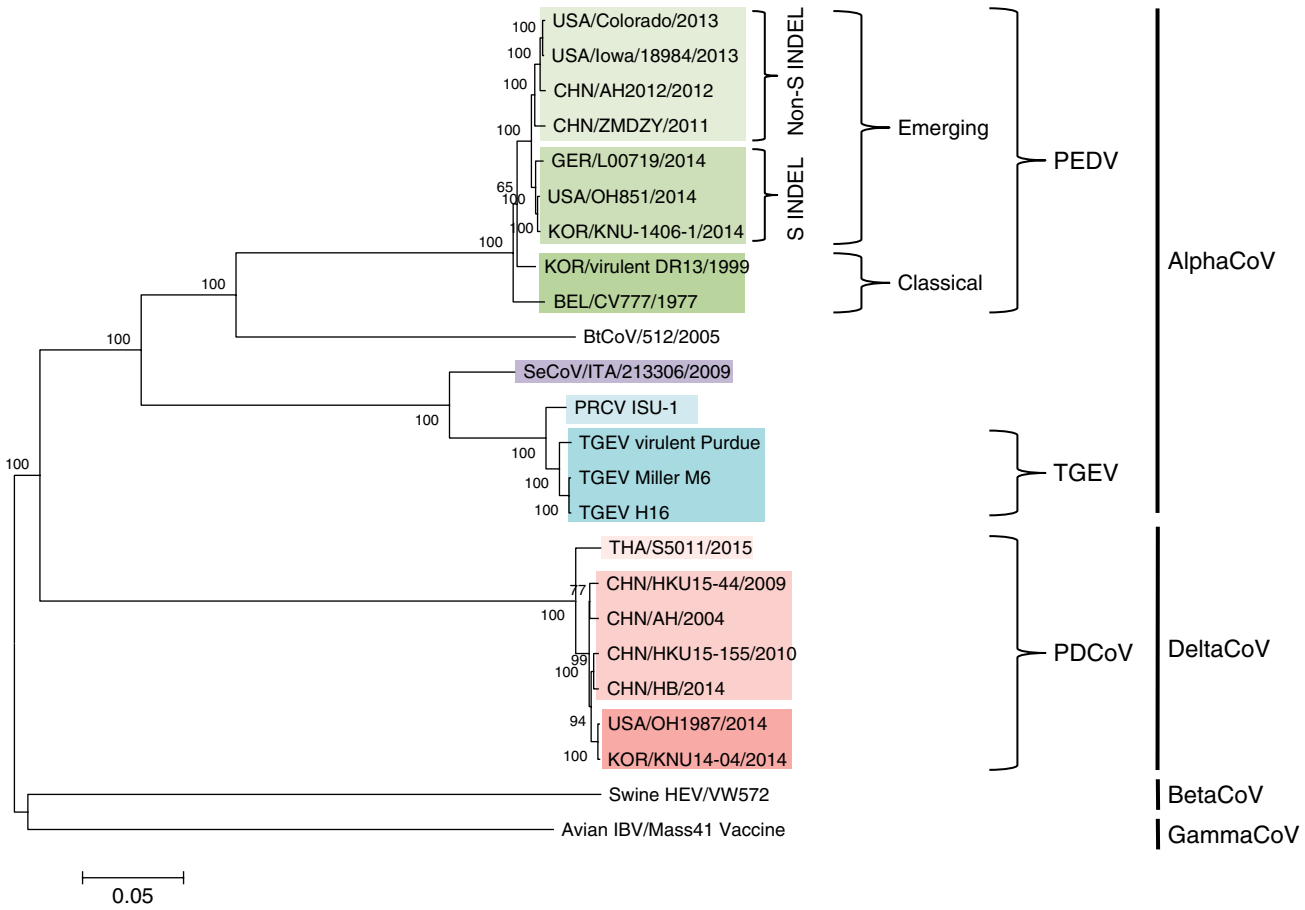


Figure 31.1 Phylogenetic tree of coronaviruses. The complete genomes of TGEV, PEDV, and PDCoV strains, closely related PRCV and a bat alphacoronavirus, a porcine HEV strain, and an avian IBV strain were selected. Multiple sequence alignments were performed using ClusterW, and a neighbor-joining phylogenetic tree supported with a bootstrap test of 1000 replicates was constructed using MEGA 6.0 software. The number on each branch indicates the bootstrap value. The scale represents the nucleotide substitutions per site. Color-shaded areas denote different swine virus clusters.

The N protein (47 kDa) interacts with viral RNA to form a helical ribonucleoprotein complex. This structure, in association with M protein, forms an internal icosahedral core in TGEV. The 29–36 kDa M glycoprotein is embedded in the viral envelope by 3–4 membrane-spanning regions. In TGEV, the hydrophilic N-terminus with a single accessible glycosylation site is responsible for interferon (IFN) induction (Charley and Laude 1988). Epitopes on protruding N- and C-terminal ends of the M protein of TGEV bind complement-dependent neutralizing monoclonal antibodies (MAbs) (Laude et al. 1992; Woods et al. 1988).

The TGEV S glycoprotein (220 kDa) occurs as trimer complexes (Delmas and Laude 1990) and functions in virus neutralization (complement independent), virus-cell attachment, membrane fusion, and hemagglutination. The large deletion in the S gene of PRCV results in a smaller S protein (170–190 kDa) (Figure 31.3). During fusion of TGEV with host cell membranes, two highly conserved heptad repeat regions (HR1 and HR2) of the S protein undergo conformational changes important for fusion (Ma et al. 2005). Entry of TGEV into the cell is likely associated with cholesterol-rich membrane microdomains (Ren et al. 2008), since exogenous cholesterol rescued virus infectivity.

Epitope mapping of the S glycoprotein of TGEV revealed four antigenic sites (A, B, C, D) (Figure 31.3). Site A–B, the conserved immunodominant epitope, is recognized strongly by neutralizing MAbs (Correa et al. 1990; Delmas and Laude 1990; Simkins et al. 1992, 1993), although other sites (D, C) can also induce virus-neutralizing (VN) antibodies (Delmas and Laude 1990). The S protein mutations in attenuated TGEV strains or the natural TGEV deletion mutant PRCV include a serine/alanine mutation at amino acid (aa) 585 position associated with induction of VN antibodies, as well as receptor (aminopeptidase N) binding (Zhang et al. 2007).

Porcine aminopeptidase N (pAPN) has been identified as the TGEV cell receptor (Delmas et al. 1992). The receptor-binding and major neutralizing site (site A) on the S

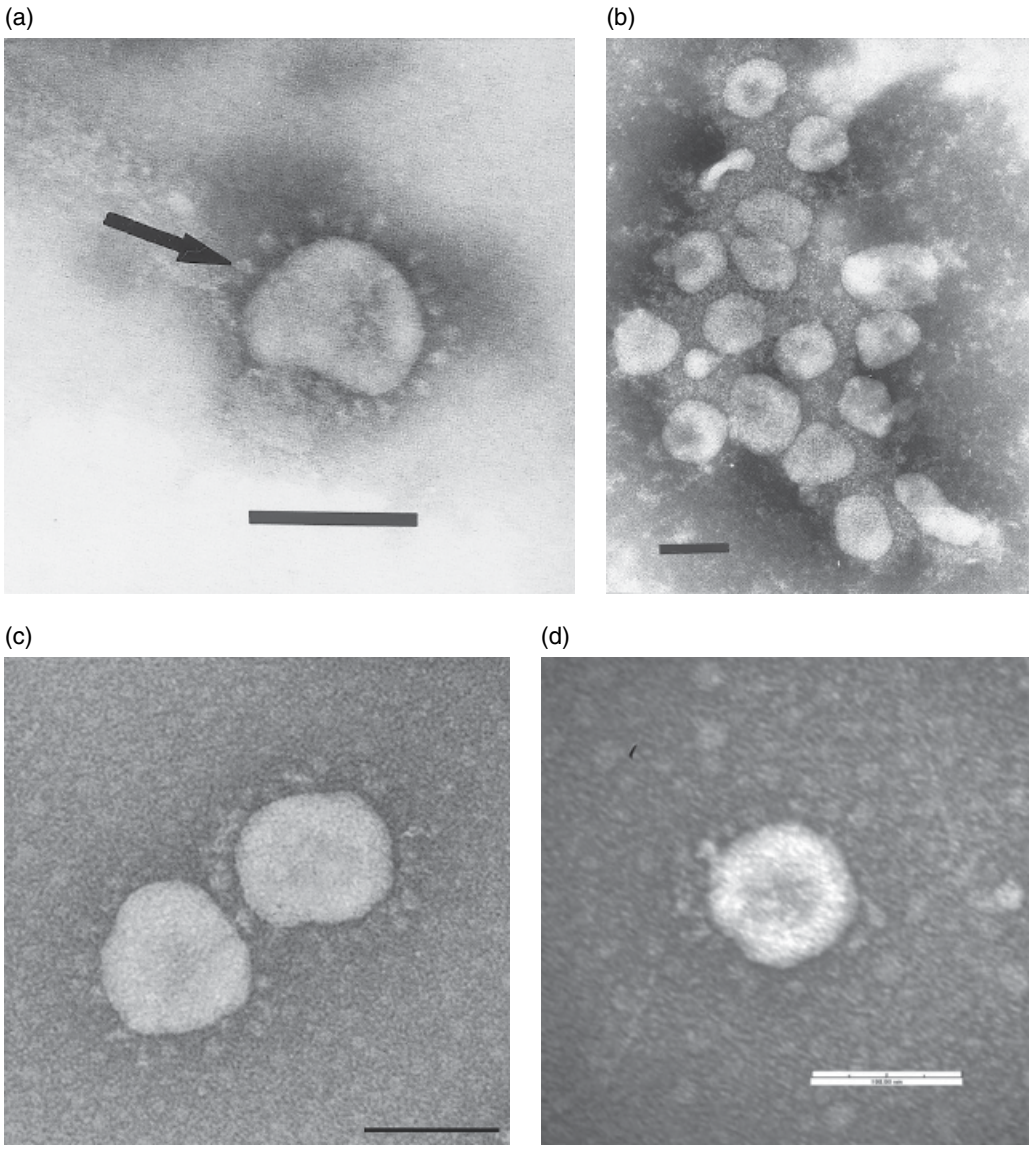


Figure 31.2 Electron micrographs. (a) A TGEV particle showing typical coronavirus morphology. Arrow points to the virus peplomers or spikes. Bar = 100 nm. (b) Typical virus–antibody aggregates observed by immunoelectron microscopy of TGEV and gnotobiotic pig anti-TGEV serum. Bar = 100 nm. (c) Two particles of emerging non-S INDEL PEDV PC22A strain, bar = 100 nm. *Source:* Oka et al. (2014). Reproduced with permission of Elsevier. (d) A PDCoV particle, bar = 100 nm. *Source:* Jung et al. (2015b).

protein of TGEV are located within the same domain (Figure 31.3) (Godet et al. 1994). TGEV binding to sialic acid residues on glycoproteins of target cells was proposed to initiate infection of intestinal enterocytes (Schwegmann-Wessels et al. 2002). Treatment of TGEV with sialidase enhanced hemagglutinating activity (Noda et al. 1987; Schultze et al. 1996). The hemagglutinating activity resides in the N-terminal region of the TGEV S protein, a region that is missing from the PRCV S protein; thus, determination of hemagglutinating activity (Schultze et al. 1996) could potentially differentiate PRCV and TGEV strains.

TGEV and PEDV as well as PDCoV also encode 1–2 accessory proteins encoded by open reading frame (ORF) 3 (TGEV and PEDV), ORF6 (PDCoV), and ORF7 (TGEV and PDCoV). The overall genome organization is 5′UTR-ORF1ab, S, ORF3, E, M, ORF6, N, ORF7-3′UTR.

Contrasts and comparisons

Seven CoVs are related antigenically or by their genomic sequences (Enjuanes and Van der Zeijst 1995): TGEV, PRCV, canine coronavirus (CCoV), feline infectious peritonitis virus (FIPV), feline enteric coronavirus (FECoV), PEDV, and human CoV 229E. CoVs within the species *Alphacoronavirus 1* (TGEV, PRCV, CCoV, FIPV, FECoV) are antigenically related, based on cross-reactivity in VN and immunofluorescence (IF) tests and with MAbs to the S, N, or M proteins, and all share the antigenic subsite

Ac on the S protein (Enjuanes and Van der Zeijst 1995). As members of the same species, these viruses likely represent host range mutants of an ancestral virus strain (de Groot et al. 2008). TGEV and CCoV could be distinguished *in vitro* by two-way cross-neutralization tests and other biological differences (Reynolds et al. 1980), i.e. both TGEV and CCoV grow in canine kidney cells and a feline cell line, whereas TGEV, but neither CCoV nor FIPV, grows in swine cells.

For the S glycoprotein that confers host range specificity, the 300 aa residues at the N-terminus are the most variable. In this domain, CCoV and FIPV are more similar to each other than to TGEV (Wesseling et al. 1994). Differentiation of the TGEV-related CoVs is possible using specific MAbs to the S glycoprotein of TGEV that recognize TGEV but not PRCV, FIPV, or CCoV strains (Callebaut et al. 1989; Laude et al. 1993; Sanchez et al. 1990; Simkins et al. 1992, 1993).

Interestingly, outbreaks of fatal acute gastroenteritis associated with TGEV-related CCoV type II (CCoV-II) were reported in European dogs (Decaro et al. 2009; Erles and Brownlie 2009). The close genetic relatedness (>96% aa identity) in the key replicase domains suggested that the recently emerged CCoV-II strains are host range variants of TGEV that infect dogs (de Groot et al. 2008). Based on the S protein, the CCoV-IIb strains (TGEV-like) (Decaro et al. 2010) may represent novel recombinant viruses of mixed (TGEV/CCoV) origin.

Several researchers reported that the severe acute respiratory syndrome (SARS) CoV cross-reacts with antibodies to *Alphacoronavirus 1* species (TGEV, PRCV, CCoV, FeCoV) through the N protein (Ksiazek et al. 2003; Sun and Meng 2004) and that this cross-reactivity mapped to the N-terminal region (Vlasova et al. 2007). This discovery led to use of SARS CoV-specific N peptide fragments in serologic assays to detect SARS CoV antibodies in animal sera (Vlasova et al. 2007). One-way cross-reactivity with the N protein has also been reported for PEDV, FIPV, CCoV, TGEV, and a putative mink CoV (Have et al. 1992; Zhou et al. 2010). Although no cross-reactivity between PEDV and TGEV-related CoVs was initially reported (Enjuanes and Van der Zeijst, 1995), one-way cross-reactivity between TGEV Miller and several PEDV strains (classical CV777, emerging non-S INDEL and S INDEL US strains) was recently confirmed (Lin et al. 2015b).

In vivo biological differences in pathogenicity for neonatal pigs are evident among TGEV, CCoV, and FIPV strains. Whereas virulent FIPV caused diarrhea and intestinal lesions similar to those of virulent TGEV, CCoV caused no clinical signs and only slight villous atrophy. CCoV shed by acutely infected dogs, infected baby pigs, and induced serum VN antibodies to CCoV and TGEV (Woods and Wesley 1992). However, baby pigs and pregnant gilts infected with FIPV did not produce TGEV VN antibodies, but did develop some immunity to TGEV challenge.

PRCV strains have been characterized and sequenced (Britton et al. 1991; Costantini et al. 2004; Kim et al. 2000b; Rasschaert et al. 1990; Vaughn et al. 1995; Zhang et al. 2007). Two striking features characterize the PRCV genome: (1) a large deletion (621–681 nt) near the N-terminus of the S gene producing a smaller S protein (Figure 31.3) and (2) a variable region with deletions that compromise ORF3 downstream of the S gene. These genetic changes may account for the altered tissue tropism of PRCV (Ballesteros et al. 1997; Sanchez et al. 1999). An overall nucleotide and aa sequence identity of 96–98% between TGEV and PRCV suggests that PRCV evolved from TGEV (Zhang et al. 2007) and that this occurred on a number of independent occasions.

Disease outbreaks caused by swine CoVs are endemic or variable in swine-producing countries. Nevertheless, the diseases induced by these CoVs have resisted eradication

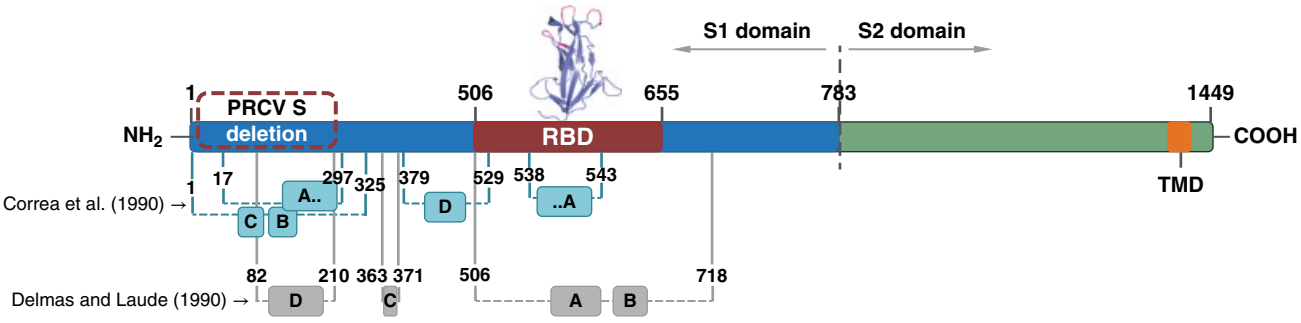


Figure 31.3 Schematic diagram of TGEV S protein. RBD, receptor-binding domain (shown in red); TMD, transmembrane domain (shown in orange). According to Correa et al.'s (1990) classification (light blue boxes), site A is discontinuous and located around positions 17 and 297 and 538 and 543, site B is located between amino acids 1 and 325, and site D is located between 379 and 529. The relative order of sites C and B and part of site A is not determined. According to Delmas and Laude's (1990) classification (light gray boxes), S protein antigenic sites D, C, and A/B are located between amino acids 82 and 210, between 363 and 371, and between 506 and 718, respectively. Dotted red box indicates a 227 aa deletion in the PRCV S protein. *Source:* Adapted from Correa et al. (1990) and Delmas and Laude (1990).

efforts, and the viruses may continue to evolve in domestic pigs, wild boars, and other potential reservoir (bats) or secondary hosts, such as carnivores, via interspecies transmission.

Transmissible gastroenteritis virus and porcine respiratory coronavirus

Relevance

TGE is a highly contagious enteric viral disease of swine characterized by vomiting, severe diarrhea, and high mortality (often 100%) in piglets less than 2 weeks of age. TGE was first described by Doyle and Hutchings (1946) in the United States and subsequently reported worldwide. Although swine of all ages are susceptible to TGEV or PRCV infection, the mortality in TGEV and/or PRCV seropositive herds and in swine over 5 weeks of age is generally low.

The appearance and widespread prevalence of PRCV, a naturally occurring deletion mutant of TGEV, lessened the clinical impact of TGE (Brown and Cartwright 1986; Laude et al. 1993; Pensaert et al. 1986, 1993; Pensaert 1989; Yaeger et al. 2002). However, sporadic outbreaks of severe diarrhea in piglets caused by TGEV in TGEV/PRCV seronegative herds are still reported in North America, Europe, and Asia. Currently, TGEV and PEDV co-circulate in Asia, Europe, and the United States, and recently, pathogenic recombinant TGEV/PEDV variants (swine enteric coronavirus [SeCoV]) were identified and characterized in Europe (Akimkin et al. 2016; Belsham et al. 2016; Boniotti et al. 2016). SeCoV that contains PEDV S gene on a TGEV backbone reportedly causes disease clinically indistinguishable from that caused by TGEV and PEDV. This epidemiological situation requires frequent monitoring and development of reliable tools for differential diagnosis (Kim et al. 2001; Masuda et al. 2016).

Etiology

TGEV antigen can be demonstrated by IF staining in the cytoplasm of infected cells 4–5 hours post infection (Pensaert et al. 1970). Maturation of virus occurs in the cytoplasm by budding through the endoplasmic reticulum, and viral particles (65–90 nm in diameter) are observed within cytoplasmic vacuoles (Figure 31.4a) (Pensaert et al. 1970; Thake 1968). Virus may line host cell membranes after exit from infected cells (Figure 31.4b). A similar intracellular replication scenario has been described for PEDV (Figure 31.4c). TGEV glycoproteins are also evident on the surface of infected ST cells (Laviada et al. 1990).

TGEV is stable when stored frozen, but labile at room temperature or higher. Infectious virus persisted in liquid manure slurry for more than 8 weeks at 5 °C (41 °F), 2 weeks at 20 °C (68 °F), and 24 hours at 35 °C (95 °F) (Haas et al. 1995). In recent studies that used TGEV as a surrogate for SARS CoV (Casanova et al. 2009), it remained infectious in water and sewage for several days at 25 °C (77 °F) and for several weeks at 4 °C (39 °F).

TGEV is highly photosensitive. Fecal material containing 1×10^5 pig infectious doses (PID) was inactivated within 6 hours when exposed to sunlight or to ultraviolet light (Cartwright et al. 1965; Haelterman 1962). TGEV is inactivated by exposure to 0.03% formalin, 1% Lysovet (phenol and aldehyde), 0.01% beta-propiolactone, 1 mM binary ethylenimine, sodium hypochlorite, NaOH, iodines, quaternary ammonium compounds, ether, and chloroform (Brown 1981; VanCott et al. 1993). TGEV field strains are trypsin resistant, relatively stable in pig bile, and stable at pH 3 (Laude et al. 1981), allowing virus to survive in the stomach and small intestine. However, properties of attenuated and field strains of TGEV vary.

Public health

Pigs are the main species naturally susceptible to TGEV and PRCV. No infection of humans has been reported.

Epidemiology

On a herd basis, two epidemiologic forms of TGE are recognized: epidemic and endemic. Infections with the TGEV deletion mutant PRCV present a different pattern, greatly complicating seroprevalence studies of the epidemiology of TGEV (Pensaert 1989).

Epidemic versus endemic TGE

Epidemic TGE occurs when most of the animals in a herd are TGEV/PRCV seronegative and susceptible. After introduction, the disease spreads rapidly to swine of all ages, especially during winter. Inappetence, vomiting, or diarrhea occurs in most animals. Suckling pigs show marked clinical signs and rapidly dehydrate. Mortality is very high in pigs under 2–3 weeks of age but decreases in older pigs. Lactating sows often develop anorexia and agalactia, with reduced milk production, which further contributes to piglet mortality.

Endemic TGE refers to the persistence of the virus and disease in a herd perpetuated by the continual or frequent influx of susceptible swine. Endemic TGE is a common sequel to a primary outbreak and occurs in seropositive herds that have frequent farrowings (Stepanek et al. 1979), herd additions, or commingling of susceptible pigs. In endemically infected herds, TGEV

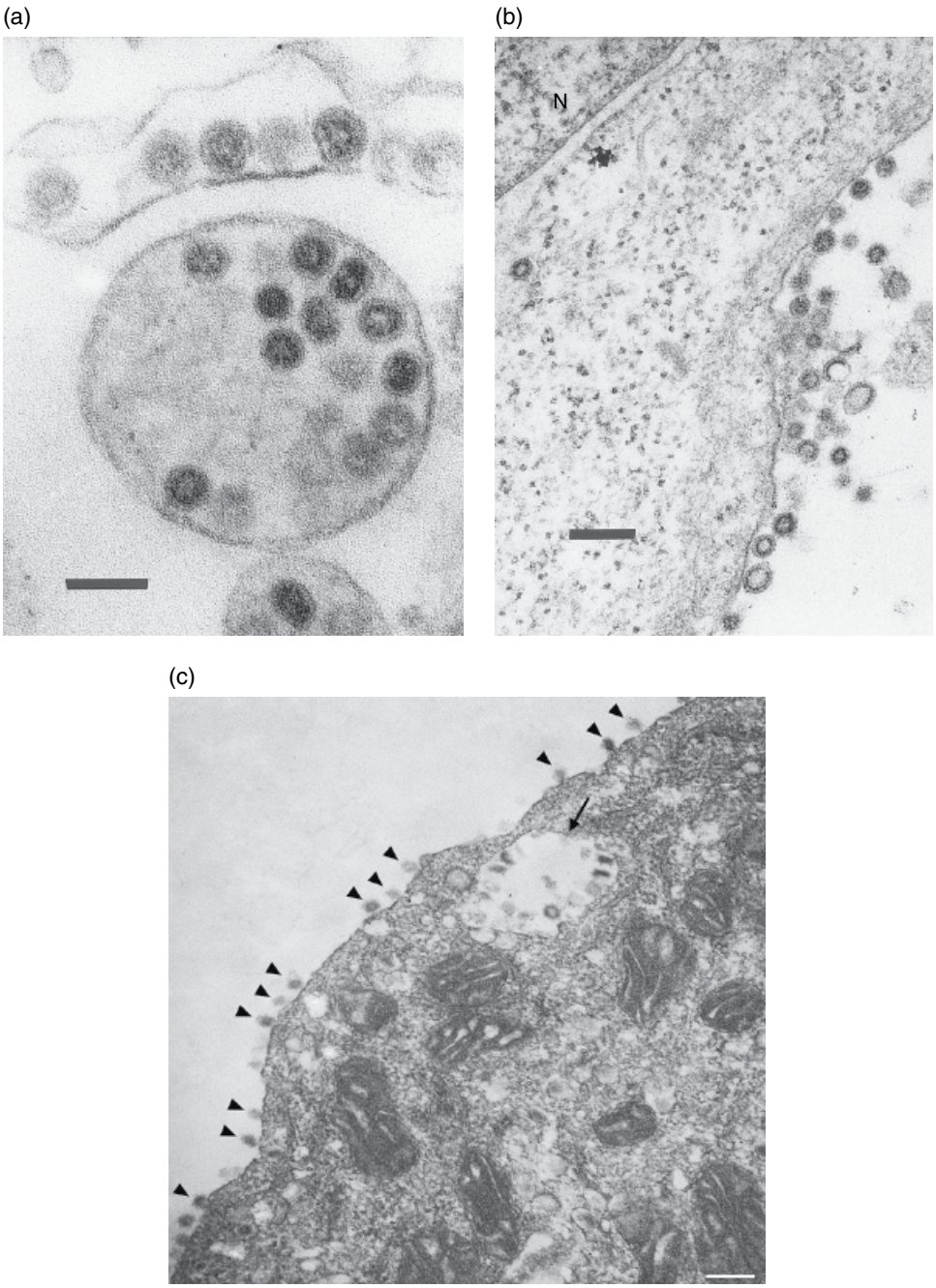


Figure 31.4 Electron micrographs. (a) TGEV in vesicles of the endoplasmic reticulum of a pig kidney cell (36 hours post infection). Bar = 100 nm. (b) TGEV lining the cell membrane of a pig kidney cell (36 hours post infection). N = nucleus; bar = 200 nm. (c) A PEDV-infected Vero cell. PEDV particles (arrow heads) on the cell surface and inside a vesicle (arrow) of the infected Vero cell. Scale bar = 200 nm. For (c), *Source:* Oka et al. (2014). Reproduced with permission of Elsevier.

spreads slowly among adult swine (Pritchard 1987). Sows are frequently immune and asymptomatic and will transfer a variable degree of passive lactogenic immunity to their progeny. In these herds, mild TGEV diarrhea occurs, and mortality is usually under 10–20% in pigs from approximately 6 days of age until approximately 2 weeks post weaning. The age-related effects are influenced by the management system and the degree of passive immunity from the sow.

Endemic TGE in suckling or recently weaned pigs can be difficult to diagnose and must be differentiated from other types of endemic diarrheal pathogens common in young pigs, such as PEDV, PDCoV, rotavirus, and *Escherichia coli*. Endemic TGE persists in the herd as long as susceptible or partially immune swine are exposed to TGEV. It is unclear whether the source of virus is from reactivation of virus shedding in carrier swine or reintroduction of virus into the herd from an external source.

Porcine respiratory coronavirus

PRCV is a TGEV variant that infects the respiratory tract with limited or no shedding in feces (Pensaert 1989). However, PRCV-infected pigs produce antibodies that neutralize TGEV. The first isolation of PRCV was in Belgium in 1984 (Pensaert et al. 1986). In 1989, PRCV was detected in two herds in the United States with no history of TGEV vaccination or clinical disease (Hill et al. 1990; Wesley et al. 1990).

Swine population density, distance between farms, and season influence PRCV epidemiology (Have 1990; Pensaert 1989). PRCV infects pigs of all ages by contact or airborne transmission. PRCV infections are often subclinical. The risk of PRCV spread increases in areas of high swine density, where the virus can travel several kilometers. The virus has spread rapidly and extensively in pigs in Europe (Brown and Cartwright 1986; Have 1990; Laude et al. 1993; van Nieuwstadt et al. 1989) and became endemic even in TGEV-free countries (Laude et al. 1993; Pensaert 1989; Pensaert et al. 1993). A limited serological survey in 1995 in the United States suggested that many asymptomatic herds in Iowa were seropositive for PRCV (Wesley et al. 1997).

PRCV circulates in the herd, infecting pigs before the age of 10–15 weeks after passively acquired maternal antibodies have declined. Introduction of pigs into fattening units and commingling of PRCV-negative and PRCV-positive pigs from diverse sources result in seroconversion to PRCV in pigs shortly after introduction into most units.

Susceptible pigs experimentally infected with PRCV shed virus from nasal secretions for less than 2 weeks (Onno et al. 1989; VanCott et al. 1993; Wesley et al. 1990). There is no evidence for the fecal–oral transmission of PRCV. PRCV persists in closed breeding farms by regularly infecting newly weaned pigs, even in the presence of maternal antibodies (Pensaert et al. 1993). PRCV can persist in the herd throughout the year, or it can disappear in summer and reappear in the nursery and fattening units in winter. Coincident with the widespread dissemination of PRCV, the seroprevalence of TGEV in Europe has decreased, to a low prevalence (Brown and Paton 1991; Pensaert et al. 1993).

Transmission and reservoirs

An epidemiological feature of TGE is its seasonal appearance during winter. Haelterman (1962) suggested that this is because the virus is stable when frozen and more labile when exposed to heat or to sunlight. This would allow virus transmission between herds in winter on fomites or animals. He proposed at least three possible reservoirs for TGEV between seasonal epidemics: (1) pig farms in which the virus spreads subclinically, (2) hosts other than swine, and (3) carrier pigs. There is evidence for the existence of TGEV in non-porcine hosts. Cats, dogs, and foxes have been suggested as possible carriers of TGEV from one herd to another, since they can shed virus in their feces for variable periods (Haelterman 1962; McClurkin et al. 1970) and virus excreted by dogs was infectious for pigs (Haelterman 1962; Reynolds and Garwes 1979).

The concentration of starlings (*Sturnus vulgaris*) in winter in feeding areas of swine may foster mechanical spread of TGEV among farms. Pilchard (1965) reported that TGEV was detected in the droppings of starlings for up to 32 hours after feeding TGEV. Houseflies (*Musca domestica*) have also been proposed as possible mechanical vectors for TGEV. TGEV antigen was detected in flies within a swine herd, and experimentally inoculated flies excreted TGEV for 3 days (Gough and Jorgenson 1983). According to surveys conducted in Central Europe, antibodies against TGEV are also present in approximately 30% of the feral pig population (Sedlak et al. 2008).

The third possibility relating to TGE transmission is the duration of TGEV shedding and the role of the carrier pig. Nasal shedding of PRCV in experimentally infected pigs occurs through 10 days post infection (DPI) (Onno et al. 1989; Wesley et al. 1990). However, how long pigs clinically recovered from TGEV and PRCV infection in the field remain infectious is unknown. One report indicated chronic and/or persistent TGEV fecal shedding for up to 18 months, suggesting a possible role for the long-term carrier hog in transmitting TGEV (Woods and Wesley 1998). Although TGEV has been detected in the intestinal and respiratory tracts for periods of up to 104 DPI (Underdahl et al. 1975), it is unknown whether infectious virus is shed or transmitted. Addition of sentinel pigs to a herd at 3, 4, and 5 months after a previous TGE outbreak resulted in no infections in the introduced pigs, as determined by serologic tests (Derbyshire et al. 1969).

Pathogenesis

Intestinal and extraintestinal replication of TGEV

Jejunal enterocytes undergo massive necrosis within 12–24 hours after infection, resulting in marked reduction in enzymatic activity (alkaline phosphatase, lactase, etc.) in the small intestine. This disrupts digestion and cellular transport of nutrients and electrolytes (including sodium), thereby causing an accumulation of liquid in the intestinal lumen and acute malabsorptive diarrhea (Moon 1978) that leads to severe and fatal dehydration in piglets (Butler et al. 1974) and loss of extravascular protein. Dehydration is also related to metabolic acidosis coupled with abnormal cardiac function due to hyperkalemia.

The severe villous atrophy in the jejunum (Figure 31.5a and b) and to a lesser extent in the ileum of TGEV-infected pigs is often absent in the proximal duodenum (Hooper and Haelterman 1966a). Villous atrophy is more

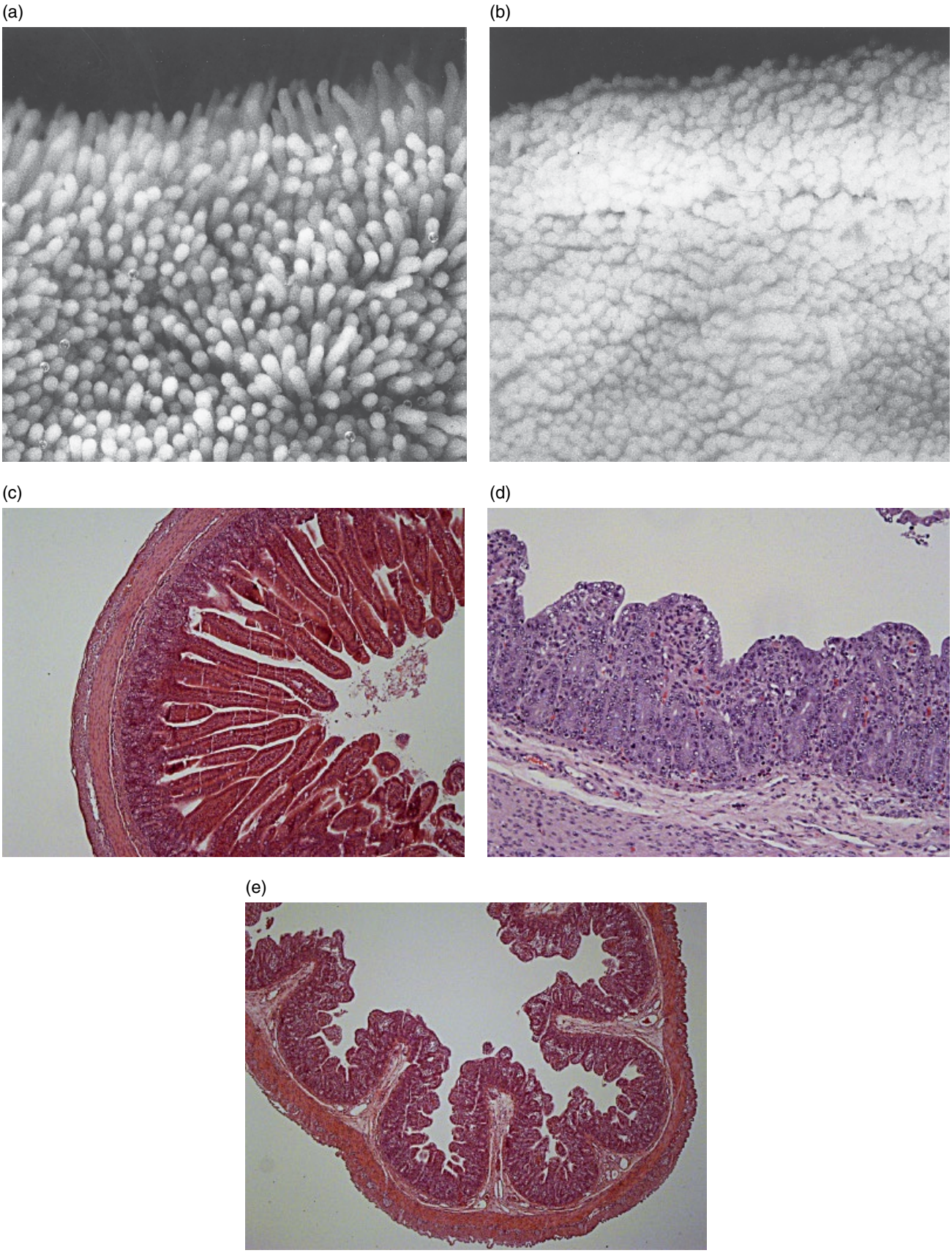


Figure 31.5 Villi of the jejunum from a normal pig (a) and from a TGEV-infected pig (b), as viewed through a dissecting microscope (approximately ×10). Hematoxylin and eosin (H&E)-stained jejunum of a normal gnotobiotic pig (17 days of age), showing normal villi (×80) (c) (*Source:* Jung et al. 2015b); of a PEDV-infected gnotobiotic pig (26 days of age) at 46 hours post inoculation (at onset of clinical signs), showing acute diffuse, severe atrophic jejunitis (×200) (d) (*Source:* Jung et al. 2014); and of a PDCoV-infected gnotobiotic pig (17 days of age) at 3 days post inoculation (×40) (e) (*Source:* Jung et al. 2015b).

severe in newborn pigs than in 3-week-old pigs (Moon 1978), suggesting higher susceptibility of neonates to TGEV infection. A similar degree and distribution of small intestinal villous atrophy is also evident for PEDV (Figure 31.5c and d) and PDCoV (Figure 31.5c and e).

Mechanisms to account for age-dependent susceptibility to clinical disease include the slower replacement in newborn pigs of infected villous epithelial cells by migration of cells from crypts (Moon 1978). These newly replaced villous enterocytes are reportedly resistant to TGEV infection, possibly due to induction of innate immunity and intestinal IFN (Abou-Youssef and Ristic 1972) or the inability of the regenerating cells to support virus growth.

The exposure dose of infectious virus plays a major role in age-dependent susceptibility. The infectious dose of TGEV needed to infect a 6-month-old market hog was 10^4 times greater than that needed to infect a 2-day-old piglet (Witte and Walther 1976). Moreover, the severity of clinical signs due to TGEV increased when pigs were injected with a synthetic corticosteroid, dexamethasone (Shimizu and Shimizu 1979), similar to dexamethasone-aggravated lung pathology in PRCV infection (Jung et al. 2007; Zhang et al. 2008), indicating the possible effect of stress on TGEV/PRCV disease severity. In addition, TGEV in combination with other enteric pathogens, such as *E. coli* or porcine rotavirus, caused more severe enteritis than either infection alone (Underdahl et al. 1972). Likewise, PRCV respiratory infection and lung lesions were exacerbated by preexisting porcine reproductive and respiratory syndrome virus (PRRSV) infection (Jung et al. 2009; van Reeth et al. 1996).

Extraintestinal sites for TGEV replication include lungs (alveolar macrophages) and mammary tissues (Kemeny et al. 1975). Oronasal infection of pigs with TGEV caused pneumonia (Underdahl et al. 1975). Cell culture-attenuated but not virulent TGEV replicated in cultures of alveolar macrophages *in vitro*, suggesting a possible role for these cells in lung infection (Laude et al. 1984). Moreover, TGEV was detected in nasal secretions of infected piglets (VanCott et al. 1993) and lactating sows exposed to infected piglets (Kemeny et al. 1975). Cell-cultured strains of TGEV generally showed reduced virulence in pigs, with less replication in the gut and higher levels of replication in the upper respiratory tract compared with virulent TGEV (Frederick et al. 1976; VanCott et al. 1993).

TGEV replicated in mammary tissues of lactating sows (Saif and Bohl 1983) and infected sows shed virus in milk (Kemeny and Woods 1977). The clinical or epidemiological significance of mammary gland infection with TGEV under field conditions is unclear, but agalactia is often seen in TGEV-infected sows and TGEV spreads rapidly among pigs.

Replication of PRCV in the respiratory tract

PRCV has a tropism for the respiratory tract. It replicates to high titers in porcine lungs (1×10^7–10^8 $TCID_{50}$) in type 1 and 2 pneumocytes and infects epithelial cells of the nares, trachea, bronchi, bronchioles, alveoli, and, occasionally, alveolar macrophages (Atanasova et al. 2008; Jung et al. 2007, 2009; O'Toole et al. 1989; Pensaert et al. 1986). PRCV induces necrosis of infected cells, increasing innate immune responses at the infection sites, including high levels of IFN-α and nitric oxide in lungs (Jung et al. 2009, 2010). Innate cytokines inhibit initial viral replication and modulate Th1/Th2 responses with the latter enhancing B-cell responses, leading to secretion of VN antibodies.

Virus shedding in nasal secretions lasted for 4–6 days after experimental PRCV infection. The severity of PRCV-induced pneumonia and viral replication in lung peaked at 8–10 DPI, coinciding with increased numbers of T and B cells and frequency of lymphocytic inflammation. Thereafter, pulmonary lesions and clinical signs resolved concurrently with increased VN antibody titers (Atanasova et al. 2008; Jung et al. 2009).

Depending on the experimental conditions and the virus strains used, PRCV may be detected in blood, tracheobronchial lymph nodes, and occasionally the small intestines of infected pigs. However, virus in infected enterocytes does not spread to adjacent cells (Cox et al. 1990a,b), and fecal shedding is low or undetectable. The limited intestinal replication of PRCV may be related to the deletion in the S gene. When fecal and nasal isolates of PRCV from the same pigs were compared genetically, only point mutations, but not additional deletions, were noted in the S gene (Costantini et al. 2004).

Clinical signs

Epidemic TGE

Typical clinical signs of TGE in seronegative piglets are vomiting and profuse watery, yellowish diarrhea, with rapid loss of weight, dehydration, and high morbidity and mortality in pigs under 2 weeks of age. The severity of clinical signs, duration of disease, and mortality are inversely related to the age of the pig. Most pigs under 7 days of age will die in 2–7 days after onset of clinical signs. Most suckling pigs over 3 weeks of age will survive, but may remain stunted. Clinical signs of TGE in finishing swine and in sows include inappetence, transient diarrhea, and vomiting.

The incubation period is short, usually 18 hours to 3 days. Infection generally spreads rapidly through the entire group, and most swine are affected in 2–3 days, but this is more likely to occur in winter than summer (Haelterman 1962).

Endemic TGE

Endemic TGE occurs in large herds that farrow frequently and in TGEV or PRCV seropositive herds. Clinical signs are usually less severe than those in seronegative pigs of the same age. Mortality is low, especially if pigs are kept warm. The clinical signs in suckling pigs can resemble rotavirus, PEDV, or PDCoV diarrhea (Bohl et al. 1978; Pensaert and de Bouck 1978; Wang et al. 2014a). In some herds, endemic TGE is manifested primarily in weaned pigs and may be confused with PEDV (Madson et al. 2014), *E. coli*, coccidia, or rotavirus infections (Pritchard 1987).

Porcine respiratory coronavirus

Experimentally, PRCV infection of pigs is mostly subclinical with self-limiting respiratory infection. The early antiviral effects of innate immune responses to PRCV infection, followed by cell-mediated and antibody responses, likely effectively control the infection (Atanasova et al. 2008; Jung et al. 2007, 2009, 2010; Zhang et al. 2008). Clinical signs include (1) respiratory signs (e.g. coughing, abdominal breathing, dyspnea), (2) depression and/or anorexia, and (3) slightly decreased growth rates (Lanza et al. 1992; van Reeth et al. 1996; Wesley and Woods 1996).

The severity and frequency of clinical signs are influenced by the presence of other bacterial or viral pathogens in the herd. For example, coinfection with PRRSV can alter the severity of either PRCV or PRRSV infections. Inoculation with PRRSV followed by PRCV resulted in prolonged fever with respiratory disease, reduced weight gain, and prolonged severe pneumonia (Jung et al. 2009; van Reeth et al. 1996). Ongoing or preexisting PRRSV infection significantly suppressed innate immune responses (reduced IFN-α levels in lung and blood natural killer [NK] cell cytotoxicity) during early PRCV infection, which may exacerbate PRCV pneumonia (Jung et al. 2009).

TGEV lesions

TGE gross lesions are confined to the gastrointestinal tract. The stomach is distended with curdled milk and may have petechial hemorrhages (Hooper and Haelterman 1966b). The small intestine is distended with yellow fluid and curdled, undigested milk. The wall is thin and transparent, due to villous atrophy. A major lesion of TGE is markedly shortened villi of the jejunum and ileum (Figure 31.5a and b), similar to PEDV and PDCoV lesions (Figure 31.5c–e) (Debouck et al. 1981; Jung et al. 2015b), but usually more severe and extensive than that seen in rotavirus diarrhea (Bohl et al. 1978). Infections with some strains of *E. coli* and coccidia may produce similar lesions (Hornich et al. 1977). Transmission EM of TGEV-infected villous enterocytes has revealed alterations in the microvilli, mitochondria, endoplasmic reticulum, and other cytoplasmic components. Virus particles, primarily in cytoplasmic vacuoles, were observed in villous enterocytes and in M cells, lymphocytes, and macrophages in the dome regions of Peyer's patches (Chu et al. 1982; Thake 1968).

Pathologic findings and the extent of villous atrophy are highly variable in pigs from endemically infected herds (Pritchard 1987). Moxley and Olson (1989) showed that the level of passive immunity in TGEV-infected pigs influenced both the degree of villous atrophy and its segmental distribution. Villous atrophy was minimal in pigs nursing sows previously infected with virulent TGEV, compared with pigs nursing seronegative sows or sows given live attenuated vaccines. In partially protected pigs, villous atrophy was primarily in the ileum and not the jejunum. Similar observations were noted in pigs from herds with endemic TGE.

PRCV lesions

PRCV primarily causes upper and lower respiratory tract disease. The PRCV-induced lesions are generally limited to the lungs and commonly observed as consolidation of the lung and bronchointerstitial pneumonia, with frequent peribronchiolar and perivascular lymphohistiocytic cuffing (Atanasova et al. 2008; Cox et al. 1990a; Halbur et al. 1993; Jabrane et al. 1994; Jung et al. 2007, 2009). PRCV-induced bronchointerstitial pneumonia is characterized by (1) thickening of the alveolar septa by infiltration of inflammatory leucocytes, principally macrophages and lymphocytes; (2) type 2 pneumocyte hypertrophy and hyperplasia; (3) accumulation of necrotic cells and inflammatory leucocytes in alveolar and bronchiolar lumina due to airway epithelial necrosis; and (4) peribronchiolar or perivascular lymphohistiocytic inflammation. Within 10 days of PRCV infection, the virus simultaneously induces inflammatory (cell necrotizing) and proliferative (alveolar septal thickening) chronic-active bronchointerstitial pneumonia (Jung et al. 2007, 2009).

Diagnosis

The collection and preservation of appropriate clinical specimens is necessary for reliable diagnosis. Because clinical signs and atrophic enteritis caused by TGEV are frequently observed in other enteric infections (rotavirus, PEDV, PDCoV, and coccidia), laboratory diagnosis of TGE must be accomplished by one or more of the following procedures: detection of viral antigen or nucleic acids in feces or lesions, virus isolation from specimens, or detection of TGEV antibodies.

Diagnosis of PRCV requires similar procedures, but with a focus on respiratory specimens. Evaluation of

clinical signs, histologic lesions, and tissue distribution of viral antigen may provide a presumptive diagnosis. PRCV does not cause diarrhea or villous atrophy and replicates almost exclusively in respiratory tissues (Pensaert 1989). Thus, PRCV is suspected if there is antigen in lung tissues, seroconversion to TGEV/PRCV, and no signs of enteric disease.

Detection of viral antigens or nucleic acids

Detection of TGEV antigen in small intestinal enterocytes is commonly used to diagnose TGE. Either IF (Pensaert et al. 1970) or immunohistochemical (IHC) (Shoup et al. 1996) techniques using MAb against the highly conserved N protein of TGEV may be used in frozen or formalin-fixed tissues (Figure 31.6a), but they require pigs in the early stage of infection. A similar viral antigen distribution is seen in the small intestine of PEDV- (Figure 31.6b) and PDCoV-infected pigs (Figure 31.6c). An exception for PEDV and PDCoV is the occasional detection of viral antigens in the crypt epithelial cells and the colon.

An enzyme-linked immunosorbent assay (ELISA) using MAb or polyclonal antibodies to TGEV is used to detect TGEV antigens in cell culture, feces, and intestinal contents (Lanza et al. 1995; Sestak et al. 1996, 1999a; van Nieuwstadt et al. 1988) or PRCV antigen in cell culture, nasal swabs, or lung homogenates (Lanza et al. 1995).

RT-PCR or real-time RT-PCR is currently used for diagnosis of TGEV and differentiation of TGEV, PRCV, PDCoV, and PEDV (Costantini et al. 2004; Kim et al.

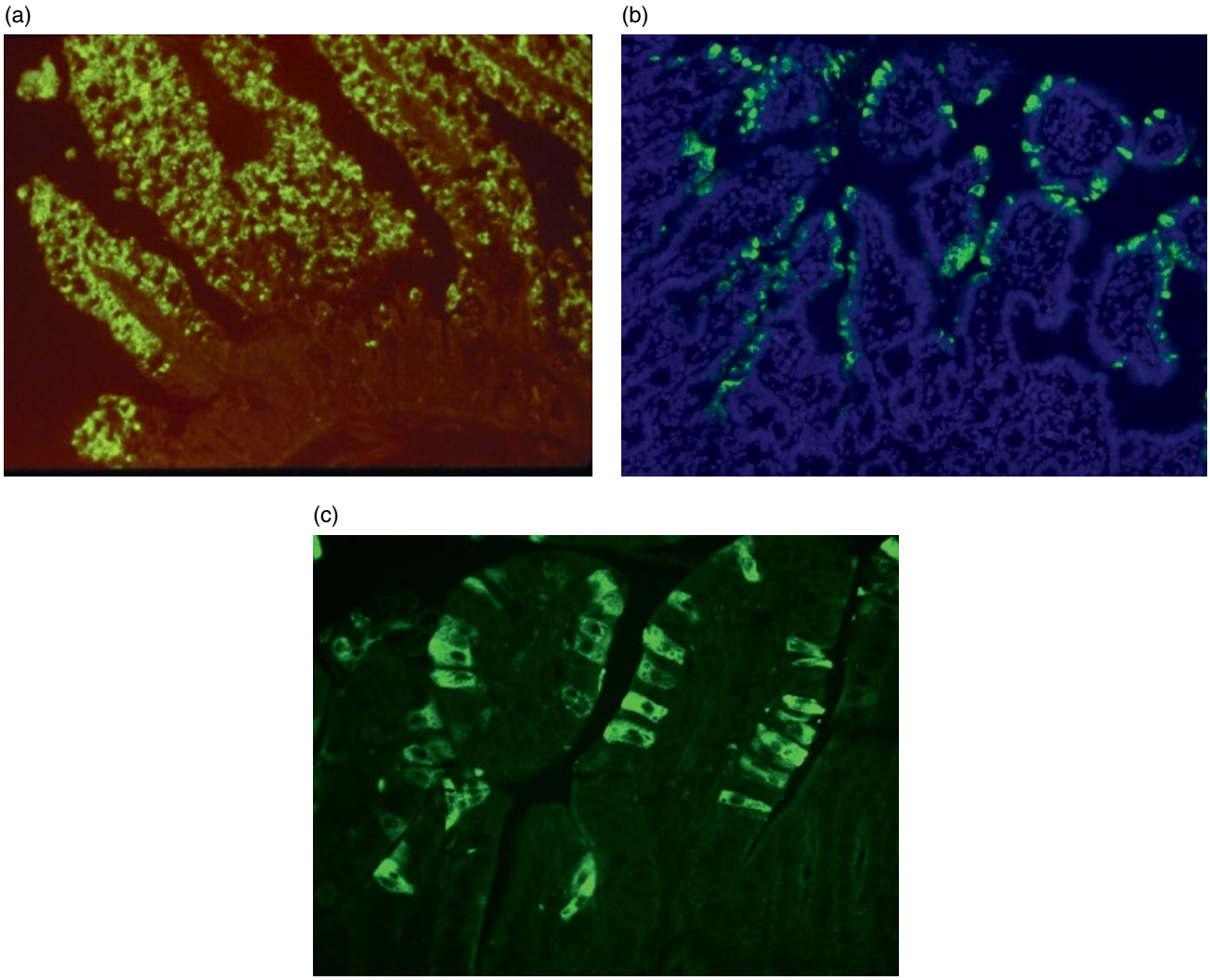


Figure 31.6 Immunofluorescent staining (green) of (a) TGEV antigens in almost 100% of the ileal enterocytes lining the villi of a TGEV-infected piglet. Note absence of TGEV antigens in the crypt epithelial cells. (b) PEDV antigens in the enterocytes of the jejunum of a piglet at 67 hours post inoculation with the emerging non-S INDEL PEDV strain PC21A (37–41 hours after onset of clinical signs), indicating that the epithelial cells lining atrophied villi are positive for PEDV (×200). *Source:* Jung et al. 2014. (c) PDCoV antigens in the jejunum of a gnotobiotic pig at 3 days post inoculation with PDCoV strain OH-FD22, showing similar localization of PDCoV antigens in the cytoplasm of villous epithelial cells (×400).

2000a, 2001, 2007; Masuda et al. 2016; Ogawa et al. 2009). PRCV/TGEV differentiation is accomplished using PCR primers targeting the S gene deletion region in PRCV strains. Multiplex RT-PCR and real-time RT-PCR assays have been developed for the simultaneous detection of major porcine viruses associated with diarrhea including rotavirus, TGEV, PDCoV, and PEDV (Masuda et al. 2016; Ogawa et al. 2009). These assays permit detection of up to nine viruses in a sample. Moreover, multiplex microarray hybridization was employed for the rapid differential diagnosis of eight CoVs including TGEV (Chen et al. 2005).

Electron microscopy (EM)

TGEV can be demonstrated in the intestinal contents and feces of infected pigs by negative contrast transmission EM (Figure 31.2a). Immune electron microscopy (IEM) has advantages over conventional EM in being more sensitive for detecting TGEV and distinguishing it from PEDV, PDCoV, and enveloped membranous debris, as well as concurrently detecting the presence of other enteric viruses (Figure 31.2b) (Saif et al. 1977).

Virus isolation

Primary and secondary pig kidney (PK) cells (Bohl and Kumagai 1965) or cell lines (Laude et al. 1981), porcine thyroid cells (Witte 1971), and the McClurkin swine testicle (ST) cell line (McClurkin and Norman 1966) are recommended for the isolation of TGEV from feces or gut contents of infected pigs. Distinct cytopathic effects (CPE) may be negligible upon primary isolation of field strains, requiring additional passages. The CPE consists of enlarged, rounded cells with a balloon-like appearance (Bohl and Kumagai 1965). For detecting viral CPE or plaques, the sensitivity of ST cells can be further enhanced by adding pancreatin or trypsin to cell culture media (Bohl 1979) and using older cells.

Pig kidney and ST cells are preferred for isolating PRCV from nasal swab fluids or lung tissue homogenates. PRCV- and TGEV-induced CPE are similar, with syncytia frequently observed as also reported for PEDV and SARS CoV grown in Vero cells (Hofmann and Wyler 1988; Ksiazek et al. 2003). Identification of cell culture virus can be done by VN, IF staining, or IEM using specific TGEV antiserum or differential MAbs (Garwes et al. 1988) and RT-PCR using virus-specific primers (Enjuanes and Van der Zeijst 1995; Kim et al. 2000a; Laude et al. 1993).

Serology

TGEV antibodies can be detected by several serologic tests. However, TGEV serology is complicated by the fact that both TGEV and PRCV induce VN antibodies that are qualitatively and quantitatively similar (Pensaert 1989). A blocking ELISA test can differentiate between these antibodies based on using MAbs to TGEV antigenic sites that are absent on the PRCV S protein (Bernard et al. 1989; Callebaut et al. 1989; Delmas and Laude 1990; Garwes et al. 1988; Sanchez et al. 1990; Sestak et al. 1999b; Simkins et al. 1992, 1993). Blocking ELISAs should only be applied on a herd basis because some pigs with low TGEV or PRCV antibody titers may not be detected (Callebaut et al. 1989; Sestak et al. 1999b; Simkins et al. 1993) and the accuracy of commercial ELISAs for differentiating US strains of PRCV and TGEV is low (Sestak et al. 1999b). ELISA tests were used to differentiate not only between TGEV and PRCV antibodies but also between TGEV and TGEV-like CCoVs or classical CCoV-II antibodies (Elia et al. 2010; Lopez et al. 2009).

A rise in antibody titer between acute and convalescent serum samples provides retrospective evidence for TGEV or PRCV infection. To determine the presence of endemic TGE or PRCV, serum samples from 2- to 6-month-old swine (often free of passively acquired antibodies at this age) can be tested for antibodies (Derbyshire et al. 1969). The VN test using cell culture-adapted viruses has been the most widely used (Bohl 1979; Bohl and Kumagai 1965). VN antibodies to TGEV are detectable in serum by 7–8 DPI and persist for at least 18 months. Little is known regarding the persistence of VN antibodies to PRCV within a herd. Antibody ELISA tests (Bernard et al. 1989; Berthon et al. 1990; Callebaut et al. 1989; Garwes et al. 1988; Sestak et al. 1999a, b; van Nieuwstadt et al. 1989) have been reported, but they require concentrated purified virus or S or N protein for coating ELISA plates.

Immunity

Active immunity to TGEV

The duration of active immunity in swine after oral infection with virulent TGEV has not been well characterized. Intestinal infection of breeding-age swine results in detectable serum antibodies that persist for at least 6 months and possibly several years (Stepanek et al. 1979). Although serum antibodies provide serologic evidence of TGEV or PRCV infection, they afford little indication of the degree of active immunity to TGEV. Swine that have recovered from TGE are immune to subsequent short-term challenge, presumably due to local immunity within the intestinal mucosa (Brim et al. 1995; Saif et al. 1994; VanCott et al. 1993, 1994). The age and immune status of the animal at initial infection and the severity of the challenge influence the completeness and duration of active immunity.

The mechanism of active immunity in the gut relates to stimulation of the secretory IgA (sIgA) immune system with production of sIgA antibodies by intestinal plasma cells (Saif et al. 1994; VanCott et al. 1993, 1994). IgA TGEV antibodies and antibody-secreting cells (ASCs)

have been detected in the intestine and serum of pigs after oral, but not parenteral inoculation with TGEV (Kodama et al. 1980; Saif et al. 1994; VanCott et al. 1993, 1994). Kodama et al. (1980) proposed that detection of IgA antibody in the serum, presumably intestinally derived, might serve as an indicator of active immunity to TGE. Enzyme-linked immunospot (ELISPOT) assay was used to investigate the kinetics of IgA and IgG TGEV antibody production by the pig's systemic and local gut-associated lymphoid tissues (GALT). High numbers of IgA ASCs were induced in GALT only by virulent TGEV. In contrast, live attenuated (vaccine) TGEV or PRCV strains induced significantly fewer IgA ASCs (Berthon et al. 1990; Saif et al. 1994; VanCott et al. 1993, 1994). Besides local antibody-mediated immunity, cell-mediated immunity (CMI) may also be important in active immunity against TGEV infections. However, only indirect evidence exists concerning the role of CMI in resistance to TGEV infection. CMI was demonstrated with lymphocytes obtained from GALT of swine orally infected with virulent TGEV (Brim et al. 1995; Frederick et al. 1976; Shimizu and Shimizu 1979), whereas swine parenterally or oronasally inoculated with attenuated TGEV or PRCV developed CMI mainly in systemic sites. Lymphoproliferative responses to TGEV persisted within GALT, but not systemic lymphocytes, for at least 110 days after oral infection of 6-month-old swine (Shimizu and Shimizu 1979), but for only about 14–21 days after infection of younger (7- to 11-day-old) pigs (Brim et al. 1995). CD4 T helper cells are involved in lymphoproliferative responses to TGEV (Anton et al. 1995). Potent production of antiviral IFN-α by plasmacytoid dendritic cells (DCs) derived from TGEV-infected swine was observed upon stimulation of these cells *in vitro* with TGEV antigens (Calzada-Nova et al. 2010).

A correlation between lymphoproliferative responses and lactogenic immunity to TGEV was described in sows vaccinated with attenuated or recombinant TGEV vaccines (Park et al. 1998). Although T-cell epitopes were identified by lymphoproliferation studies for each of the three major proteins of TGEV, a dominant functional T helper epitope was defined on the N protein (N321) (Anton et al. 1995). The N321 peptide-induced T cells collaborated in the *in vitro* synthesis of TGEV VN antibodies specific for the S protein. Maximal responses were induced by native S protein combined with recombinant N protein. Such findings have important implications for design of CoV subunit or other recombinant CoV vaccines.

Because lymphocyte cytotoxicity was absent in newborn piglets and decreased in parturient sows, it was proposed that a lack of NK cell activity against TGEV-infected cells might correlate with the increased susceptibility of newborn piglets and parturient sows to TGEV infection (Cepica and Derbyshire 1984). Thus, CMI or innate immunity may play a role in either recovery from TGEV infection or resistance to reinfection via the rapid elimination of TGEV-infected epithelial cells. Some TGEV strains can also downregulate host immune responses. A virulent (SHXB) but not attenuated (STC3) TGEV strain impaired the ability of porcine intestinal DCs or monocyte-derived DCs to recognize antigen, migrate, and induce T-cell proliferation *in vivo* and *in vitro* (Zhao et al. 2014).

PRCV-induced active immunity to TGEV

The dramatic decline in epidemic outbreaks of TGE in Europe following the widespread dissemination of PRCV prompted researchers to examine if respiratory PRCV infection could induce protective intestinal immunity against TGEV. The consensus from several studies was that prior infection of nursing or weaned pigs with PRCV provided partial immunity against TGEV challenge, as evidenced by a reduced duration and level of virus shedding and diarrhea in most pigs studied (Brim et al. 1995; Cox et al. 1993; VanCott et al. 1994; Wesley and Woods 1996).

This partial immunity presumably is related to the rapid increase in TGEV VN antibodies (Cox et al. 1993; Wesley and Woods 1996) and numbers of IgG and IgA ASCs in the intestines of PRCV-exposed pigs after TGEV challenge (Saif et al. 1994; VanCott et al. 1994). The altered tissue tropism of PRCV was also linked to a shift in antibody responses; that is, in TGEV-infected pigs, more IgA ASCs were found in gut, whereas PRCV predominantly induced IgG ASCs in the lung (VanCott et al. 1994). Migration of PRCV IgG and IgA ASCs from the bronchus-associated lymphoid tissues (BALT) to the gut of the PRCV-exposed pigs after TGEV challenge might explain the rapid anamnestic response and the partial protection induced (VanCott et al. 1994). However, neonatal pigs required at least 6–8 days after PRCV exposure to develop partial immunity to TGEV challenge (Wesley and Woods 1996).

Passive immunity to TGEV

Passive lactogenic immunity is critical to provide newborn piglets with immediate protection against TGEV infection. Circulating passive antibodies, acquired after absorption of colostral immunoglobulin (primarily IgG), protect the neonate against systemic but generally not intestinal infection (Hooper and Haelterman 1966a; Saif and Sestak 2006). Mechanisms of passive immunity to TGEV infections have been reviewed (Chattha et al. 2015; Saif and Bohl 1979; Saif and Jackwood 1990; Saif and Sestak 2006). Swine recovered from TGE transmit passive immunity to their suckling pigs by the frequent ingestion of colostrum or milk (lactogenic immunity) that contains TGEV VN antibodies (Hooper and Haelterman 1966a). Such antibodies in the lumen of the

intestine neutralize the ingested TGEV and protect the susceptible small intestinal enterocytes. This is accomplished naturally when piglets suckle immune sows frequently or by continuous feeding of antiserum to piglets. During the first week of lactation, IgA becomes dominant in milk and IgG decreases.

TGEV IgA antibodies in milk are stable in the gut and provide the most effective protection, but IgG antibodies are also protective if high titers are maintained in milk after vaccination (Bohl and Saif 1975) or by artificial feeding of colostral IgG antibodies (Stone et al. 1977). TGEV IgG antibodies are produced in the sow's milk after parenteral or systemic immunization, whereas TGEV IgA antibodies occur in milk after intestinal infection. It is postulated that IgA immunocytes migrate to the mammary gland after antigenic stimulation in the gut where they localize and secrete IgA antibodies into colostrum and milk that play a key role in passive intestinal immunity of suckling pigs (Bohl and Saif 1975; Saif and Bohl 1979; Saif and Jackwood 1990; Saif and Sestak 2006). The "gut–mammary" immunologic axis, first proposed in relation to TGEV infections in swine (Bohl et al. 1972; Saif et al. 1972), provided the initial concept for a common mucosal immune system. This concept continues to be important in the design of maternal vaccines that are capable of providing effective lactogenic immunity against enteric pathogens.

PRCV-induced passive immunity to TGEV

The incidence and severity of TGE in countries with PRCV has declined since PRCV has become widespread (Schwegmann-Wessels and Herrler 2006). This suggests that prior exposure of swine to PRCV imparts partial immunity to TGEV (Laude et al. 1993; Pensaert 1989).

Prior natural exposure of sows to PRCV induced a variable degree of passive protection (44–53% mortality) against experimental TGEV challenge of suckling pigs (Bernard et al. 1989; Paton and Brown 1990). Variable protection in the field during TGE outbreaks was also noted among litters of PRCV-exposed sows (Pensaert 1989; Sanchez et al. 1990). Similar variable levels of protection (30–67% mortality) were reported after TGEV challenge of piglets suckling sows that had been experimentally infected or reinfected with PRCV during pregnancy (De Diego et al. 1992; Lanza et al. 1995; Sanchez et al. 1990; Sestak et al. 1996; Wesley and Woods 1993). In the latter two studies, litter mortality was lowest (range = 0–27%), and IgA and IgG milk antibody titers were highest in sows multiply exposed to PRCV during two subsequent pregnancies. These experimental findings agreed with field reports that naturally PRCV-exposed sows reinfected with PRCV during pregnancy secreted PRCV IgA antibodies in milk and provided a high degree of protection (0–12.5% mortality) to TGEV challenge (Sanchez et al. 1990). Besides PRCV IgA antibodies in milk, a hallmark of protection in these and other studies (Wesley and Woods 1993) was induction of active immunity to TGEV in the sow preventing clinical disease or agalactia.

Besides quantitative differences in the levels of IgA antibodies induced in milk of sows after exposure to TGEV or PRCV, researchers have investigated potential differences in virus epitopes recognized by the milk IgA antibodies (De Diego et al. 1992, 1994). In TGEV-infected sows, antigenic subsite A (Aa, Ab, Ac), followed by antigenic subsite D, was the best inducer of IgA antibodies, while after PRCV infection, antigenic site D and subsite Ab were immunodominant (Figure 31.3). Thus, only IgA recognizing at least antigenic sites A and D conferred protection *in vivo*, whereas any immunoglobulin isotype reactive to one antigenic site neutralized virus *in vitro*.

Prevention and control

Treatment

No antiviral drugs have been developed for treatment of TGE. After the discovery of SARS CoV, studies were conducted with various surrogate viruses, including TGEV, to develop anti-CoV agents. Ortego et al. (2007) used TGEV deletion mutants to show that absence of the E protein blocks virus trafficking in the endoplasmic reticulum and prevents virus maturation. RNA interference (RNAi) targeting the viral RNA polymerase was studied *in vitro* as a strategy to prevent TGEV infection (Zhou et al. 2007). Although protective *in vitro*, the results of analogous *in vivo* experiments were less convincing (Zhou et al. 2010).

Studies suggest that IFN may activate NK cells in newborn pigs, contributing to resistance to challenge with TGEV (Lesnick and Derbyshire 1988; Loewen and Derbyshire 1988). In addition, during a field outbreak of TGE, 1- to 12-day-old piglets treated orally for 4 days with 1–20 IU of human IFN-α had significantly greater survival rates than placebo-treated piglets (Cummins et al. 1995).

The only available treatment for TGE is to alleviate starvation, dehydration, and acidosis. Parenteral treatment with fluids, electrolytes, and nutrients are effective in treating young pigs, but not practical under farm conditions. Oral therapy with balanced electrolyte or glucose solutions is contraindicated in young pigs (Moon 1978). The following measures are suggested: provide a warm (above 32 °C [90 °F]), draft-free, dry environment and provide water or nutrient solutions freely to TGEV-infected pigs. Such measures reduced mortality in pigs that were infected at more than 3–4 days of age. Antibacterial therapy is beneficial in 2- to 5-week-old pigs if there is concurrent infection with bacterial pathogens. Cross-fostering of infected or susceptible litters

onto TGE-immune sows was useful in some field outbreaks (Stepanek et al. 1979).

Management

Biosecurity

Swine in the incubative or viral shedding stage of the disease or possibly carriers can transmit TGEV. To introduce swine into a herd, precautions are needed to assure that swine originate from herds free of TGE, are serologically negative, and/or have been placed in isolation on the farm for 2–4 weeks before being added to the herd. After a TGE outbreak, at least 4 weeks should elapse from the last sign of disease before introducing such animals into a "clean" herd. Feces from TGEV-infected swine can be carried on boots, shoes, clothing, truck beds, feeds, and so on and can be a source of infection to other herds, requiring strict disinfection regimes, especially in winter.

After onset of TGE and endemic TGEV

When TGE occurs on a farm and pregnant animals have not yet been exposed, two procedures may minimize losses of newborn pigs: (1) If the sows are due to farrow in at least 2 weeks, use feedback methods to orally expose them to virulent autogenous virus, such as a slurry of minced intestines of acutely infected pigs, so that they will be immune at farrowing. (2) If the sows will farrow in under 2 weeks, attempt to provide facilities and management procedures to avoid exposure to TGEV until at least 3 weeks post farrowing.

Some success has been achieved in elimination of TGEV from epidemically infected closed breeder herds without depopulation by the following procedures (Harris et al. 1987): (1) bring in breeding stock replacements for the next 4–6 months; (2) in the face of an outbreak, feedback TGEV acutely infected minced piglet intestines simultaneously to all pigs in the herd (including replacement stock) to eliminate susceptible hosts, shorten the time the disease progresses through the herd, and ensure more uniform exposure levels in all pigs; (3) maintain strict all-in/all-out production in farrowing and nursery units; and (4) add sentinel seronegative pigs about 2 months after clinical signs of TGE disappear and monitor these pigs for seroconversion to TGEV. Potential hazards associated with feedback control of TGE include possible spread of other pathogens to pregnant sows and throughout the herd.

Other approaches to control or terminate endemic TGE include the following. First, pregnant seropositive sows can be vaccinated intramuscularly late in gestation or shortly after farrowing with live attenuated TGEV vaccine to boost immunity, increase milk antibody levels, and maintain longer passive immunity in suckling pigs (Saif and Sestak 2006; Stepanek et al. 1979). Although this procedure may only delay onset of TGE in exposed pigs, the delay itself can reduce mortality. Second, break the cycle of infection by eliminating reservoirs of susceptible pigs in a unit: prevent the continual influx of susceptible animals into the herd temporarily (alter farrowing schedule as possible), utilize other facilities, and create smaller farrowing and nursing units to achieve an all-in/all-out system.

Immunoprophylaxis

Vaccines and vaccinations

There are several licensed TGEV vaccines. All contain inactivated or live attenuated TGEV and are approved for use in pregnant or neonatal swine. These vaccines and their efficacy have been reviewed (Saif and Sestak 2006) but will be briefly summarized.

Many variables complicate the evaluation of both experimental and commercial TGEV vaccines, resulting in conflicting data. These include the challenge dose and strain of TGEV, the age of the pig at challenge, environmental conditions (especially temperature), the health status and milking efficiency of the vaccinated sow, and the immune status (for TGEV or PRCV antibodies) of the dam at vaccination. If previously infected sows were unknowingly used in vaccine challenge studies, this could account for discrepant results seen in immune responses and piglet protection. This possibility can only be eliminated by using sensitive tests (such as VN) to measure TGEV/PRCV antibodies and by knowing the herd history of test animals since occurrence of PRCV in herds further complicates TGEV vaccine studies.

TGE vaccination of the seronegative pregnant dam

A variety of viral vaccines (virulent, attenuated, inactivated, and subunit) and routes of administration (oral, intranasal, intramuscular, subcutaneous, and intramammary) (Bohl and Saif 1975; Moxley and Olson 1989; Saif and Bohl 1979; Saif and Jackwood 1990; Saif and Sestak 2006) have been tested for induction of lactogenic immunity. Only oral administration of live virulent virus to pregnant sows consistently stimulated high levels of protective immunity for the sow and persisting TGEV IgA antibodies in milk that passively protected piglets.

The generally poor results for oral or intranasal vaccination of sows using attenuated TGEV strains (Moxley and Olson 1989; Saif and Bohl 1979; Saif and Sestak 2006) may be attributed to the limited replication of most attenuated strains in the sow's intestine (Frederick et al. 1976). This results in little antigenic stimulation of intestinal IgA immunocytes and correspondingly little IgA antibody secretion in milk. Thus, the dilemma is how to develop commercial TGEV vaccines that are capable of stimulating IgA in the gut of sows, but sufficiently attenuated so as not to produce disease in newborn pigs.

Parenteral TGEV vaccines induced even lower or inconsistent protection rates in TGEV/PRCV seronegative swine. They have two major disadvantages: (1) Vaccinated swine develop little or no gut immunity and often get sick when exposed to TGEV, depriving their suckling pigs of milk. (2) The low titer IgG and no IgA TGEV antibodies in milk of vaccinated sows fail to provide optimal passive protection to suckling pigs. Currently available parenterally administered TGEV vaccines may be more effective in boosting immunity in pregnant swine previously infected with TGEV or PRCV than in initiating immunity in seronegative pregnant swine. These vaccines may be especially useful in herds in which endemic TGE is a problem (Stepanek et al. 1979).

TGE vaccination of neonatal or weaned pigs

Active immunization of suckling or feeder pigs could be important for control of endemic infections, especially in newly weaned pigs, in which TGEV infections result in increased mortality. Live attenuated and inactivated TGEV vaccines have been licensed in the United States for oral or intraperitoneal administration, respectively, shortly after birth. However, the presence of maternal antibodies in vaccinated pigs decreased or completely suppressed (Furuuchi et al. 1978; Hess et al. 1982; Lanza et al. 1995; Sestak et al. 1996) active antibody production following oral administration of attenuated TGEV vaccines. Other approaches using recombinant TGEV proteins (reviewed in next section) have been used in attempts to actively immunize young pigs against TGEV.

Recombinant vaccine approaches

Among the major structural proteins of TGEV, the S protein contains immunodominant epitopes recognized by VN antibodies. Epitopes for continuous domains (Delmas and Laude 1990) were incorporated into synthetic peptides derived from the S protein (Posthumus et al. 1991). However, a peptide containing the major T helper cell epitope derived from the N protein has been reported to cooperate with the S protein for *in vitro* induction of TGEV antibody (Anton et al. 1996).

To express the TGEV S (or S epitopes), M, or N proteins, several prokaryotic and eukaryotic systems such as *E. coli*, *Salmonella*, adenovirus, vaccinia virus, pox virus, baculovirus, DNA vectors, and plants were used (Enjuanes et al. 1992; Godet et al. 1991; Gomez et al. 2000; Meng et al. 2013; Park et al. 1998; Shoup et al. 1997; Smerdou et al. 1996; Torres et al. 1996; Tuboly et al. 2000; Yuan et al. 2015). In some studies (Torres et al. 1996), but not others (Gomez et al. 2000; Smerdou et al. 1996; Tuboly et al. 2000), protective antibodies were induced in inoculated animals correlating with partial protection (Park et al. 1998; Shoup et al. 1997). A novel approach to passive immunization was suggested by feeding the recombinant immunoproteins capable of inducing TGEV VN antibodies to sows to confer passive immunity to piglets (Bestagno et al. 2007). The approach may be cost effective by expressing these proteins in plants (Monger et al. 2006).

Various levels of VN antibodies and protection were induced using eukaryotic vectors to express the TGEV S glycoprotein encoding the glycosylation-dependent antigenic determinants (sites A and B) with or without sites C and D (Figure 31.3). The baculovirus- or vaccinia virus-expressed S glycoprotein of TGEV induced low titers of VN antibodies in serum, colostrum, and milk, but low or no protection (Godet et al. 1991; Hu et al. 1985; Shoup et al. 1997; Tuboly et al. 1995). Only S glycoprotein constructs containing antigenic site A induced high VN antibody titers. Sites C and D induced only low titer VN antibodies, but interestingly, they primed pigs for secondary serum antibody responses after challenge (Shoup et al. 1997).

Similar findings were evident in studies using the same baculovirus-expressed S constructs administered IM to boost antibody responses in sows vaccinated orally with attenuated TGEV vaccines: the partial protection rates were comparable with IM boosting with attenuated TGEV vaccine (Park et al. 1998). Baculovirus-expressed TGEV structural proteins (S, N, and M) coadministered IP with *E. coli* mutant LT adjuvant induced TGEV IgA antibody responses associated with reduced TGEV shedding in challenged pigs (Sestak et al. 1999b).

Recent studies have used molecular approaches to develop vectored TGEV vaccines and test them in porcine and murine models. A human adenovirus engineered to express the TGEV or PRCV S proteins (Callebaut et al. 1996; Torres et al. 1996; Tuboly and Nagy 2001) elicited variable protection against TGEV mortality and little protection against TGEV or PRCV infection. An oral *Lactobacillus casei*-based vaccine expressing repetitive (20X and 40X) peptides of the antigenic D site of TGEV S protein induced humoral and T-cell-based immune responses and Th17 polarization in mice (Jiang et al. 2014, 2016). Additionally, an attenuated *Salmonella typhimurium* vector expressing TGEV N or M proteins and a recombinant *Bacillus subtilis* strain expressing TGEV S protein induced T-cell proliferation, antibody and cytokine responses in mice, and antibody responses in pigs, respectively (Mou et al. 2016; Qing et al. 2016; Zhang et al. 2016a). Yuan et al. (2015) expressed the A epitope of the S protein in swinepox virus and demonstrated that this vaccine administered to sows was immunogenic and protected piglets against clinical disease. DNA plasmids were generated for PEDV and TGEV for the development of DNA vaccines that were immunogenic in mice, but not tested in pigs (Meng et al. 2013).

An effective TGEV vaccine should primarily elicit an intestinal immune response (Saif and Jackwood 1990; Saif and Sestak 2006; VanCott et al. 1993). Further improvements of TGEV vaccines might be achieved by the use of mucosal adjuvants/delivery systems such as immunostimulating complexes (ISCOMs), vitamin A, probiotic bacteria, biodegradable microspheres, or infectious recombinant TGEV clones engineered to enhance TGEV immunogenicity and reduce pathogenicity (Chattha et al. 2015; Enjuanes et al. 2005). Studies of TGEV infectious cDNA minigenomes indicate that this approach also can be used for targeted delivery of immunogens derived from other pathogens to the intestine or respiratory tract.

Porcine epidemic diarrhea virus

Relevance

In 1971, acute outbreaks of diarrhea in feeder and finishing pigs were observed in England (Oldham 1972). The disease spread to other European countries, and the name "epidemic viral diarrhea" (EVD) was adopted. In 1976, similar outbreaks were observed, but in swine of all ages, including suckling pigs (Wood 1977) and, in 1978, a CoV-like agent was associated with the outbreaks in piglets (Chasey and Cartwright 1978; Pensaert and de Bouck 1978). Experimental inoculations with the Belgian isolate (CV777) revealed its enteropathogenicity for piglets and growing pigs (Debouck and Pensaert 1980), and the names "porcine epidemic diarrhea" (PED) and PED virus (PEDV) were adopted (Debouck et al. 1982). In the 1970s and 1980s, PEDV caused widespread epidemics in Europe, with severe losses in suckling pigs. Since then, PEDV has been associated more often with isolated outbreaks and recurrent diarrheic problems in weaned and feeder pigs. However, epidemics also occurred, as in Italy in 2005–2006.

In Asia, PEDV epidemics were first reported in 1982 and outbreaks continued through the 1990s and 2000s. The situation changed in 2010 when PED outbreaks in China caused by highly virulent PEDV strains resulted in the loss of >1 million piglets in 1 year (Sun et al. 2012). In 2013, PEDV outbreaks were reported in the United States, likewise with severe losses (Stevenson et al. 2013).

The initial PEDV strains are referred to as classical PEDV strains (Chen et al. 2013). The strains identified since 2010 are considered emerging PEDV strains. Variants of the emerging PEDV strains containing insertions and deletions in the S gene ("S INDEL" strains) were first detected in the United States (Lin et al. 2016; Wang et al. 2014b). By the end of 2016, the emerging PEDV strains, both non-S INDEL and S INDEL, had spread throughout North and South America, Asia, and Europe.

The situation continues to evolve. For example, recombinant enteric CoVs between TGEV and PEDV have been detected in Italy (2009–2012), in Germany (2012), and in Eastern Europe (2016) (Akimkin et al. 2016; Belsham et al. 2016; Boniotti et al. 2016).

Etiology

Morphologic and physicochemical properties of PEDV (Figure 31.2c) are similar to those of other members of the family *Coronaviridae* (Figure 31.2). Based on genetic and antigenic criteria, PEDV is included in the genus *Alphacoronavirus* together with bat coronavirus (BtCoV)/512/2005, TGEV, PRCV, FECoV, FIPV, CCoV, and HCoV 229E. Based on phylogenetic analysis of the complete genomes, the global PEDV strains are divided into two major groups: the classical PEDV strains that first emerged in the 1970s in Europe and the PEDV strains appeared after 2010 (Lin et al. 2016) (Figure 31.1). The emerging PEDV strains are further divided into "non-S INDEL" (mainly highly virulent) and "S INDEL" subgroups (Figure 31.1) because the former and latter cause severe and mild PED, respectively, in the field and in experimental pig challenge studies (see "Pathogenesis"). The S INDEL strains contain insertions and deletions similar to the classical PEDV strains in the S1 subunit of the S protein (Vlasova et al. 2014). They likely resulted from multiple recombination events between the classical and emerging PEDV strains in Asia, perhaps related to widespread use of live classical PEDV vaccine strains in swine. Other minor PEDV variants have been reported, e.g. US TC-PC177, USA/OK10240-8/2017, and Japanese TTR-2 strains, bearing large deletions (194–200 aa) in the N-terminal domain (NTD) of the S protein (Oka et al. 2014; Suzuki et al. 2015; Zhang et al. 2018). The 197-aa deletion (residues 34–230) of PC177 strain occurred during Vero cell adaptation, whereas the 194-aa deletion (residues 23–216) of the TTR-2 strain and the 200-aa deletion (residues 31–230) of the OK10240-8 strain were detected in clinical swine samples in Japan and the United States, respectively. Unlike the altered tissue tropism seen for PRCV (enteric to respiratory), the two PEDV strains (PC177 and TTR-2) retained their enteric tropism, but with reduced virulence (Lin et al. 2016; Suzuki et al. 2016).

Although PEDV variants have different insertions or deletions in the S glycoprotein and variation in the S glycoprotein of PEDV may be related to pathogenesis and cross-neutralizing activity, there appears to be only one PEDV serotype (Choudhury et al. 2016; Lin et al. 2016). There is no cross-neutralization between PEDV and TGEV or between PEDV and PDCoV (Lin et al. 2015b; Ma et al. 2016). However, a low degree of cross-reactivity

was observed between PEDV and other animal alphacoronavirus antibodies. For example, a TGEV MAb recognized PEDV N protein (Lin et al. 2015b), TGEV Miller antiserum reacted with PEDV N protein (Gimenez-Lirola et al. 2017), TGEV and PRCV antisera reacted with PEDV M protein (Gimenez-Lirola et al. 2017), and mink alphacoronavirus antiserum reacted with PEDV M and N proteins (Have et al. 1992).

Vero (African green monkey kidney) cells support the growth of PEDV in culture medium supplemented with trypsin (Figure 31.4c). CPE consists of vacuolation and large multinucleated syncytia (Hofmann and Wyler 1988). PEDV also grows in various swine cell lines, including bladder and kidney cells (Shibata et al. 2000; Wang et al. 2016b), ST cells (Liu et al. 2015a), alveolar macrophage cell line 3D4 (Park and Shin 2014), and small intestinal epithelial cells (IECs) (Cao et al. 2015; Cong et al. 2015). As reviewed by Teeravechyan et al. (2016), PEDV can also replicate in bat lung cell line Tb1-Lu (Liu et al. 2015a), duck IEC line MK-DIEC (Khatri 2015), and human liver cell line HuH-7 (Wang et al. 2016b).

pAPN, the cell receptor used by TGEV, was initially considered to be the putative receptor of PEDV with some supporting evidence (Cong et al. 2015; Li et al. 2007; Nam and Lee 2010); however, some recent studies argue that pAPN may not be a functional receptor for PEDV (Li et al. 2017; Shirato et al. 2016).

Public health

PEDV is only infectious for swine and does not play a known role in public health.

Epidemiology

Classical PEDV regularly caused epidemics in Europe from 1971 until the late 1980s, but reports after 2000 are rare. An epidemic in Italy (2005–2006) affected 63 herds, but mortality was largely restricted to suckling piglets (Martelli et al. 2008). Until the emergence of new PED outbreaks in 2014, PEDV was not considered important, and therefore, the prevalence of classical PEDV in Europe is unknown. Except for an outbreak associated with the emerging non-S INDEL strain in Ukraine in 2014, subsequent outbreaks in France, Germany, Belgium, Slovenia, and the Netherlands were due to the emerging S INDEL strains (Lin et al. 2016).

In Asia, classical PED appeared in China in the late 1970s, causing serious losses in many provinces (Wang et al. 2016a; Xuan et al. 1984). PED was recognized in Japan in 1982 (Kuwahara et al. 1988; Sueyoshi et al. 1995; Takahashi et al. 1983) and Korea in 1993 (Chae et al. 2000; Hwang et al. 1994; Kweon et al. 1993), but is known to be present in India (Barman et al. 2003) and Thailand (Puranaveja et al. 2009). In 2010, despite the widespread use of PEDV strain CV777 vaccines, severe PEDV outbreaks due to non-S INDEL strains occurred in China (Sun et al. 2012, 2016; Wang et al. 2016a). Later, emerging S INDEL strains were also detected in China (Wang et al. 2016a).

Since 2013, the emerging non-S INDEL PEDV strains have been detected in other Asian countries/regions outside of China, including Japan (Masuda et al. 2015), South Korea (Kim et al. 2015), Vietnam (Vui et al. 2014), Thailand (Cheun-Arom et al. 2015), Taiwan (Lin et al. 2014), and the Philippines (Kim et al. 2016). The S INDEL PEDV was also detected in Japan in 2013 (Suzuki et al. 2015) and Korea in 2014 (Lee et al. 2014).

The first highly virulent PED outbreak caused by non-S INDEL PEDV occurred in swine farms in the United States in April 2013 (Stevenson et al. 2013), followed by the detection of milder PED outbreaks caused by the S INDEL PEDV in January 2014 (Wang et al. 2014b). From 2013 to 2014, PEDV killed approximately 7 million piglets in the United States. In January 2017, PEDV had spread to 39 US states and to Puerto Rico. PEDV has also spread to other countries (e.g. Canada and Mexico) in the Western Hemisphere (Lin et al. 2016). PEDV has not been reported in Africa or Australia.

Direct or indirect fecal–oral transmission is the main route of PEDV transmission. Contaminated equipment, feed and feed ingredients, transportation, or personnel may serve as vehicles for PEDV transmission (Dee et al. 2014, 2016; Schumacher et al. 2016). Evidence of PEDV aerosol transmission has been reported in some (Alonso et al. 2014), but not other studies (Niederwerder et al. 2016). In emerging non-S INDEL PEDV experimentally infected 4-week-old pigs, infectious virus excretion assessed by PEDV transmission to susceptible sentinel pigs lasted 14–16 days (Crawford et al. 2015). However, at 42 days post-initial oral exposure, some pigs still shed PEDV RNA in feces, illustrating discordance between prolonged detection of PEDV RNA in feces and the transmission of infectious PEDV to susceptible pigs.

After an outbreak on a breeding farm, PEDV can become endemic through a cycle of infection of consecutive litters as they lose lactogenic immunity at weaning. Although a study from South Korea showed a PEDV infection rate of 9.75% in wild boars (Lee et al. 2016a), their role in the maintenance and transmission of PEDV is unknown.

Pathogenesis

The pathogenesis of PED is related to the age of pigs at the time of infection, virus strain virulence, inoculation routes, and doses.

The PEDV pathogenesis was first studied in piglets (3 days of age) orally inoculated with the classical PEDV

CV777 isolate (Coussement et al. 1982; Debouck et al. 1981) (Table 31.1). Clinical signs were observed after 22–36 hours. Viral replication occurred mainly in the cytoplasm of villous epithelial cells throughout the small intestine as early as 12–18 hours post inoculation (PI), peaking at 24–36 hours. Infection resulted in degeneration of enterocytes, leading to a reduction in the villous height/crypt depth (VH/CD) ratios from the normal 7 : 1 to ≤4 : 1. The pathogenic features of classical PEDV in the small intestine of piglets were very similar to those of TGEV, but somewhat less pronounced (Figure 31.5). PEDV replication was also observed in the colonic epithelium where slight cell degeneration was seen (Ducatelle et al. 1982). Occasionally, PEDV-positive crypt cells were also observed by IHC or IF staining, but the enterocyte regeneration capacity was preserved (Debouck et al. 1981; Sueyoshi et al. 1995). Shibata et al. (2000) showed that SPF pigs inoculated with field PEDV between the ages of 2 days and 12 weeks developed age-dependent resistance. That is, mortality was only observed in 2- to 7-day-old piglets. Pathogenic features of PED caused by classical PEDV strains described in Korea and Japan are very similar to those reported in Europe (Kim and Chae 2003; Sueyoshi et al. 1995).

Lohse et al. (2016) studied PEDV pathogenicity in 5-week-old pigs using classical PEDV strain (TC Br1/87, P3), an emerging S INDEL strain in Germany, and a non-S INDEL strain in the United States. Unfortunately, the S INDEL PEDV failed to infect pigs. Compared with the classical PEDV-infected pigs, the non-S INDEL PEDV-infected pigs had more severe clinical signs and histopathological changes, higher peak viral RNA shedding titers in feces, and longer detection of viral RNA in serum. These results suggested that the emerging non-S INDEL PEDV was more virulent than classical PEDV. However, concerns related to data interpretation include the following: (1) The Br1/87 inoculum was the Vero cell culture-adapted virus at passage 3, whereas the non-S INDEL was the wild-type virus from pigs and its infectious dose was not determined, so the disease outcomes may be due to different infectious doses. (2) The non-S INDEL inoculum contained a low amount of rotavirus by conventional RT-PCR. Therefore, coinfection with PEDV and rotavirus may influence disease severity. Generally, the pathogenesis and the age-dependent resistance of the emerging non-S INDEL PEDV strains were similar to those of the classical PEDV strains (Table 31.1) (Jung et al. 2014, 2015a; Madson et al. 2014; Niederwerder et al. 2016; Pensaert and Martelli 2016; Stevenson et al. 2013). Compared with the emerging non-S INDEL PEDV strains, S INDEL PEDV-infected piglets had lower mortality rates and less severe histopathological changes (milder villous atrophy) and less antigen in the small intestine (Table 31.1) (Chen et al. 2016a; Lin et al. 2015a). The pathogenicity of PEDV TTR-2 and TC-PC177 strains that have a large deletion in the NTD of the S protein was milder compared with that of the emerging non-S INDEL PEDV strains (Lin et al. 2016; Suzuki et al. 2016).

The infectious doses of PEDV differ for different ages of pigs: 100- to 1000-fold less PEDV was needed to infect younger pigs compared with the dose required to infect 3-week-old pigs (Thomas et al. 2015). The infectious dose of an emerging non-S INDEL PEDV strain (PC22A) was as low as 0.1 plaque-forming unit (PFU)/pig in 4-day-old Cesarean-derived colostrum-deprived (CDCD) piglets (Liu et al. 2015b). Doses of 0.1 PFU/pig and 1–10,000 PFU/pig caused diarrhea in 40 and 100% piglets, respectively. Thomas et al. (2015) compared the infectious doses for another emerging non-S INDEL PEDV strain (USA/IN19338/2013) in 5-day-old and 3-week-old pigs: 0.056 and 0.56–5600 $TCID_{50}$/pig caused diarrhea in 25 and 100% of neonatal piglets, respectively, and at least 100-fold higher doses (56–5600 $TCID_{50}$/pig) caused diarrhea in 100% of 3-week-old pigs. However, the infectious dose for older pigs, such as finisher pigs, has not been determined, but is expected to be higher than that needed to infect weaned pigs, as was observed for TGEV (Witte and Walther 1976).

During the acute phase of PEDV infection, viral RNA was detected transiently in the serum of PEDV-infected suckling and weaned pigs (Chen et al. 2016a; Jung et al. 2014, 2015a; Lohse et al. 2016; Suzuki et al. 2016). Peak RNA titers in serum were low (7–8 $\log_{10}$ GE/mL) compared with concurrent high peak RNA titers in feces (11–12 $\log_{10}$ GE/mL) (Jung et al. 2015a). Whether detection of viral RNA in serum represents infectious virus and the role of viremia in PEDV pathogenesis is unknown. In general, PEDV RNA titers are about 4–6 $\log_{10}$ higher than infectious titers (PFU or $TCID_{50}$) depending on different PEDV strains and/or the real-time RT-PCR assays (Jung et al. 2014; Song et al. 2016; Thomas et al. 2015).

Low levels of PEDV RNA were also detected in other tissues, such as the lung, liver, spleen, and muscle of pigs euthanized during acute PEDV infection (Chen et al. 2016a; Lohse et al. 2016; Park and Shin 2014). However, because the blood was not drained before collecting each tissue, the viral RNA was most likely from blood, except for the lungs, where PEDV antigens were detected by IHC (Park and Shin 2014). In the later study, the researchers found that a wild-type Korean PEDV non-S INDEL strain CNU-091222-01/2009 replicated in alveolar macrophages of infected pigs. Because no others have reported the detection of PEDV in the lungs, whether this is a unique characteristic of earlier emerging non-S INDEL strains (pre-2010) is unknown and needs to be investigated. In addition, PEDV RNA was detected from 40.8% (20/49) of sow milk samples during the emerging PEDV epidemics (Sun et al. 2012). TGEV replicated in the mammary glands of sows injected intramammarily

Table 31.1 Comparative pathogenesis of different clusters of PEDV in experimentally infected piglets (younger than 6 days of age).

PEDV strain	Inoculum/dose per pig	Pig type/age (day) at inoculation	Villous atrophy (VH:CD ratios)	Onset of clinical signs (hpi)	Vertical location of PEDV		Longitudinal distribution of PEDV		References
					Villous	Crypt	D, J, I	C	
Classical									
CV777	Fecal suspension/4 $\log_{10}$ PID	CDCD/2–3	Moderate to severe (1.5–4.2)	22–36	+++ (entire)	+	D, J, I (cont)	+	Coussement et al. (1982)
SNUVR971496	Cell culture (P3)/6.8 $\log_{10}$ $TCID_{50}$	Colostrum-deprived/1	Severe (1.1–3.3)	12–36	+++ (entire)	–	D, J, I (cont)	–	Kim and Chae (2003)
Non-S INDEL									
IN19338	Cell culture (P7)/0.056–5600 $TCID_{50}$	Conventional/5	Severe (1.2–1.7)	24	+++ (entire)	NR	D, J, I (cont)	NR	Thomas et al. (2015)
PC22A	Cell culture (P3)/1–4 $\log_{10}$ PFU	CDCD, conventional/3–4	Severe (0.8–2.3)	< 24	+++ (entire)	+	D, J, I (cont)	+	Liu et al. (2015b)
S INDEL									
Iowa106	Fecal suspension/10–12 $\log_{10}$ GE	Conventional/4	Moderate to severe (1.4–5.4)	24–72	++ (entire)	–	D (patchy), J, I (cont)	–	Lin et al. (2015)
IL20697	Cell culture/5 $\log_{10}$ $TCID_{50}$	Conventional with milk replacer/5	Mild	NR	+/++	NR	D, J (NR), I (patchy)	+	Chen et al. (2016a)

Source: This table is adapted/updated from Saif (1989). Reproduced with permission of Taylor and Francis.
PID, pig infectious dose; $TCID_{50}$, 50% tissue culture infectious dose; PFU, plaque-forming unit; GE, genomic equivalent; CDCD, Cesarean-derived colostrum-deprived; SPF, specific pathogen free; VH: CD, villous height/crypt depth ratio; hpi, hours' post inoculation; D, duodenum; J, jejunum; I, ileum; cont, continuous; NR, not reported; –, +, ++, and +++ denotes none, less than 30%, 30–60%, and more than 60% of villous enterocytes that were positive for PEDV antigens, respectively.

with live TGEV during lactation (Saif and Bohl 1983). Whether PEDV replicates in the mammary glands of sows, whether the PEDV RNA represents infectious PEDV, or whether the PEDV RNA in milk is from the saliva of their infected nursing piglets is unclear. Although some PEDV strains may replicate at low levels outside of the intestine, it remains unclear whether it contributes to PEDV pathogenesis.

PEDV infection results in massive loss of enterocytes and the malfunction of infected enterocytes, leading to maldigestive and malabsorptive diarrhea (Coussement et al. 1982; Debouck et al. 1981; Jung et al. 2006). During acute PEDV infection, gut integrity was reduced, leading to loss of water into the intestinal lumen and high osmotic pressure (Annamalai et al. 2015). The following factors may contribute to the more severe clinical signs, higher mortality rates, and slower recovery in PEDV-infected neonatal piglets compared with weaned pigs:

1) Slower turnover of villous enterocytes in neonatal piglets (5–7 days) compared with 2–3 days in 3-week-old weaned pigs (Jung et al. 2015a; Moon et al. 1975).
2) Increased numbers of intestinal stem cells and proliferation of crypt cells occurred later (3 days post-PEDV) in neonatal piglets than in weaned pigs (1 day post-PEDV) (Jung et al. 2015a).
3) Deficiency in innate immunity in suckling pigs compared with weaned pigs (Annamalai et al. 2015) (see "Immunity").

Clinical signs

PED shares most clinical features with TGE including watery diarrhea, vomiting, anorexia, and depression. On breeding farms, pigs of all ages become sick. Morbidity approaches 100% in piglets, but can vary in sows. Piglets up to 1 week of age may die from dehydration, and mortality ranges from 50 to 100%. Older pigs recover after about 1 week. In sows, diarrhea is variable, and they may only show depression and anorexia. In fattening pigs, all pigs in the unit may have watery feces within a week and often show severe anorexia and depression.

The disease on a breeding farm is self-limiting and stops when the pregnant sows develop lactogenic immunity to protect their offspring. The interval between onset and cessation of the disease is generally 3–4 weeks, but may be much longer in large breeding farms with multiple separated units. After the acute outbreak has passed, diarrhea may persist on the farm in weaned pigs and become recurrent (Martelli et al. 2008). PEDV may also be involved in a multi-etiologic diarrhea syndrome in feeder pigs appearing 2–3 weeks after they enter the fattening units, particularly when the pigs originate from different sources and when new pigs are continuously added to the fattening unit (van Reeth and Pensaert 1994).

Lesions

Lesions have been described in experimentally and naturally infected suckling piglets for classical PEDV (Coussement et al. 1982; Kim and Chae 2003; Pospischil et al. 1981; Sueyoshi et al. 1995), emerging non-S INDEL (Chen et al. 2016a; Jung et al. 2015a; Madson et al. 2015; Stevenson et al. 2013), and S INDEL PEDV infections (Chen et al. 2016a; Lin et al. 2015a). Lesions are confined to the small intestine that is distended with watery, yellowish fluid. Microscopically, vacuolation, syncytia, and exfoliation of small intestinal enterocytes occur mainly on the proximal villi. The small intestinal villi are reduced in length (Figure 31.5d), and the enzymatic activity of the intestine is markedly decreased. This pathology is very similar to that observed in TGE and PDCoV (Figure 31.5). No histopathologic changes have been observed in the colon, although PEDV antigens were detected in vacuolated colonic epithelial cells (Chen et al. 2016a; Debouck et al. 1981; Jung et al. 2014).

Diagnosis

Diagnosis should be made based on both clinical signs and laboratory detection of viral RNA, viral antigens, or increased PEDV antibodies. For the detection of PEDV RNA, the most widely used laboratory diagnostic method is RT-PCR (Ishikawa et al. 1997; Kim et al. 2001; Kubota et al. 1999; Liu and Wang 2016) or real-time RT-PCR (Kim et al. 2007; Wang et al. 2014d; Zhang et al. 2016b). Loop-mediated isothermal amplification (LAMP) assays (Ren and Li 2011; Yu et al. 2015) have been developed for the detection of PEDV RNA but are used less in diagnostic laboratories. Newer technology such as the specific primer-independent metagenomic sequencing (next-generation sequencing) can be used to determine the nearly complete (lacking 5′ and 3′ ends) viral genome sequences from clinical specimens (Chen et al. 2014; Marthaler et al. 2013). *In situ* hybridization can be used to detect PEDV RNA in fixed tissues (Kim and Chae 2000; Stadler et al. 2015).

Diagnosis can be made by direct demonstration of PEDV and/or its antigens using IF or IHC tests on the small intestinal tissues of pigs euthanized acutely near the onset of diarrhea and prior to the desquamation of enterocytes (Debouck et al. 1981; Guscetti et al. 1998; Jung et al. 2014; Stevenson et al. 2013; Sueyoshi et al. 1995) (Figure 31.6b). PEDV particles can be demonstrated using direct EM or IEM of feces of pigs collected acutely after diarrhea onset. Virus particles are difficult to recognize when the virion spikes are lost or not clearly visible. Furthermore, IEM must be applied to differentiate PEDV from TGEV and PDCoV because the CoVs have identical morphology (Figure 31.2).

Isolation of field strains of PEDV from intestinal contents/homogenates or feces is done in Vero cells or in other cell types. Trypsin treatment and blind passages

may be needed before CPE appears, but early detection can be done by IF staining (Hofmann and Wyler 1988; Shibata et al. 2000). Successful isolation of PEDV in Vero cells is higher with intestinal contents/homogenates than with feces (Chen et al. 2014; Oka et al. 2014).

Antigen-capture ELISAs have been developed for detection of PEDV antigens in feces using polyclonal antibodies and MAbs (Callebaut et al. 1982; Carvajal et al. 1995), but they are not widely used.

Paired serum samples are required for serologic diagnosis of endemic PEDV. Recently, IgG and IgA antibodies to PEDV were detected in oral fluids, suggesting they may be suitable to monitor prior herd exposure to PEDV (Bjustrom-Kraft et al. 2016). PEDV antibodies have been demonstrated with indirect ELISAs using antigens consisting of cell-cultivated virus (Carvajal et al. 1995; Hofmann and Wyler 1990; Kweon et al. 1994; Thomas et al. 2015), or S and N viral proteins extracted from infected Vero cells (Knuchel et al. 1992; Oh et al. 2005), or expressed in bacteria or using mammalian expression systems (Chen et al. 2016b; Gerber et al. 2014; Gerber and Opriessnig 2015; Hou et al. 2007; Okda et al. 2015; Paudel et al. 2014; Wang et al. 2015). Blocking and competitive ELISAs have also been developed for the detection of PEDV antibodies using MAbs or polyclonal antibodies as competitive antibodies (Carvajal et al. 1995; Okda et al. 2015; van Nieuwstadt and Zetstra 1991). Serum IgG antibodies against the N proteins of PEDV can be detected by 9–14 DPI, with titers peaking around 21 DPI and then declining gradually (Okda et al. 2015). Recently, a fluorescent microsphere immunoassay (FMIA) was developed (Gimenez-Lirola et al. 2017; Okda et al. 2015), but it is not widely used due to the need for specific equipment. The VN test in Vero cells is critical to assess VN antibodies to PEDV (Oh et al. 2005; Okda et al. 2015; Paudel et al. 2014; Thomas et al. 2015). These serological assays have been widely used to monitor prior exposure to the virus and to evaluate the efficacy of vaccines.

PEDV infections must be differentiated from TGE, SeCoV, and PDCoV, which in the case of acute diarrhea in swine of all ages can only be done through laboratory testing. Since SeCoVs are recombinants between TGEV (backbone) and PEDV (mainly S protein), only assays targeting both TGEV (any genes except for S gene) and PEDV (S gene) fragments can identify those viruses. In neonatal colibacillosis or rotavirus diarrhea, adult animals are not affected, and sick pigs usually are born from gilts or young sows. Laboratory techniques must be used to differentiate PED from other causes of diarrhea in weaned or feeder pigs.

Immunity

Evidence shows that PEDV has the ability to evade host IFN responses. Of 21 PEDV-encoded proteins, at least 11 proteins have been identified as IFN antagonists, which include both ORF1ab-encoded NS proteins (nsp1, nsp3, nsp5, nsp7, nsp14, nsp15, nsp16), structural proteins (E, M, N), and the accessory protein ORF3 (Ding et al. 2014; Wang et al. 2015; Zhang et al. 2016c). Identification of the virus-encoded IFN antagonists and understanding their mechanism of action may lead to novel therapeutic targets and more effective vaccines.

PEDV-infected suckling pigs had significantly lower NK cell frequencies, undetectable NK cell activity, and lower IFN-γ-producing $CD3^{-}CD4^{-}CD8^{+}$ NK cells in blood and ileum compared with PEDV-infected weaned pigs (Annamalai et al. 2015). Deficiency in innate immune function of neonatal NK cells may contribute to the more severe PEDV infection in suckling pigs compared with weaned pigs as also reported for TGEV infections (Derbyshire et al. 1969).

Inflammatory responses play a significant role in the pathogenesis of enteric CoVs. Compared with suckling pigs, weaned pigs had a delayed proinflammatory cytokine induction that coincided with the delayed onset of infection, disease, and shedding of PEDV RNA in feces (Annamalai et al. 2015). Toll-like receptor 2 (TLR2), TLR3, and TLR9 may contribute to NF-κB activation in response to PEDV infection in small IECs *in vitro* (Cao et al. 2015). The viral proteins E and N upregulated IL-8 expression by inducing endoplasmic reticulum stress and subsequent activation of the NF-κB pathway (Xu et al. 2013a,b).

Humoral immune responses to PEDV infection are very similar to those described for TGEV [reviewed in TGEV section (Chattha et al. 2015; Saif and Sestak 2006)]. VN antibodies are detectable in the serum, but may not play an important role because protection against enteric disease is primarily dependent on the presence of sIgA antibodies in the intestinal mucosa (Chattha et al. 2015; Langel et al. 2016). Immunity may not be long lasting, but a rapid anamnestic response upon reexposure may prevent reoccurrence of disease.

Although PED occurs in pigs of all ages, piglets up to 1 week of age may experience high mortality and need to be protected by maternal antibodies, especially VN and sIgA, via colostrum and milk from immunized dams. The mechanisms of lactogenic protection described for TGEV infection apply to PED as well (reviewed in TGEV section [Chattha et al. 2015; Langel et al. 2016]). Lactogenic immunity is induced in sows by intestinal infection with PEDV, which then activates the gut–mammary–sIgA axis. Pigs lose lactogenic protection at weaning and soon become susceptible to PEDV infection. Cell-mediated immunity likely plays a role in viral clearance, but there is no experimental data on this topic. PEDV may persist on the farm in susceptible pigs as part of recurring weaning diarrhea after an acute outbreak.

Prevention and control

PEDV is highly contagious, and strict sanitation and biosecurity are required to prevent virus entrance. Do not commingle sources or groups of pigs; ensure facilities and transportation vehicles are thoroughly washed, disinfected, and dried before pigs enter; and do not share boots, clothing, or equipment between different ages of pigs.

Feedback (intentional exposure of sows to virus using feces or small intestines from acutely infected piglets) will stimulate lactogenic immunity in the sow herd, reduce clinical signs in piglets, and shorten clinical outbreaks. Feedback may also be used in the nursery, grower, or finisher pigs, but nose-to-nose contact and fecal–oral spread will quickly contaminate the entire facility. It should be recognized that other pathogens present in clinically affected animals can be transmitted via the feedback process.

In Europe, the disease has been of insufficient economic importance to develop vaccines. In China, various inactivated and/or attenuated bivalent (TGEV and PEDV) or trivalent (TGEV, PEDV, and rotavirus strain NX) vaccines based on the classical PEDV strains have been available as early as in 1999 (Ma et al. 1995; Sun et al. 2016; Wang et al. 2016a). However, classical PEDV vaccines were not efficacious in protecting pigs against the highly virulent non-S INDEL PEDV infection (Li et al. 2012; Sun et al. 2012; Wang et al. 2013) and newer vaccines (e.g. a bivalent inactivated PEDV and TGEV vaccine based on the emerging non-S INDEL PEDV strain AJ1102) have appeared in the market. In Japan, a commercial attenuated PEDV vaccine based on the classical PEDV 83P-5 strain has been in use since 1997 (Sato et al. 2011). In Korea, attenuated vaccines based on strains KPEDV-9 (Kweon et al. 1999) or DR13 (Song et al. 2007) were commercialized in 1999 and 2004, respectively. Reportedly, not all sows given the vaccines developed protective lactogenic immunity (Song et al. 2015a). Two commercial vaccines are available in the United States. The first vaccine (June 2014) was developed using a replication-deficient Venezuelan equine encephalitis virus packaging system to express the PEDV S protein (Crawford et al. 2016). The second vaccine (September 2014) is an inactivated whole virus vaccine based on an emerging non-S INDEL PEDV strain (Crawford et al. 2016).

Porcine deltacoronavirus

Relevance

In February 2014, acute outbreaks of diarrhea associated with PDCoV were observed in sows and their piglets on five Ohio farms in the United States (Wang et al. 2014a). Previously, PDCoV had been reported in the feces of domestic pigs in China in 2012 (Woo et al. 2012), but the role of the virus as an enteric pathogen was unclear at that time. PDCoV has spread nationwide in the United States (Wang et al. 2014c) and caused deaths among suckling pigs (Anon 2014). Experimental studies verified that US PDCoV isolates are enteropathogenic in nursing pigs, as evident by acute, watery diarrhea and severe intestinal lesions (Figure 31.5c and e) (Chen et al. 2015; Jung et al. 2015b). However, the clinical impact and disease severity of PDCoV is less than that of epidemic PEDV and TGEV (Anon 2014).

Since the PDCoV outbreaks in the United States, it has also been identified on swine farms in Canada, Korea, China, Thailand, Vietnam, and Laos PDR, but in Canada and Korea, PDCoV failed to spread nationwide (Lee et al. 2016b; Marthaler et al. 2014b). Differential diagnosis of PDCoV, PEDV, and TGEV is critical to control CoV diarrheas in pig farms, especially in the regions where these CoVs have emerged or reemerged.

Etiology

PDCoV belongs to the genus *Deltacoronavirus* of the family *Coronaviridae*. Morphologic and physicochemical properties of PDCoV are similar to those of other members in the family *Coronaviridae* (Figure 31.2).

All global PDCoV strains overall share high nucleotide identities (Zhang 2016) (see "Epidemiology"). However, a comprehensive genetic analysis of global strains revealed that US/Korean PDCoV strains clustered together, Chinese strains clustered separately, and Thai strains formed another cluster (Zhang 2016) (Figure 31.1). Chinese PDCoV strains had multiple mutation or deletion sites in their S, NSP, or 3′ untranslated region (UTR) genes, whereas these mutations were not found in the genomes of US PDCoV strains (Wang et al. 2016c). Several investigators reported no cross-reactivity of PDCoV with antibodies to either PEDV or TGEV (Chen et al. 2015; Ma et al. 2015). However, another study reported antigenic cross-reactivity between US PDCoV and PEDV strains, possibly sharing at least one epitope on their N proteins (Ma et al. 2016).

LLC porcine kidney (LLC-PK) and ST cells supplemented with exogenous trypsin or pancreatin support the isolation and serial propagation of PDCoV in cell culture (Hu et al. 2015). The CPE consisted of enlarged and rounded cells and then cell shrinkage and detachment.

Recent studies have demonstrated that PDCoV employs pAPN as a major receptor for cellular entry, although it remains to be elucidated whether another receptor is involved in PDCoV infection (Li et al. 2018; Wang et al. 2018; Zhu et al. 2018).

Public health

There is no evidence that PDCoV is infectious for humans or plays a role in public health.

Epidemiology

The ancestral origin of PDCoV is unclear, but considering that PDCoV emerged recently, PDCoV may be incompletely adapted to pigs. Molecular surveillance in China and Hong Kong in 2007–2011 detected DCoVs only in pigs and wild birds (Woo et al. 2012). However, DCoVs were previously isolated from rectal swabs of small mammals, including Asian leopard cats and Chinese ferret badgers, at Chinese live animal markets in 2005–2006 (Dong et al. 2007). Their helicase and S genes were closely related to those of PDCoV. The data suggest the potential interspecies transmission of DCoVs between these wild small mammals, pigs, and birds. A recent study also revealed that PDCoV-inoculated gnotobiotic (Gn) calves exhibited an acute infection without disease or intestinal lesions, but with persisting fecal viral RNA shedding and seroconversion (Jung et al. 2017). Consequently, the potential ability of PDCoV and other DCoV isolates from birds or small mammals to infect different species should be investigated.

In February 2014, PDCoV was detected in US swine. Among 42 fecal or intestinal samples collected from diarrheic sows and piglets on five Ohio farms, 39 (92.9%) were positive for PDCoV by RT-PCR (Wang et al. 2014a). The PDCoV Ohio strain HKU15-OH1987 had a 99% nucleotide identity to the two prototype strains of PDCoV, HKU15-44 and HKU15-155, reported in Chinese pigs in 2012. During a similar period, genetically similar strains, USA/IA/2014/8734 and SDCV/USA/Illinois121/2014, were identified by other US diagnostic laboratories (Li et al. 2014; Marthaler et al. 2014a). Among PDCoV-positive premises, coinfection with PEDV is common (Zhang 2016). The origin of PDCoV in US swine is unknown, although there was serologic and virologic evidence suggesting its presence in the United States prior to its detection in February 2014 (Sinha et al. 2015; Thachil et al. 2015).

PDCoV has also been reported in Canada, Korea, mainland China, Thailand, Vietnam, and Lao PDR (Dong et al. 2015; Janetanakit et al. 2016; Lee et al. 2014; Lorsirigool et al. 2016; Marthaler et al. 2014a; Saeng-Chuto et al. 2017; Song et al. 2015b). The Korean PDCoV strains (KUN14-04, SL2, and SL5) had high nucleotide identities (98.7–99.2%) to US PDCoV strains (Lee et al. 2014, 2016b). In mainland China, coinfections with PDCoV and PEDV were common (Dong et al. 2015; Song et al. 2015b). Chinese PDCoV strains had ≥98.6% nucleotide identities with each other and ≥97.1% nucleotide identities with the global PDCoV strains (Zhang 2016). The Thai PDCoV strains were highly similar to each other but formed a novel phylogenetic cluster separated from US and Chinese PDCoVs (Janetanakit et al. 2016; Saeng-Chuto et al. 2017). PDCoVs identified in Lao PDR were more closely related to Thai PDCoVs, whereas PDCoVs detected in Vietnam were more closely related to US PDCoVs (Saeng-Chuto et al. 2017).

Fecal–oral is the main PDCoV transmission route. Feces and/or vomitus and other contaminated fomites are major transmission sources of the virus. Based on experimental findings (Hu et al. 2016; Ma et al. 2015; Zhang 2016), diarrhea in infected piglets was observed for approximately 5–10 days, with persisting viral RNA shedding for up to 19–28 days in feces and for up to 42 days in oral fluids.

Pathogenesis

The pathogenesis of PDCoV has been studied in Gn or conventional piglets orally inoculated with US and/or Chinese PDCoV isolates at 5–21 days of age (Chen et al. 2015; Dong et al. 2016; Hu et al. 2016; Jung et al. 2015b; Ma et al. 2015). Clinical signs (diarrhea and/or vomiting) occurred at 1–3 DPI. Replication of PDCoV is confined to the small and large intestinal epithelia. PDCoV-infected enterocytes rapidly undergo acute necrosis (Jung et al. 2016a), leading to marked villous atrophy in the small intestine (Figure 31.5c and e), but not in the large intestine. During acute infection, PDCoV antigens are detected mainly in the villous epithelium of the atrophied mid-jejunum (Figure 31.6c) to ileum and, to a lesser extent, in duodenum, proximal jejunum, and cecum/colon. Occasionally, a few PDCoV antigens are detected in crypt epithelial cells of the jejunum and ileum (Jung et al. 2016a) and immune cells in the intestinal lamina propria, Peyer's patches, and mesenteric lymph nodes (Hu et al. 2016). Frequently, acute viremia with low PDCoV RNA titers in serum was observed (Chen et al. 2015; Hu et al. 2016; Ma et al. 2015). After pigs recovered from clinical disease, larger amounts of PDCoV antigens were detected in the gut lymphatic tissues (Hu et al. 2016). PDCoV antigens were not detected in other organs, including the respiratory tract of pigs (Jung et al. 2016b). However, by real-time RT-PCR, PDCoV RNA could be detected in low to moderate quantities in multiple organs, possibly due to viremia (Chen et al. 2015; Ma et al. 2015).

Clinical signs

Clinical signs of PDCoV infection in suckling and older pigs are similar, but milder, than those of PEDV and TGEV infections. In suckling piglets, PDCoV induces acute, watery diarrhea, frequently accompanied by vomiting, leading to dehydration, loss of body weight,

lethargy, and death. Experimentally, the onset of diarrhea coincided with, or was detected 1–2 days later than, the first detection of viral RNA in feces (Jung et al. 2015b; Ma et al. 2015).

Diarrhea is probably a consequence of malabsorption due to massive loss of absorptive enterocytes, resulting in decreased brush border membrane-bound digestive enzymes, similar to PEDV infection (Jung et al. 2006). Mild vacuolation observed in the infected colonic epithelial cells may interfere with the reabsorption of water and electrolytes (Jung et al. 2015b). Dehydration is also exacerbated by vomiting.

Seronegative pigs of all ages are susceptible to PDCoV infection. On seronegative farrowing farms, morbidity can reach up to 100% in piglets but can vary in sows. Based on field observations in US swine in 2014 (Anon 2014), PDCoV infection caused a number of deaths (up to a 40% mortality) among suckling pigs. Similarly, PDCoV diarrhea outbreaks in breeding farms in China and Thailand resulted in 64–80% mortality among suckling piglets. PDCoV infection is more severe and more likely to result in mortality in piglets as compared with older pigs. On many farms, morbidity and mortality may be affected by coinfections with other enteric viruses, such as PEDV and rotavirus (Marthaler et al. 2014b; Song et al. 2015b). The disease on breeding farms is self-limiting and stops when pregnant sows develop lactogenic immunity to protect their offspring.

PDCoV infection shares several clinical features with TGEV and PEDV infections, but the virus likely spreads more slowly among pigs, possibly due to its lower adaptation to pigs. Relative to PEDV infections, PDCoV-infected pigs shed less PDCoV RNA in the feces (Jung et al. 2015b), indicating lower replication of PDCoV in the intestine of pigs. This aspect of PDCoV infection may be a contributing factor to its lower mortality in nursing piglets, as compared with PEDV infections.

Lesions

Lesions have been described in suckling piglets experimentally and naturally infected with US, Chinese, or Thai PDCoV strains (Chen et al. 2015; Dong et al. 2016; Hu et al. 2016; Janetanakit et al. 2016; Jung et al. 2015b; Ma et al. 2015; Wang et al. 2016c). Lesions resemble those observed in TGEV and PEDV infections (Figure 31.5), but are usually less extensive.

Gross lesions are limited to the gastrointestinal tract and are characterized by thin and transparent intestinal walls (proximal jejunum to colon) with accumulation of large amounts of yellow fluid. The stomach is frequently filled with curdled milk. The transparency and fragility of affected intestines are milder, as compared with PEDV and TGEV infections. Histological lesions are characterized by acute, multifocal to diffuse, mild to severe atrophic enteritis in the proximal jejunum to ileum (Figure 31.5c and e), occasionally accompanied by mild vacuolation of the superficial epithelial cells in the cecum and colon (Jung et al. 2015b). No villous atrophy or histologic lesions were evident in the duodenum, which coincided with few PDCoV antigen-positive duodenal epithelial cells (Chen et al. 2015; Jung et al. 2015b). During acute infection, vacuolated enterocytes or massive cell exfoliation was seen on the tips or the entire villi in the jejunum and ileum. Atrophied villi were frequently fused and covered with a degenerated or regenerated flattened epithelium. Infiltration of inflammatory cells, such as macrophages, lymphocytes, and neutrophils, was evident in the lamina propria. No lesions were seen in other organs.

Diagnosis

The diagnostic approaches described earlier for TGEV and PEDV also apply to PDCoV diagnosis. Laboratory techniques should be used to differentiate PDCoV infection from PEDV, TGEV, and rotavirus diarrhea in pigs. Definitive diagnosis of PDCoV infection includes detection of PDCoV RNA or antigens in the feces or intestinal tissues from diarrheic pigs. Diagnosis can be made by RT-PCR assays that target a conserved region of PDCoV M or N genes (Marthaler et al. 2014b; Wang et al. 2014a; Zhang et al. 2016b), IF or IHC using virus-specific MAbs or polyclonal antibodies (Chen et al. 2015; Jung et al. 2015b; Ma et al. 2015), and *in situ* hybridization (Jung et al. 2015b). A real-time duplex RT-PCR assay for detection of PDCoV and/or differentiation of the virus from PEDV in intestines and feces was developed (Zhang et al. 2016b).

Direct EM can be used to demonstrate PDCoV particles in feces collected from diarrheic pigs (Figure 31.2d), but IEM using hyperimmune or convalescent sera is essential to differentiate PDCoV from PEDV or TGEV (Jung et al. 2015b). Isolation of PDCoV from feces or intestinal tissues was attempted in LLC-PK or ST cells, but the success rate was low except for a few strains (OH-FD22) (Hu et al. 2015). Serologic diagnosis of PDCoV can be conducted by IFA, VN, and ELISA assays. Isotypes of PDCoV antibodies in serum and milk can be quantitated by ELISA using antigens consisting of cell culture-grown virus (Ma et al. 2016) or S1 and N viral proteins (Okda et al. 2016; Su et al. 2016; Thachil et al. 2015).

Immunity

The immune responses of pigs to PDCoV infection are largely undefined, but they are likely similar to those described earlier for TGEV and PEDV. Hu et al. (2016) reported the development of PDCoV antibodies in serum of PDCoV-infected pigs (Hu et al. 2016). Gn pigs orally

inoculated with the original or tissue culture-grown PDCoV strain OH-FD22 had detectable serum IgG, IgA, and VN antibodies by 14 DPI that peaked at 24 DPI, when the pigs had recovered from clinical disease and fecal virus shedding. While PDCoV infection is epidemic, young piglets can be protected by transfer of maternal antibodies via colostrum and milk from immune dams, especially IgA and VN antibodies that neutralize PDCoV in the gut. Lactogenic immunity is expected to be strongly induced in sows by oral infection with PDCoV, which then activates the gut–mammary link, as described earlier for TGEV (Bohl et al. 1972; Saif et al. 1972).

Prevention and control

The prevention and control measures described earlier for TGEV and PEDV infections also apply to PDCoV infection. There are no treatments or vaccines to control PDCoV infection. Preventive or therapeutic antibiotic therapy can be implemented if there is concurrent infection with enteric bacterial pathogens. Symptomatic treatment of suckling pigs with diarrhea includes intraperitoneal administration of bicarbonate fluids and free access to water to alleviate acidosis and dehydration. If mortality is substantial among suckling piglets, feedback methods (intentional exposure of pregnant sows to virus-positive minced intestines from acutely infected piglets) will stimulate lactogenic immunity and reduce the high mortality if administered to sows at least 2 weeks pre-farrowing. During PDCoV epidemics, high-level biosecurity procedures to reduce PDCoV transmission via contaminated fomites are essential.

Hemagglutinating encephalomyelitis virus (vomiting and wasting disease)

Relevance

In 1962, Greig and coworkers isolated a viral pathogen from the brains of suckling pigs with encephalomyelitis in Canada. Designated hemagglutinating encephalomyelitis virus (HEV), the virus was later classified as a CoV (Greig et al. 1971). In 1969, an antigenically identical virus was isolated in England from suckling pigs showing anorexia, depression, vomiting, and stunting, but without signs of encephalomyelitis (Cartwright et al. 1969). The condition was called vomiting and wasting disease (VWD). Both forms of the disease were experimentally reproduced by Mengeling and Cutlip (1976) using isolates from the same farm. pHEV is widespread among swine, but the infection is generally subclinical, although some outbreaks may cause losses (Alsop 2006; Quiroga et al. 2008).

Etiology

pHEV belongs to the genus *Betacoronavirus* of the family *Coronaviridae* (Figure 31.1). The virus agglutinates erythrocytes of mice, rats, chickens, and several other animals. The natural host of pHEV is the pig. Although pHEV may show different clinical manifestations, there is only one serotype. Age-related susceptibility of the pigs, possible strain differences in virulence, and variation in pathogenesis may influence clinical signs. pHEV shows a strong tropism for neural tissues in pigs. Likewise, the virus displays neurotropism in mice and Wistar rats (Hirano et al. 2004; Yagami et al. 1993).

In vitro, only porcine cells are susceptible to pHEV. pHEV was first isolated in primary PK cells with CPE characterized by syncytia (Greig et al. 1962). pHEV was also shown by IF staining to propagate in other porcine cell cultures: adult thyroid gland, embryonic lung, and cell lines such as ST, PK-15, $IBRS_2$, SK, SK-K, and KSEK6 swine embryo kidney.

Public health

Pigs are the only species known to be susceptible to pHEV and pHEV has no public health significance.

Epidemiology

Serologic surveys (1960–1990) revealed that pHEV infection in swine occurs worldwide and is endemic in both breeding and fattening swine (Pensaert 2006). The presence of pHEV, as detected by isolation or serology, was reported in Europe, in the Western Hemisphere (United States, Canada, Argentina), in Asia (Japan, Taiwan), and in Australia.

pHEV is maintained in swine populations by infecting successive groups of pigs after replacement or weaning. The virus is excreted oronasally (Hirahara et al. 1989; Pensaert and Callebaut 1974) for 8–10 days. Transmission occurs via nasal secretions, via nose-to-nose contact, and aerogenically. Persistent virus carriers are not known to exist.

Generally, pigs will only develop disease when they become infected oronasally prior to 3–4 weeks of age and if originating from nonimmune mothers (Appel et al. 1965). Pigs with maternally derived pHEV antibodies that prevent the virus from reaching neural target tissues are clinically unaffected when exposed to pHEV (Appel et al. 1965). Pigs infected at later ages normally do not develop clinical disease. Since pHEV is endemic in most swine populations, most sows are immune and protect their offspring by maternal antibodies. Thus, clinical outbreaks are rare and usually occur in litters from nonimmune mothers, often first-parity sows. Three outbreaks are notable. In 2001, pHEV was isolated from

newborn and early weaned pigs with vomiting and posterior paralysis on a Canadian farm (Sasseville et al. 2001). Alsop (2006) described a clinically diagnosed outbreak of VWD in 2002 in a 650-sow genetic nucleus herd. Quiroga et al. (2008) described a VWD outbreak with motor disorders in Argentina in 2006. It occurred in a three-site herd with 6,000 sows where the breeder stock consisted of 55% gilts and first- or second-parity sows.

Pathogenesis

The type and severity of clinical signs vary and are related to age, possible differences in virus virulence (Mengeling and Cutlip 1976), and the course of viral pathogenesis.

The primary site of replication of pHEV in pigs is the respiratory tract (Andries and Pensaert 1980b; Hirahara et al. 1987; Mengeling et al. 1972). IF staining revealed that epithelial cells of nasal mucosa, tonsils, lungs, and some unidentified cells in the small intestine were infected. Primary replication may result in mild or subclinical signs.

Experimental studies in colostrum-deprived piglets inoculated oronasally with pHEV provided insight into pHEV pathogenesis (Andries and Pensaert 1980a). From the primary sites of replication, the virus spread via the peripheral nervous system to the CNS via different pathways. One pathway led from the nasal mucosa and tonsils to the trigeminal ganglion and the trigeminal sensory nucleus in the brain stem. A second pathway was along the vagal nerves via the vagal sensory ganglion to the vagal sensory nucleus in the brain stem. A third pathway led from the intestinal plexuses to the spinal cord, also after replication in local sensory ganglia. Viremia was of little or no importance in the pathogenesis of the disease (Andries and Pensaert 1980b).

In the CNS, the infection started in well-defined nuclei of the medulla oblongata, but progressed into the entire brain stem, the spinal cord, and sometimes also the cerebrum and cerebellum. IF staining in the brain was always restricted to the perikaryon and processes of neurons. Vomiting was induced by viral replication in the vagal sensory ganglion (ganglion distale vagi) or by impulses to the vomiting center produced by infected neurons at different sites (Andries 1982). To elucidate the pathogenesis of wasting, Andries (1982) suggested that virus-induced lesions in the intramural plexuses of the stomach may contribute to gastric stasis and delayed stomach emptying.

Clinical signs

Sneezing or coughing may be the first sign of infection because of primary pHEV replication in the upper respiratory tract. Body temperature can be elevated at disease onset, but returns to normal in 1–2 days. The incubation period for the appearance of more specific signs is 4–7 days. Two main clinical manifestations associated with pHEV neurotropism are possible in pigs below 3–4 weeks of age: (1) typical VWD with frequent vomiting leading to death or subsequent wasting and (2) acute encephalomyelitis with motor disorders. However, signs of both clinical forms may occur in the same herd during an outbreak.

For VWD, clinical signs are repeated retching and vomiting. Pigs start suckling, but withdraw from the sow and vomit the milk. The persistent vomiting and decreased food intake results in constipation and a rapid decline of condition. Neonatally infected pigs become severely dehydrated after a few days, exhibit dyspnea and cyanosis, lapse into coma, and die. Older pigs lose their appetite and become emaciated. They continue to vomit, although less frequently than in the acute stage. Wasting, often with distension of the cranial abdomen, may appear. This "wasting" state persists for several weeks and may be post weaning, requiring euthanasia. During the acute stage of VWD outbreaks, some pigs may show neurologic signs, such as abnormal gait, dullness, tremors, and nystagmus.

At the herd or farrowing unit level, morbidity varies greatly and probably depends on the proportion of nonimmune neonatal litters present at the time of infection. In litters without maternal protection, morbidity is litter dependent and may approach 100% when the infection occurs near birth. Morbidity decreases markedly with increasing age at infection. Mortality is variable, but may be 100% in neonatally infected litters.

In the Argentina outbreak (Quiroga et al. 2008), only suckling pigs were involved. Vomiting and wasting were the main signs, with slight motor disorders. Disease occurred in 27.6% of pigs <1 week old and declined to 1.6% in pigs 3 weeks of age. In this pHEV outbreak, an estimated 12.6% (3683) of the suckling pigs in the affected farrowing units died or were euthanized. After weaning, a mean of 29% (15–40%) of the pigs coming from affected farrowing units showed wasting.

Outbreaks of the motor encephalomyelitis disease in suckling pigs may start with sneezing, coughing, and vomiting 4–7 days after birth. Vomiting continues intermittently for 1–2 days, but is rarely severe. In some outbreaks, the first sign is acute depression and huddling. After 1–3 days, pigs exhibit various combinations of nervous disorders. Generalized muscle tremors and hyperesthesia are common. Pigs may have a jerky gait and walk backward, ending in a dog-sitting position. They become weak, are unable to rise, and paddle their limbs. Blindness, opisthotonus, and nystagmus may also occur. Finally, the animals become dyspneic and lie prostrate on their sides. In most cases, coma precedes death.

Morbidity and mortality in neonatal pigs is usually 100%, but older pigs show a mild transient illness in which posterior paralysis may be the most common sign. Outbreaks described in Taiwan (Chang et al. 1993) in 30- to 50-day-old pigs were characterized by fever, constipation, hyperesthesia, muscular tremor, progressive anterior paresis, posterior paresis, prostration, recumbency, and paddling movements with a morbidity of 4% and a mortality approaching 100%. The pigs died 4–5 days after the onset of clinical signs.

Lesions

The only gross lesions reported in pHEV infections are cachexia, stomach dilatation, and distension of the abdomen in some chronically affected pigs (Schlenstedt et al. 1969).

Microscopic lesions of epithelial degeneration and inflammatory cell infiltration are found in the tonsils and respiratory system of acutely diseased pigs (Cutlip and Mengeling 1972; Narita et al. 1989). A nonsuppurative encephalomyelitis was reported in 70–100% of pigs with nervous signs and in 20–60% of pigs showing VWD. The lesions are characterized by perivascular cuffing, gliosis, and neuronal degeneration (Alexander 1962; Chang et al. 1993; Richards and Savan 1960). They are most pronounced in the gray matter of the pons Varolii, medulla oblongata, and the dorsal horns of the upper spinal cord.

Microscopic changes in the stomach wall were found only in pigs showing VWD. Degeneration of the ganglia of the stomach wall and perivascular cuffing were present in 15–85% of diseased animals. The lesions were most pronounced in the pyloric gland area (Schlenstedt et al. 1969).

Diagnosis

Diagnosis can be made by virus isolation, IHC, or RT-PCR (Quiroga et al. 2008). Tonsils, brain stem, and lungs dissected aseptically from young acutely diseased piglets can be used for testing. It is difficult to isolate the virus from pigs that have been sick for more than 2–3 days. For virus isolation, suspensions are inoculated onto primary PK cells, secondary pig thyroid cells, or porcine cell lines. pHEV is detected by the presence of syncytia, by hemadsorption, or by hemagglutination. One or more blind passages may be needed since specimens often contain small amounts of infectious virus.

Antibodies to pHEV can be detected by VN or hemagglutination inhibition (HI) tests. The HI and VN tests were almost equally diagnostic in swine sera, but VN is more specific (Sasaki et al. 2003). Antibody titer results must be evaluated carefully because subclinical infections with pHEV are very common. Moreover, a significant rise in antibody titer can be detected only if acute sera are taken near the onset of clinical signs. Pigs may develop antibody titers as early as 6–7 DPI, which often coincides with early disease, making an interpretation of paired serology more difficult.

Differential diagnosis must be made between pHEV encephalomyelitis, Teschen–Talfan disease, and pseudorabies (Aujeszky's) disease. In the latter infections, clinical signs of encephalomyelitis, including motor disorders, are more severe and may appear in piglets and older pigs. Aujeszky's disease in non-vaccinated animals also induces respiratory signs in older pigs and abortions in sows. All these viruses can be grown in PK cells and pig thyroid cells, but the type of CPE differs and only pHEV causes hemadsorption and hemagglutination. They can be further differentiated by virus-specific tests.

Immunity

After infection, pigs develop detectable protective circulating antibodies (HI, VN) to pHEV in 7–9 days. The duration of antibodies has not been determined. The duration of immunity is less important in pHEV because of the resistance to disease that develops with age. Neonatal pigs born to immune mothers are fully protected by maternally derived antibodies that persist until the age of 4–18 (mean 10.5) weeks (Paul and Mengeling 1984).

Prevention and control

On most breeding farms, pHEV infection persists endemically by pig-to-pig transmission and through subclinical respiratory infections. Gilts usually contract the virus before their first farrowing and then provide protection to their offspring via colostral antibodies. When sows are not immune at farrowing, (e.g. in newly populated farms, well-isolated gilts, or small farms in which the virus is not maintained), infection of pigs within the first weeks after birth results in clinical signs. Promoting virus circulation in the farm so that gilts are immune at farrowing prevents disease in piglets.

Once clinical signs are evident, the disease will run its course; spontaneous recoveries are rare. Litters born 2–3 weeks after the onset of disease are usually protected because nonimmune gestating sows should have become infected and immune by farrowing. Piglets born to nonimmune sows early in the outbreak can be passively protected by parenteral inoculation with specific immune serum shortly after birth. Hyperimmune serum is not commercially available, but pooled serum collected from older sows (at the slaughterhouse) should be filter sterilized and tested to confirm presence of pHEV antibodies. No vaccines against pHEV are currently available.

Porcine torovirus

Relevance

Torovirus (ToV) particles were initially detected by EM in the feces of a 3-week-old piglet with diarrhea in England. Subsequent studies revealed a high seroprevalence (81–100%) in adults or young nursing piglets and high detection rates (50–75%) among subclinically infected weaned pigs (Kroneman et al. 1998; Pignatelli et al. 2010). Latter reports from Europe, North America, and South Africa suggested that ToV was endemic. In contrast, in Korea, only 6.4% (19 of 295) of diarrheic feces from 3- to 45-day-old piglets were positive for porcine ToV (Shin et al. 2010). Of these, about 74% also contained other enteric pathogens. Consequently, the link between porcine ToV and enteric disease is unclear, and there are no reports confirming porcine ToV pathogenicity or gut lesions.

Etiology

Porcine ToV represents a species within the genus *Torovirus* of the subfamily *Torovirinae* in the family *Coronaviridae*. The genomic organization, replication strategy, and properties resemble other members in the family *Coronaviridae* (de Groot et al. 2008). Like some betacoronaviruses, porcine ToVs also possess an HE protein. Notable differences from CoVs include a smaller N protein (approximately 18.7 kD) and a tubular nucleocapsid, leading to differences in ToV particle morphology (spherical, elongated, or kidney shaped) (Kroneman et al. 1998). Multiple clusters of porcine ToVs have been identified based on gene sequence analysis (de Groot et al. 2008; Pignatelli et al. 2010; Shin et al. 2010).

Epidemiology and immunity

Based on serologic and shedding data from clinically normal pigs or sows in European herds, ToVs were endemic in 14 farms tested. High seroprevalence rates (81%) were detected in sows on 10 Dutch farms by testing for cross-reactive VN antibodies to equine ToV (Kroneman et al. 1998). Similarly, 100% of sows, nursing, and older pigs in three farms in Spain were seropositive for ToV antibodies using an ELISA based on porcine ToV N protein (Pignatelli et al. 2010). Longitudinal studies revealed fecal shedding (80%) (RT-PCR or real-time RT-PCR) post weaning at 4–14 days for 1–9 days (Kroneman et al. 1998) or at 4 and 8 weeks post weaning (50–75%) (Pignatelli et al. 2010). In both studies, maternal antibody titers were initially high in piglets, declined at weaning, and then increased post infection at 11 or 15 weeks of age.

Because most pigs become infected with ToVs post weaning, maternal antibodies apparently provide at least partial protection. However, the immune correlates of protection to porcine ToV infection are not known. In one of four farms, it was postulated that ToV infection of suckling piglets in the presence of maternal antibodies delayed development of active immune responses such that these pigs, but not pigs from the other farms, shed the same ToV strain pre- and post weaning (Pignatelli et al. 2010). Genetically diverse ToV strains were detected within herds in the latter study and in Korean farms. The porcine ToVs were associated with sporadic infections among diarrheic pigs from 65 Korean farms surveyed (6.2% of farms positive) (Shin et al. 2010). Based on phylogenetic analysis of the S and N genes, the Korean ToV strains formed distinct branches with clusters corresponding to the farm of origin.

Diagnosis

Methods to propagate porcine ToVs in cell culture have not been described. For serologic studies, a cell culture-adapted equine ToV has been used to assess cross-reactive VN antibodies in swine (Kroneman et al. 1998; Pignatelli et al. 2010). Recently an indirect ELISA using recombinant purified porcine ToV N protein as antigen was developed (Pignatelli et al. 2010). In most, but not all cases, there was a good correlation between ELISA and VN tests. Discrepancies observed could reflect use of heterologous equine ToV antigen in VN, compromising detection of low titer antibodies.

Porcine ToVs have been detected in feces using IEM to identify antibody-aggregated ToV particles and differentiate them from other fecal porcine CoVs (TGEV, PEDV, and PDCoV) (Kroneman et al. 1998). For detection of ToV-specific viral RNA, RT-PCR and real-time RT-PCR targeting conserved regions of the porcine ToV N gene or the 3′ UTR of the genome have been described (Kroneman et al. 1998; Pignatelli et al. 2010; Shin et al. 2010).

Prevention and control

Based on the limited data available, the stress of transport, movement, and redistribution of pigs, even within multisite farms, could precipitate porcine ToV infection with similar or distinct co-circulating strains (Pignatelli et al. 2010). Thus, management practices applicable to control of other enteric CoV infections should be implemented for control of porcine ToVs.

References

Abou-Youssef MH, Ristic M. 1972. *Am J Vet Res* 33:975–979.

Akimkin V, Beer M, Blome S, et al. 2016. *Emerg Infect Dis* 22:1314–1315.

Alexander TJ. 1962. *Am J Vet Res* 23:756–762.

Alonso C, Goede DP, Morrison RB, et al. 2014. *Vet Res* 45:73.

Alsop JE. 2006. *J Swine Health Prod* 14:97–100.

van Reeth K, Pensaert M. 1994. *Vet Rec* 135:594–597.

van Reeth K, Nauwynck H, Pensaert M. 1996. *Vet Microbiol* 48:325–335.

Andries K. 1982. Pathogenese en epizoötiologie van "vomiting and wasting disease," een virale infektie bij het varken. PhD dissertation, Med Fac Diergeneeskd Rijksuniv. *Ghent* 24:164.

Andries K, Pensaert MB. 1980a. *Am J Vet Res* 41:1372–1378.

Andries K, Pensaert MB. 1980b. *Am J Vet Res* 41:215–218.

Annamalai T, Saif LJ, Lu Z, et al. 2015. *Vet Immunol Immunopathol* 168:193–202.

Anon. 2014. *J Am Vet Med Assoc* 244:1234.

Anton IM, Sune C, Meloen RH, et al. 1995. *Virology* 212:746–751.

Anton IM, Gonzalez S, Bullido MJ, et al. 1996. *Virus Res* 46:111–124.

Appel M, Greig AS, Corner AH 1965. *Res Vet Sci* 6:482–489.

Atanasova K, Van Gucht S, Barbé F, et al. 2008. Lung cell tropism and inflammatory cytokine-profile of porcine respiratory coronavirus infection. *Open Vet Sci J* 2:117–126.

Ballesteros ML, Sanchez CM, Enjuanes L. 1997. *Virology* 227:378–388.

Barman N, Barman B, Sarma K, et al. 2003. *Indian J Anim Sci* 73:576–578.

Belsham GJ, Rasmussen TB, Normann P, et al. 2016. *Transbound Emerg Dis* 63:595–601.

Bernard S, Bottreau E, Aynaud JM, et al. 1989. *Vet Microbiol* 21:1–8.

Berthon P, Bernard S, Salmon H, et al. 1990. *J Immunol Methods* 131:173–182.

Bestagno M, Sola I, Dallegno E, et al. 2007. *J Gen Virol* 88:187–195.

Bjustrom-Kraft J, Woodard K, Gimenez-Lirola L, et al. 2016. *BMC Vet Res* 12:99.

Bohl E. 1979. Diagnosis of diarrhea in pigs due to transmissible gastroenteritis virus or rotavirus. In Bricout F, Scherrer R, eds. *Viral Enteritis in Humans and Animals*. Paris, France: INSERM, pp. 341–343.

Bohl EH, Kumagai T. 1965. The use of cell cultures for the study of swine transmissible gastroenteritis virus. In United States Livestock Sanitary Association Meeting, pp. 343–350.

Bohl EH, Saif LJ. 1975. *Infect Immun* 11:23–32.

Bohl EH, Gupta RK, Olquin MV, et al. 1972. *Infect Immun* 6:289–301.

Bohl EH, Kohler EM, Saif LJ, et al. 1978. *J Am Vet Med Assoc* 172:458–463.

Boniotti MB, Papetti A, Lavazza A, et al. 2016. *Emerg Infect Dis* 22:83–87.

Brim TA, VanCott JL, Lunney JK, et al. 1995. *Vet Immunol Immunopathol* 48:35–54.

Britton P, Mawditt KL, Page KW. 1991. *Virus Res* 21:181–198.

Brown I, Cartwright S. 1986. *Vet Rec* 119:282–283.

Brown TT Jr. 1981. *Am J Vet Res* 42:1033–1036.

Brown IH, Paton DJ. 1991. *Vet Rec* 128:500–503.

Butler DG, Gall DG, Kelly MH, et al. 1974. *J Clin Invest* 53:1335–1342.

Callebaut P, Debouck P, Pensaert M. 1982. *Vet Microbiol* 7:295–306.

Callebaut P, Pensaert MB, Hooyberghs J. 1989. *Vet Microbiol* 20:9–19.

Callebaut P, Enjuanes L, Pensaert M. 1996. *J Gen Virol* 77 (Pt 2):309–313.

Calzada-Nova G, Schnitzlein W, Husmann R, et al. 2010. *Vet Immunol Immunopathol* 135:20–33.

Cao L, Ge X, Gao Y, et al. 2015. *J Gen Virol* 96:1757–1767.

Cartwright SF, Harris HM, Blandford TB, et al. 1965. *J Comp Pathol* 75:387–396.

Cartwright SF, Lucas M, Cavill JP, et al. 1969. *Vet Rec* 84:175–176.

Carvajal A, Lanza I, Diego R, et al. 1995. *J Vet Diagn Invest* 7:60–64.

Casanova L, Rutala WA, Weber DJ, et al. 2009. *Water Res* 43:1893–1898.

Cepica A, Derbyshire JB. 1984. *Can J Comp Med* 48:258–261.

Chae C, Kim O, Choi C, et al. 2000. *Vet Rec* 147:606–608.

Chang G, Chang T, Lin S, et al. 1993. Isolation and identification of hemagglutinating encephalomyelitis virus from pigs in Taiwan. *J Chin Soc Vet Sci* 19:147–158.

Charley B, Laude H. 1988. *J Virol* 62:8–11.

Chasey D, Cartwright SF. 1978. *Res Vet Sci* 25:255–256.

Chattha KS, Roth JA, Saif LJ. 2015. *Annu Rev Anim Biosci* 3:375–395.

Chen W, Yan M, Yang L, et al. 2005. *Emerg Infect Dis* 11:446–448.

Chen J, Liu X, Shi D, et al. 2013. *Viruses* 5:2601–2613.

Chen Q, Li G, Stasko J, et al. 2014. *J Clin Microbiol* 52:234–243.

Chen Q, Gauger P, Stafne M, et al. 2015. *Virology* 482:51–59.

Chen Q, Gauger PC, Stafne MR, et al. 2016a. *J Gen Virol* 97:1107–1121.

Chen Q, Thomas JT, Gimenez-Lirola LG, et al. 2016b. *BMC Vet Res* 12:70.
Cheun-Arom T, Temeeyasen G, Srijangwad A, et al. 2015. *Genome Announc* 3:e00634-15.
Choudhury B, Dastjerdi A, Doyle N, et al. 2016. *Virus Res* 226:40–49.
Chu RM, Glock RD, Ross RF. 1982. *Am J Vet Res* 43:67–76.
Cong Y, Li X, Bai Y, et al. 2015. *Virology* 478:1–8.
Correa I, Gebauer F, Bullido MJ, et al. 1990. *J Gen Virol* 71 (Pt 2):271–279.
Costantini V, Lewis P, Alsop J, et al. 2004. *Arch Virol* 149:957–974.
Coussement W, Ducatelle R, Debouck P, et al. 1982. *Vet Pathol* 19:46–56.
Cox E, Hooyberghs J, Pensaert MB. 1990a. *Res Vet Sci* 48:165–169.
Cox E, Pensaert MB, Callebaut P, et al. 1990b. *Vet Microbiol* 23:237–243.
Cox E, Pensaert MB, Callebaut P. 1993. *Vaccine* 11:267–272.
Crawford K, Lager K, Miller L, et al. 2015. *Vet Res* 46:49.
Crawford K, Lager KM, Kulshreshtha V, et al. 2016. *Virus Res* 226:108–116.
Cummins JM, Mock RE, Shive BW, et al. 1995. *Vet Immunol Immunopathol* 45:355–360.
Cutlip RC, Mengeling WL. 1972. *Am J Vet Res* 33:2003–2009.
De Diego M, Rodriguez F, Alcaraz C, et al. 1994. *J Gen Virol* 75 (Pt 10):2585–2593.
Debouck P, Pensaert M. 1980. *Am J Vet Res* 41:219–223.
Debouck P, Pensaert M, Coussement W. 1981. *Vet Microbiol* 6:157–165.
Debouck P, Callebaut P, Pensaert M. 1982. Prevalence of porcine epidemic diarrhea (PED) virus in the pig population of different countries. In Proceedings of the 7th International Pig Veterinary Congress, p. 53.
Decaro N, Mari V, Campolo M, et al. 2009. *J Virol* 83:1532–1537.
Decaro N, Mari V, Elia G, et al. 2010. *Emerg Infect Dis* 16:41–47.
Dee S, Clement T, Schelkopf A, et al. 2014. *BMC Vet Res* 10:176.
Dee S, Neill C, Singrey A, et al. 2016. *BMC Vet Res* 12:51.
Delmas B, Laude H. 1990. *J Virol* 64:5367–5375.
Delmas B, Gelfi J, L'Haridon R, et al. 1992. *Nature* 357:417–420.
Derbyshire JB, Jessett DM, Newman G. 1969. *J Comp Pathol* 79:445–452.
De Diego M, Laviada MD, Enjuanes L, et al. 1992. *J Virol* 66:6502–6508.
Ding Z, Fang L, Jing H, et al. 2014. *J Virol* 88:8936–8945.
Dong BQ, Liu W, Fan XH, et al. 2007. *J Virol* 81:6920–6926.
Dong N, Fang L, Zeng S, et al. 2015. *Emerg Infect Dis* 21:2254–2255.
Dong N, Fang L, Yang H, et al. 2016. *Vet Microbiol* 196:98–106.
Doyle LP, Hutchings LM. 1946. *J Am Vet Med Assoc* 108:257–259.
Ducatelle R, Coussement W, Debouck P, et al. 1982. *Vet Pathol* 19:57–66.
Elia G, Decaro N, Martella V, et al. 2010. *J Virol Methods* 163:309–312.
Enjuanes L, Van der Zeijst B. 1995. Molecular basis of transmissible gastroenteritis virus epidemiology. In Siddell SG, ed. *The Coronavidae*. New York, NY: Plenum Press, pp. 337–376.
Enjuanes L, Sune C, Gebauer F, et al. 1992. *Vet Microbiol* 33:249–262.
Enjuanes L, Sola I, Alonso S, et al. 2005. *Curr Top Microbiol Immunol* 287:161–197.
Erles K, Brownlie J. 2009. *Virus Res* 141:21–25.
Frederick GT, Bohl EH, Cross RF. 1976. *Am J Vet Res* 37:165–169.
Furuuchi S, Shimizu M, Shimizu Y. 1978. Field trials on transmissible gastroenteritis live virus vaccine in newborn piglets. *Natl Inst Anim Health Q* 18:135–142.
Garwes DJ, Stewart F, Cartwright SF, et al. 1988. *Vet Rec* 122:86–87.
Gerber PF, Opriessnig T. 2015. *MethodsX* 2:368–373.
Gerber PF, Gong Q, Huang YW, et al. 2014. *Vet J* 202:33–36.
Gimenez-Lirola LG, Zhang J, Carrillo-Avila JA, et al. 2017. *J Clin Microbiol* 55:1426–1436.
Godet M, Rasschaert D, Laude H. 1991. *Virology* 185:732–740.
Godet M, Grosclaude J, Delmas B, et al. 1994. *J Virol* 68:8008–8016.
Gomez N, Wigdorovitz A, Castanon S, et al. 2000. *Arch Virol* 145:1725–1732.
Gong L, Li J, Zhou Q, et al. 2017. *Emerg Infect Dis* 23:1607–1609.
Gonzalez JM, Gomez-Puertas P, Cavanagh D, et al. 2003. *Arch Virol* 148:2207–2235.
Gough PM, Jorgenson RD. 1983. *Am J Vet Res* 44:2078–2082.
Greig AS, Mitchell D, Corner AH, et al. 1962. *Can J Comp Med Vet Sci* 26:49–56.
Greig AS, Johnson CM, Bouillant AM. 1971. *Res Vet Sci* 12:305–307.
de Groot R, Ziebuhr J, Poon L, et al. 2008. Revision of the family Coronaviridae. Taxonomic proposal of the Coronavirus Study Group to the ICTV Executive Committee. http://talk.ictvonline.org/media/p/1230.aspx. Accessed January 27, 2017.
Guscetti F, Bernasconi C, Tobler K, et al. 1998. *Clin Diagn Lab Immunol* 5:412–414.
Haas B, Ahl R, Bohm R, et al. 1995. *Rev Sci Tech* 14:435–445.

Haelterman EO. 1962. Epidemiological studies of transmissible gastroenteritis of swine. In United States Livestock Sanitary Association Meeting, pp. 305–315.
Halbur PG, Paul PS, Vaughn EM, et al. 1993. *J Vet Diagn Invest* 5:184–188.
Harris D, Bevier G, Wiseman B. 1987. Eradication of transmissible gastroenteritis virus without depopulation. In American Association of Swine Practitioners Meeting, p. 555.
Have P. 1990. *Adv Exp Med Biol* 276:435–439.
Have P, Moving V, Svansson V, et al. 1992. *Vet Microbiol* 31:1–10.
Hess R, Chen Y, Bachmann PA. 1982. Active immunization of feeder pigs against transmissible gastroenteritis (TGE): Influence of maternal antibodies. In International Pig Veterinary Congress, p. 1.
Hill H, Biwer J, Woods R, et al. 1990. Porcine respiratory coronavirus isolated from two U.S. swine herds. In American Association of Swine Practitioners Meeting, p. 333.
Hirahara T, Yasuhara H, Kodama K, et al. 1987. Isolation of hemagglutinating encephalomyelitis virus from respiratory tract of pigs in Japan. *Nihon Juiqaku Zasshi* 49:85–93.
Hirahara T, Yamanaka M, Yasuhara H, et al. 1989. Experimental infection of pigs with porcine hemagglutinating encephalomyelitis virus. *Nihon Juiqaku Zasshi* 51:827–830.
Hirano N, Nomura R, Tawara T, et al. 2004. *J Comp Pathol* 130:58–65.
Hofmann M, Wyler R. 1988. *J Clin Microbiol* 26:2235–2239.
Hofmann M, Wyler R. 1990. *Vet Microbiol* 21:263–273.
Hooper BE, Haelterman EO. 1966a. *J Am Vet Med Assoc* 149:1580–1586.
Hooper BE, Haelterman EO. 1966b. *Am J Vet Res* 27:286–291.
Hornich M, Salajka E, Stepanek J. 1977. *Zentralbl Veterinarmed B* 24:75–86.
Hou XL, Yu LY, Liu J. 2007. *Vet Microbiol* 123:86–92.
Hu S, Bruszewski J, Smalling R, et al. 1985. *Adv Exp Med Biol* 185:63–82.
Hu H, Jung K, Vlasova AN, et al. 2015. *J Clin Microbiol* 53:1537–1548.
Hu H, Jung K, Vlasova AN, et al. 2016. *Arch Virol* 161:3421–3434.
Hwang EK, Kim JH, Jean YH, et al. 1994. Current occurrence of porcine epidemic diarrhea in Korea. *RDA J Agric Sci* 36:587–596.
Ishikawa K, Sekiguchi H, Ogino T, et al. 1997. *J Virol Methods* 69:191–195.
Jabrane A, Girard C, Elazhary Y. 1994. *Can Vet J* 35:86–92.
Janetanakit T, Lumyai M, Bunpapong N, et al. 2016. *Emerg Infect Dis* 22:757–759.
Jiang X, Yu M, Qiao X, et al. 2014. *Appl Microbiol Biotechnol* 98:8301–8312.
Jiang X, Hou X, Tang L, et al. 2016. *Appl Microbiol Biotechnol* 100:7457–7469.
Jung K, Ahn K, Chae C. 2006. *Res Vet Sci* 81:310–315.
Jung K, Alekseev KP, Zhang X, et al. 2007. *J Virol* 81:13681–13693.
Jung K, Renukaradhya GJ, Alekseev KP, et al. 2009. *J Gen Virol* 90:2713–2723.
Jung K, Gurnani A, Renukaradhya GJ, et al. 2010. *Vet Immunol Immunopathol* 136:335–339.
Jung K, Wang Q, Scheuer KA, et al. 2014. *Emerg Infect Dis* 20:662–665.
Jung K, Annamalai T, Lu Z, et al. 2015a. *Vet Microbiol* 178:31–40.
Jung K, Hu H, Eyerly B, et al. 2015b. *Emerg Infect Dis* 21:650–654.
Jung K, Hu H, Saif LJ. 2016a. *Vet Microbiol* 182:57–63.
Jung K, Hu H, Saif LJ. 2016b. *Virus Res* 226:50–59.
Jung K, Hu H, Saif LJ. 2017. *Arch Virol* 162:2357–2362.
Kemeny LJ, Woods RD. 1977. *Am J Vet Res* 38:307–310.
Kemeny LJ, Wiltsey VL, Riley JL. 1975. *Cornell Vet* 65:352–362.
Khatri M. 2015. *Emerg Infect Dis* 21:549–550.
Kim O, Chae C. 2000. *Vet Pathol* 37:62–67.
Kim O, Chae C. 2003. *J Comp Pathol* 129:55–60.
Kim L, Chang KO, Sestak K, et al. 2000a. *J Vet Diagn Invest* 12:385–388.
Kim L, Hayes J, Lewis P, et al. 2000b. *Arch Virol* 145:1133–1147.
Kim SY, Song DS, Park BK. 2001. *J Vet Diagn Invest* 13:516–520.
Kim SH, Kim IJ, Pyo HM, et al. 2007. *J Virol Methods* 146:172–177.
Kim SH, Lee JM, Jung J, et al. 2015. *Arch Virol* 160:1055–1064.
Kim YK, Cho YY, An BH, et al. 2016. *Arch Virol* 161:1323–1328.
Knuchel M, Ackermann M, Muller HK, et al. 1992. *Vet Microbiol* 32:117–134.
Kodama Y, Ogata M, Simizu Y. 1980. *Am J Vet Res* 41:740–745.
Kroneman A, Cornelissen LA, Horzinek MC, et al. 1998. *J Virol* 72:3507–3511.
Ksiazek TG, Erdman D, Goldsmith CS, et al. 2003. *N Engl J Med* 348:1953–1966.
Kubota S, Sasaki O, Amimoto K, et al. 1999. *J Vet Med Sci* 61:827–830.
Kuwahara H, Nunoya T, Samejima T, et al. 1988. *J Jpn Vet Med Assoc* 41:169–173.
Kweon C, Kwon BJ, Jung TS, et al. 1993. *Korean J Vet Res* 33:249–254.
Kweon CH, Kwon BJ, Kang YB, et al. 1994. *Korean J Vet Res* 34:321–326.
Kweon CH, Kwon BJ, Lee JG, et al. 1999. *Vaccine* 17:2546–2553.
Langel SN, Paim FC, Lager KM, et al. 2016. *Virus Res* 226:93–107.

Lanza I, Brown IH, Paton DJ. 1992. *Res Vet Sci* 53:309–314.
Lanza I, Shoup DI, Saif LJ. 1995. *Am J Vet Res* 56:739–748.
Laude H, Gelfi J, Aynaud JM. 1981. *Am J Vet Res* 42:447–449.
Laude H, Charley B, Gelfi J. 1984. *J Gen Virol* 65 (Pt 2):327–332.
Laude H, Gelfi J, Lavenant L, et al. 1992. *J Virol* 66:743–749.
Laude H, van Reeth K, Pensaert M. 1993. *Vet Res* 24:125–150.
Laviada MD, Videgain SP, Moreno L, et al. 1990. *Virus Res* 16:247–254.
Lee S, Park GS, Shin JH, et al. 2014. *Genome Announc* 2:e01116-14.
Lee DU, Kwon T, Je SH, et al. 2016a. *Vet Microbiol* 192:90–94.
Lee JH, Chung HC, Nguyen VG, et al. 2016b. *Transbound Emerg Dis* 63:248–252.
Lesnick CE, Derbyshire JB. 1988. *Vet Immunol Immunopathol* 18:109–117.
Li BX, Ge JW, Li YJ. 2007. *Virology* 365:166–172.
Li W, Li H, Liu Y, et al. 2012. *Emerg Infect Dis* 18:1350–1353.
Li G, Chen Q, Harmon KM, et al. 2014. *Genome Announc* 2:e00278–e00214.
Li W, Luo R, He Q, et al. 2017. *Virus Res* 235:6–13.
Li W, Hulswit RJG, Kenney SP, et al. 2018. *Proc Natl Acad Sci U S A* 115:E5135–E5143.
Lin CN, Chung WB, Chang SW, et al. 2014. *J Vet Med Sci* 76:1297–1299.
Lin CM, Annamalai T, Liu X, et al. 2015a. *Vet Res* 46:134.
Lin CM, Gao X, Oka T, et al. 2015b. *J Virol* 89:3332–3342.
Lin CM, Saif LJ, Marthaler D, et al. 2016. *Virus Res* 226:20–39.
Liu X, Wang Q. 2016. *J Virol Methods* 234:137–141.
Liu C, Tang J, Ma Y, et al. 2015a. *J Virol* 89:6121–6125.
Liu X, Lin CM, Annamalai T, et al. 2015b. *Vet Res* 46:109.
Loewen KG, Derbyshire JB. 1988. *Can J Vet Res* 52:149–153.
Lohse L, Krog JS, Strandbygaard B, et al. 2016. *Transbound Emerg Dis* 64:1380–1386.
Lopez L, Venteo A, Garcia M, et al. 2009. *J Vet Diagn Invest* 21:598–608.
Lorsirigool A, Saeng-Chuto K, Temeeyasen G, et al. 2016. *Arch Virol* 161:2909–2911.
Ma SQ, Wang M, Feng L, et al. 1995. *Chin Anim Infect Dis* 6:23–27.
Ma G, Feng Y, Gao F, et al. 2005. *Biochem Biophys Res Commun* 337:1301–1307.
Ma Y, Zhang Y, Liang X, et al. 2015. *MBio* 6:e00064.
Ma Y, Zhang Y, Liang X, et al. 2016. *Vet Microbiol* 186:90–96.
Madson DM, Magstadt DR, Arruda PH, et al. 2014. *Vet Microbiol* 174:60–68.
Madson DM, Arruda PH, Magstadt DR, et al. 2015. *Vet Pathol* 53:44–52.
Martelli P, Lavazza A, Nigrelli AD, et al. 2008. *Vet Rec* 162:307–310.
Marthaler D, Jiang Y, Otterson T, et al. 2013. *Genome Announc* 1:e00555–13.
Marthaler D, Jiang Y, Collins J, et al. 2014a. *Genome Announc* 2:e00218–00214.
Marthaler D, Raymond L, Jiang Y, et al. 2014b. *Emerg Infect Dis* 20:1347–1350.
Masuda T, Murakami S, Takahashi O, et al. 2015. *Arch Virol* 160:2565–2568.
Masuda T, Tsuchiaka S, Ashiba T, et al. 2016. *Jpn J Vet Res* 64:5–14.
McClurkin AW, Norman JO. 1966. *Can J Comp Med Vet Sci* 30:190–198.
McClurkin AW, Stark SL, Norman JO. 1970. *Can J Comp Med* 34:347–349.
Meng F, Ren Y, Suo S, et al. 2013. *PLoS One* 8:e57468.
Mengeling WL, Cutlip RC. 1976. *J Am Vet Med Assoc* 168:236–239.
Mengeling WL, Boothe AD, Ritchie AE. 1972. *Am J Vet Res* 33:297–308.
Monger W, Alamillo JM, Sola I, et al. 2006. *Plant Biotechnol J* 4:623–631.
Moon HW. 1978. *J Am Vet Med Assoc* 172:443–448.
Moon HW, Kemeny LJ, Lambert G, et al. 1975. *Vet Pathol* 12:434–445.
Mou C, Zhu L, Xing X, et al. 2016. *Antiviral Res* 131:74–84.
Moxley RA, Olson LD. 1989. *Am J Vet Res* 50:111–118.
Nam E, Lee C. 2010. *Vet Microbiol* 144:41–50.
Narita M, Kawamura H, Tsuboi T, et al. 1989. *J Comp Pathol* 100:305–312.
Niederwerder MC, Nietfeld JC, Bai J, et al. 2016. *J Vet Diagn Invest* 28:671–678.
van Nieuwstadt AP, Zetstra T. 1991. *Am J Vet Res* 52:1044–1050.
van Nieuwstadt AP, Cornelissen JB, Zetstra T. 1988. *Am J Vet Res* 49:1836–1843.
van Nieuwstadt AP, Zetstra T, Boonstra J. 1989. *Vet Rec* 125:58–60.
Noda M, Yamashita H, Koide F, et al. 1987. *Arch Virol* 96:109–115.
O'Toole D, Brown I, Bridges A, et al. 1989. *Res Vet Sci* 47:23–29.
Ogawa H, Taira O, Hirai T, et al. 2009. *J Virol Methods* 160:210–214.
Oh JS, Song DS, Yang JS, et al. 2005. *J Vet Sci* 6:349–352.
Oka T, Saif LJ, Marthaler D, et al. 2014. *Vet Microbiol* 173:258–269.
Okda F, Liu X, Singrey A, et al. 2015. *BMC Vet Res* 11:180.
Okda F, Lawson S, Liu X, et al. 2016. *BMC Vet Res* 12:95.
Oldham J. 1972. Letter to the editor. *Pig Farming* (Suppl Oct):72–73.

Onno M, Jestin A, Cariolet R, et al. 1989. *Zentralbl Veterinarmed B* 36:629–634.
Ortego J, Ceriani JE, Patino C, et al. 2007. *Virology* 368:296–308.
Pan Y, Tian X, Qin P, et al. 2017. *Vet Microbiol* 211:15–21.
Park JE, Shin HJ. 2014. *Virus Res* 191:143–152.
Park S, Sestak K, Hodgins DC, et al. 1998. *Am J Vet Res* 59:1002–1008.
Paton DJ, Brown IH. 1990. *Vet Res Commun* 14:329–337.
Paudel S, Park JE, Jang H, et al. 2014. *Vet Q* 34:218–223.
Paul PS, Mengeling WL. 1984. *Am J Vet Res* 45:932–934.
Pensaert M. 1989. Transmissible gastroenteritis virus (respiratory variant). In Pensaert M, ed. *Virus Infections of Porcines*. Amsterdam: Elsevier Science Publishers, pp. 154–165.
Pensaert MB. 2006. Hemagglutinating encephalomyelitis virus. In Straw BL, Zimmerman JJ, D'Allaire S, et al., eds. *Diseases of Swine*. Ames, IA: Blackwell Publishing, pp. 353–358.
Pensaert MB, Callebaut PE. 1974. Characteristics of a coronavirus causing vomition and wasting in pigs. *Arch Gesamte Virusforsch* 44:35–50.
Pensaert MB, de Bouck P. 1978. *Arch Virol* 58:243–247.
Pensaert MB, Martelli P. 2016. *Virus Res* 226:1–6.
Pensaert M, Haelterman EO, Burnstein T. 1970. *Arch Gesamte Virusforsch* 31:321–334.
Pensaert M, Callebaut P, Vergote J. 1986. *Vet Q* 8:257–261.
Pensaert M, Cox E, van Deun K, et al. 1993. *Vet Q* 15:16–20.
Penzes Z, Gonzalez JM, Calvo E, et al. 2001. *Virus Genes* 23:105–118.
Pignatelli J, Grau-Roma L, Jimenez M, et al. 2010. *Vet Microbiol* 146:260–268.
Pilchard EI. 1965. *Am J Vet Res* 26:1177–1179.
Pospischil A, Hess RG, Bachmann PA. 1981. *Zentralbl Veterinarmed B* 28:564–577.
Posthumus WP, Lenstra JA, van Nieuwstadt AP, et al. 1991. *Virology* 182:371–375.
Pritchard GC. 1987. *Vet Rec* 120:226–230.
Puranaveja S, Poolperm P, Lertwatcharasarakul P, et al. 2009. *Emerg Infect Dis* 15:1112–1115.
Qing Y, Liu J, Huang X, et al. 2016. *Virus Genes* 52:218–227.
Quiroga MA, Cappuccio J, Pineyro P, et al. 2008. *Emerg Infect Dis* 14:484–486.
Rasschaert D, Duarte M, Laude H. 1990. *J Gen Virol* 71 (Pt 11):2599–2607.
Ren X, Li P. 2011. *Virus Genes* 42:229–235.
Ren X, Glende J, Yin J, et al. 2008. *Virus Res* 137:220–224.
Reynolds DJ, Garwes DJ. 1979. *Arch Virol* 60:161–166.
Reynolds DJ, Garwes DJ, Lucey S. 1980. *Vet Microbiol* 5:283–290.
Richards W, Savan M. 1960. *Cornell Vet* 50:132–155.
Saeng-Chuto K, Lorsirigool A, Temeeyasen G, et al. 2017. *Transbound Emerg Dis* 64:3–10.
Saif LJ 1989. Comparative aspects of enteric viral infections. In Saif LJ, Theil KW, eds. *Viral Diarrhea of Man and Animals*. Boca Raton, FL: CRC, pp. 9–34.
Saif LJ, Bohl EH. 1979. Role of SIgA in passive immunity of swine to enteric viral infections. In Ogra P, Dayton D, eds. *Immunology of Breast Milk*. New York, NY: Raven Press, pp. 237–248.
Saif LJ, Bohl EH. 1983. *Ann N Y Acad Sci* 409:708–723.
Saif L, Jackwood DJ. 1990. Enteric virus vaccines: Theoretical considerations, current status, and future approaches. In Saif L, Theil KW, eds. *Viral Diarrheas of Man and Animals*. Boca Raton, FL: CRC Press, Inc., pp. 313–329.
Saif LJ, Sestak K. 2006. Transmissible gastroenteritis virus and porcine respiratory coronavirus. In Straw BE, Zimmerman JJ, D'Allaire D, et al., eds. *Diseases of Swine*. Ames, IA: Blackwell Publishing Company, pp. 489–516.
Saif LJ, Bohl EH, Gupta RK. 1972. *Infect Immun* 6:600–609.
Saif LJ, Bohl EH, Kohler EM, et al. 1977. *Am J Vet Res* 38:13–20.
Saif LJ, van Cott JL, Brim TA. 1994. *Vet Immunol Immunopathol* 43:89–97.
Sanchez CM, Jimenez G, Laviada MD, et al. 1990. *Virology* 174:410–417.
Sanchez CM, Izeta A, Sanchez-Morgado JM, et al. 1999. *J Virol* 73:7607–7618.
Sasaki I, Kazusa Y, Shirai J, et al. 2003. *J Vet Med Sci* 65:381–383.
Sasseville AM, Gelinas AM, Sawyer N, et al. 2001. *Adv Exp Med Biol* 494:57–62.
Sato T, Takeyama N, Katsumata A, et al. 2011. *Virus Genes* 43:72–78.
Schlenstedt D, Barnikol H, Plonait H. 1969. Vomiting and distress in suckling pigs (short clinical report). *Dtsch Tierarztl Wochenschr* 76:694–695.
Schultze B, Krempl C, Ballesteros ML, et al. 1996. *J Virol* 70:5634–5637.
Schumacher LL, Woodworth JC, Jones CK, et al. 2016. *Am J Vet Res* 77:1108–1113.
Schwegmann-Wessels C, Herrler G. 2006. Transmissible gastroenteritis virus infection: A vanishing specter. *Dtsch Tierarztl Wochenschr* 113:157–159.
Schwegmann-Wessels C, Zimmer G, Laude H, et al. 2002. *J Virol* 76:6037–6043.
Sedlak K, Bartova E, Machova J. 2008. *J Wildl Dis* 44:777–780.
Sestak K, Lanza I, Park SK, et al. 1996. *Am J Vet Res* 57:664–671.
Sestak K, Meister RK, Hayes JR, et al. 1999a. *Vet Immunol Immunopathol* 70:203–221.
Sestak K, Zhou Z, Shoup DI, et al. 1999b. *J Vet Diagn Invest* 11:205–214.
Shibata I, Tsuda T, Mori M, et al. 2000. *Vet Microbiol* 72:173–182.

Shimizu M, Shimizu Y. 1979. *Infect Immun* 23:239–243.
Shin DJ, Park SI, Jeong YJ, et al. 2010. *Arch Virol* 155:417–422.
Shirato K, Maejima M, Islam MT, et al. 2016. *J Gen Virol* 97:2528–2539.
Shoup DI, Swayne DE, Jackwood DJ, et al. 1996. *J Vet Diagn Invest* 8:161–167.
Shoup DI, Jackwood DJ, Saif LJ. 1997. *Am J Vet Res* 58:242–250.
Simkins RA, Weilnau PA, Bias J, et al. 1992. *Am J Vet Res* 53:1253–1258.
Simkins RA, Weilnau PA, Van Cott J, et al. 1993. *Am J Vet Res* 54:254–259.
Sinha A, Gauger P, Zhang J, et al. 2015. *Vet Microbiol* 179:296–298.
Smerdou C, Urniza A, Curtis R 3rd, et al. 1996. *Vet Microbiol* 48:87–100.
Song DS, Oh JS, Kang BK, et al. 2007. *Res Vet Sci* 82:134–140.
Song D, Moon H, Kang B. 2015a. *Clin Exp Vaccine Res* 4:166–176.
Song D, Zhou X, Peng Q, et al. 2015b. *Transbound Emerg Dis* 62:575–580.
Song Q, Stone S, Drebes D, et al. 2016. *Virus Res* 226:85–92.
Stadler J, Zoels S, Fux R, et al. 2015. *BMC Vet Res* 11:142.
Stepanek J, Mensik J, Franz J, et al. 1979. Epizootiology, diagnosis and prevention of viral diarrhea in piglets under intensive husbandry conditions. In 21st World Veterinary Congress, Moscow, p. 43.
Stevenson GW, Hoang H, Schwartz KJ, et al. 2013. *J Vet Diagn Invest* 25:649–654.
Stone SS, Kemeny LJ, Woods RD, et al. 1977. *Am J Vet Res* 38:1285–1288.
Su M, Li C, Guo D, et al. 2016. *J Vet Med Sci* 78:601–606.
Sueyoshi M, Tsuda T, Yamazaki K, et al. 1995. *J Comp Pathol* 113:59–67.
Sun ZF, Meng XJ. 2004. *J Clin Microbiol* 42:2351–2352.
Sun RQ, Cai RJ, Chen YQ, et al. 2012. *Emerg Infect Dis* 18:161–163.
Sun D, Wang X, Wei S, et al. 2016. *J Vet Med Sci* 78:355–363.
Suzuki T, Murakami S, Takahashi O, et al. 2015. *Infect Genet Evol* 36:363–368.
Suzuki T, Shibahara T, Yamaguchi R, et al. 2016. *J Gen Virol* 97:1823–1828.
Takahashi K, Okada K, Ohshima K. 1983. An outbreak of swine diarrhea of a new-type associated with coronavirus-like particles in Japan. *Nihon Juigaku Zasshi* 45:829–832.
Teeravechyan S, Frantz PN, Wongthida P, et al. 2016. *Virus Res* 226:152–171.
Thachil A, Gerber PF, Xiao CT, et al. 2015. *PLoS One* 10:e0124363.
Thake DC. 1968. *Am J Pathol* 53:149–168.
Thomas JT, Chen Q, Gauger PC, et al. 2015. *PLoS One* 10:e0139266.
Torres JM, Alonso C, Ortega A, et al. 1996. *J Virol* 70:3770–3780.
Tuboly T, Nagy E. 2001. *J Gen Virol* 82:183–190.
Tuboly T, Nagy E, Derbyshire JB. 1995. *Can J Vet Res* 59:70–72.
Tuboly T, Yu W, Bailey A, et al. 2000. *Vaccine* 18:2023–2028.
Underdahl NR, Mebus CA, Stair EL, et al. 1972. *Can Vet J* 13:9–16.
Underdahl NR, Mebus CA, Torres-Medina A. 1975. *Am J Vet Res* 36:1473–1476.
VanCott JL, Brim TA, Simkins RA, et al. 1993. *J Immunol* 150:3990–4000.
VanCott JL, Brim TA, Lunney JK, et al. 1994. *J Immunol* 152:3980–3990.
Vaughn EM, Halbur PG, Paul PS. 1995. *J Virol* 69:3176–3184.
Vlasova AN, Zhang X, Hasoksuz M, et al. 2007. *J Virol* 81:13365–13377.
Vlasova AN, Marthaler D, Wang Q, et al. 2014. *Emerg Infect Dis* 20:1620–1628.
Vui DT, Tung N, Inui K, et al. 2014. *Genome Announc* 2:e00753–14.
Wang XM, Niu BB, Yan H, et al. 2013. *Arch Virol* 158:2487–2494.
Wang L, Byrum B, Zhang Y. 2014a. *Emerg Infect Dis* 20:1227–1230.
Wang L, Byrum B, Zhang Y. 2014b. *Emerg Infect Dis* 20:917–919.
Wang L, Byrum B, Zhang Y. 2014c. *Emerg Infect Dis* 20:1594–1595.
Wang L, Zhang Y, Byrum B. 2014d. *J Virol Methods* 207:154–157.
Wang D, Fang L, Shi Y, et al. 2015. *J Virol* 90:2090–2101.
Wang D, Fang L, Xiao S. 2016a. *Virus Res* 226:7–13.
Wang J, Deng F, Ye G, et al. 2016b. *Virol Sin* 31:49–56.
Wang L, Hayes J, Sarver C, et al. 2016c. *Arch Virol* 161:171–175.
Wang B, Liu Y, Ji CM, et al. 2018. *J Virol* 92:e00318-18.
Wesley RD, Woods RD. 1993. *Vet Microbiol* 38:31–40.
Wesley RD, Woods RD. 1996. *Am J Vet Res* 57:157–162.
Wesley RD, Woods RD, Hill HT, et al. 1990. *J Vet Diagn Invest* 2:312–317.
Wesley RD, Woods RD, McKean JD, et al. 1997. *Can J Vet Res* 61:305–308.
Wesseling JG, Vennema H, Godeke GJ, et al. 1994. *J Gen Virol* 75 (Pt 7):1789–1794.
Witte KH. 1971. *Zentralbl Veterinarmed B* 18:770–778.
Witte KH, Walther C. 1976. Age-dependent susceptibility of pigs to infections with the virus of transmissible gastroenteritis. In International Pig Veterinary Society Congress, Ames, IA, K3.

Woo PC, Lau SK, Lam CS, et al. 2012. *J Virol* 86:3995–4008.
Wood EN. 1977. *Vet Rec* 100:243–244.
Woods RD, Wesley RD. 1992. *Can J Vet Res* 56:78–80.
Woods RD, Wesley RD. 1998. *Adv Exp Med Biol* 440:641–647.
Woods RD, Wesley RD, Kapke PA. 1988. *Am J Vet Res* 49:300–304.
Xu X, Zhang H, Zhang Q, et al. 2013a. *Virol J* 10:26.
Xu X, Zhang H, Zhang Q, et al. 2013b. *Vet Microbiol* 164:212–221.
Xuan H, Xing D, Wang D, et al. 1984. *China J Vet Sci* 4:202–208.
Yaeger M, Funk N, Hoffman L. 2002. *J Vet Diagn Invest* 14:281–287.
Yagami K, Izumi Y, Kajiwara N, et al. 1993. *J Comp Pathol* 109:21–27.
Yu X, Shi L, Lv X, et al. 2015. *Virol J* 12:76.
Yuan X, Lin H, Fan H. 2015. *Vaccine* 33:3900–3906.
Zhang J. 2016. *Virus Res* 226:71–84.
Zhang X, Hasoksuz M, Spiro D, et al. 2007. *Virology* 358:424–435.
Zhang X, Alekseev K, Jung K, et al. 2008. *J Virol* 82:4420–4428.
Zhang D, Huang X, Zhang X, et al. 2016a. *J Virol Methods* 227:6–13.
Zhang J, Tsai YL, Lee PY, et al. 2016b. *J Virol Methods* 234:34–42.
Zhang Q, Shi K, Yoo D. 2016c. *Virology* 489:252–268.
Zhang J, Yim-Im W, Chen Q, et al. 2018. *Virus Genes* 54:323–327.
Zhao S, Gao Q, Qin T, et al. 2014. *Vet Microbiol* 171:74–86.
Zhou JF, Hua XG, Cui L, et al. 2007. *Antiviral Res* 74:36–42.
Zhou J, Huang F, Hua X, et al. 2010. *Virus Res* 149:51–55.
Zhou P, Fan H, Lan T, et al. 2018. *Nature* 556:255–258.
Zhu X, Liu S, Wang X, et al. 2018. *Emerg Microbes Infect* 7:65.

32

Filoviruses

Hana M. Weingartl and Glenn A. Marsh

Relevance

In late 2008, outbreaks of severe disease were reported on several pig farms in the Philippines. The Philippine authorities sought help from the US Department of Agriculture (USDA) Plum Island Foreign Animal Disease Diagnostic Laboratory (FADDL) for diagnosis, and porcine reproductive and respiratory syndrome virus (PRRSV) was detected and isolated from the samples along with porcine circovirus 2 (PCV2). In addition, an unexpected cytopathic effect (CPE) was detected in Vero cell cultures, and, using a panviral microarray RNA, Reston virus (RESTV) was detected (Barrette et al. 2009). Follow-up studies of farmworkers in the Philippines detected antibodies to RESTV, suggesting human exposure. Experimental studies demonstrated that domestic swine are susceptible to infection with RESTV (Marsh et al. 2011), with the virus replicating to high titer in the absence of clinical signs.

Natural infection of pigs with Ebola virus (EBOV) has not been reported, although experimental inoculation study showed that pigs are susceptible to EBOV infection (Kobinger et al. 2011). However, the role of domestic and/or feral pigs as either reservoirs or amplifying host is still unclear.

Etiology

The family *Filoviridae* falls under the order *Mononegavirales* and is divided into three genera: *Marburgvirus*, *Ebolavirus*, and *Cuevavirus* (Afonso et al. 2016). The *Ebolavirus* genus contains five species, namely, *Reston ebolavirus*, *Bundibugyo ebolavirus*, *Sudan ebolavirus*, *Zaire ebolavirus*, and *Taï Forest ebolavirus* with respective viruses, such as Reston virus (RESTV), Bundibugyo virus (BDBV), Sudan virus (SUDV), Ebola virus (EBOV), and Taï Forest virus (TAFV) (Kuhn et al. 2010). The RESTV isolates from the Philippine outbreak (Reston-08 A, C, and E) were approximately 97% identical at the nucleotide level to the original 1989 RESTV isolate (Barrette et al. 2009) and, therefore, were not assigned a new species designation.

Filovirus virions have a characteristic filamentous shape of uniform diameter (80 nm) and variable length (800–1400 nm). This permits the use of electron microscopy for preliminary determination of the virus family (Figure 32.1). The structure of filovirus virions is the most complex in the order *Mononegavirales*. This complexity provides for highly flexible virions, presumably to allow the long particles to bend without breaking the genome (Booth et al. 2013).

Ebolaviruses are negative-strand RNA viruses with approximately 19 kb non-segmented genome, composed of seven genes (3′-NP-VP35-VP40-GP-VP30-VP24-L-5′), each coding for one protein, except for the GP gene. As a result of RNA editing and subsequent cleavage of the protein products by cellular proteases, four proteins are expressed from the GP gene: sGP, delta peptide, ssGP, and $GP_{1,2}$. The RNA genome is protected by a helical protein capsid with the major component being the nucleocapsid protein (NP), while the L (large) protein (RNA-dependent RNA polymerase) represents a minor component. The NP and virus protein 30 (VP30) (transcription factor) form an inner nucleocapsid directly associated with the outer nucleocapsid, which is composed of VP35 (polymerase cofactor) and VP24. The outer nucleocapsid is connected via putative low density projections to the matrix VP40 layer on the cytoplasmic side of the viral envelope, derived from host plasma membrane. Glycoprotein $GP_{1,2}$ trimers are either embedded in the outer layer of the lipid envelope or secreted after cleavage by the host TNF-α-converting enzyme (Booth et al. 2013; Martin et al. 2016).

Following viral attachment to one of the numerous proteins in the cell membrane that can serve as a cellular attachment factor, the filoviruses enter the cells mainly by micropinocytosis, and the $GP_{1,2}$ is proteolytically

Diseases of Swine, Tenth Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson.

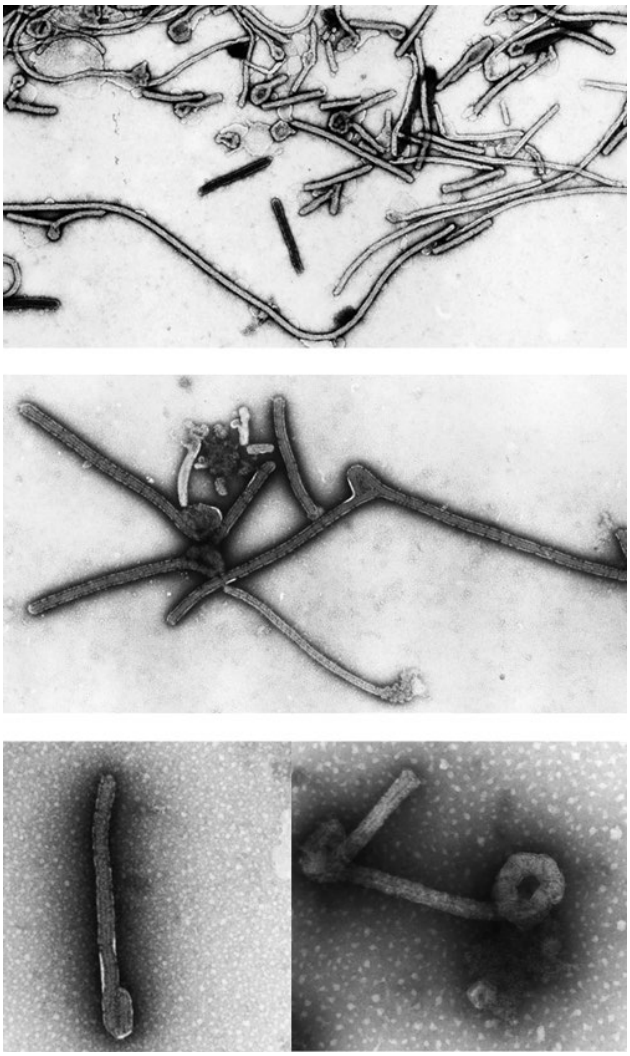


Figure 32.1 Electron micrographic images of different preparations of RESTV. Negative stain, increasing magnification from the top to the bottom. *Sources:* Two upper images from Roland et al. (2012); bottom images of individual particles courtesy of Lynn Burton.

activated in downstream endosomal vesicles. Interaction of cleaved GP_1 with NCP1 (Niemann–Pick C1 protein) anchored in the late endosomes/lysosomes triggers conformational changes in the GP_2, which leads to membrane fusion and release of the nucleocapsid into the cytoplasm. Filoviruses replicate in the cytoplasm in typical negative-stranded RNA virus fashion: primary transcription by the L protein and the VP30 transcription factor from the negative-sense genome is followed by translation, which leads to secondary transcription and production of more viral proteins. Once the viral proteins accumulate, RNA synthesis switches to genome replication. The eventual accumulation of nucleocapsid proteins results in the genome encapsidation. The $GP_{1,2}$ is glycosylated during transport through the endoplasmic reticulum and Golgi apparatus. The virion maturation is driven by the VP40 embedded in the internal layer of the plasma membrane, and the mature virions are generated by budding through the lipid bilayer of the host plasma membrane (Martin et al. 2016).

Several cell lines from different species were found to be susceptible to ebolavirus; however Vero (e.g. E6) cells have been found to be the most permissive, with the highest infectious titers in the range of 1×10^6 PFU/mL.

Public health

RESTV does not cause disease in humans and, therefore, is not a significant public health issue. However, because the mechanisms of attenuation in humans are not understood, RESTV is handled at Biosafety Level 4 (BSL-4). The concern is that outbreaks of RESTV with multiple pig-to-pig transmissions could result in virus mutations and the emergence of pathogenic strains able to cause disease in pigs and possibly humans. Identification and control of outbreaks is a key to prevent ongoing replication in pigs.

EBOV is a zoonotic virus and Ebola virus disease (EVD) outbreaks are of high public health significance. Historically, the number of outbreaks, cases, and fatalities was limited, and outbreaks were readily controlled. In December 2013, the first case of EVD occurred in Guinea. The outbreak, mainly in Guinea, Sierra Leone, and Liberia, lasted until 2016 and resulted in approximately 29,000 cases with over 11,000 fatalities. Although EVD was formerly known as Ebola hemorrhagic fever, bleeding was present in only approximately 30% of patients. In this outbreak, EVD was characterized by multiple nonspecific symptoms, such as fever and fatigue, followed by anorexia, headache, and gastrointestinal symptoms (Cherif et al. 2017).

Due to the apparent transmission of RESTV from pigs to humans in the 2008/2009 outbreak of highly pathogenic PRRSV, there is a concern that African ebolaviruses could also infect pigs (both domestic and wild) and the pigs then can serve as a source of infection for humans (Atherstone et al. 2017). In fact, increased die-outs in red river hogs (*Potamochoerus porcus*) in areas with EBOV outbreaks (Lahm et al. 2007) suggest that wild pigs could be infected with ebolavirus during outbreaks.

Reston virus

Epidemiology

Prior to the diagnosis of RESTV infection in swine in 2008, RESTV had only been associated with outbreaks of disease in nonhuman primates from the Philippines.

In addition to swine in the Philippines (Barrette et al. 2009; Sayama et al. 2012), RESTV infection of pigs in China has also been reported (Pan et al. 2014). The natural reservoir of RESTV in the Asian region has not been identified; however, several bat species in the region have been demonstrated to have antibodies that react to the virus.

Nonhuman primates are the only animals that have been demonstrated to develop fatal disease following infection with RESTV. Several humans have developed antibodies to the virus following exposure; however, disease associated with this exposure has never been recorded. This is consistent with observations in swine.

The mechanism of RESTV transmission in pigs is unknown. In experimentally infected pigs, high level of viral shedding from the nasopharynx was documented following oronasal challenge, with virus detected in nasal and tonsil swabs for up to a week after challenge (Marsh et al. 2011). This finding suggests that transmission via a droplet or aerosol route is possible; however, transmission studies have not been reported for pigs following exposure to RESTV. Virus has also been detected on rectal swabs from some animals on days 6 and 8 after exposure.

RESTV stability in the environment and susceptibility to disinfectant have not been reported, but it is expected to be similar to other filoviruses.

Pathogenesis, clinical signs, and immunity

Following exposure of 5-week-old pigs to RESTV, virus replication was observed in many different tissues. The highest levels of virus were observed in the lung and lymphoid tissues; however, virus was also detected in muscle tissue of some animals (Marsh et al. 2011). The infection profile was consistent with an acute infection, with no evidence indicating virus persistence in any tissue at necropsy on 28 days post inoculation (DPI). Clearance of virus was associated with seroconversion and occurred within 10–12 DPI. Antibodies (IgG) have been detected in pigs experimentally inoculated with RESTV within 6 DPI, and by 10 DPI all pigs had developed detectable antibody levels (Marsh et al. 2011).

In two studies of eight pigs each (Marsh et al. 2011), differences were observed in levels of viremia and virus shedding. The reason for these differences has not been determined; however, it was hypothesized that this difference may have been a consequence of season and underlying health status of animals. It is possible, although unproven, that a higher prevalence of subclinical respiratory infections in the winter may predispose animals to higher levels of virus replication. Importantly, despite the high levels of RESTV replication observed in animals following exposure, all animals remained clinically healthy, with no evidence of fever, respiratory disease, or skin lesions (Marsh et al. 2011).

Lesions

Lesions in pigs following exposure to RESTV are confined to the lymphoid and respiratory systems. Lymphadenomegaly affecting submandibular, retropharyngeal, and bronchial lymph nodes was a common finding in pigs necropsied on day 6 or 8 after exposure. Consolidation of lung lobes was also observed in some pigs, with RESTV antigen associated primarily with alveolar macrophages and also bronchoalveolar epithelial cells. Mild acute rhinitis was identified in all pigs, but antigen was only detected in submucosal lymphoid tissue and not the respiratory epithelium. Focal necrosis of tonsillar epithelium associated with neutrophil infiltrates has also been reported. By day 28 after exposure, no gross or histologic abnormalities are detected in animals (Marsh et al. 2011).

Diagnosis

Despite the fact that RESTV has not been associated with human disease, work involving infectious virus is restricted to BSL-4 laboratories in most countries. Standard culture techniques are suitable for RESTV, with Vero cells being the choice for virus isolation. Virus replication in Vero cells produces low-level CPE in 7–10 days, with immunostaining being a useful technique to confirm virus replication. Real-time RT-PCR assays targeting the L or the NP gene have been developed for the detection of RESTV in samples from pigs (Barrette et al. 2009; Marsh et al. 2011; Pan et al. 2014). Immunohistochemical staining of tissue samples can also be utilized to detect virus replication (Barrette et al. 2009; Marsh et al. 2011).

ELISA assays have been developed to detect antibodies to RESTV from swine samples, but assay performance has not been fully characterized (Marsh et al. 2011; Sayama et al. 2012). Other assays, such as virus neutralization, can be used to confirm ELISA results, although these assays require BSL-4 containment.

Prevention and control

There are no mechanisms to prevent or control RESTV (or other filovirus) infections in pigs. Given that the infection is subclinical, RESTV infections on pig farms would only be detected if routine screening for RESTV antibodies were conducted and positive results confirmed. Although the risk to humans is low, farms would probably be quarantined and depopulated. Due to the infrequency of infection in swine and the lack of disease, vaccines are unlikely to be available.

Ebola virus

Epidemiology

Human outbreaks of the four ebolaviruses – EBOV, SUDV, BDBV, and TAFV – so far occurred in a belt roughly corresponding to the tropical rain forest. Even the 2013–2016 outbreak started in a forest area of Guinea but spread through human-to-human contact throughout the West Africa, including major urban centers.

Several species of bats were determined to be disease-free ebolavirus reservoirs. Other species, such as duiker, and under laboratory conditions guinea pigs, mice, and pigs can also be infected by EBOV. Humans, great apes, and several species of nonhuman primates can be fatally infected by ebolaviruses, and there is a major concern that EBOV infections contribute to the severe decline of great ape populations, namely, gorillas, chimpanzees, and bonobos (Gonzalez et al. 2012). It has to be noted that the great apes eat pig carcasses and vice versa, which can contribute to wildlife ebolavirus transmission cycle, if the pigs are infected by EBOV in wild.

Field infections of swine with EBOV have not been reported, but pigs can be infected experimentally and transmit the virus to co-housed pigs and nonhuman primates (Weingartl et al. 2012). Shedding in pigs occurs predominantly via the respiratory tract, and infectious virus can be recovered from nasal washes and oral swabs for 3–14 DPI (Nfon et al. 2013).

Transmission of the virus to nonhuman primates housed in cages in the same room as EBOV-infected pigs is thought to occur via droplets, either by inhalation or fomites (Weingartl et al. 2012). Low amount of virus was also detected in feces, but only at 5 DPI. The virus in nasal wash (10^2–10^3 $TCID_{50}$/mL) was sufficient to infect contact piglets. A subsequent shedding in contact piglets in the range of 1×10^1–10^2 $TCID_{50}$/mL raises a question of whether virus spread in swine herds could be self-limiting (Kobinger et al. 2011).

EBOV can remain viable in liquid samples (such as blood) for many days, depending on temperature, but heating samples for 1 hour at 60 °C leads to inactivation. EBOV persisted in dried human blood for 7–10 days in the West African climate and remained viable on surfaces for days, including personal protective equipment, again depending on temperature and humidity. The virus can be inactivated with 70% ethanol or 0.5% sodium hypochlorite in minutes, and sodium hypochlorite was widely used for disinfection during the 2013–2016 outbreak. All filoviruses are also susceptible to lipid solvents and phenolic compounds. Animal facilities may be decontaminated by first removing organic material and then introducing formaldehyde vapor. A solution of 1% formaldehyde will also inactivate EBOV. On the other hand, EBOV is only moderately sensitive to ultraviolet (254 nm) irradiation (Cook et al. 2016; European Food Safety Authority 2014).

Pathogenesis, clinical signs, and lesions

EBOV pathogenesis in swine was described for experimental infections only. Four- to six-week-old pigs (Landrace) experimentally inoculated with 1×10^6 PFU/animal of EBOV-Kikwit via oronasal route developed clinical disease characterized by labored breathing, reluctance to move, and high rectal temperature (40–41.5 °C) starting at 4 DPI (Kobinger et al. 2011). In pigs, lungs appear to be the primary virus target. Virus antigen was detected in epithelial cells, endothelial cells, and alveolar macrophages, and massive infiltration of noninfected immune cells into the lungs was observed. This observation was different from the one made in the nonhuman primates co-housed with the pigs where virus antigen was also detected in lungs, primarily in macrophages; however there was no recruitment of other immune cells into the lungs (Weingartl et al. 2012).

Transient viremia (RNA detection) was observed only in some pigs at 5 or 6 DPI, and the infectious virus was detected in the lung, bladder, heart, liver, and trachea and also in draining lymph nodes of the respiratory tract, while viral RNA was present at 7 DPI in lung-associated submandibular and mesenteric lymph nodes, spleen, lung, tonsil, trachea, nasal turbinates, heart, liver, spleen, and intestine (Kobinger et al. 2011).

Age-related difference between 4- and 6-week-old piglets was observed in terms of severity of the respiratory signs: while older piglets suffered from severe acute respiratory distress, only some of the younger piglets had labored breathing. However, other age groups were not experimentally inoculated in these studies (Kobinger et al. 2011; Nfon et al. 2013; Weingartl et al. 2012).

Infection in pigs can be inapparent or with range in severity of respiratory clinical signs (rate increasing from 35 to more than 80 breaths per minute – labored with strong abdominal component) and can resemble other diseases of swine that affect the respiratory tract such as swine influenza or porcine reproductive and respiratory syndrome. Incubation period following high dose challenge is about 3 days, when the shedding starts, followed by clinical signs about a day later. Due to low number and narrow age span of the experimental animals, it is difficult to comment on morbidity and mortality rates, but it appears that both can be low in comparison with nonhuman primates, as recovery was observed early post inoculation in younger animals that were not euthanized (Weingartl et al. 2012).

Gross lesions were observed only in lungs (Figure 32.2a) and included lobe consolidation with individual lobules

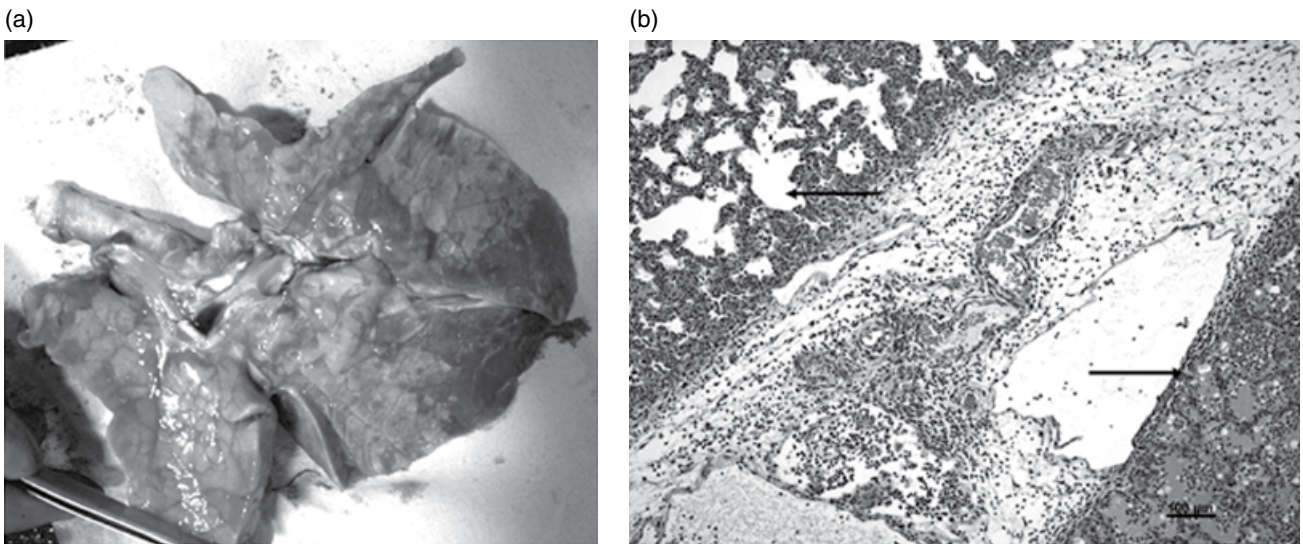


Figure 32.2 Lungs of 6-week-old piglet 5 days after inoculation with EBOV. (a) Macroscopic lesions. Lungs with consolidated lobes and dark-colored severely affected lobules. (b) Microscopic lesions. Left arrow indicates a relatively unaffected lobule; right arrow indicates a severely affected lobule with alveoli filled with fluid and inflammatory cell exudate. The interlobular septum is edematous, with vasculitis and inflammatory cell infiltrate. *Source:* Courtesy of James Neufeld.

of darker (plum) color. This pattern corresponded with bronchointerstitial pneumonia; the microscopic lesions localized to affected lobules (Figure 32.2b).

Diagnosis

Since ebolavirus infections of swine can resemble a number of respiratory diseases, diagnosis will require laboratory confirmation. Methods of virus detection include virus isolation and virus RNA and antigen detection that could be performed on inactivated samples under lower biosafety conditions.

Vero cells (E6, CV1, V76) are commonly used for virus isolation and propagation. Productive infection produces CPE in 9–10 DPI. Confirmation of virus isolation can be also performed by plaque assay combined with immunostaining, which can distinguish between the virus species (e.g. SUDV from EBOV). Diagnostic work involving live EBOV is, however, restricted to BSL-4 laboratories.

Viral RNA in samples can be detected using real-time RT-PCR, validated for swine samples (Pickering et al. 2018), and the presence of viral antigen in tissues can be confirmed by immunohistochemistry (Kobinger et al. 2011).

Methods of antibody detection available for the BSL-4 laboratories include virus neutralization, for example, microtiter plaque reduction assay (mPRNT), followed by immunohistochemical detection. Indirect IgG ELISA detecting antibodies against recombinant NP validated for swine serum samples heat inactivated at 60 °C for 1 hour is possible to perform under lower biosafety conditions. Confirmatory testing to exclude false positive samples can be done preferably by immunoblot using recombinant NP antigen, or at BSL-4 laboratory by mPRNT (Pickering et al. 2018).

Immunity

Under experimental infections with EBOV, striking differences in severity of the disease were observed between the 4-week-old and 6-week-old piglets (Kobinger et al. 2011; Nfon et al. 2013; Weingartl et al. 2012). This may be linked to the rapid development of the immune system that occurs in pigs between 3 and 6 weeks of age. It appears that dysregulation/over-activation of the pulmonary proinflammatory response causes the immunopathogenesis of ZEBOV infection in 6-week-old piglets (Nfon et al. 2013). Infiltration of neutrophils and monocytes/macrophages into the lungs, where only the macrophages are infected, was observed in all inoculated piglets, but the degree of the infiltration was far more severe in the 6-week-old animals. Genes for proinflammatory cytokines, acute-phase proteins, and chemokines were upregulated in lungs. Systemic effects included a decline in the proportion of monocyte/dendritic cells and B cells (Nfon et al. 2013).

The first IgM response was detected at 5 or 6 DPI (IgM capture ELISA using whole EBOV antigen) (Nfon et al. 2013). IgG against the recombinant NP was first detected at 7 DPI, depending on the animal. Virus neutralizing antibodies became detectable starting at 10 DPI (Pickering et al. 2018).

References

Afonso CL, Amarasinghe GK, Bányai K, et al. 2016. *Arch Virol* 161:2351–2360.

Atherstone C, Smith E, Ochungo P, et al. 2017. *Transbound Emerg Dis* 64:333–343.

Barrette RW, Metwally SA, Rowland JM, et al. 2009. *Science* 325:204–206.

Booth TF, Rabb MJ, Beniac DR. 2013. *Trends Microbiol* 21:583–593.

Cherif MS, Koonrungsesomboon N, Diallo MP, et al. 2017. *Eur J Clin Microbiol Infect Dis* 36:689–695.

Cook BWM, Cutts TA, Nikiforuk AM, et al. 2016. *Sci Rep* 6:38293.

European Food Safety Authority. 2014. An update on the risk of transmission of Ebola virus (EBOV) via the food chain. *EFSA J* 12:3884.

Gonzalez JP, Prugnolle F, Leroy E. 2012. *Curr Top Microbiol Immunol* 365:337–353.

Kobinger GP, Leung A, Neufeld J, et al. 2011. *J Infect Dis* 204:200–208.

Kuhn JH, Becker S, Ebihara H, et al. 2010. *Arch Virol* 155:2083–2103.

Lahm SA, Kombila M, Swanepoel R, et al. 2007. *Trans R Soc Trop Med Hyg* 101:64–78.

Marsh GA, Haining J, Robinson R, et al. 2011. *J Infect Dis* 204(Suppl 3):S804–S809.

Martin B, Hoenen T, Canard B, et al. 2016. *Antiviral Res* 135:1–14.

Nfon CK, Leung A, Smith G, et al. 2013. *PLoS One* 8:e61904.

Pan Y, Zhang W, Cui L, et al. 2014. *Arch Virol* 159:1129–1132.

Pickering B, Collingnon B, Smith G, et al. 2018. *Transbound Emerg Dis* 65:77–84.

Roland JM, Geisbert TW, Rowland RRR. 2012. Filovirus. In Zimmerman JJ, Karriker LA, Ramirez A, et al., eds. *Diseases of Swine*, 10th ed. New York: Wiley, pp. 525–527.

Sayama Y, Demetria C, Saito M, et al. 2012. *BMC Vet Res* 8:82.

Weingartl HM, Embury-Hyatt C, Nfon C, et al. 2012. *Sci Rep* 2:811.

33

Flaviviruses

David T. Williams, John S. MacKenzie, and John Bingham

Overview

Flaviviruses are a diverse group of viruses that include several important pathogens, such as yellow fever virus (YFV), dengue virus (DENV), Japanese encephalitis virus (JEV), West Nile virus (WNV), Murray Valley encephalitis virus (MVEV), Saint Louis encephalitis virus (SLEV), and Zika virus (ZIKV). Taxonomically, they belong to the family *Flaviviridae*, genus *Flavivirus*, which includes 53 recognized species and 19 unclassified viruses (ICTV 2017). Flaviviruses are divided into three groups based on vector association: tick-borne, mosquito-borne, and no known vector. This chapter will focus on JEV, the most important flavivirus pathogen of swine. We will also consider two other flaviviruses, WNV and MVEV, which can infect pigs and may play a role in the transmission cycle of these viruses. Japanese encephalitis (JE) and West Nile (WN) fever are OIE-listed diseases, in recognition of their propensity to spread and zoonotic potential.

Flavivirus infections cause a range of diseases in animals. Most infections lead to subclinical infection consisting of viremia, which may be low level and of short duration. A proportion of infections lead to more serious outcomes, including neurological disease, kidney disease, reproductive failure, and, in humans, hemorrhagic syndrome. It is likely that the underlying pathogenesis is similar across the viral lineages and host species and that there is a high degree of commonality in potential disease presentation. Therefore, while it is important to consider the disease entities reported for swine, it is worthwhile also to bear in mind that disease syndromes similar to those found in other species may occur. Indeed, new flaviviral syndromes and presentations have been described in recent years (Murray et al. 2010; Ricklin et al. 2016b), leading to the belief that the flaviviruses and the diseases they cause are probably more varied than originally thought. As flaviviruses move into new geographical ranges, as they infect new species, and as they infect hosts under varied physiological and pathological profiles, further new disease outcomes are likely to emerge.

The flavivirus genome is a single-stranded positive-sense RNA molecule approximately 11 kilobases in length. A single open reading frame encodes three structural proteins (capsid, pre-membrane [prM], envelope [Env]) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) and is flanked by noncoding regions (Lindenbach et al. 2013). Flavivirus virions are icosahedral in shape and are approximately 50 nm in diameter. Virus particles are enveloped by a host-derived lipid membrane containing the prM/M and Env proteins and have a buoyant density of 1.19–1.23 g/cm^3 (Russell et al. 1980). The envelope surrounds the nucleocapsid, which consists of capsid proteins and genomic RNA. The prM and Env proteins are involved in virus entry and particle assembly during cellular infection. The prM protein is enzymatically cleaved during virus egress to produce the M protein and mature virions. Flavivirus NS proteins have functional roles in viral polyprotein processing, viral RNA synthesis, and evasion of innate immunity. The noncoding regions contain conserved elements and secondary structures that are involved in viral RNA replication and translation.

Flaviviruses can be inactivated with various chemicals and disinfectants, including 3 mM binary ethylenimine, 3–8% paraformaldehyde, 2% glutaraldehyde, 1% hypochlorite, ethanol, isopropanol, and 0.05% Tween 20, as well as UV light (Mayo and Beckwith 2002; Muller et al. 2016; OIE 2013; Pyke et al. 2004a). The pH of the infected matrix or medium is also important for viability; ZIKV infectivity was highest at pH 9, whereas infectivity was lost at pH ≤4 or ≥12 (Muller et al. 2016). The thermal inactivation point of flaviviruses is 40 °C (OIE 2013), and virus present in serum or media can be inactivated by heating at 56 °C for 30 minutes (Fang et al. 2009). Incubation at 28 °C reduced the infectivity of WNV by 1000-fold after 4 days, while no reduction was found in

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

virus held at 4 °C (Mayo and Beckwith 2002). An evaluation of the environmental stability of ZIKV showed that drying virus culture for 18 hours reduced infectivity by 1000-fold; however, virus remained infectious for over 3 days (Muller et al. 2016). In infected mosquitoes, WNV or JEV RNA was detectable for up to 2 weeks after mosquito death under a variety of conditions, including high temperatures and humidity, but the ability to culture virus was lost after 24–48 hours (Johansen et al. 2002; Turell et al. 2002). These studies underline the importance of surface disinfection after handling potentially infected material, as well as the need for appropriate sample handling and transport for diagnostic testing.

Japanese encephalitis virus

Relevance

Epidemics of human encephalitis suggestive of JE occurred in Japan as early as 1871, and large summer outbreaks occurred in the 1920s and 1930s (Mackenzie et al. 2007). JEV was identified as the etiological agent in 1933 in Japan (Hayashi 1934) and subsequently isolated from *Culex tritaeniorhynchus* mosquitoes (Mitamura et al. 1938). The ecology of JEV was elucidated largely by early studies in Japan that demonstrated the importance of pigs in the amplification cycle (Scherer et al. 1959b). JEV is a major cause of human viral encephalitis and childhood viral neurological infection and disability in southern, eastern, and southeastern Asia, particularly affecting rural communities (Campbell et al. 2011; Mackenzie et al. 2007). JEV can also cause fatal encephalitis in horses. In pigs, infection with JEV can result in reproductive failure and can occasionally cause neurological disease in piglets.

Etiology

JEV exists as a single serotype and two major immunotypes can be recognized by various serological methods. There are five genotypes of JEV (Schuh et al. 2013; Solomon et al. 2003). Genotype 1 comprises strains from Cambodia, China, Korea, northern Thailand, Vietnam, Japan, India, Australia, and Taiwan. Genotype 2 is composed of strains from Indonesia, Malaysia, southern Thailand, Papua New Guinea, and Australia. Genotype 3 contains isolates from the known geographic range of JEV, with the exception of the Australasian region. Genotype 4 isolates have only been found in Indonesia. Genotype 5 strains have been found in Malaysia, China (Tibet), and South Korea. Over the past two decades, viruses belonging to genotype 1 have replaced circulating genotype 2 and genotype 3 viruses in several regions.

Public health

An estimated 68,000 human cases of JE occur annually in JE-endemic areas, with a combined population of over 3 billion (Campbell et al. 2011). Of these cases, approximately 75% occur in children aged 0–14 years. The majority of human infections are asymptomatic, with 1 in 50 to 1 in 1000 infections progressing to clinical disease (Vaughn and Hoke 1992). Approximately 20–30% of clinical JE cases are fatal, 30–50% develop long-term neuropsychological sequelae, and the remaining cases fully recover. Amplification of JEV in pigs is generally thought to precede epidemic transmission. However, epidemic activity in human populations can occur in the absence of large populations or high densities of pigs (Lord et al. 2015).

Epidemiology

JEV is a zoonotic virus maintained in nature by transmission cycles involving *Culex* sp. mosquitoes and certain species of wild and domestic birds and pigs as the vertebrate hosts (Burke and Leake 1988; Endy and Nisalak 2002). Humans become infected when bitten by an infected mosquito, but they are incidental, dead-end hosts. Of particular importance in the ecology of JEV is the interplay between rice cultivation, vector densities, and pig rearing in close proximity to human habitation (Gajanana et al. 1997; Kanojia et al. 2003; Le Flohic et al. 2013).

Pigs are the principal amplification hosts of JEV, especially in epidemic areas, and the maintenance hosts in endemic areas. They develop a high and prolonged viremia following natural infection, lasting 2–4 days and capable of infecting various mosquito species. In serological surveys, pigs consistently display higher virus titers than other domestic or wild animals. They are particularly attractive to the major mosquito vectors as the source of blood meals, providing a sensitive indicator of virus transmission in endemic areas (Burke et al. 1985b). Despite the potential risk to humans if raised in open pens near human habitation, pigs have been used as sentinel animals to monitor for virus activity in a number of countries, essentially serving as an early warning system. Recent results have suggested that JEV can transmit between pigs in the absence of arthropod vectors; virus was shown to be shed in oronasal secretions, and in-contact pigs were highly susceptible to infection by this route. Furthermore, it was found that virus replicated to high levels in the tonsils, and could persist in that site for at least 25 days, even in the presence of neutralizing antibody (Ricklin et al. 2016a). These results have considerable significance for JEV epidemiology and ecology, and further studies are urgently needed to verify the reports of direct transmission.

Horses, cattle, buffaloes, and goats are dead-end hosts (Mackenzie et al. 2007), but because they attract a number of the major vector species, especially *C. tritaeniorhynchus*, they make good potential hosts for surveillance and may act as "dampers" in an outbreak situation (Johnsen et al. 1974; Peiris et al. 1993). Other animals may have relatively high seroprevalence rates, such as sheep and dogs, but viremia levels are believed to be too low to infect mosquitoes (Banerjee et al. 1979; Johnsen et al. 1974).

JEV has been isolated from a number of bats belonging to the families *Pteropodidae*, *Rhinolophidae*, *Hipposideridae*, *Miniopteridae*, and *Vespertilionidae* (Mackenzie et al. 2016; Sulkin and Allen 1974), but most experimental studies of their potential role in maintenance and transmission have been carried out in microbats, in which viremia lasts as long as 25–30 days at a level sufficiently high to infect mosquitoes.

Wild birds, particularly ardeid species, are believed to be important maintenance hosts of JEV and may act as amplifiers in epidemics (Scherer et al. 1959a; Soman et al. 1977). JEV has been isolated from a number of avian species, but the most important is undoubtedly the black-crowned night heron (*Nycticorax nycticorax*). Chickens are rarely infected and may have a limited role in transmission or surveillance. Wild birds have been implicated in the spread of JEV to new areas (Scott 1988; Solomon et al. 2003).

JEV has been isolated from many mosquito species, but not all are believed to be competent to transmit the virus. There is a consensus that *C. tritaeniorhynchus* is the major vector throughout most of Asia, but other species may be locally important, such as *C. gelidus*, *C. vishnui*, *C. fuscocephala*, *C. pseudovishnui*, *C. bitaeniorhynchus*, *C. annulirostris*, *C. quinquefasciatus*, and *Mansonia uniformis* (Burke and Leake 1988; Rosen 1986; van den Hurk et al. 2009; Vaughn and Hoke 1992). The major *Culex* vectors of JEV are rice field-breeding species that bite during the night, particularly at dusk and dawn, and prefer animals to humans for blood meals.

The means by which JEV "survives" between epidemics and overwinters in its northern range has been the subject of speculation. There are four major hypotheses: (1) the virus overwinters in mosquito eggs after vertical transmission; (2) the virus survives in hibernating mosquitoes; (3) the virus survives in hibernating animals, such as reptiles or bats; and (4) the virus is reintroduced each year by migratory birds or even by mosquitoes blown by prevailing winds from endemic areas. Possibly, each of the four methods contributes to overwintering in different situations. In support of the first hypothesis, vertical transmission occurs in several *Aedes* and *Culex* species mosquitoes in nature and under laboratory conditions (Rosen et al. 1989).

Pathogenesis

Much of our knowledge of flavivirus pathogenesis comes from model species infected with various viruses, particularly JEV, WNV, SLEV, tick-borne encephalitis virus, and MVEV. The pathogenesis of flaviviruses that have been studied to date is essentially similar. Flavivirus infections are initiated following the bite of an infected mosquito or tick, although recent evidence suggests that contact transmission between pigs may also play a significant role (Ricklin et al. 2016a). The virus is believed to initially replicate in the keratinocytes of the skin and is transferred to the lymph nodes within dendritic cells before transfer to the circulation (Johnston et al. 2000). Viremia leads to dissemination within a range of parenchymal tissues. In mammals, viral replication has been demonstrated by histological assay in neural cell bodies (brain and ganglia), in connective tissue, and in skeletal, cardiac, and smooth muscle (Bingham et al. 2014). Replication probably also occurs in lymphoid tissues, particularly tonsil, as high viral RNA loads have been detected therein (Ricklin et al. 2016b), although the precise cell type in which replication takes place has not been determined. The cell and tissue tropism range and replication amount are markedly extended in immunocompromised hosts (Armah et al. 2007; Guarner et al. 2004). The incubation period (time from exposure to first clinical signs) is 2–9 days for 75% of human cases (Rudolph et al. 2014). Viremia is detectable usually within 1–5 days postinfection (DPI) and persists for approximately 4–5 days (Huang and Wong 1963; Ricklin et al. 2016b), falling with the rise of circulating antibody. Virus can reach the central nervous system (CNS) as early as 3 DPI (Monath et al. 1983; Yamada et al. 2004). How the virus enters the brain is not known, but is believed to be either via the olfactory mucosa, where there is no blood–brain barrier (Monath et al. 1983; Yamada et al. 2009), via direct disruption of the blood–brain barrier (Chang et al. 2015), or by transneuronal spread (Samuel et al. 2007). The severity of disease is, in part, determined by immunological factors of the host, as immunoincompetence results in high levels of disseminated virus in tissues (Armah et al. 2007; Guarner et al. 2004). Neonates and young animals appear to be more likely than adults to develop severe CNS infection.

JEV infection in pigs usually results in subclinical infection with viremia (Ricklin et al. 2016a). The circumstances under which clinical disease develops in pigs are not clearly understood, but it is likely that young age, underlying incidental disease processes, and lack of prior exposure to flaviviruses are factors that increase the likelihood that infection becomes established in the CNS and other organs. JEV in pigs may infect neurons, leading to a mononuclear cell meningoencephalitis with neurological signs (Ricklin et al. 2016b; Yamada et al. 2004),

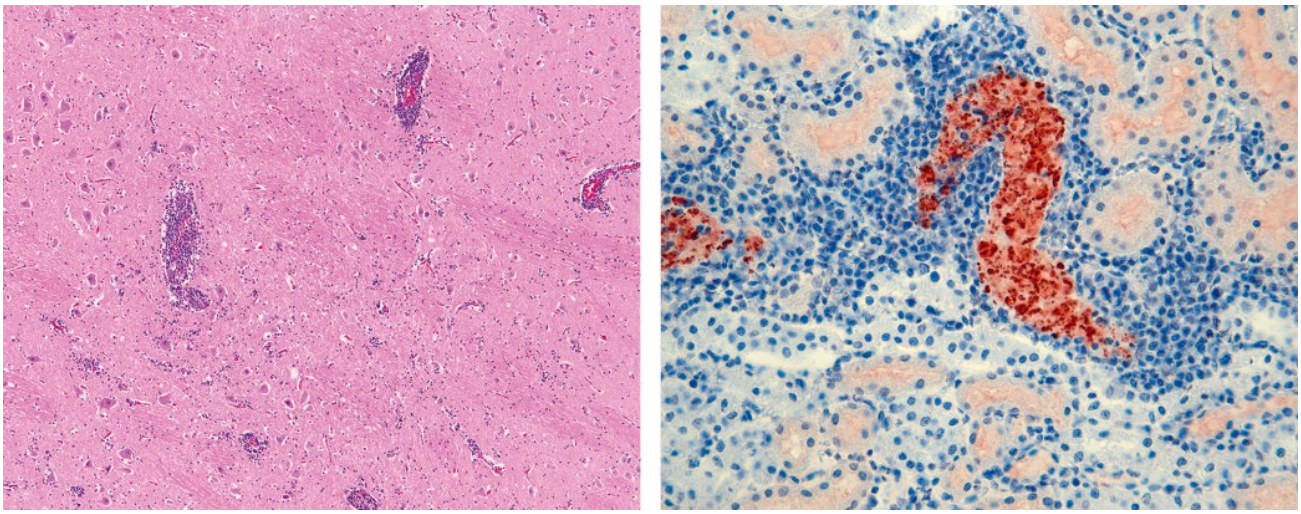


Figure 33.1 Japanese encephalitis virus infection in pigs, showing nonsuppurative inflammation in the brain (left; hematoxylin and eosin stain) and flaviviral antigen in a kidney tubule, with mononuclear cell interstitial nephritis (right; immunohistochemistry, flavivirus NS1 protein). No viral antigen was detected in affected brain. JEV was confirmed by viral sequence analysis. *Source:* Regional Animal Health Office 6, Ho Chi Minh City, Vietnam; laboratory diagnosis at Australian Animal Health Laboratory, Geelong, Australia.

or kidney tubules, resulting in interstitial nephritis (Figure 33.1; unpublished data from CSIRO, Australian Animal Health Laboratory, Australia and Regional Animal Health Office 6, Vietnam). Reproductive failure is also a recognized outcome of infection of adult pigs. JEV crosses the placenta to infect fetuses, causing abortion, stillbirths, mummification, and neonatal deaths (Burns 1950; Shimizu et al. 1954; Takashima et al. 1988). It may also infect testicular tissues in boars, causing infertility (Hashimura et al. 1954; Ogasa et al. 1977).

Clinical signs

Adult pigs do not generally show overt signs of infection. The most common disease manifestation in infected pregnant sows or gilts is reproductive failure leading to abortion and litters that contain stillborn or mummified fetuses or live, weak piglets (Burns 1950; Platt and Joo 2006; Takashima et al. 1988). Reproductive failure occurs in sows infected with JEV before 60–70 days of gestation; infections later in gestation do not appear to affect piglets. Natural infection of piglets with JEV is normally inapparent, but recent reports suggested that infection of young pigs 2–40 days of age may occasionally lead to a wasting syndrome with histological evidence of meningoencephalitis and with varying degrees of depression and hind limb tremors (Yamada et al. 2004).

JEV has also been associated with infertility in boars (Hashimura et al. 1976; Ogasa et al. 1977). Infection of boars may lead to edematous, congested testicles, resulting in lowered motile sperm counts and abnormal spermatozoa. These effects are usually temporary and complete recovery occurs in most instances.

Lesions

There are no characteristic lesions of JEV infection in sows that manifest reproductive failure. In boars, there is thickening of the tunica vaginalis and epididymis, with edema and inflammation of the epididymis, tunica vaginalis, and testis (Hashimura et al. 1976; Ogasa et al. 1977). Stillborn fetuses or live, weak piglets often present with hydrocephalus, subcutaneous (SC) edema, cerebellar hypoplasia, and spinal hypomyelinogenesis (OIE 2013). Hydrothorax, ascites, serosal petechiae, necrotic foci in the liver and spleen, and congestion in the lymph nodes, meninges, and spinal cord may also be found (Burns 1950). Infected piglets may present with diffuse nonsuppurative encephalitis, characterized by neuronal necrosis, neuronophagia, glial nodules, and perivascular cuffing in the brain and spinal cord (Yamada et al. 2004). Mononuclear cell interstitial nephritis is associated with viral replication in renal tubules (Figure 33.1).

Diagnosis

Agents causing clinical diseases characterized by abortion, fetal mummification or stillbirth, and encephalitis in pigs up to 6 months of age should be considered in the differential diagnosis. These should include pseudorabies (Aujeszky's disease) virus, classical swine fever virus, hemagglutinating encephalomyelitis virus, rubulavirus (blue eye paramyxovirus), Menangle virus, porcine brucellosis, porcine teschovirus, porcine parvovirus, porcine reproductive and respiratory syndrome virus, and salt poisoning (OIE 2013).

Laboratory diagnosis of JE normally involves virus isolation, reverse transcription polymerase chain reaction

(RT-PCR) and immunohistochemistry (IHC) for agent detection, and serological tests for antibody detection (Yang 2016). For specimen submission to the laboratory, placental tissues from fetuses, stillbirths, and neonates from cases of reproductive disease should be collected; virus may be detected in the brain, spleen, liver, or placenta. A full range of tissues should also be collected into 10% formalin for histopathology. Whole blood and serum samples should be collected for virus detection and serology. Paired serum should be collected 2–4 weeks apart. Unfixed tissues and blood/serum should be chilled and transported under refrigerated conditions. If delays in transit of ≥48 hours are anticipated, specimens should be frozen and shipped on dry ice.

Definitive diagnosis depends on the isolation or detection of JEV in clinical specimens. Since viremia lasts only a few days and virus can rarely be isolated from either blood or cerebrospinal fluid (CSF), infected brain, spleen, liver, or placental tissues may provide the best chance of virus isolation. This can be achieved by inoculating tissue homogenates (or whole blood and CSF) intracerebrally into suckling mice or onto susceptible cells. A range of cells including primary chicken embryo, the continuous *Aedes albopictus* mosquito cell line C6/36, African green monkey kidney (Vero), and baby hamster kidney (BHK) cells are permissive for JEV infection. JEV does not normally cause cytopathic effect (CPE) in C6/36 cells. Therefore, further culture in vertebrate cells and/or detection of viral antigen or RNA may be required to confirm virus isolation. Confirmatory testing of brain homogenates of mice that succumb to inoculation should be performed in a similar manner.

Of the molecular methods available for detection of JEV, RT-PCR is most commonly employed in diagnostic laboratories to identify viral genome in clinical specimens or following culture. Several assays have been reported, including both conventional and real-time tests (Pyke et al. 2004b; Tanaka 1993; Yang et al. 2004). RT-loop-mediated isothermal amplification (LAMP) assays have been reported for molecular testing of JEV infections. LAMP offers a simplified and convenient assay format for nucleic acid testing without the need for sophisticated equipment or skilled personnel (Liu et al. 2012; Parida et al. 2006). When compared with real-time RT-PCR, RT-LAMP has been shown to perform with comparable levels of sensitivity for testing swine specimens (Liu et al. 2012). Multiplex PCR assays targeting JEV and other swine pathogens may be useful in achieving a diagnosis by the process of exclusion or for surveillance activities. These include conventional assays for detecting coinfecting DNA or RNA swine viruses (Xu et al. 2012), microsphere array assays for detection of closely related flaviviruses (Foord et al. 2014), or "syndromic" real-time PCRs for diagnosis of swine viruses associated with respiratory and reproductive disease (Wu et al. 2014; Zhang et al. 2015).

JEV antigen can be detected in serum specimens using flavivirus-specific monoclonal antibodies (MAb) and in fetal tissues using IHC (Iwasaki et al. 1986; Yamada et al. 2004). Detection of antigen in infected mouse brains can be performed by hemagglutination assay using sucrose/acetone extracts, followed by a hemagglutination inhibition (HI) test using JEV reference antiserum for typing (Yang 2016).

As for other flaviviruses, serological diagnosis of JEV infections is often based on detection of serum antibodies using ELISA, HI, immunofluorescent antibody (IFA), and virus neutralization (VN) assays (Beaty et al. 1995; Burke et al. 1987; Clarke and Casals 1958; Yang 2016).

In older pigs, interpretation of serological tests needs to take a number of factors into consideration, including vaccination history and age. Maternal antibody persists in some pigs for as long as 8 months (Hale et al. 1957). IgM antibody appears 2–3 days after infection and persists for at least 2 weeks (Burke et al. 1985a) and, in some animals, several months. There is a high degree of serological cross-reactivity between flaviviruses, especially among members of the JEV group, and care must be taken in interpretation of serological results (Williams et al. 2001). In locations where related flaviviruses are known to co-circulate, these should be included in parallel testing.

Various ELISA formats have been reported or are available commercially. IgM capture (MAC-) ELISA may be used for diagnosing acute or recent infections (Pant et al. 2006). Competitive and indirect ELISAs have been developed and applied to the detection of antibody responses to JEV, including for seroprevalence studies (Kolhe et al. 2015; Pant et al. 2006; Williams et al. 2001; Yang et al. 2006). The HI test has also been used in serosurveys of pigs or wild boars (Nidaira et al. 2014; Yamanaka et al. 2010). To resolve cross-reacting antibody responses, the more specific VN test is recommended. Of the different formats described, the plaque reduction neutralization test (PRNT) is the most specific, particularly when the 90% threshold is applied. The specificity of NT antibody response can be confirmed by the demonstration of a rise in titers between paired serum samples. Results may be considered significant for the identification of JEV if titers are ≥ fourfold than a reaction against any related flavivirus. However, VN tests are technically difficult, are time consuming, and may not always yield clear results. Measurement of the magnitude of antibody response and relative differential of serum antibody titers against different flaviviruses can also be used to evaluate the significance of serological results (Pant et al. 2006). Since the viral NS1 protein is only produced by natural infection, ELISAs to detect NS1 antibodies can be used to differentiate infected from vaccinated animals (DIVA), as described for horses (Konishi et al. 2004).

Immunity

The flavivirus proteins Env, prM, and NS1 are immunogenic and the most important for inducing protective immune responses (Pierson and Diamond 2013). The Env protein is a major component of the virion surface and the main target of neutralizing antibodies. Uncleaved prM is present in immature or partially mature virus particles released from infected cells and can also elicit host immunity. NS1 is absent from the flavivirus virion, but is expressed on the surface of infected cells and is secreted in high levels, mainly as a hexamer. Antibodies to NS1 can protect against infection *in vivo* via the complement pathway or Fc-γ receptor-mediated immune complex clearance (Chung et al. 2006; Schlesinger et al. 1987).

Cross-neutralizing antibody responses to JEV have been observed in pigs following experimental infection with closely related flaviviruses. Prior exposure to MVEV prevented or suppressed the level and duration of viremia following challenge with JEV and correlated with heightened levels of existing cross-reactive virus-neutralizing antibodies to JEV (Lunt et al. 2001; Williams et al. 2001). Similar booster responses occurred if infection with WNV was followed by challenge with JEV. However, levels of JEV viremia varied, ranging from undetected or low (Ilkal et al. 1994; Williams et al. 2001) to virus titers equivalent to primary infected animals (Lunt et al. 2001). This variation may reflect differences in virus strains, breed, or age of the pigs used. In regions hyperendemic for flaviviruses, cross-reactive immunity in pigs from prior infection with related flaviviruses may be expected to inhibit subsequent infection with JEV; thus, amplification and spread of the virus may be restricted, along with a reduced risk of porcine reproductive disease.

Prevention and control

The current JEV vaccines and future directions have been extensively reviewed (Halstead and Thomas 2011; Wilder-Smith and Halstead 2010; Yun and Lee 2014). Vaccine development has largely focused on human vaccines rather than vaccines for pigs. The most commonly used JEV vaccine for human use in endemic areas of Asia is a live attenuated cell culture-derived vaccine based on the SA14-14-2 strain (genotype 3) produced in China. Elsewhere, two other vaccines have been used: a Vero cell-derived inactivated (SA-14-14-2) vaccine in use since 2009, especially for travelers, and a chimeric YFV/JEV vaccine based on the YFV 17D vaccine strain, but with the prM and E protein genes of the JEV SA14-14-2 strain. The latter virus has been licensed for use in Thailand and Australia.

Inactivated, mouse brain-derived, or live attenuated vaccines have been used to prevent virus amplification or reproductive disease in pigs in Japan (Igarashi 2002), Taiwan (Fan et al. 2013), Nepal (Pant et al. 2006), and Korea (Nah et al. 2015). The live attenuated vaccine is more efficacious than the inactivated vaccine against natural and experimental infection (Daniels et al. 2002; Ueba et al. 1978). Vaccination of swine against JEV is not widely used for two reasons: (1) immunization of large numbers of newborn animals each year is costly, and (2) the period for effective immunization using live attenuated JE vaccine is limited by the presence of maternal antibodies (Igarashi 2002). However, in areas of high JEV endemicity, pig vaccination may be efficacious in reducing the incidence of pig infections and therefore the risk of human disease (Khan et al. 2014). The emergence of genotype 1 throughout much of Asia has raised concerns about the efficacy of genotype 3-based vaccines. A study of cross-protection based on serum from humans vaccinated with genotype 3 inactivated vaccines demonstrated cross-neutralizing responses to genotypes 1–4 using a PRNT, thus implying cross-protective capacity (Erra et al. 2013). However, similar studies using horse or pig sera from genotype 3 JEV-vaccinated animals from Korea and Taiwan showed little or no cross-neutralization to a local isolate belonging to genotype 1 (Fan et al. 2013; Kang et al. 2016). This highlights the importance of further cross-protection studies to determine whether vaccination with current genotype 3 vaccine strains can prevent viremia and reproductive disease in pigs following challenge with heterologous genotype strains.

JEV infection of pigs can also be controlled by preventing their exposure to infected mosquitoes, but this is usually not practical unless pigs are maintained in a mosquito-free environment. Mosquito management and control programs implemented for public health purposes in proximity of pig farming activities may also be expected to reduce mosquito biting activity and therefore swine infections.

West Nile virus

Relevance

WNV was first isolated in Uganda in 1937 from the blood of a febrile woman (Smithburn et al. 1940). It is one of the most widely distributed flaviviruses, with a geographic range encompassing Africa, the Middle East, Europe, Asia, and Australasia (Hubalek and Halouzka 1999; Murgue et al. 2002). WNV was detected in North America in 1999 when its introduction caused an outbreak in New York City (Nash et al. 2001). Since then, it has rapidly spread throughout mainland United States, to Canada, and to Latin America and the Caribbean.

Historically, WNV infections were associated with sporadic cases or occasional outbreaks of mild disease in humans, birds, and horses; only rarely were these

associated with neurological disease. However, from the mid-1990s, large outbreaks of severe disease involving high incidences of neurological infection in humans occurred in Europe, the Mediterranean Basin, and the United States (Hayes and Gubler 2006; McLean et al. 2002; Murgue et al. 2002). These were accompanied by large numbers of equine encephalitis cases and avian mortalities (Castillo-Olivares and Wood 2004; CDC 2002). In equines, approximately 10% of infected animals develop neurological disease, with mortality ranging from 10–20% to 40–60% (Castillo-Olivares and Wood 2004; Roche et al. 2013). Large numbers of mortalities in wild birds and commercial flocks have also occurred during epidemics in the United States and Europe; however mass mortality of susceptible bird species has occurred less frequently in Europe (Komar et al. 2003; Weinberger et al. 2001). Rare cases of WNV neurological disease have also been reported in sheep, alpacas, seals, alligators, squirrels, white-tailed deer, and reindeer (Heinz-Taheny et al. 2004; Miller et al. 2003, 2005; Palmer et al. 2004; Yaeger et al. 2004). Although WNV is a significant pathogen of human, birds, and horses, it is not known to be pathogenic for pigs.

Etiology

A wide spectrum of genetic diversity exists within the WNV species, with seven distinct phylogenetic lineages recognized (Rizzoli et al. 2015). Lineages 1 and 2 were the first described (Lanciotti et al. 2002; Scherret et al. 2001). Viruses belonging to these lineages are the dominant circulating types and are responsible for human and animal disease. Lineage 1 is divided into three clades: lineage 1a comprises strains from Africa, the Middle East, Europe, the Americas, and India, lineage 1b is made up of the Australian subtype Kunjin (KUNV), and lineage 1c is composed of human and mosquito isolates from India. Lineage 2 consists of the prototype Ugandan strain and strains from Africa, Madagascar, and Europe. Additional lineages of WNV comprise isolates from Europe, Malaysia, and West Africa. These have not yet been associated with disease. Within and between lineages and clades, virulent and attenuated strains have been characterized (Perez-Ramirez et al. 2017; Prow et al. 2016).

Public Health

Approximately 20% of human infections are symptomatic, typically manifesting as a self-limited WN fever (Rizzoli et al. 2015; Sejvar 2014). Less than 1% of infections develop into disease of the CNS (meningoencephalitis, encephalomyelitis). The incidence of severe disease increases with age, with the highest risk occurring in the elderly. Case fatality rates can range between 10 and 30% during outbreaks, and neurological sequelae can persist in over half of survivors 1 year after infection.

Epidemiology

WNV exists in endemic transmission cycles involving ornithophilic mosquitoes and birds. The virus can infect a remarkable range of vectors and vertebrate hosts, thus facilitating its rapid spread throughout the world. In the United States alone, the virus has been isolated from approximately 60 species of mosquitoes, >300 species of birds, and >30 species of non-avian hosts (Gubler 2007). *Culex* species mosquitoes are the most common vector, particularly those of the *Culex pipiens* complex, but WNV has also been isolated from species of *Aedes*, *Anopheles*, *Coquillettidia*, *Culiseta*, *Mimomyia*, and *Ochlerotatus* (Hall et al. 2002; Hubalek and Halouzka 1999; Komar et al. 2003). Evidence from serological surveys and experimental infections has established birds as the primary vertebrate hosts of WNV (Komar et al. 2003). Passerine species such as the house sparrow, blue jay, and common grackle are thought to be important for epidemic transmission in urban settings, while migratory species such as storks (order *Ciconiiformes*) may be important for long-range virus transmission and spread.

The majority of non-avian species infected by WNV, including humans and horses, are incidental hosts and are thought to play only minor roles, if any, in transmission. Serological evidence of WNV infection in domestic and feral pigs has been reported in Australia, Asia, the United States, and Europe (Escribano-Romero et al. 2015; Gard et al. 1976; Geevarghese et al. 1987; Gibbs et al. 2006; Pant et al. 2006), suggesting that pigs may be useful sentinels for WNV activity. There are limited reports of WNV isolation from pigs in the field (Ilkal et al. 1994). Furthermore, experimental infections indicate that pigs are generally poor amplifying hosts of WNV (Ilkal et al. 1994; Lunt et al. 2001; Platt 2004; Teehee et al. 2005). Viremic responses can vary from undetectable to moderate and appear to depend on the age of animals and virus strain. In weanling pigs experimentally infected subcutaneously with a NY99 strain, virus titers reached sufficient levels (10^4–$10^{5.5}$ $TCID_{50}$/mL) to infect blood-feeding *C. pipiens* mosquitoes (Platt 2004). However, the duration of peak viremia was relatively short, ranging from 0.2 to 1.1 days. This contrasts with that observed in passerine birds infected with a NY99 strain, in which titers exceeded 10^8 plaque-forming units/mL for 4 days (Komar et al. 2003). Unlike JEV in pigs, transmission between infected and noninfected control pigs housed together in experimental conditions was not found (Platt 2004; Teehee et al. 2005). Adult pigs also failed to become infected by ingestion when fed WNV-infected mice (Teehee et al. 2005), in contrast to cats.

Similar to JEV transmission, WNV occurs year-round in tropical and subtropical regions, but is epidemic in temperate regions. WNV can overwinter in hibernating adult mosquitoes (Nasci et al. 2001), and evidence of vertical transmission in *Culex* species mosquitoes has also been reported (Anderson and Main 2006), indicating potential mechanisms by which the virus can survive in temperate areas. Reintroduction of WNV by viremic migratory birds or perpetuation in persistently infected birds has also been proposed (Kramer et al. 2008). In addition, ticks may play a role in WNV maintenance and overwintering (Lawrie et al. 2004; Mumcuoglu et al. 2005).

Pathogenesis, clinical signs, and lesions

There is little information available on the pathogenesis of WNV in pigs. In experimentally infected domestic pigs, no clinical signs were observed (Platt 2004; Teehee et al. 2005; unpublished observations from CSIRO, Australian Animal Health Laboratory). Platt (2004) observed moderate perivascular cuffing in the brain and spinal cord tissue of infected weanling pigs in the absence of viral antigen. In a proportion of pigs with perivascular cuffing, meningoencephalitis was also evident. In the same study, a pregnant sow was infected during the second trimester of gestation. Fetus development was unaffected by maternal infection, no abnormal histological features were observed, and no viral antigen was detected in fetal CNS tissue.

Diagnosis

Serological diagnosis is the primary means of determining WNV infections (Dauphin and Zientara 2007; Ostlund and Monaco 2013; Sambri et al. 2013). Human and veterinary specimens may be tested by ELISA, HI, and PRNT for the diagnosis of clinical cases or for serosurveillance of domestic animals and wildlife populations, including domestic and feral pigs or wild boar. IgM and IgG ELISA test kits are commercially available. In many instances, serological testing involves initial screening by ELISA or HI, followed by PRNT to confirm positive samples. As described above for JEV, serological diagnosis of WNV can be complicated by the presence of cross-reactive antibody to closely related flaviviruses, such as JEV (India), Usutu (Europe), SLEV (North America), and MVEV (Australia); therefore, these should be included in parallel testing where relevant. Serological diagnosis of horses should also take into account WNV vaccination status.

WNV can be detected in clinical specimens (e.g. CSF, blood, or tissues) or mosquitoes following isolation in cell culture using conventional or real-time RT-PCR and by immunoassay. Cell lines used for virus isolation include Vero, rabbit kidney (RK-13), pig kidney, or mosquito cells (C6/36 or AP-61). Confirmation of WNV isolation can be done by testing culture extracts by immunoassay using WNV-specific antibodies or by RT-PCR.

Antigen-capture assays can be used to detect WNV particles in field-collected avian swabs and mosquito pools and in human serum (Burkhalter et al. 2006; Hunt et al. 2002) or secreted WNV NS1 protein in serum (Saxena et al. 2013). IHC staining of fixed tissue samples from infected birds and mammals has also been used to detect WNV antigen. Caution should be exercised when testing mammalian tissues using this method because many equine WN encephalitis cases yield false-negative results (Ostlund and Monaco 2013) and nonspecific binding of primary antibody to mammalian tissues has been reported (Kauffman et al. 2003). A method for IHC staining of pig brain and spinal cord tissue has been reported (Platt 2004).

Molecular diagnostic assays have been reported for the detection of WNV RNA in infected mosquitoes and clinical specimens, including conventional (Johnson et al. 2001) or real-time RT-PCR assays (Eiden et al. 2010; Lanciotti et al. 2000; Tang et al. 2006) and RT-LAMP assays (Parida et al. 2004). Specificity for selected WNV lineages is an important regional consideration. The real-time assays cited above can detect lineage 1 and 2 viruses co-circulating in Europe. In Australia, an RT-PCR was developed for the specific detection of WNV-KUNV (lineage 1b) (Pyke et al. 2004b), found only in the Australasian region where other WNV lineages are exotic. Fall et al. (2016) described a real-time RT-PCR assay for the detection and genotyping of lineages circulating in Africa. A pan-WNV real-time RT-PCR assay has also been recently reported for the detection of viruses belonging to all of the recognized lineages (Vazquez et al. 2016).

Prevention and control

There is no specific treatment available for WNV infection. Several veterinary vaccines have been licensed (Brandler and Tangy 2013; McVey et al. 2015). In the United States and Europe, cell culture-derived inactivated whole virus vaccines have been approved for use in horses. Live canary poxvirus-vectored vaccines expressing the WNV prM and Env genes have also been approved in the United States and Europe. In the United States, a chimeric live attenuated vaccine comprising WNV prM and Env genes in a YFV 17D genome backbone was licensed for equine use, but discontinued because of a high incidence of adverse events. A DNA vaccine encoding the prM and Env genes was approved for use in horses, but also discontinued. In addition, an inactivated human cell-derived whole virus vaccine was

approved for use in geese in Israel, and a cell culture-derived inactivated whole virus vaccine was licensed for use in alligators.

Vector control activities include reducing mosquito populations through source reduction and insecticides and public education to limit exposure to mosquito bites (Kramer et al. 2008). WNV surveillance involves identifying infection in humans, horses, wildlife, and bird populations (especially corvids and sentinel chickens), by either passive or active means, as well as mosquito trapping to monitor vector abundance and virus activity (CDC 2013). The use of predictive models that include climate data (temperature, rainfall), prior disease incidence, vector density, and avian mortality can predict the relative risk of virus activity (McVey et al. 2015).

Murray Valley encephalitis

Relevance

MVEV is the most important cause of arboviral neurological disease in humans in Australia. MVEV can cause fatal encephalitis in horses (Gard et al. 1977; Gordon et al. 2012; Roche et al. 2013) and has been associated with morbidity and mortality in young farmed ducks (unpublished data from CSIRO, Australian Animal Health Laboratory). Although pigs can be infected with MVEV, no disease association has been reported (Kay et al. 1985; Lunt et al. 2001). Pigs may, however, play a role in the maintenance of virus transmission.

Etiology

Isolates of MVEV are closely related antigenically and cannot be differentiated by standard serological techniques. Four genotypes of MVEV are recognized (Williams et al. 2015). Genotype 1 is the dominant type on mainland Australia, and the most recent strains from Papua New Guinea belong to this type. There are two distinct sublineages of G1 (A and B) that recently evolved and appear to have different patterns of transmission in Australia: G1B strains have been isolated from across the mainland, whereas G1A strains have not been detected outside the northwest of the country. Genotype 2 consists only of mosquito isolates from the northwestern Australia. The remaining genotypes each comprise single isolates from Papua New Guinea made in 1956 (G3) and 1966 (G4).

Public health

Epidemics of severe encephalitis were reported between 1917 and 1925 in eastern and southeastern Australia, but no etiology could be determined. MVEV was isolated for the first time from fatal cases during a major outbreak of encephalitis in the Murray–Darling river system of southeastern Australia in 1951 (French 1952). A second major outbreak occurred in 1974, and although 58 cases (13 fatal) were reported from all mainland Australian states, the majority of cases were observed again in southeastern Australia (Marshall 1988). In 2011, following record levels of rainfall, widespread virus activity occurred across Australia, which led to 17 human cases of MVE disease and 3 deaths (Selvey et al. 2014). Sporadic cases of disease occur between outbreaks, with almost all occurring in northern and northwestern Australia, where the virus is believed to be endemic. Most human infections are asymptomatic or cause mild febrile illness, and only approximately 1:150 to 1:1000 infected individuals develop encephalitis, with fatality and morbidity rates similar to those for JE (Knox et al. 2012).

Epidemiology

Although the major epidemics of MVE occurred in southeastern Australia in the last century, MVEV is now endemic in northern Australia. The virus is also found in New Guinea and probably the eastern Indonesian archipelago.

The principal vector, *C. annulirostris*, accounts for the majority of isolates. Based on serosurveys and experimental evidence, the main vertebrate hosts are thought to be water birds belonging to the orders *Ciconiiformes*, *Pelecaniformes*, and *Anseriformes* (Marshall 1988). In particular, herons have been implicated as having a prominent role in MVEV transmission. Experimental infections of animals indicated that other vertebrates might also contribute to the transmission cycle, including gray kangaroos and rabbits (high levels of viremia), dogs, and chickens (moderate viremia) (Kay et al. 1985). MVEV has also been isolated from sentinel chickens, suggesting a potential role in the transmission cycle (Campbell and Hore 1975).

The role of pigs in the ecology of MVEV remains unclear. Serosurveys demonstrated a high prevalence of seropositive feral pigs in eastern Australia (Gard et al. 1976). However, following experimental infections, pigs were found to produce only moderate to low-grade viremia (Kay et al. 1985). Since feral pig densities are extremely high in parts of northern and eastern Australia (Choquenot et al. 1996), it has been proposed that even if only a small proportion respond to infection with viremia capable of infecting mosquito vectors, a significant contribution may be made to the maintenance or amplification of MVEV (Marshall 1988). However, a study of host-feeding preferences of *C. annulirostris* in northern Australia showed that the majority of blood meals were obtained from marsupials (>60%), suggesting

that host-seeking vectors are diverted away from feral pigs (van den Hurk et al. 2003).

It is not well understood how MVEV resurfaces in regions where occasional or rare activity is found. Virus may be reintroduced by migratory viremic water birds or windblown mosquitoes. There is also evidence that MVEV can be maintained over dry periods in desiccation-resistant eggs of *A. normensis* (Broom et al. 1989). Thus, environmental factors such as wind, rainfall, and temperature are likely to affect transmission and maintenance.

Pathogenesis and clinical signs

No disease or clinical signs following MVEV infection of pigs have been reported. There is also no available evidence that infection leads to secondary amplification in reproductive organs or fetuses. Kay et al. (1985) performed experimental infections on domestic and feral pigs aged between 6 and 20 weeks. These animals responded with low to moderate, but variable, levels of viremia lasting from 1 to 5 DPI. In some pigs, titers were considered sufficient to infect *C. annulirostris*; however this was not empirically tested. Of significance was the infection of a single pregnant sow that farrowed 14 weeks after inoculation. No mummified fetuses, stillbirths, or birth defects in the neonates were reported. Although the sow did not develop detectable viremia, an HI antibody response was observed. Surprisingly, no antibodies were detected in the offspring, and half of these developed trace viremia after challenge with MVEV, indicating little or no protection from maternal antibody. Further studies involving pregnant sows are needed to fully assess the potential for reproductive disease following MVEV infection.

Diagnosis

The majority of MVEV infections are diagnosed using serologic assays (e.g. HI, VN, IgM-IFA, and competitive ELISA) (Williams et al. 2010). HI, VN assays, and ELISA have been used for antibody detection in serological surveys of feral swine or following experimental infections of pigs (Gard et al. 1976; Lunt et al. 2001; Williams et al. 2001). IgM capture ELISA may also be used to detect antibodies following recent infection.

MVEV can be cultivated using the same range of cell lines as JEV. Isolation of MVEV from clinical specimens or mosquitoes is typically achieved using Vero cells or C6/36 cells, followed by passage in Vero cells or other vertebrate cell lines. Intracerebral (IC) inoculation of suckling mice or chorioallantoic membranes of embryonated chicken eggs has also been used. A fixed cell enzyme immunoassay with NS1-specific antibody has been used for detection of MVEV antigen following culture (Broom et al. 1998). Conventional and real-time RT-PCR assays for the detection of viral RNA in extracts of culture isolates, human clinical samples, and infected mosquitoes have been reported (Niven et al. 2017; Pyke et al. 2004b; Williams et al. 2010). A RT-LAMP assay specific for MVEV has also been described (Gong et al. 2015). In addition, MVEV has been incorporated as a target for a multiplexed microsphere array assay for detecting viral RNA (Foord et al. 2014). IHC for detecting viral antigen has been described (Barton et al. 2015).

Prevention and control

There is no vaccine to protect against, or specific antiviral drugs to treat, infections with MVEV. Prevention is largely focused on public health measures informed by arbovirus surveillance and vector management and control programs (Selvey et al. 2014). In Australia, monitoring of sentinel chickens for seroconversion to MVEV is routinely carried out in most states. Mosquito trapping in the summer and autumn months is also performed to determine vector abundance and test for the presence of virus. These programs enable early detection and warning of MVEV activity.

Other flaviviruses

There are few reports of other flaviviruses causing infections in pigs. Notably, pigs have been used in preliminary studies to develop animal models of human infections caused by DENV and ZIKV (Cassetti et al. 2010; Darbellay et al. 2017). The anatomical and physiological similarities of pigs to humans, availability of immunological reagents, and genomic, transcriptomic, and proteomic tools make pigs an attractive alternative to nonhuman primates for studying human infectious diseases (Bassols et al. 2014).

Yucatan miniature pigs infected with DENV serotype 1 (DENV1) via the SC or intravenous routes produced virus NT antibodies; however viremia was only observed following SC infection (Cassetti et al. 2010). When SC-inoculated pigs were subsequently challenged with DENV1, rash and dermal edema were observed, accompanied by DENV immune complexes in the serum, suggestive of antibody-dependent enhancement (ADE) of infection.

Neonatal pigs were inoculated with ZIKV to evaluate their susceptibility and potential to model infection (Darbellay et al. 2017). IC, intradermal (ID), or intraperitoneal routes of inoculation resulted in the development of low-level viremia, as well as IgM and NT antibody responses, in piglets. Viral RNA was also detected in the urine, brain (IC only), and spleen of infected pigs. Clinical

signs (leg weakness, ataxia, and tremor) were only observed in 2/11 IC-inoculated pigs.

The results from these studies suggest that pigs could be used to investigate the pathogenesis of DENV and ZIKV infections and test human vaccines and antivirals, but additional research is required to further develop and refine these models.

The potential for pigs to serve as amplifying or maintenance hosts for ZIKV was also investigated using 3-month-old pigs inoculated by SC and ID routes (Ragan et al. 2017). No clinical signs were observed and viremia was not detected, nor was infectious virus in tissues collected at necropsy (7 DPI). All pigs seroconverted by day 7. Therefore, although it would appear unlikely that pigs could serve as possible reservoirs for ZIKV, they might prove useful as sentinel animals.

The possibility that other flaviviruses, including many obscure ones, may emerge to cause disease in swine should not be discounted.

References

Anderson JF, Main AJ. 2006. *J Infect Dis* 194:1577–1579.

Armah HB, Wang G, Omalu BI, et al. 2007. *Brain Pathol* 17:354–362.

Banerjee K, Ilkal MA, Bhat HR, et al. 1979. *Indian J Med Res* 70:364–368.

Barton AJ, Prow NA, Hall RA, et al. 2015. *Aust Vet J* 93:53–57.

Bassols A, Costa C, Eckersall PD, et al. 2014. *Proteomics Clin Appl* 8:715–731.

Beaty BJ, Calisher CH, Shope RE. 1995. Arboviruses. In Lennette E, Lennette DA, Lennette ET, eds. *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*. Washington, DC: American Public Health Association, pp. 189–212.

Bingham J, Payne J, Harper J, et al. 2014. *J Gen Virol* 95:1221–1232.

Brandler S, Tangy F. 2013. *Viruses* 5:2384–2409.

Broom AK, Wright AE, Mackenzie JS, et al. 1989. *J Med Entomol* 26:100–103.

Broom AK, Hall RA, Johansen CA, et al. 1998. *Pathology* 30:286–288.

Burke DS, Leake CJ. 1988. Japanese encephalitis. In Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Boca Raton, FL:CRC Press, pp. 63–92.

Burke DS, Tingpalapong M, Elwell MR, et al. 1985a. *Am J Vet Res* 46:2054–2057.

Burke DS, Tingpalapong M, Ward GS, et al. 1985b. *Southeast Asian J Trop Med Public Health* 16:199–206.

Burke DS, Nisalak A, Gentry MK. 1987. *J Med Virol* 23:165–173.

Burkhalter KL, Lindsay R, Anderson R, et al. 2006. *J Am Mosq Control Assoc* 22:64–69.

Burns KF. 1950. *Proc Soc Exp Biol Med* 75:621–625.

Campbell J, Hore DE. 1975. *Aust Vet J* 51:1–3.

Campbell GL, Hills SL, Fischer M, et al. 2011. *Bull World Health Organ* 89:766–774.

Cassetti MC, Durbin A, Harris E, et al. 2010. *Vaccine* 28:4229–4234.

Castillo-Olivares J, Wood J. 2004. *Vet Res* 35:467–483.

CDC. 2002. *Morb Mortal Wkly Rep* 51:1129–1133.

CDC. 2013. West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control, pp. 1–69. https://www.cdc.gov/westnile/resourcepages/pubs.html. Accessed July 4, 2017.

Chang CY, Li JR, Chen WY, et al. 2015. *Glia* 63:1915–1932

Choquenot D, McIlroy J, Korn T. 1996. *Managing Vertebrate Pests: Feral Pigs*. Canberra: Australian Government Publishing Service.

Chung KM, Nybakken GE, Thompson BS, et al. 2006. *J Virol* 80:1340–1351.

Clarke DH, Casals J. 1958. *Am J Trop Med Hyg* 7:561–573.

Daniels PW, Williams DT, MacKenzie JS. 2002. Japanese encephalitis. In Morilla A, Yoon K-J, Zimmerman JJ, eds. *Trends in Emerging Viral Diseases of Swine*. Ames, USA: Iowa State Press, pp. 249–263.

Darbellay J, Lai K, Babiuk S, et al. 2017. *Emerg Microbes Infect* 6:e6.

Dauphin G, Zientara S. 2007. *Vaccine* 25:5563–5576.

Eiden M, Vina-Rodriguez A, Hoffmann B, et al. 2010. *J Vet Diagn Invest* 22:748–753.

Endy TP, Nisalak A. 2002. *Curr Top Microbiol Immunol* 267:11–48.

Erra EO, Askling HH, Yoksan S, et al. 2013. *Clin Infect Dis* 56:267–270.

Escribano-Romero E, Lupulovic D, Merino-Ramos T, et al. 2015. *Vet Microbiol* 176:365–369.

Fall G, Faye M, Weidmann M, et al. 2016. *Vector Borne Zoonotic Dis* 16:781–789.

Fan YC, Chen JM, Chen YY, et al. 2013. *Vet Microbiol* 163:248–256.

Fang Y, Brault AC, Reisen WK. 2009. *Am J Trop Med Hyg* 80:862–863.

Foord AJ, Boyd V, White JR, et al. 2014. *J Virol Methods* 203:65–72.

French EL. 1952. *Med J Aust* 1:100–103.

Gajanana A, Rajendran R, Samuel PP, et al. 1997. *J Med Entomol* 34:651–659.

Gard GP, Giles JR, Dwyer-Grey RJ, et al. 1976. *Aust J Exp Biol Med Sci* 54:297–302.

Gard GP, Marshall ID, Walker KH, et al. 1977. *Aust Vet J* 53:61–66.

Geevarghese G, Shaikh BH, Jacob PG, et al. 1987. *Indian J Med Res* 86:413–418.

Gibbs SE, Marlenee NL, Romines J, et al. 2006. *Vector Borne Zoonotic Dis* 6:261–265.

Gong R, Wang HH, Qin H, et al. 2015. *Biomed Environ Sci* 28:227–30.

Gordon AN, Marbach CR, Oakey J, et al. 2012. *J Vet Diagn Invest* 24:431–436.
Guarner J, Shieh WJ, Hunter S, et al. 2004. *Hum Pathol* 35:983–990.
Gubler DJ. 2007. *Clin Infect Dis* 45:1039–1046.
Hale JH, Lim KA, Colless DH. 1957. *Ann Trop Med Parasitol* 51:374–379.
Hall RA, Broom AK, Smith DW, et al. 2002. *Curr Top Microbiol Immunol* 267:253–269.
Halstead SB, Thomas SJ. 2011. *Expert Rev Vaccines* 10:355–364.
Hashimura K, Uemiyada S, Komemura S, et al. 1954. Experimental stillbirth in pregnant swine infected with Japanese encephalitis virus. In Summary 81st Meeting Japanese Society of Veterinary Science, p. 176.
Hashimura WM, Uemiyada S, Komemra S, et al. 1976. Isolation of Japanese encephalitis virus from orchitis in pigs. In Summary 81st Meeting Japanese Society of Veterinary Science, p. 136.
Hayashi M. 1934. Ubertragung des virus von encephalitis epidemica aud affen. *Proc Imp Acad Tokyo* 10:41–44.
Hayes EB, Gubler DJ. 2006. *Annu Rev Med* 57:181–194.
Heinz-Taheny KM, Andrews JJ, Kinsel MJ, et al. 2004. *J Vet Diagn Invest* 16:186–190.
Huang CH, Wong C. 1963. *Acta Virologica* 7:322–330.
Hubalek Z, Halouzka J. 1999. *Emerg Infect Dis* 5:643–650.
Hunt AR, Hall RA, Kerst AJ, et al. 2002. *J Clin Microbiol* 40:2023–2030.
van den Hurk AF, Johansen CA, Zborowski P, et al. 2003. *Med Vet Entomol* 17:403–411.
van den Hurk AF, Smith CS, Field HE, et al. 2009. *Am J Trop Med Hyg* 81:457–462.
ICTV. 2017. The Online (10th) Report of the International Committee on Taxonomy of Viruses.
Igarashi A. 2002. *Curr Top Microbiol Immunol* 267:139–152.
Ilkal MA, Prasanna Y, Jacob PG, et al. 1994. *Acta Virol* 38:157–161.
Iwasaki Y, Zhao JX, Yamamoto T, et al. 1986. *Acta Neuropathol* 70:79–81.
Johansen CA, Hall RA, van den Hurk AF, et al. 2002. *Am J Trop Med Hyg* 67:656–661.
Johnsen DO, Edelman R, Grossman RA, et al. 1974. *Am J Epidemiol* 100:57–68.
Johnson DJ, Ostlund EN, Pedersen DD, et al. 2001. *Emerg Infect Dis* 7:739–741.
Johnston LJ, Halliday GM, King NJ. 2000. *J Invest Dermatol* 114:560–568.
Kang BK, Hwang JM, Moon H, et al. 2016. *Clin Exp Vaccine Res* 5:26–30.
Kanojia PC, Shetty PS, Geevarghese G. 2003. *Indian J Med Res* 117:104–110.
Kauffman EB, Jones SA, Dupuis AP 2nd, et al. 2003. *J Clin Microbiol* 41:3661–3667.
Kay BH, Young PL, Hall RA, et al. 1985. *Aust J Exp Biol Med Sci* 63:109–126.
Khan SU, Salje H, Hannan A, et al. 2014. *PLoS Negl Trop Dis* 8:e3166.
Knox J, Cowan RU, Doyle JS, et al. 2012. *Med J Aust* 196:322–326.
Kolhe RP, Bhilegaonkar KN, Dubbal ZB, et al. 2015. *Indian J Anim Res* 49:343–349.
Komar N, Langevin S, Hinten S, et al. 2003. *Emerg Infect Dis* 9:311–322.
Konishi E, Shoda M, Ajiro N, et al. 2004. *J Clin Microbiol* 42:5087–5093.
Kramer LD, Styer LM, Ebel GD. 2008. *Annu Rev Entomol* 53:61–81.
Lanciotti RS, Kerst AJ, Nasci RS, et al. 2000. *J Clin Microbiol* 38:4066–4071.
Lanciotti RS, Ebel GD, Deubel V, et al. 2002. *Virology* 298:96–105.
Lawrie CH, Uzcategui NY, Gould EA, et al. 2004. *Emerg Infect Dis* 10:653–657.
Le Flohic G, Porphyre V, Barbazan P, et al. 2013. *PLoS Negl Trop Dis* 7:e2208.
Lindenbach BD, Murray CL, Thiel H-J, et al. 2013. *Flaviviridae*. In Knipe DM, Howley PM, eds. *Fields Virology*, 6th ed. Philadelphia, USA: Lippincott Williams & Wilkins, pp. 712–746.
Liu H, Liu ZJ, Jing J, et al. 2012. *Vector Borne Zoonotic Dis* 12:1042–1052.
Lord JS, Gurley ES, Pulliam JR. 2015. *PLoS Negl Trop Dis* 9:e0004074.
Lunt RA, Boyle DG, Middleton DJ, et al. 2001. *Arbovirus Res Aust* 8:220–224.
Mackenzie JS, Williams DT, Smith DW. 2007. Japanese encephalitis virus: The geographic distribution, incidence and spread of a virus with a propensity to emerge in new areas. In Tabor E, ed. *Perspectives in Medical Virology: Emerging Viruses in Human Populations*. Amsterdam: Elsevier, pp. 201–268.
Mackenzie JS, Childs JE, Field HE, et al. 2016. The role of bats as reservoir hosts of emerging neuroviruses. In Reiss SC, ed. *Neurotropic Viral Infections*. Switzerland: Springer International Publishing, pp. 403–454.
Marshall ID. 1988. Murray Valley and Kunjin encephalitis. In Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Boca Raton, FL: CRC Press, pp. 151–189.
Mayo DR, Beckwith WH. 2002. *J Clin Microbiol* 40:3044–3046.
McLean RG, Ubico SR, Bourne D, et al. 2002. *Curr Top Microbiol Immunol* 267:271–308.
McVey DS, Wilson WC, Gay CG. 2015. West Nile virus. *Rev Sci Tech Off Int Epiz* 34:431–439.
Miller DL, Mauel MJ, Baldwin C, et al. 2003. *Emerg Infect Dis* 9:794–799.
Miller DL, Radi ZA, Baldwin C, et al. 2005. *J Wildl Dis* 41:246–249.
Mitamura T, Kitaoka M, Mori K, et al. 1938. Isolation of the virus of Japanese epidemic encephalitis from mosquitoes caught in nature. *Tokyo Iji Shinshi* 62:820.
Monath TP, Cropp CB, Harrison AK. 1983. *Lab Invest* 48:399–410.
Muller JA, Harms M, Schubert A, et al. 2016. *Emerg Infect Dis* 22:1685–1687.

Mumcuoglu KY, Banet-Noach C, Malkinson M, et al. 2005. *Vector Borne Zoonotic Dis* 5:65–71.
Murgue B, Zeller H, Deubel V. 2002. *Curr Top Microbiol Immunol* 267:195–221.
Murray K, Walker C, Herrington E, et al. 2010. *J Infect Dis* 201:2–4.
Nah JJ, Yang DK, Kim HH, et al. 2015. *Clin Exp Vaccine Res* 4:130–136.
Nasci RS, Savage HM, White DJ, et al. 2001. *Emerg Infect Dis* 7:742–744.
Nash D, Mostashari F, Fine A, et al. 2001. *N Engl J Med* 344:1807–1814.
Nidaira M, Kyan H, Taira K, et al. 2014. *Epidemiol Infect* 142:856–860.
Niven DJ, Afra K, Iftinca M, et al. 2017. *Emerg Infect Dis* 23:280–283.
Ogasa A, Yokoki Y, Fujisaki Y, et al. 1977. Reproductive disorders in boars infected experimentally with Japanese encephalitis virus. *Jpn J Anim Reprod* 23:171–175.
OIE. 2013. Japanese encephalitis. In OIE Technical Disease Card. World Organisation for Animal Health (OIE). http://www.oie.int/animal-health-in-the-world/technical-disease-cards. Accessed June 30, 2017.
Ostlund EN, Monaco F. 2013. Chapter 2.1.24: West Nile Fever. In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2017. World Organisation for Animal Health (OIE), pp. 1–13 (Adopted by the OIE in 2013).
Palmer MV, Stoffregen WC, Rogers DG, et al. 2004. *J Vet Diagn Invest* 16:219–222.
Pant GR, Lunt RA, Rootes CL, et al. 2006. *Comp Immunol Microbiol Infect Dis* 29:166–175.
Parida M, Posadas G, Inoue S, et al. 2004. *J Clin Microbiol* 42:257–263.
Parida MM, Santhosh SR, Dash PK, et al. 2006. *J Clin Microbiol* 44:4172–4178.
Peiris JS, Amerasinghe FP, Arunagiri CK, et al. 1993. *Trans R Soc Trop Med Hyg* 87:541–548.
Perez-Ramirez E, Llorente F, Del Amo J, et al. 2017. *J Gen Virol* 98:662–670.
Pierson TC, Diamond MS. 2013. Flaviviruses. In Knipe DM, Howley PM, eds. *Fields Virology*, 6th ed. Philadelphia, USA: Lippincott Williams and Wilkins, pp. 747–794.
Platt KB 2004. Characterization of West Nile Virus Infection in Swine. Research Report for US National Pork Board (NPB# 02-118), pp. 1–13.
Platt KB, Joo HS. 2006. Japanese encephalitis and West Nile viruses. In Straw BE, Zimmerman JJ, D'Allaire S, et al., eds. *Diseases of Swine*, 9th ed.. Ames, IA: Blackwell Publishing, pp. 359–365.
Prow NA, Edmonds JH, Williams DT, et al. 2016. *Emerg Infect Dis* 22:1353–1362.
Pyke AT, Phillips DA, Chuan TF, et al. 2004a. *BMC Microbiol* 4:3.
Pyke AT, Smith IL, van den Hurk AF, et al. 2004b. *J Virol Methods* 117:161–167.
Ragan IK, Blizzard EL, Gordy P, et al. 2017. *Vector Borne Zoonotic Dis* 17:161–164.
Ricklin ME, Garcia-Nicolas O, Brechbuhl D, et al. 2016a. *Nat Commun* 7:10832.
Ricklin ME, Garcia-Nicolas O, Brechbuhl D, et al. 2016b. *Vet Res* 47:34.
Rizzoli A, Jimenez-Clavero MA, Barzon L, et al. 2015. *Euro Surveill* 20.
Roche SE, Wicks R, Garner MG, et al. 2013. *Aust Vet J* 91:5–13.
Rosen L. 1986. *Annu Rev Microbiol* 40:395–414.
Rosen L, Lien JC, Shroyer DA, et al. 1989. *Am J Trop Med Hyg* 40:548–556.
Rudolph KE, Lessler J, Moloney RM, et al. 2014. *Am J Trop Med Hyg* 90:882–891.
Russell PK, Brandt WE, Dalrymple JM. 1980. Chemical and antigenic structure of flaviviruses. In Schlesinger RW, ed. *The Togaviruses: Biology, Structure, Replication*. New York: Academic Press, pp. 503–529.
Sambri V, Capobianchi MR, Cavrini F, et al. 2013. *Viruses* 5:2329–2348.
Samuel MA, Wang H, Siddharthan V, et al. 2007. *Proc Natl Acad Sci USA* 104:17140–17145.
Saxena D, Kumar JS, Parida M, et al. 2013. *J Clin Virol* 58:528–534.
Scherer WF, Buescher EL, McClure HE. 1959a. *Am J Trop Med Hyg* 8:689–697.
Scherer WF, Moyer JT, Izumi T, et al. 1959b. *Am J Trop Med Hyg* 8:698–706.
Scherret JH, Poidinger M, Mackenzie JS, et al. 2001. *Emerg Infect Dis* 7:697–705.
Schlesinger JJ, Brandriss MW, Walsh EE. 1987. *J Gen Virol* 68:853–857.
Schuh AJ, Ward MJ, Brown AJ, et al. 2013. *PLoS Negl Trop Dis* 7:e2411.
Scott TW. 1988. Vertebrate host ecology. In Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Boca Raton, FL: CRC Press, p. 257.
Sejvar JJ. 2014. *Viruses* 6:606–623.
Selvey LA, Dailey L, Lindsay M, et al. 2014. *PLoS Negl Trop Dis* 8:e2656.
Shimizu T, Kawakami Y, Fukuhara S, et al. 1954. *Jpn J Exp Med* 24:363–375.
Smithburn KC, Hughes TP, Burke AW, et al. 1940. *Am J Trop Med Hyg* 20:471–473.
Solomon T, Ni H, Beasley DW, et al. 2003. *J Virol* 77:3091–3098.
Soman RS, Rodrigues FM, Guttikar SN, et al. 1977. *Indian J Med Res* 66:709–718.
Sulkin SE, Allen R. 1974. *Virus Infections in Bats*, Vol 8. Houston, TX: Buchdruckerei Merkur AG.
Takashima I, Watanabe T, Ouchi N, et al. 1988. *Am J Trop Med Hyg* 38:420–427.
Tanaka M. 1993. *J Virol Methods* 41:311–322.
Tang Y, Anne Hapip C, Liu B, et al. 2006. *J Clin Virol* 36:177–182.
Teehee ML, Bunning ML, Stevens S, et al. 2005. *Arch Virol* 150:1249–1256.
Turell MJ, Spring AR, Miller MK, et al. 2002. *J Med Entomol* 39:1–3.

Ueba N, Kimura T, Nakajima S, et al. 1978. *Biken J* 21:95–103.
Vaughn DW, Hoke CH. 1992. *Epidemiol Rev* 14:197–221.
Vazquez A, Herrero L, Negredo A, et al. 2016. *J Virol Methods* 236:266–270.
Weinberger M, Pitlik SD, Gandacu D, et al. 2001. *Emerg Infect Dis* 7:686–691.
Wilder-Smith A, Halstead SB. 2010. *Curr Opin Infect Dis* 23:426–431.
Williams DT, Daniels PW, Lunt RA, et al. 2001. *Am J Trop Med Hyg* 65:379–387.
Williams DT, Johansen CA, Harnett GB, et al. 2010. Murray Valley encephalitis virus. In Liu D, ed. *Molecular Detection of Human Viral Pathogens*. Boca Raton, FL: Taylor & Francis CRC Press, pp. 219–229.
Williams DT, Diviney SM, Niazi AU, et al. 2015. *PLoS Negl Trop Dis* 9:e0004240.
Wu H, Rao P, Jiang Y, et al. 2014. *Mol Cell Probes* 28:264–270.
Xu XG, Chen GD, Huang Y, et al. 2012. *J Virol Methods* 183:69–74.
Yaeger M, Yoon KJ, Schwartz K, et al. 2004. *J Vet Diagn Invest* 16:64–66.
Yamada M, Nakamura K, Yoshii M, et al. 2004. *Vet Pathol* 41:62–67.
Yamada M, Nakamura K, Yoshii M, et al. 2009. *J Comp Pathol* 141:156–162.
Yamanaka A, Mulyatno KC, Susilowati H, et al. 2010. *Jpn J Infect Dis* 63:58–60.
Yang DK. 2016. Chapter 2.1.10: Japanese encephalitis. In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2017. World Organisation for Animal Health (OIE), pp. 1–14 (Adopted by the OIE in 2016).
Yang DK, Kweon CH, Kim BH, et al. 2004. *J Vet Sci* 5:345–351.
Yang DK, Kim BH, Lim SI, et al. 2006. *J Vet Sci* 7:271–275.
Yun SI, Lee YM. 2014. *Hum Vaccin Immunother* 10:263–279.
Zhang M, Xie Z, Xie L, et al. 2015. *J Virol Methods* 224:9–15.

34

Hepatitis E Virus

Xiang-Jin Meng, Patrick G. Halbur, and Tanja Opriessnig

Relevance

Hepatitis E virus (HEV), the causative agent of human hepatitis E, is an important public health problem in many developing countries in Asia and Africa. Hepatitis E is also endemic in industrialized countries. As a fecal–orally transmitted disease, contaminated water or water supplies are major sources of HEV infections in humans. The mortality associated with HEV infection in humans is typically low (<1%), but can be up to 25% during pregnancy. Meng et al. (1997) isolated and characterized the first animal strain of HEV, swine hepatitis E virus (swine HEV), from piglets in the United States. Although swine HEV only causes microscopic lesions of hepatitis in pigs and no signs of clinical disease (Halbur et al. 2001), it poses a zoonotic risk to humans via direct contact with infected pigs (Meng et al. 2002; Withers et al. 2002) or through consumption of undercooked pork (Feagins et al. 2007, 2008; Yazaki et al. 2003) or pork products (Colson et al. 2010; Cossaboom et al. 2016).

Etiology

HEV is classified in the family *Hepeviridae* (Meng 2010a,b, 2016), which consists of two genera: *Orthohepevirus* (all mammalian and avian HEV isolates) and *Piscihepevirus* (cutthroat trout virus) (Smith et al. 2014). Among the four species in the genus *Orthohepevirus*, the species *Orthohepevirus A* consists of at least seven genotypes: genotype 1 and 2 HEV are restricted to humans; genotype 3 and 4 HEV infect humans, pigs, and several other species (Meng 2016); genotype 5 and 6 HEV infect wild boars; and genotype 7 HEV infects camels (Smith et al. 2014). HEV is a spherical, non-enveloped virus particle of approximately 32–34 nm in diameter. Swine HEV cannot be efficiently cultivated in cell culture.

The genome of swine HEV is a polyadenylated, single-stranded, positive-sense RNA molecule of approximately 7.2 kb (Meng et al. 1998). The genome consists of three open reading frames (ORFs), a short 5′ noncoding region (NCR), and a short 3′ NCR. ORF1 encodes nonstructural proteins, ORF2 encodes an immunogenic capsid protein, and ORF3 encodes a small multifunctional protein. ORF2 and ORF3 are translated from a single bicistronic mRNA and overlap each other, but neither overlaps ORF1 (Huang et al. 2007).

Public health

Hepatitis E is a zoonotic disease for which pigs are a reservoir (Meng 2010b, 2016). Concerns for zoonotic infections include occupational exposures, consumption of contaminated pork, and transmission of swine HEV from pig xenografts to human recipients.

Genotypes 3 and 4 strains of swine HEV infected both rhesus monkeys and a chimpanzee. Conversely, genotype 3 and 4 strains of human HEV infected pigs (Meng et al. 1998; Meng 2010a,b). Today it is recognized that genotype 3 and 4 swine HEV strains are zoonotic and infect humans (Meng 2016), although the zoonotic potential for genotype 5 and 6 strains of swine HEV from wild boars remains unknown. Pig caretakers and swine veterinarians in both developing and industrialized countries are at an increased risk of acquiring HEV infection. For example, swine veterinarians in the United States were 1.51 times more likely to be positive for anti-HEV antibodies than other blood donors (Meng et al. 2002). Withers et al. (2002) reported that swine workers in a major swine-producing state (North Carolina) in the United States had a 4.5-fold higher anti-HEV antibody prevalence rate (10.9%) than control subjects (2.4%).

Infected pigs excrete large amounts of HEV in feces, thus posing a concern for environmental safety (Yugo and Meng 2013). HEV-containing pig manure and feces

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

could contaminate irrigation or coastal water, with concomitant contamination of produce or shellfish. HEV strains of swine origin have been detected in sewage water (Pina et al. 2000).

Sporadic cases of acute hepatitis E have been linked to the consumption of contaminated raw and undercooked pig liver (Yazaki et al. 2003). Chronic hepatitis E cases in organ transplant recipients are almost exclusively linked to the zoonotic genotype 3 and 4 HEV strains of swine origin (Kamar et al. 2015). Approximately 2% of the pig liver sold in grocery stores in Japan and 11% in the United States tested positive for swine HEV RNA. Furthermore, the contaminating virus present in pig liver from the grocery stores in the United States was fully infectious (Feagins et al. 2007). HEV RNA was also detected from pork products such as sausages and chitterlings (Colson et al. 2010; Cossaboom et al. 2016). These data provide compelling evidence for zoonotic HEV transmission via direct contact with infected pigs or via consumption of contaminated pig meats (Meng 2010b).

Epidemiology

Swine HEV infection is ubiquitous in pigs worldwide, regardless of whether HEV is endemic in the respective human population. Besides domestic pigs, swine HEV also infects wild boars. Under experimental conditions, rhesus monkeys and chimpanzees were susceptible to infection by both genotype 3 and 4 swine HEV (Meng et al. 1998).

HEV seroprevalence in pigs is age dependent: one study showed that most pigs younger than 2 months of age were seronegative, whereas the majority of pigs older than 3 months of age were seropositive (Meng et al. 1997). Infection generally occurs at 2–3 months of age, shortly after maternally derived antibodies wane. Infected pigs generally have a transient viremia lasting for 1–2 weeks and shed viruses in feces for 3–7 weeks (Meng et al. 1997; Takahashi et al. 2003).

Transmission of swine HEV among pigs is presumably fecal–oral, with feces from infected pigs probably the primary source of the virus. It is believed that pigs acquire infection through direct contact with infected pigs or through ingestion of feces-contaminated feed or water. However, experimental reproduction of swine HEV infection in pigs via the oral route of inoculation proved to be difficult (Kasorndorkbua et al. 2003). Therefore, other routes of transmission cannot be ruled out.

Infectious swine HEV present in commercial pig livers is completely inactivated by adequate cooking (e.g. frying or boiling for 5 minutes). However, incubation of the contaminated pig liver homogenates at 56 °C (133 °F) for 1 hour did not abolish virus infectivity (Feagins et al. 2008). HEV is thought to resist inactivation by acidic and mild alkaline conditions in the intestinal tract.

Pathogenesis

The pathogenesis of swine HEV is largely unknown. It is assumed that HEV replicates in the gastrointestinal tract after ingestion and subsequently spreads to its target organ, the liver. Virus replication in the liver has been demonstrated (Meng et al. 1998). Extrahepatic sites of swine HEV replication have also been identified in various tissues of pigs including small intestines, colon, and hepatic and mesenteric lymph nodes (Williams et al. 2001).

Clinical signs

HEV produces subclinical infection in pigs infected under natural or experimental conditions. The incubation period, from the time of infection to virus shedding in feces, ranged from 1 to 4 weeks (Halbur et al. 2001). The percentage of HEV-infected pigs within a herd is very high (up to 80–100% in some herds); however, the morbidity and mortality attributable to swine HEV infection is not known.

Lesions

Specific-pathogen-free (SPF) pigs experimentally infected with swine HEV remained clinically normal, but mild to moderate enlargement of hepatic and mesenteric lymph nodes was observed from 7 to 55 days post inoculation (DPI) (Halbur et al. 2001). Microscopic lesions characterized by mild to moderate multifocal lymphoplasmacytic hepatitis and focal hepatocellular necrosis were also common (Figure 34.1). Hepatic inflammation and hepatocellular necrosis peaked in severity at 20 DPI (Halbur et al. 2001). Pregnant gilts infected with swine HEV had mild multifocal lymphohistiocytic hepatitis, and individual hepatocellular necrosis was observed in some gilts. No HEV-associated lesions in the reproductive tract or fetuses were observed (Kasorndorkbua et al. 2003).

Diagnosis

Swine HEV is difficult to work with because it does not grow in cell culture or cause clinical disease in pigs. Currently, the diagnosis of swine HEV infection is based on polymerase chain reaction (PCR) and ELISA. A

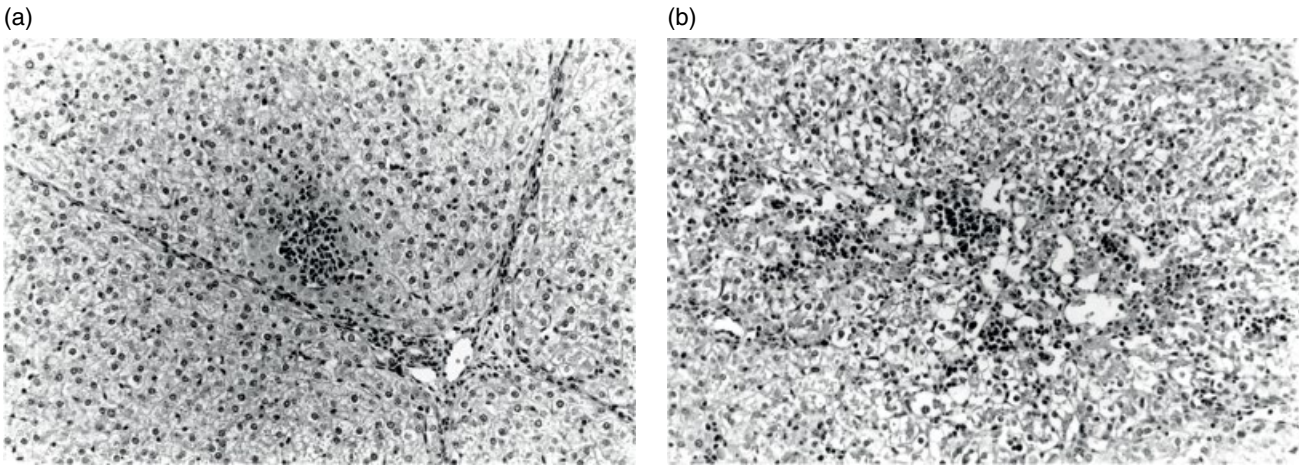


Figure 34.1 Histological liver lesions in pigs experimentally infected with swine HEV or human HEV. (a) Liver of a pig experimentally infected with the swine HEV showing mild focal infiltration of lymphocytes, plasma cells, and macrophages and mild diffuse inflammation in hepatic sinusoids at 14 days post inoculation (DPI). Hepatocytes are mildly swollen and vacuolated. (b) Liver of a pig experimentally infected with the US-2 strain of human HEV showing moderate lymphoplasmacytic and histiocytic hepatitis and severe vacuolar degeneration and swelling of hepatocytes at 14 DPI. Hematoxylin and eosin stain. *Source:* Halbur et al. (2001). Reproduced with permission of the American Society for Microbiology.

fluorescent microbead-based immunoassay has also been descried for the detection of HEV IgG antibody (Owolodun et al. 2013). The results of an ELISA based on swine HEV capsid antigen correlated well with those obtained with a genotype 1 human HEV capsid antigen (Meng et al. 2002; Zhang et al. 2011). Specific RT-PCR and real-time PCR assays have also been developed (Meng 2010a).

Immunity

The capsid protein of swine HEV is immunogenic and induces protective immunity. The capsid protein of swine HEV shares common antigenic epitopes with human and avian HEVs. Cross-challenge experiments in primates have demonstrated cross-protection following infection with different genotypes of human HEV strains (Emerson and Purcell 2003). Prior infection of pigs with a genotype 3 swine HEV protects against subsequent challenges with human genotype 3 and 4 HEV strains (Sanford et al. 2011). Piglets born to seropositive sows had maternal antibodies lasting 7–9 weeks (Meng et al. 1997). It is believed that maternal antibodies confer protective immunity to the piglets against swine HEV infection.

Prevention and control

The major concern for swine HEV is its zoonotic potential and associated pork safety concerns. A commercial vaccine against HEV ("Hecolin® HEV 239") is currently available for human use in China, but not in other countries. It may be advantageous to vaccinate pigs in order to minimize potential zoonotic transmission and eliminate pork safety concerns. Adequate personal and public hygiene can minimize HEV transmission.

References

Colson P, Borentain P, Queyriaux B, et al. 2010. *J Infect Dis* 202:825–834.

Cossaboom CM, Heffron CL, Cao D, et al. 2016. *J Med Virol* 88:1641–1645.

Emerson SU, Purcell RH. 2003. *Rev Med Virol* 13:145–154.

Feagins AR, Opriessnig T, Guenette DK, et al. 2007. *J Gen Virol* 88:912–917.

Feagins AR, Opriessnig T, Guenette DK, et al. 2008. *Int J Food Microbiol* 123:32–37.

Halbur PG, Kasorndorkbua C, Gilbert C, et al. 2001. *J Clin Microbiol* 9:918–923.

Huang YW, Opriessnig T, Halbur PG, et al. 2007. *J Virol* 81:3018–3026.

Kamar N, Abravanel F, Lhomme S, et al. 2015. *Clin Res Hepatol Gastroenterol* 39:20–27.

Kasorndorkbua C, Thacker BJ, Halbur PG, et al. 2003. *Can J Vet Res* 67:303–306.

Meng XJ. 2010a. *J Viral Hepat* 17:153–161.

Meng XJ. 2010b. *Vet Microbiol* 140:256–265.
Meng XJ. 2016. *PLoS Pathog* 12(8):e1005695.
Meng XJ, Purcell RH, Halbur PG, et al. 1997. *Proc Natl Acad Sci USA* 94:9860–9865.
Meng, XJ, Halbur PG, Shapiro MS, et al. 1998. *J Virol* 72:9714–9721.
Meng XJ, Wiseman B, Elvinger F, et al. 2002. *J Clin Microbiol* 40:117–122.
Owolodun OA, Giménez-Lirola LG, Gerber PF, et al. 2013. *J Virol Methods* 193:278–83.
Pina S, Buti M, Cotrina M, et al. 2000. *J Hepatol* 33:826–833.
Sanford BJ, Dryman BA, Huang YW, et al. 2011. *Virus Res* 159:17–22.
Smith DB, Simmonds P, Jameel S, et al. 2014. *J Gen Virol* 95:2223–2232.
Takahashi M, Nishizawa T, Miyajima H, et al. 2003. *J Gen Virol* 84:851–862.
Williams TP, Kasorndorkbua C, Halbur PG, et al. 2001. *J Clin Microbiol* 39:3040–3046.
Withers MR, Correa MT, Morrow M, et al. 2002. *Am J Trop Med Hyg* 66:384–388.
Yazaki Y, Mizuo H, Takahashi M, et al. 2003. *J Gen Virol* 84:2351–2357.
Yugo DM, Meng XJ. 2013. *Int J Environ Res Public Health* 10:4507–4533.
Zhang H, Mohn U, Prickett JR, et al. 2011. *J Virol Methods* 175:156–162.

35

Herpesviruses

Thomas C. Mettenleiter, Bernhard Ehlers, Thomas Müller, Kyoung-Jin Yoon, and Jens Peter Teifke

Overview

Herpesviruses infect a wide variety of animals from mollusks to mammals, and more than 200 distinct herpesvirus species have been recognized so far. Herpesviruses belong to the most complex and most widespread viruses. They are grouped in the order *Herpesvirales* based on the morphology of the virion and biological characteristics of their replication cycle (Davison 2010; Davison et al. 2009).

The order *Herpesvirales* includes three families: *Herpesviridae*, *Alloherpesviridae*, and *Malacoherpesviridae*. Herpesviruses infecting reptiles, birds, and mammals, which show a significant homology in their genomic sequence and gene arrangement, constitute the family *Herpesviridae*, whereas the phylogenetically more distant herpesviruses infecting amphibia and fish make up the family *Alloherpesviridae*. Herpesviruses from mollusks (ostreid herpesvirus 1 and haliotid herpesvirus 1) represent the type and only members of the *Ostreavirus* and *Aurivirus* genera of the family *Malacoherpesviridae*.

In addition to virion morphology, all herpesviruses share the capacity to establish a state of latency, resulting in lifelong association with the infected host. During latency, viral gene expression is restricted and only serves to maintain the latent state without production of infectious virus. After reactivation, infectious virus is again produced and spreads to infect other susceptible individuals. This striking biological feature is a hallmark of herpesvirus infections and explains their evolutionary success.

Within the family *Herpesviridae*, three subfamilies have been established based on distinct biology and genetic analyses: *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*. Herpesviruses relevant for swine are present in all three subfamilies (Table 35.1).

Members of the *Alphaherpesvirinae* have a rapid lytic replication cycle and establish latency (primarily) in neurons of sensory ganglia. They are grouped into five genera: genus *Simplexvirus*, which includes species *Human alphaherpesvirus 1* (human herpes simplex virus 1 [HSV-1]), species *Human alphaherpesvirus 2* (HSV-2), species *Bovine alphaherpesvirus 2* (bovine herpesvirus 2 [BoHV-2] [bovine mammillitis virus]), and other species; genus *Varicellovirus*, which includes species *Human alphaherpesvirus 3* (human varicella-zoster virus [VZV]), species *Bovine alphaherpesvirus 1* (BoHV-1, infectious bovine rhinotracheitis virus), species *Bovine alphaherpesvirus 5* (BoHV-5, bovine encephalitis herpesvirus), species *Equid alphaherpesvirus 1* (EHV-1), species *Equid alphaherpesvirus 3* (EHV-3), species *Equid alphaherpesvirus 4* (EHV-4), species *Suid alphaherpesvirus 1* (pseudorabies virus [PRV]), and other species; genus *Iltovirus* with infectious laryngotracheitis virus in the species *Gallid alphaherpesvirus 1*; genus *Mardivirus* with Marek's disease virus in the species *Gallid alphaherpesvirus 2*; and genus *Scutavirus* with Chelonid alphaherpesvirus 5 as type member.

Members of the *Betaherpesvirinae* generally specify larger genomes than either the alpha- or gammaherpesviruses and are characterized by extended replication cycles and strict host specificity. Genera *Cytomegalovirus* (type member human cytomegalovirus or human betaherpesvirus 5), *Muromegalovirus* (type member murid betaherpesvirus 1 or murine cytomegalovirus), *Roseolovirus* (type member human betaherpesvirus 6), and *Proboscivirus* (type member elephantid betaherpesvirus 1) have been recognized.

The *Gammaherpesvirinae* comprises herpesviruses with transforming potential and preferential association with lymphocytes grouped into genera *Lymphocryptovirus* (type member Epstein–Barr virus [EBV] or human gammaherpesvirus 4), *Rhadinovirus* (type member Kaposi's sarcoma herpesvirus [KSHV] or human gammaherpesvirus 8), *Macavirus* (type member suid gammaherpesvirus 3 [porcine lymphotropic herpesvirus 1, PLHV-1]; suid gammaherpesvirus 4 [porcine lymphotropic her-

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 35.1 Herpesviruses of swine.[a]

New taxonomic name	Trivial name	Genus	Subfamily
Suid alphaherpesvirus 1	Pseudorabies virus (Aujeszky's disease virus)	*Varicellovirus*	*Alphaherpesvirinae*
Suid betaherpesvirus 2	Porcine cytomegalovirus	Unassigned	*Betaherpesvirinae*
Suid gammaherpesvirus 3	Porcine lymphotropic herpesvirus 1	*Macavirus*	*Gammaherpesvirinae*
Suid gammaherpesvirus 4	Porcine lymphotropic herpesvirus 2	*Macavirus*	*Gammaherpesvirinae*
Suid gammaherpesvirus 5	Porcine lymphotropic herpesvirus 3	*Macavirus*	*Gammaherpesvirinae*
Ovine gammaherpesvirus 2	Sheep-associated malignant catarrhal fever virus	*Macavirus*	*Gammaherpesvirinae*

[a] Whereas pigs are main/sole hosts for suid alphaherpesvirus 1, suid betaherpesvirus 2, and suid gammaherpesviruses 3-5, OvHV-2 is primarily found in sheep but causes spillover infections in pigs.

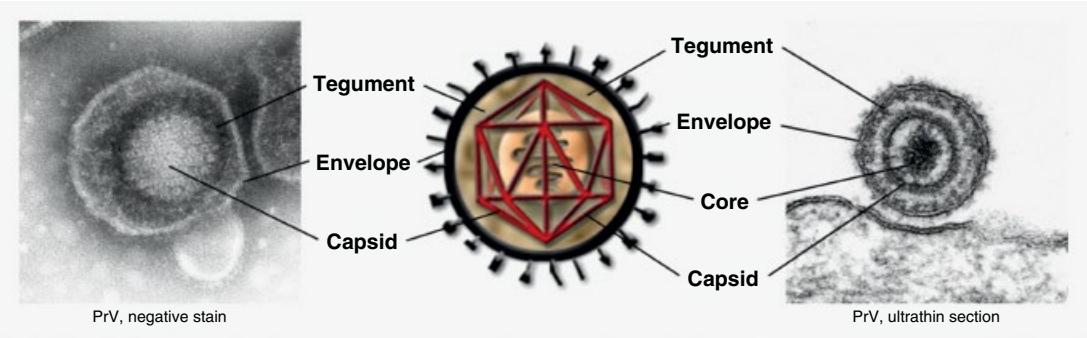


Figure 35.1 Morphology of a typical herpes virion (PRV). *Source:* Mettenleiter et al. (2008). Reproduced with permission of Elsevier.

pesvirus 2, PLHV-2]; suid gammaherpesvirus 5 [porcine lymphotropic herpesvirus 3, PLHV-3]; malignant catarrhal fever [MCF] virus, which can be alcelaphine gammaherpesvirus 1 [AlHV-1], ovine gammaherpesvirus 2 [OvHV-2], or caprine gammaherpesvirus 2 [CpHV-2], depending on species), and *Percavirus* (derived from perissodactyl and carnivore) containing equid gammaherpesvirus 2 (EHV-2), equid gammaherpesvirus 5 (EHV-5), and mustelid gammaherpesvirus 1.

Herpesvirus morphology

Herpesvirus particles consist of four structural elements: the core, which contains the linear, double-stranded DNA genome; the icosahedral nucleocapsid; a lipid envelope derived from the host cell into which viral proteins, mostly glycoproteins, are embedded; and the tegument, a proteinaceous structure linking the envelope and nucleocapsid similar to the matrix in RNA viruses (Figure 35.1). Whereas the basic morphology of the herpes virion appears identical in all members of the *Herpesvirales*, the diameter of the virus particle varies around approximately 200 nm depending on the amount of tegument. The approximately 100 nm diameter capsid shell exhibits icosahedral symmetry with a triangulation number of 16, thus consisting of 162 capsomeres with 150 hexons and 12 pentons.

Receptor-binding proteins as well as major immunogens are located in the viral envelope. Most of them are modified by the addition of carbohydrates and, thus, represent glycoproteins. Within the *Herpesviridae* glycoproteins B (gB), gH, gL, gM, and gN are conserved. Glycoprotein gB and a heterodimer of gH and gL constitute the core machinery mediating fusion between virion envelope and the host cell membrane, whereas the gM–gN heterodimeric complex functions during virion assembly.

Genomic organization and gene expression

The genomes of herpesviruses consist of linear, double-stranded DNA molecules varying in size between approximately 123,000 base pairs (simian varicellovirus) and approximately 300,000 base pairs (koi herpesvirus) encoding between 70 (VZV) and 229 (rhesus cytomegalovirus [RhCMV] [Macacine betaherpesvirus 3]) predicted proteins. Many herpesvirus genomes contain, besides singular "unique" sequences, extensive repetitive sequences that can either occur as tandem repeats at one site or in different locations in the viral genome in parallel or antiparallel orientation. Approximately 40 genes and gene products are conserved in all members of the *Herpesviridae* (Mettenleiter et al. 2008). They are mostly arranged in gene blocks and encompass proteins required for capsid structure and maturation, several tegument

and envelope proteins, the replication, cleavage, and packaging machinery for the viral genome, and proteins with enzymatic functions in nucleotide metabolism. Approximately half of the genes/proteins of each herpesvirus are not essential for viral replication in cell culture, including those relevant for transmission and spread *in vivo* and for immune modulation and evasion.

Herpesvirus replication

Herpesvirus replication is a complex process (Figure 35.2). Adsorption of free virions to target cells is mediated by virally encoded glycoproteins embedded in the virion envelope. This interaction triggers fusion between the virion envelope and the host cell membrane. Fusion primarily occurs at the cell surface, but may also be executed after endocytosis. After the release of the nucleocapsid into the cytosol, it is transported by cellular motor proteins along microtubules to the nuclear pore, where it docks with one vertex oriented toward the pore and viral genomic DNA is released into the nucleus through the nuclear pore.

In the nucleus, the linear viral genome circularizes, and viral gene expression ensues in a cascade-like fashion in the order: immediate-early (α-), early (β-), and late (γ-) gene expression. Capsid assembly occurs in the nucleus, and the resulting nucleocapsids leave the nucleus for final maturation in the cytosol by budding at the inner nuclear membrane, thereby acquiring a primary envelope that fuses with the outer nuclear membrane to release nucleocapsids into the cytosol. Viral tegument proteins then attach to the nucleocapsid prior to final (secondary) envelopment by a second budding process into vesicles of the trans-Golgi network, resulting in the formation of a complete virion within a cellular secretory vesicle. Release of mature virus particles then occurs by fusion of the vesicle with the plasma membrane. This prototypic replication cycle appears to be valid for all herpesviruses, although more or less subtle deviations may apply (Mettenleiter et al. 2009).

The spread of herpesvirus infections occurs not only via free virions but also by direct cell-to-cell spread. It is unclear whether complete virions or subviral particles (e.g. nucleocapsids) mediate this direct spread.

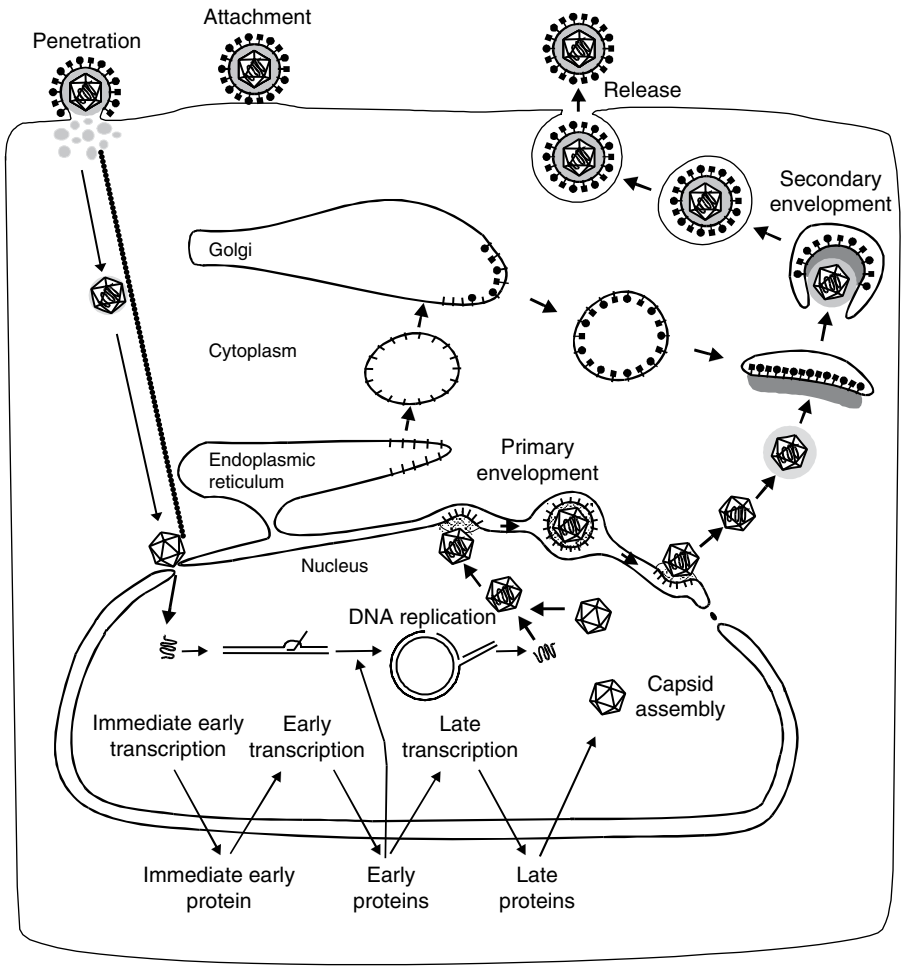


Figure 35.2 Herpesvirus replication cycle. *Source:* Mettenleiter (2004). Reproduced with permission of Elsevier.

The success of herpesviruses is mainly due to their ability to establish lifelong latency in the infected host. Thus, the viral genome persists indefinitely, even after clinical signs disappear. Spontaneous reactivation results in the formation and shedding of infectious virions able to infect susceptible animals. Alphaherpesviruses establish latency primarily in sensory neurons after replication in peripheral epithelial cells, whereas latency of betaherpesviruses is established in extraneural sites, for example, secretory glands. Latent gammaherpesviruses are mostly present in the cells of the lymphoreticular system, primarily B and T cells. Establishment and maintenance of the latent state are achieved by different mechanisms in the different herpesvirus subfamilies. However, they have in common a restricted expression of specific latency-related genes with a concomitant absence of lytic viral gene expression.

Suid alphaherpesvirus 1 (Aujeszky's disease virus; pseudorabies virus)

Relevance

Although the taxonomic species name *Suid alphaherpesvirus 1* indicates that the natural host of PRV is pigs, clinical PRV was first described in 1813 in cattle. This was because PRV infection in swine, particularly in older animals, may produce only innocuous respiratory signs or may be clinically inapparent, whereas productive infection in other susceptible species is invariably fatal and characterized by severe central nervous signs. Thus, the rabies-like clinical picture in cattle prompted the use of the term "pseudorabies" in Switzerland in 1849. Likewise, "mad itch" was used to describe the disease in cattle in the United States in the first half of the nineteenth century because PRV causes excessive pruritus.

In 1902, the Hungarian veterinary pathologist Aladár Aujeszky reported the isolation of the infectious agent from a diseased ox, a dog, and a cat and differentiated it from rabies (Aujeszky 1902). It could be passaged in rabbits reproducing the typical clinical signs. Guinea pigs and mice were also found to be susceptible, whereas chicken and doves were resistant. Thus, the disease became widely known as Aujeszky's disease (AD). It was not until 1931 that Richard Shope established that the agent of "mad itch" was also present in domestic pig holdings in the United States. Erich Traub in Germany was the first to cultivate PRV *in vitro* in organ explants in 1933. One year later, Sabin and Wright reported a serological relationship between PRV and herpes simplex virus, resulting in the inclusion of PRV into the herpesvirus group.

Role in swine health

Whereas PRV exhibits a wide host range capable of infecting basically all mammals except higher primates, only pigs are able to survive a productive infection and are thus considered the natural host. PRV infections in swine soared after the Second World War, particularly in Europe, when intensive pig breeding and farrowing were established. In the 1970s, PRV became a major scourge of pigs worldwide, distributed primarily by global movement of animals and animal products.

Although field isolates and strains differ in virulence, they can cause devastating losses by fatal infection of piglets and abortions in pregnant animals. Pigs exhibit a pronounced age resistance against PRV, with younger animals more susceptible to fatal infections characterized by neuronal signs, such as ataxia, convulsions, and sudden death. In contrast, older animals (>1 year) primarily present with respiratory distress or even subclinical infection. In pregnant animals, infection of fetuses results in resorption, mummification, or abortion.

Early eradication programs focused on elimination (culling) of infected herds met with some success, for example, in Great Britain or Scandinavian countries. However, advances in molecular biology produced the first so-called marker vaccines that allowed serological differentiation between vaccinated and infected animals. These vaccines provided the basis for cost-effective control and, eventually, PRV eradication (van Oirschot 1999).

Etiology

PRV belongs to the genus *Varicellovirus* in the subfamily *Alphaherpesvirinae* of the family *Herpesviridae* (Davison 2010). Only a single serotype is known. Isolates were initially differentiated by the restriction pattern of viral genomes after cleavage with sequence-specific endonucleases, such as Bam HI or Kpn I (Herrmann et al. 1984).

In 2004, the complete genome sequence of PRV assembled from various strains was described (Klupp et al. 2004) and recently supplemented by complete isogenic genome sequences (Szpara et al. 2011). The PRV genome encompasses approximately 140–143 kbp and contains at least 72 open reading frames (ORFs) encoding 70 different proteins (Figure 35.3). The viral genome consists of two unique portions, the long (U_L) and short (U_S) unique regions, and two large inverted repeats (terminal repeat [TR] and internal repeat [IR]), which bracket the U_S. This results in inversion of the U_S relative to the U_L and the existence of two isomeric forms of the viral genome, that is, a class D herpesvirus genome.

Laboratory cultivation

Due to its broad host range, many cell lines and primary cell cultures from different species are permissive for PRV. Cell lines generally used for PRV replication and

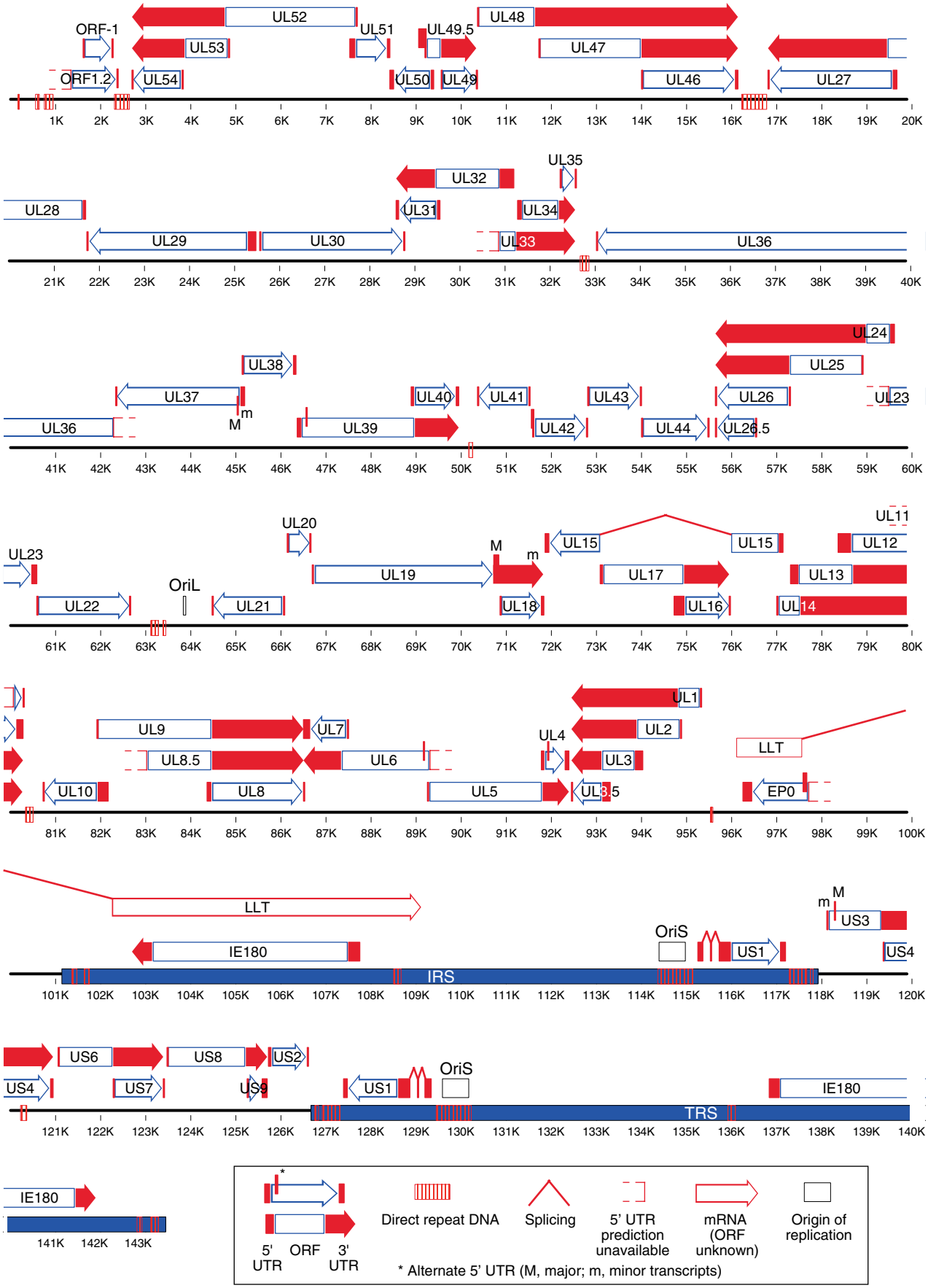


Figure 35.3 Predicted PRV transcript and gene organization. The linear form of the PRV genome is shown with U_L being followed by IR, U_S, and TR. The predicted locations of open reading frames, 5′ and 3′ untranslated regions, DNA repeats, splice sites, and origins of replication (Ori) are depicted. *Source:* Klupp et al. (2004). LLT, large latency transcript; UTR, untranslated region.

experimental analysis include rabbit kidney (RK-13), porcine kidney (PK-15), Madin–Darby bovine kidney (MDBK), or primate Vero cells. PRV undergoes lytic replication, resulting in cytopathic effect (CPE) manifested either by immediate cell destruction (plaque formation) or by formation of syncytia due to the fusion activity of viral glycoproteins exposed at the cell surface. The appearance of CPE depends on the infectious dose but is routinely seen in 6–24 hours. Infected cells can be unambiguously identified by immunostaining using PRV-specific antibodies. Genetically engineered viral variants expressing reporter proteins such as β-galactosidase or fluorescent proteins can be used to track virus infection in cell culture and in experimental animal infections. These viruses are also used for neuronal circuit tracing based on the capacity of PRV to spread transsynaptically in neuronal networks (Curanovic and Enquist 2009).

Public health

Although isolated reports describe putative infections of humans with PRV, they are not conclusive, and it is generally accepted, and has been demonstrated even by self-inoculation, that humans are resistant against natural PRV infection (Jentzsch and Apostoloff 1970).

Epidemiology

AD has an almost worldwide distribution, except for Norway, Australia, and most of the Southeast Asian islands. It occurs particularly in regions with dense pig populations. The disease had not existed widely outside Eastern Europe before the 1960s, but had spread nearly globally by the end of the 1980s assisted by the emergence of more virulent PRV strains and changes in swine management, notably total confinement of large numbers of pigs and continuous farrowing. As a result, PRV became one of the most important infectious diseases of domestic pigs.

Due to increased control efforts and the strict implementation of national eradication programs, AD virtually disappeared from domestic pigs in several parts of the world in recent decades. In Europe, PRV has been eliminated from domestic pig populations in Austria, Cyprus, the Czech Republic, Denmark, Finland, France, Germany, Hungary, Luxembourg, the Netherlands, Sweden, Switzerland, Slovakia, and Great Britain (England, Scotland, and Wales). Canada, New Zealand, and the United States are also free of AD in domestic pigs (Hahn et al. 2010; MacDiarmid 2000; Müller et al. 2003). In PRV-free countries, vaccination is prohibited. AD is still endemic in eastern and southeastern Europe, Latin America, Africa, and Asia. Nonetheless, more countries are implementing national eradication programs to eliminate PRV. However, it is noteworthy that emergence of novel PRV variants could occur and bring additional challenges to eradicate PRV. For example, since late 2011, PRV variants have emerged in a large number of swine herds in many regions of China, and the traditional Bartha-K61 vaccines did not appear to be very effective against the PRV variants (Sun et al. 2016; Yu et al. 2014).

Despite successful elimination of PRV from domestic pigs, the disease is widespread in populations of nondomestic swine, including feral pigs, wild boar, and hybrids, around the world (Müller et al. 2000, 2011). Although PRV in wild boar generally has not impacted the AD-free status of domestic pigs, infected wild boars represent a constant danger for reintroduction of PRV into free herds and regions.

In Europe, PRV is present in wild boar in many countries, for example, France, Germany, Spain, Italy, Slovenia, Croatia, the Czech Republic, Poland, Austria, and Serbia. Seroprevalence in these populations ranges from 4 to 66% at the national level (Lipowski et al. 2002; Milicevic et al. 2016; Müller et al. 2010, 2011; Steinrigl et al. 2012). PRV is also endemic in feral swine populations in the United States (Hahn et al. 2010). Although the prevalence of PRV infection in wild boar in other parts of the world is not known, isolation of PRV from wild boar (and hunting dogs) has been reported across Europe, as well as from feral pigs in the United States (Hahn et al. 2010; Müller et al. 2010).

Molecular characterization of a large number of PRV isolates using Bam HI restriction fragment length polymorphism revealed four major genome types. Type I is found predominantly in the United States and central Europe, whereas types II and III circulate in central Europe and northern Europe, respectively. Type IV is restricted to Asia. Within the major genome types, several subtypes can be distinguished (Christensen 1995; Herrmann et al. 1984) aided by phylogenetic analyses of PRV field strains using partial sequence analysis of the gC gene (Fonseca et al. 2010; Hahn et al. 2010; Müller et al. 2010; Sozzi et al. 2014). In addition, full genome sequences from strains isolated in different countries are becoming available (Mathijs et al. 2016; Yu et al. 2016).

Susceptible species

Pigs are the only natural host for PRV, but the virus can naturally infect cattle, sheep, cats, dogs, mice, and rats, causing fatal disease (Pensaert and Kluge 1989). Dogs seem to be highly susceptible, and there are numerous reports of PRV infection of dogs, particularly hunting dogs, either due to consuming raw meat or by direct contact with PRV-infected swine (Müller et al. 2011; Steinrigl et al. 2012; Moreno et al. 2015). Infections have also been reported in brown bear, black bear, Florida panther, raccoon, coyote, deer, and farm fur animal species (mink and foxes) (Banks et al. 1999; Bitsch and Munch 1971;

Glass et al. 1994). Only swine (*Suidae*) are able to survive a productive PRV infection (Enquist et al. 1998). The susceptibility of other members of the artiodactyl suborder *Suina*, for example, *Tayassuidae* (peccaries) and possibly hippopotamuses, has not been established. Therefore, reports of low PRV seroprevalence (<1%) in free-ranging peccaries in the southwestern United States should be interpreted with caution (Corn et al. 1987). Of the laboratory species, the rabbit is the most susceptible and develops intense local pruritus at the inoculation site. Guinea pigs are less susceptible and may resist subcutaneous inoculation, but succumb to intracerebral or intraperitoneal inoculation (Ashworth et al. 1980).

Transmission

In general, rather high quantities of virus, for example, $>1 \times 10^4$–10^5 median tissue culture infectious dose ($TCID_{50}$), are necessary to infect animals, except piglets (1×10^2 $TCID_{50}$). Thus, PRV is not very contagious (Wittmann 1991). Larger quantities of virus are necessary for oral than for intranasal infection (Jakubik 1977).

The virus is spread primarily by direct contact between swine or by contact with PRV-contaminated fomites, for example, contaminated bedding and water, meat products, and carcasses of rats, raccoons, swine, and other infected animals. The mucosae of the nasal and oral cavities are the main entry points (Donaldson 1983). Conjunctival infection may also lead to rapid onset of disease. Transmission among pigs can also occur during breeding from exposure to contaminated vaginal mucosa or semen (Beran 1991). Within feral swine and wild boar populations, PRV appears to be preferentially transmitted by the venereal route. This mode of transmission differs from that seen in natural transmission in domestic swine, where contaminated secretions, excretions, and aerosols are responsible (Romero et al. 2001). During gestation, PRV can be vertically transmitted transplacentally, mainly in the last third of gestation. The virus can also spread via colostrum to suckling piglets (Beran 1991).

Under favorable circumstances (high virus load in the air, ventilation), PRV is disseminated by the movement of air within buildings and for short distances outside, depending on the climatic conditions (Schoenbaum et al. 1990; Vannier 1988). Long-distance airborne transmission of PRV (Christensen et al. 1990) is still disputed. Although dogs, cats, and wild animals, for example, raccoons, skunks, and rats, are considered potential carriers within an endemic area (Kirkpatrick et al. 1980), their role in virus transmission may be limited due to excretion of only very low amounts of virus and rapid death (Wittmann 1991).

PRV-infected nondomestic swine pose a limited risk to domestic animals, unless they come into direct contact, for example, during breeding or feeding. Movement (trade and transport) of infected pigs during the incubation or latent phase of infection poses the greatest risk of spreading the disease, as pigs that shed virus may immediately infect new units (Blaha 1989; Wittmann 1991). After recovery, pigs remain carriers of latent virus to be reactivated, for example, by transport stress. For this reason, the World Organization for Animal Health (OIE) set international standards and recommendations for movement of pigs between areas with differing disease status (free, provisionally free, endemic; OIE 2009).

Duration and routes of shedding

Infected swine shed PRV in high concentrations in almost all body secretions, excretions, and aerosols. Virus titers in nasal and pharyngeal secretions may reach maximum values of 1×10^6–10^8 $TCID_{50}$. Virus can be isolated from oropharyngeal swabs for 18–25 days with virus titers of up to 1×10^6 $TCID_{50}$. At the peak of virus excretion, one pig may excrete $1 \times 10^{5.3}$ $TCID_{50}$ into the air during a 24 hour period (Müller et al. 2001; Wittmann 1991). Virus shedding starts 1–2 days after infection, prior to the onset of viremia and clinical signs, reaching a peak at 2–5 days and lasting up to 17 days. Transplacental transmission leads to considerable virus shedding during abortion and birth (Beran 1991; Blaha 1989). Virus can also be found in vaginal and preputial secretions, in ejaculate for up to 12 days, and in milk for 2–3 days. It is occasionally shed in urine and has been detected in rectal swabs, but not in feces, for up to 10 days (Wittmann 1991).

Persistence in the environment

Infectious PRV is rather resistant to environmental conditions, depending on pH, humidity, and temperature (Pejsak and Truszczynski 2006; Wittmann 1991). On average, about 50% infectivity is destroyed within 24 hours (Schoenbaum et al. 1990). Ultraviolet light and dry or arid conditions inactivate the virus. However, it is stable at pH 4–12, and even at extreme pH values of 2.0 and 13.5, complete inactivation can take 2–4 hours (Benndorf and Hantschel 1963). Although strain-dependent thermal sensitivity of PRV has been described (Bartha et al. 1969; Golais and Sabo 1975; Platt et al. 1980), in general, it is relatively heat resistant and stable at normal or low temperatures, remaining infectious at 25 °C (77 °F), 15 °C (59 °F), and 4 °C (39 °F) for about 6, 9, and 20 weeks, respectively. At certain subzero temperatures, for example, −18 to −25 °C (0 to −13 °F) and at −40 °C (−40 °F), PRV remains stable for years. At higher temperatures, it is inactivated rapidly between 60 and 1 minutes at 60 °C (140 °F) and 100 °C (212 °F), respectively.

Infectivity of PRV in an aerosol decreases by 50% in less than 1 hour (Schoenbaum et al. 1990). In slurry, PRV remains infectious for 1–2 months, depending on the

season (Kretzschmar 1970). At high virus doses ($10^{6.5}$ $TCID_{50}$/mL), however, infectious virus could still be detected after 27 weeks at 4 °C (39 °F) and 15 weeks at 23 °C (73 °F). Under anaerobic conditions in slurry, PRV was inactivated at 5 and 20 °C after 15 and 2 weeks and at 35 and 55 °C after 5 hours and 10 minutes, respectively (Botner 1991). In aerated slurry at pH 9.6 and 44 °C (111 °F), infectivity disappeared in 8–21 days. Infectious PRV persisted in soil for 5–6 weeks, in well water for 7 days, on hay and straw for 15 and 40 days, and on sacks and wood for 10 and 15 days in summer and in winter, respectively (Schoenbaum et al. 1991; Wittmann 1991). PRV remained infectious in swine urine and waste pit effluent for 2 weeks and less than 1 day, respectively (Schoenbaum et al. 1991).

On straw, concrete, and pelleted feed, PRV infectivity drops below detectable levels within 4 days (Schoenbaum et al. 1991). In waste food fermented by *Lactobacillus acidophilus*, the virus was inactivated at 20 °C (68 °F) and 30 °C (86 °F) within 24 hours, but remained infectious at 10 °C (50 °F) for 48 hours and at 5 °C (41 °F) for 96 hours. Heating of waste food to 70 °C (158 °F) or 80 °C (176 °F) destroyed the virus within 10 or 5 minutes, respectively. Maturation of pig meat at 4 °C (39 °F) does not inactivate the virus. However, in meat, the virus is believed to be inactivated at −18 °C (0 °F) within 40 days and after heat treatment of meat and meat products at 80 °C (176 °F) (Donaldson 1983).

Susceptibility to disinfectants

Orthophenolphenate compounds, peracetic acid, formalin, 2% sodium hydroxide, trisodium phosphate iodide disinfectants, 1–2% quaternary ammonium compounds, hypochlorites, and chlorine (chlorhexidine) solutions are suitable disinfectants (Beran 1991), with a slightly reduced effectiveness in the presence of organic matter. For large-scale disinfection, calcium chloride preparations dissolved in water, crude chloramines, and preparations containing at least 1% active formaldehyde may be used. Lime (20 kg Ca $[OH]_2/m^3$) is recommended for disinfecting slurry. PRV is relatively resistant to changes in pH between 4 and 12 (Benndorf and Hantschel 1963); hence, pure acid and lye preparations can only be used as disinfectants to a limited extent. The same applies to phenol- and alcohol-based disinfectants (Blaha 1989).

Pathogenesis

After oronasal infection of the natural host and primary replication in epithelial cells of the upper respiratory tract, the virus reaches the tonsil and local lymph nodes by way of lymph vessels. Then the virus gains access to neurons innervating the facial and oropharyngeal area, in particular the olfactory, trigeminal, and glossopharyngeal nerves. By fast axonal retrograde transport, it spreads centripetally and reaches the cell bodies of infected neurons, where either lytic or latent infection ensues. PRV is also able to cross synapses to infect neurons of higher order (Pomeranz et al. 2006). Viremia disseminates it to many organs, where the virus replicates in epithelia, vascular endothelium, lymphocytes, and macrophages (Kritas et al. 1999; Mettenleiter 2000). Recent transcriptomic analysis of PRV infection demonstrated early induction of the host innate immune response, including upregulation of interferon responsive genes, inflammatory response genes, and cytokine–cytokine receptor interactions. The expression of proinflammatory cytokines, chemokines, adhesion molecules, and inflammatory enzymes and their receptors and adaptive immune response coincide with fever and pneumonia observed during the first week postinfection. At 6 days postinfection (DPI), a significant shift toward downregulation of these transcripts including oxidative phosphorylation and RIG-I-like and Toll-like receptor pathways is noted. At 14 DPI there is a significant alteration of genes most likely involved in cell death signaling, while cell regrowth and renewal are upregulated with cell cycle mitotic signaling gene sets (Miller et al. 2016).

Replication of PRV in the central nervous system (CNS) is characterized by nonsuppurative meningoencephalitis causing severe central nervous disorders (Enquist 1994; Pensaert and Kluge 1989). Trigeminal ganglia, sacral ganglia, and tonsils are considered prime sites of latency in pigs. The demonstration of the sacral ganglia as the most common sites of PRV latency in feral swine supported the hypothesis that these viruses are primarily transmitted venereally and not by the respiratory route, as is common in domestic swine and in which the trigeminal ganglia are the predominant sites of virus latency (Romero et al. 2001). In non-porcine species, PRV is rather strictly neuroinvasive (Mettenleiter 2000).

Virulence factors

PRV strains differ widely in virulence; hence the isolate involved affects the severity of disease in pigs and the quantity and duration of virus shedding (Maes et al. 1983). Virulence also affects the tissue tropism of infecting PRV strains. Whereas highly virulent PRV strains are predominantly neuroinvasive, strains of moderate or low virulence exhibit weak neuroinvasiveness, but distinct pneumotropism. Highly adapted or attenuated PRV strains have acquired a tropism for the reproductive system (Romero et al. 2001).

PRV virulence is controlled by multiple genes (Lomniczi and Kaplan 1987; Lomniczi et al. 1984). Proteins determining virulence are found among viral membrane glycoproteins, virus-encoded enzymes, and nonessential capsid-associated proteins (Mettenleiter 2000). According to their role in viral replication in cell culture, glycoproteins (g) are either nonessential (gC,

gE, gG, gI, gM, gN) or essential (gB, gD, gH, gK, gL). In terms of virulence, glycoproteins that mediate attachment of PRV to target cells, gC and gD, are of special interest because they may directly determine viral tropism. Virus-encoded enzymes involved in nucleic acid metabolism, for example, thymidine kinase or dUTPase, are major determinants of virulence, and their inactivation leads to strong attenuation of the virus. Neuroinvasiveness and virulence are also determined by viral envelope glycoproteins (Card et al. 1992; Karger and Mettenleiter 1993). Glycoprotein gE is one of the key proteins in neuroinvasion, in both trigeminal and olfactory pathways. Deletion of the gene encoding gE significantly decreases virulence and results in restricted neuronal infection (Enquist et al. 1998). Glycoprotein gC, a major viral membrane protein required for efficient adsorption of virus in cell culture, has no obvious role in determining the pattern of neuronal infectivity, but appears to function with gE to influence neurovirulence. Besides gE and thymidine kinase, inactivation of several other PRV genes has been shown to result in the attenuation of the virus. In fact, inactivation of many genes, whose products are nonessential for viral replication in cell culture, decreases PRV virulence to a variable extent (Mettenleiter 2000).

Latency

A hallmark of herpesviruses is their capacity to persist in a latent state for the lifetime of the host (Wittmann and Rziha 1989). In PRV infection, latency is established primarily in the neurons of the trigeminal and sacral ganglia but also in the tonsils (Romero et al. 2003). No infectious virus is produced during latency, but viral genomic DNA persists extrachromosomally (Brown et al. 1995; Cheung 1995; Gutekunst 1979; Rziha et al. 1986). Under control of a latency-active promoter (LAP) (Jin et al. 2000), only a small portion of the viral genome is transcribed from part of the IR and adjoining U_L region (Cheung 1989; Priola et al. 1990) into an 8.4 kb latency-associated transcript (LAT), which overlaps in antiparallel orientation the mRNA coding for the major immediate-early protein, IE180. Thus, it has been hypothesized that hybrid formation of the two transcripts may modulate the establishment and/or maintenance of latency and reactivation (Mettenleiter et al. 2008). Within the LAT locus, a cluster of nine microRNAs has been identified that may affect the host response of porcine trigeminal ganglia during latency (Mahjoub et al. 2015). However, the exact molecular mechanism of the establishment of and reactivation from latency remains unclear.

Because of the potential to reactivate and shed infectious PRV, latently infected animals are a major threat to disease control. Reactivation can occur under stress (transport, handling, temperature) or hormonal (gestation, farrowing) stimulation. Latent virus can be detected by demonstration of viral DNA or LAT using polymerase chain reaction (PCR) or nucleic acid hybridization techniques. Since no latent antigen is produced in PRV, specific serological detection is not possible. Reactivation can be induced experimentally, for example, administration of high doses of corticosteroids produces virus shedding and transmission (Mengeling et al. 1992). Interestingly, precolonization of sites of latency appears to interfere with subsequent colonization after challenge (Schang et al. 1994). Although live attenuated vaccine strains are limited in their replication in the animal, they may also induce latency, but with reduced efficiency.

Clinical signs

The incubation period in pigs is affected by the infectious dose, the route of infection, and the host species. The incubation period normally ranges from 1 to 8 days, but may take up to 3 weeks. In other susceptible animal species, the course of disease is peracute with incubation periods of 2–3 days.

Infection of pigs with PRV produces a high fever, followed by anorexia, listlessness, dyspnea, excessive salivation, vomiting, trembling, and, eventually, marked incoordination, especially of the hind legs. Involvement of the respiratory tract with coughing, sneezing, dyspnea, and aspiration pneumonia may occur. In adult swine, high morbidity is predominantly due to respiratory involvement.

The presence and severity of clinical signs, as well as morbidity and mortality, depend on the age and immunological status of the pig (Nauwynck 1997). Furthermore, the route of infection and the virulence of the PRV strain are important factors (Schmidt et al. 2001).

In general, PRV infections in fully susceptible swine result in high morbidity and mortality, especially in juvenile animals in which meningoencephalitis and viremia-associated signs predominate. In neonatal pigs less than 7 days of age, the disease may be characterized by sudden death with few, if any, clinical signs. In 2- to 3-week-old piglets, severe signs of CNS involvement, for example, trembling, incoordination, convulsion, tremor, ataxia, and paralysis, are seen (Figure 35.4) with mortality up to 100%. Older animals (3–6 weeks of age) may show neurological signs, but usually develop age-dependent resistance. Mortality may decrease to 50% by the fourth week of age, to less than 5% in 5-month-old pigs, and even lower as the age of the infected pigs increases.

Clinical signs can be present for 6–10 days. Animals may recover within a few days, but lose weight over the course of the disease. In finishing and fattening pigs, because of the population density, clinical signs can amplify, and animals often die from secondary bacterial pneumonia. Signs in gilts and sows depend on the phase of gestation and include embryonic death,

Figure 35.4 PRV infection in piglets. Central nervous signs observed after intranasal infection of 4- to 6-week-old piglets with virulent PRV include ataxia and convulsions.

resorption of fetuses, mummified fetuses, abortion, or stillbirth, in addition to respiratory signs and fever. Pigs surviving a PRV infection become latently infected (Nauwynck 1997).

In the case of coinfections with other swine viruses, for example, porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), and swine influenza virus, a severe and often fatal proliferative and necrotizing pneumonia (PNP) may develop in weaning and post weaning pigs (Morandi et al. 2010).

Natural resistance to PRV in particular pig breeds associated with specific quantitative trait loci has been described (Reiner et al. 2002), but the mechanism is unclear.

In PRV-infected nondomestic swine, clinical signs are rare, indicating that circulating PRV variants are highly adapted to the host population (Müller et al. 2001). In Spain and Germany, rare cases of clinical AD in juvenile wild boar have been reported, indicating that these field viruses can induce disease in wild pigs that is clinically and pathologically identical to AD in domestic pigs (Gortazar et al. 2002; Schulze et al. 2010).

For other susceptible animal species, a peracute fatal course of infection is characteristic. Often, extreme pruritus resulting in severe self-mutilation is the only clinical sign observed. Dead mice, rats, dogs, or cats on farms are telltale signs for the presence of PRV prior to the onset of clinical signs in infected pigs. This also applies to hunting dogs.

Lesions

Gross lesions

There are no pathognomonic gross lesions in pigs and changes are often absent or minimal. Gross lesions may occur in non-neural tissues, including lymphoid organs, and respiratory, digestive, and reproductive tracts. Particularly in young suckling pigs lacking passive immunity,

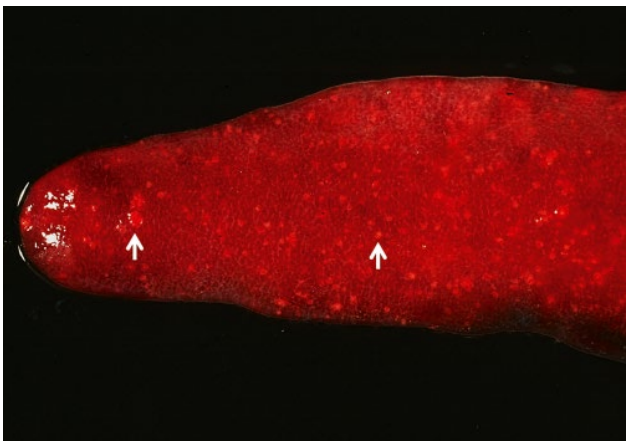


Figure 35.5 Multifocal acute coagulative necroses (two of them marked by arrows) of the spleen parenchyma after infection with PRV. *Source:* courtesy of Dr. W. Thiel, Detmold Germany.

multiple small foci (1–3 mm in diameter) of acute hemorrhagic necrosis characteristic of alphaherpesviral infections may be seen in the liver, spleen (Figure 35.5), lung, intestines, and adrenals. Typically, exudative keratoconjunctivitis, serous to fibrinonecrotic rhinitis, laryngitis, tracheitis, and necrotizing tonsillitis may be present. The CNS is free of gross lesions except for leptomeningeal hyperemia.

Gross lesions in the upper respiratory tract are most common, including rhinitis with patchy epithelial necrosis and necrotizing laryngotracheitis, often in conjunction with multifocal tonsillar necrosis. Lesions in the lower respiratory tract may include pulmonary edema and scattered small foci of necrosis, hemorrhage, or bronchointerstitial pneumonia (Becker 1964). However, the pulmonary lesions are less consistent and are composed of areas of reddening and consolidation scattered throughout the lungs, especially in the cranioventral lung lobes.

In sows, necrotizing placentitis and endometritis with thickened, edematous wall of the uterus are observed after abortion (Kluge and Maré 1978). Aborted fetuses may be macerated, or occasionally, mummified (stillbirth, mummified fetuses, embryonic death, infertility [SMEDI]). In fetuses or neonatal pigs, necrotic foci in the liver, spleen, lungs, and tonsils are common (Kluge and Maré 1976). PRV infection may also cause edema of the scrotal region.

Microscopic lesions

Microscopic lesions in pigs reflect the neuroinvasive and epitheliotropic properties of PRV. CNS lesions are characterized by a nonsuppurative meningoencephalomyelitis in the gray and white matter and ganglioneuritis of the trigeminal and paravertebral ganglia (Figure 35.6; Baskerville et al. 1973). Death may occur before neuronal degeneration or nonsuppurative inflammatory reactions in the brain become visible. If animals survive long enough, CNS injury can be marked, leading to focal neuronal degeneration and necrosis, with neuronophagia, satellitosis, and gliosis. In particular, young piglets tend to develop panencephalitis with the most severe lesions in the cerebral cortex, brain stem, spinal ganglia, and basal ganglia of the brain.

Perivascular cuffing consists predominantly of pyknotic and karyorrhectic mononuclear cells. Similar lesions exist in the spinal cord, especially in the cervical and thoracic segments. Meninges covering affected areas of the brain and cord may be thickened because of mononuclear cell infiltration.

Intranuclear eosinophilic inclusion bodies are not commonly detected in pigs, but can be present in the neurons, astrocytes, oligodendroglia, and endothelial cells. Lymphoplasmacytic inflammation with neuronal degeneration and necrosis of the myenteric plexus of the gastrointestinal tract is also described (Ezura et al. 1995).

Epithelial lesions consist of multifocal areas of coagulative or lytic, partially hemorrhagic, necrosis in the liver, tonsils, lungs, spleen, placenta, and adrenals with the presence of the amphophilic intranuclear inclusions. Viral intranuclear inclusion bodies are much more common in lesions outside the nervous system (Kluge et al. 1999). They are present in tonsil crypt epithelial cells adjacent to necrotic foci and are frequently found in the airway epithelium, within the connective tissue, and in cells sloughed into alveolar spaces. However, the specificity of the lesions must be confirmed by immunostaining.

Mucosal epithelial necrosis and submucosal infiltrations of mononuclear cells occur in the upper respiratory tract (Baskerville 1971; Baskerville et al. 1973). In the lungs, necrosis of the bronchi, bronchioli, and pneumocytes is found. Also, peribronchial mucous gland epithelium may be involved in the inflammatory process. Alveolar edema and cellular infiltration may be multifocal or diffuse. Lymphocytes, macrophages, and, less frequent, plasma cells and neutrophils are the characteristic inflammatory cells.

In the uterus, multifocal to diffuse lymphohistiocytic endometritis and vaginitis and necrotic placentitis with coagulative necrosis of chorionic fossae develop (Bolin et al. 1985; Kluge et al. 1999). Intranuclear inclusion bodies are present in degenerate trophoblasts associated with necrotic lesions (Kluge et al. 1999; Kluge and Maré 1978).

In the male reproductive tract, degeneration of seminiferous tubules and necrotic foci in the tunica albuginea

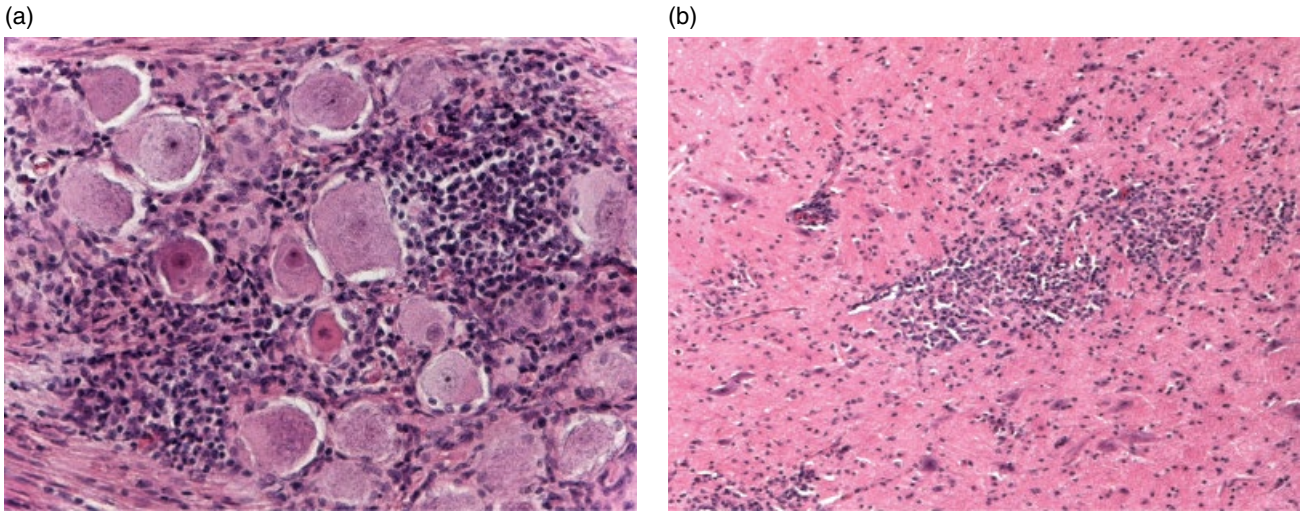


Figure 35.6 (a) Diffuse lymphoplasmacytic ganglioneuritis in the trigeminal ganglion with degeneration and necrosis of perikarya and (b) multifocal-to-coalescing nonsuppurative encephalitis in the medulla with neuronal degeneration and perivascular cuffing 8 days after PRV infection under experimental conditions.

of the testicles may be observed (Hall et al. 1984). Boars with exudative periorchitis have necrotic and inflammatory lesions in the serosa covering the genital organs. Spermatozoal abnormalities occur.

Aborted or stillborn piglets usually exhibit no evidence of encephalitis, but foci of necrosis may be found in the liver and other parenchymatous organs, together with focal bronchiolar necrosis and interstitial pneumonia. Focal necrosis of the mucosal epithelium involving the muscularis mucosa and tunica muscularis develops in the intestines (Narita et al. 1984b). Intranuclear inclusion bodies may be present in degenerative crypt epithelial cells.

The occurrence of hemorrhage and fibrin exudation results from the involvement of connective tissue and endothelium. Necrotizing vasculitis of the arterioles, venules, and lymphatic vessels around the tonsils and submaxillary lymph nodes is observed in piglets (Narita et al. 1984a). Endothelial nuclei are pyknotic and karyorrhectic, and the vessel walls are infiltrated by neutrophils. Intranuclear inclusion bodies are often present in affected endothelial cells (Kluge et al. 1999).

Diagnosis

Differential diagnosis

Several infectious and noninfectious diseases may produce clinical signs in pigs similar to pseudorabies, including rabies, porcine polioencephalomyelitis (teschovirus and sapelovirus infection), porcine astrovirus type 3 infection, classical and African swine fever, Nipah virus infection, Japanese encephalitis, hemagglutinating encephalomyelitis, encephalomyocarditis (EMC), porcine circovirus (PCV2), highly virulent strains of PRRSV, bacterial meningoencephalitis such as *Streptococcus suis* infection, swine influenza, salt poisoning, hypoglycemia, organic arsenic or mercury poisoning, congenital tremors, and other diseases causing abortion.

In species other than the pig, PRV is rather strictly neuroinvasive (Mettenleiter 2000), and diseases of the CNS, such as rabies, scrapie (sheep), and bovine spongiform encephalopathy (BSE), and diseases or conditions causing persistent itching need to be excluded.

Pathological examination

In pigs, the trigeminal ganglia, olfactory ganglia, and tonsils are the preferred tissues for isolation or detection of PRV. The virus can also be recovered from other organs, for example, lungs, spleen, liver, kidneys, lymph nodes, and pharyngeal mucosa. In latently infected pigs, virus isolation is most successful from the trigeminal ganglion in domestic pigs and the sacral ganglia in feral pigs.

PRV antigen can be detected either in cryosections by immunofluorescence or in formalin-fixed paraffin-embedded tissues by immunohistochemistry. PRV DNA can be visualized by *in situ* hybridization. In non-porcine species, the segment of the spinal cord that innervates the pruritic area of the skin should be collected. The affected area of the skin, together with the subcutaneous tissues, should also be submitted.

Samples for virus isolation should be sent to the laboratory under cold conditions. Postmortem serum can also be collected for serology. Serological tests can also be performed on muscle exudate ("meat juice") samples.

Laboratory confirmation

Rapid detection of viral infection is essential for the effective control of PRV. Clinical observations are only sufficient to lead to a suspicion of AD because the infection produces no pathognomonic clinical signs or gross postmortem lesions in swine. Therefore, laboratory confirmation is required.

Virus detection

Viral antigen can be detected using immunoperoxidase and/or immunofluorescence staining with polyclonal or monoclonal antibodies (mAbs) on impression smears and cryosections of tissues, for example, brain, lungs, and tonsils (Allan et al. 1984; Onno et al. 1988). Diagnosis is confirmed by virus isolation in conventional cell cultures requiring approximately 2–5 days, depending on the time of development of virus-specific CPE.

A variety of cells can be used for the recovery of PRV, including rabbit lung (ZP), rabbit kidney (RK-13), hamster kidney (BHK-21), porcine kidney (PK-15, SK6), African green monkey kidney (VERO), mink lung (ML), ferret kidney (FK), ovine fetal lung (OFL), bovine turbinate (BT), and turkey embryo kidney (TEK) cells (Onyekaba et al. 1987). In general, a porcine kidney cell line is employed under routine laboratory conditions.

PRV can be isolated from secretions, excretions, and tissues, for example, brain, tonsils, lungs, and spleen, of infected animals. In latently infected pigs, the trigeminal ganglia and tonsils are the most consistent sites for virus isolation. As there is no CPE characteristic of PRV and CPE may vary with the prevailing PRV strain and cell line used, virus identity is confirmed by immunofluorescence, immunoperoxidase, or neutralization assays using specific antisera or mAbs. In the absence of any obvious CPE, blind passages should be performed. Rapid detection of PRV by shell vial technique has been described (Tahir and Goyal 1995).

Detection of PRV DNA in secretions or organ samples using PCR (Jestin et al. 1990) is the method of choice. In general, primers should target regions of the genome conserved among PRV strains. Several conventional PCRs targeting genes encoding gB, gC, gD, or gE have been established (Müller et al. 2010; Schang and Osorio 1993), but there is no established international standard.

PRV-specific nested and real-time quantitative PCR assays have been described (Tombácz et al. 2009; van Rijn et al. 2004) with the capacity to differentiate between wild-type and gene-deleted vaccine viruses (Fonseca et al. 2010; Ma et al. 2008; Wernike et al. 2014).

Antibody detection

At one time, the virus neutralization (VN) test was considered the reference standard serum antibody assay (Bitsch and Ekildsen 1982), but it has been widely replaced by ELISAs. Robust and sensitive indirect or competitive ELISAs detect antibodies against the complete PRV or against distinct viral antigens (Toma 1982). The latex agglutination test (LAT) (Rodgers et al. 1996) and immunoblotting (Todd et al. 1987) are alternatives. VN and LAT are highly reliable but cannot differentiate between antibodies resulting from natural infection or vaccination. The development of ELISAs able to detect serum antibodies against gE (or gC or gG) (van Oirschot et al. 1986) allowed for the differentiation of infection from vaccination and led to the "marker" or differentiating infected from vaccinated animal (DIVA) concept. These ELISAs became a key part of PRV eradication programs.

Immunity

The major immunogens of PRV that elicit antibody-dependent and cell-mediated immunity have been identified (Mettenleiter 1996). Most are envelope glycoproteins, although the major immediate-early protein also induces an antibody response. The attachment glycoprotein gC is a major target of complement-independent neutralizing antibodies (Hampl et al. 1984; Lukacs et al. 1985) and T-cell-mediated immune responses (Ober et al. 2000; Zuckermann et al. 1990). Complement-independent neutralizing antibodies directed against gB and gD have also been identified (Hampl et al. 1984; Lukacs et al. 1985). These antibodies function by inhibiting attachment (anti-gC, anti-gD) or penetration (anti-gB) of virions. Correlated with these findings, subunit vaccines containing gB, gC, or gD, as well as DNA vaccines expressing these proteins, were able to confer at least some level of immunity against challenge virus infection (Gerdts et al. 1997; Mettenleiter 1996).

Like other herpesviruses, PRV also tries to avoid cellular immune responses by immune evasion. Binding of antibodies to viral glycoproteins exposed at the surface of infected cells results in their internalization, which may hide them from the immune system (van de Walle et al. 2003). This effect is mediated by endocytosis motifs in the C-terminal intracellular domains of gD and gB (Ficinska et al. 2005). The gE–gI complex exhibits Fc receptor activity (Favoreel et al. 1997), which may also contribute to immune evasion, as does binding of complement factor 3 by PRV gC (Huemer et al. 1992). Immune evasion is also effected by blocking major histocompatibility complex (MHC) class I-dependent antigen presentation by gN-mediated inhibition of transport of peptides from the cytosol to the endoplasmic reticulum by TAP (Koppers-Lalic et al. 2005).

Despite these immune evasion mechanisms, solid immunity against PRV infection can be induced by vaccination with inactivated viruses, modified live viruses, protein antigens, or DNA. In particular, modified live viruses attenuated either by passaging *in vitro* in cell culture or in embryonated poultry eggs have proven highly effective in decreasing clinical signs of AD (Bartha 1961), although they do not prevent infection by, and subsequent latency of, field virus. However, precolonization of ganglia by latent vaccine virus reduces latency of superinfecting wild-type PRV (Schang et al. 1994).

Immune responses

Onset of the immune response to PRV in infected and vaccinated pigs is rapid. However, slight variations in the development of the immune response may depend on the PRV strain, the route of infection, and individual pig immune competency.

PRV-specific serum antibodies are already present when the animals show clinical signs of disease (Kretzschmar 1970). Using highly sensitive serological assays (e.g. ELISAs and immunoblotting), antibodies can be detected as early as 5–7 days post inoculation, but not until 12 days post inoculation by VN. In infected animals, antibodies are almost exclusively immunoglobulin M (IgM) up to 7 days post inoculation, which persist until day 18, while in vaccinated animals, they have almost disappeared by this time (Müller et al. 2001; Rodák et al. 1987). IgG antibodies appear sooner in infected (day 7 post inoculation) than in vaccinated (day 10 post inoculation) pigs, reaching higher mean titers. In contrast, IgA antibodies seem to be present only in infected pigs from day 10 post inoculation.

Similar antibody dynamics and distribution can be detected in oropharyngeal swabs, except that IgG and IgM titers are considerably lower than in the serum, whereas IgA titers in oropharyngeal swabs are higher than in the serum (Rodák et al. 1987). About 3 weeks post inoculation, IgG antibodies reach maximum levels and, in general, persist for the life of the pig. Only in exceptional cases, virusneutralizing antibodies were reported to have disappeared after 2–3 months (Blaha 1989).

Protective immunity

Immunity after infection is durable, very stable, and protective against viremia and clinical disease. Even massive intracerebral inoculation can be resisted.

However, sterilizing immunity is not achieved. Although a significantly higher infectious dose is required to provoke exogenous infection, those virus doses are easily shed within an infected herd (Blaha 1989). In latently infected pigs, reactivation of latent PRV due to immunosuppression or new exposure to virulent virus may result in increasing virus-neutralizing antibody titers. However, there is no evidence that reactivation of latent virus is associated with any unique immunological response (Mengeling 1991).

Maternal immunity

In both domestic and nondomestic pigs, immune sows transfer PRV-specific antibodies to their offspring even years after infection. In general, maternally derived virus-neutralizing antibodies, mostly IgG, can be detected up to 14–15 weeks postpartum. Their duration is dependent on the original concentration (Iglesias and Trujano 1989; Müller et al. 2005). With 11 and 21 days in domestic pigs and wild boar, respectively, the half-life of maternal antibodies is considerably longer than known for other infections. However, anti-PRV antibodies can be detected by ELISA as long as 27 weeks postpartum, that is, twice as long as in VN assays (Müller et al. 2005; Tenhagen et al. 1995).

Maternally derived immunity prevents the transmission of PRV in newborn piglets and is able to protect against clinical disease after infection by limiting virus replication in the CNS. However, the correlation between the level of maternal immunity and protection against neuroinvasion in maternally immune neonatal pigs is dependent on the PRV strain. High virus-neutralizing antibody titers protect neonatal pigs almost completely against neural invasion, whereas low titers do not (Kritas et al. 1999). Maternally derived antibodies inhibit the ability of piglets to respond to vaccination (Tielen et al. 1981; Weigel et al. 1995), but recombinant vaccinia virus vaccines expressing PRV glycoproteins are able to circumvent maternally derived antibodies and stimulate active immunity (Brockmeier et al. 1997).

Prevention and control

Due to the rapid increase in AD in the 1970s, test and slaughter programs were initiated in several countries, including England, Switzerland, and Denmark in the early 1980s (Watson 1986). Although costly, they succeeded in eliminating AD from national pig herds, although new outbreaks did occur due to introduction of virus by trade or air. In other countries, control of disease, but not infection, was achieved by blanket vaccination with inactivated (particularly in breeding animals) and modified live virus vaccines (in finishers). In East Germany, for example, AD control was based on a combination of large-scale vaccination of breeding herds with attenuated live vaccines and rigorous stamping-out policy, especially in the final phase of eradication until successful termination of the AD eradication program in 1985 (Müller et al. 2003). Whereas inactivated and attenuated vaccines were efficacious in reducing disease, they did not lead to the elimination of virus, since none of them prevented latent infection and subsequent reactivation and shedding of virulent field virus.

Beginning in the 1980s, novel strategies in animal disease control were pioneered by the first use of genetically engineered live PRV vaccines lacking virulence-determining genes (Freuling et al. 2017). The first major improvement occurred in 1986 when the first recombinant DNA-derived modified live virus vaccine was licensed in the United States (Kit and Kit 1991). It carried a genetically engineered deletion of the thymidine kinase gene, a gene that is relevant for virulence. At about the same time, it was discovered that several classical AD vaccine strains, for example, the Bartha strain (Bartha 1961), carried deletions of the gene encoding immunogenic glycoprotein gE (Mettenleiter et al. 1985) that did not impair their potency as vaccines. After the development of an ELISA to measure anti-gE antibodies in the animal (van Oirschot et al. 1986), the combination of marker vaccine and differential ELISA made it possible to discriminate between vaccinated, PRV-uninfected animals (PRV-positive, but gE-negative) and wild-type PRV-infected (gE-positive) animals. Subsequently, additional nonessential glycoproteins, for example, gC or gG, were also deleted by genetic engineering and used as markers with appropriate serological assay systems. Since then, AD has been the prime example of using companion diagnostic ELISA tests to differentiate infected from vaccinated animals (DIVA) (Freuling et al. 2017). Thus, marker vaccines against AD were the first genetically modified live vaccines used on a wide scale (Quint et al. 1987). Detailed reviews on the development of PRV vaccines have been published elsewhere (Dong et al. 2014; Freuling et al. 2017; Mengeling et al. 1997). The combination of highly efficacious DIVA vaccines and accurate differential ELISAs has made eradication of AD from large areas of the world practical and feasible.

Porcine cytomegalovirus

Relevance

Porcine cytomegalovirus (PCMV) infection was originally designated "inclusion body rhinitis" (sometimes abbreviated as "IBR," but not to be confused with "infectious bovine rhinotracheitis") based on the histopathological observation of basophilic intranuclear inclusion bodies in cytomegalic cells of the nasal mucosa of pigs with rhinitis (Done 1955). Ultrastructural investi-

gations demonstrated herpes virions in epithelial cells of the turbinate mucous glands, lachrymal and salivary glands, and the renal tubuli. The virus grew slowly in cell culture and produced cytomegaly with large intranuclear inclusions (Yoon and Edington 2006).

Infections with PCMV are ubiquitous. PCMV exists in nearly all pig populations, but clinical disease is rare with the exception of young piglets, in which a fatal systemic disease develops. In immunologically susceptible herds, the virus causes fetal and piglet mortality, runting, rhinitis, pneumonia, and sometimes neurological signs (Yoon and Edington 2006).

Etiology

PCMV (SuHV-2) belongs to the subfamily *Betaherpesvirinae* in the family *Herpesviridae*, but is not assigned to any genus (Table 35.1; Davison 2010). Comparative genetic studies indicate that PCMV is genetically closer to human herpesviruses 6 and 7 than to cytomegaloviruses (Rupasinghe et al. 2001; Widen et al. 2001).

PCMV particles exhibit typical herpes virion morphology (Duncan et al. 1965; Valicek and Smid 1979). No distinct PCMV serotypes or genotypes have been identified, although some genetic variation was noted in polymerase and gB genes among PCMV isolates from different geographical origins (Widen et al. 2001). Possible antigenic variability has also been reported (Tajima and Kawamura 1998). The virus is sensitive to chloroform and ether. Virus infectivity is preserved at subzero temperatures (Booth et al. 1967).

PCMV can be isolated in porcine pulmonary macrophages and propagated up to maximum titers of 1×10^5–10^6 $TICD_{50}$/mL *in vitro*, for example, in primary pig lung (PL) cells, primary swine testicle (ST), PK-15 cell line, and porcine turbinate (PT) (Yoon and Edington 2006).

Basophilic intranuclear and, occasionally, small acidophilic intracytoplasmic inclusions (Figure 35.7) are present in infected cytomegalic cells (Watt et al. 1973). Since CPEs are lacking in cell culture, confirmatory immunostaining is necessary (Figure 35.8).

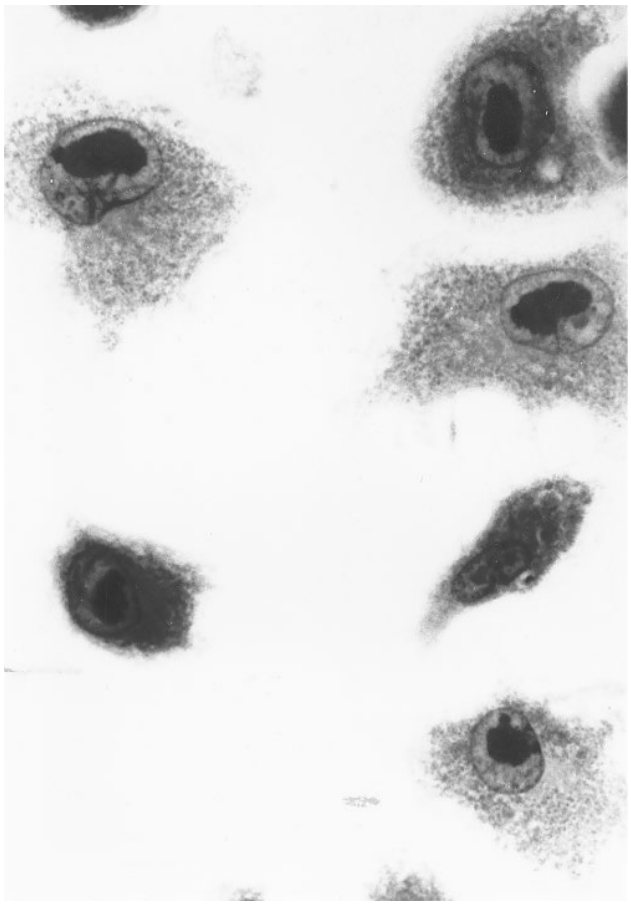


Figure 35.7 Cultured pig macrophages 11 days after inoculation with PCMV. Note the basophilic intranuclear inclusions in the enlarged cells (May–Grünwald–Giemsa; ×720). *Source:* courtesy of R.G. Watt.

Public health

PCMV is ubiquitous in pig populations and induces latent infections in pigs (Garkavenko et al. 2004; Tucker et al. 1999). Although no human infections have been reported, the potential use of live porcine cells, tissues, and organs for xenotransplantation has led to concerns regarding exposure of immunocompromised humans to infectious PCMV. Cross-species transmission of PCMV was reported in pig-to-primate xenotransplantation (Mueller et al. 2002), but a pig-to-primate islet xenotransplantation study found no evidence of the transmission of PCMV or other viruses into primate recipients (Garkavenko et al. 2008; Morozov et al. 2016a; Plotzki et al. 2015; Wynyard et al. 2014). More recent data suggested that PCMV could infect human fibroblasts *in vitro* (Whitteker et al. 2008). PCMV has also been detected in the tissues of recipient nonhuman primates, although no disease was reported (Ekser et al. 2009; Morozov et al. 2016b). PCMV plays no role in the more traditional or routine areas of concern to public health.

Epidemiology

PCMV is highly prevalent throughout the world with herd prevalence greater than 90% and more than 98% of animals positive in Europe, North America, and Japan (Collett and Roberts 2002; Deim et al. 2006; Yoon and Edington 2006).

Inclusion body rhinitis is an acute-to-subacute disease of about 4-week-old suckling piglets. Non-porcine reservoirs and arthropod vectors have not been reported.

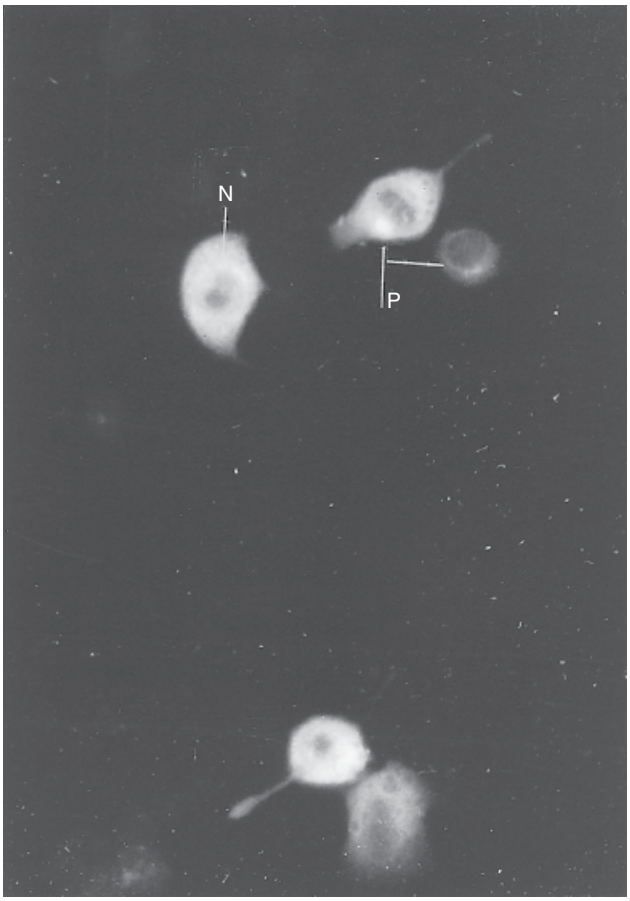


Figure 35.8 Lung macrophage cultures showing fluorescence after indirect immunofluorescence (IIF) using specific PCMV antiserum. The nuclear staining (N) is most intense at the membrane. Cytoplasmic and discrete paranuclear fluorescence (P) can also be seen (×480).

Natural infection with PCMV is limited to pigs. The virus does not replicate in mice, rabbits, dogs, cattle, or chicken embryos. However, virus replication was detected in tissues of baboons that received xenografts of porcine origin (Morozov et al. 2016a; Mueller et al. 2002).

PCMV is transmitted horizontally via the oronasal route, but congenital transmission is also well documented (Yoon and Edington 2006). Infection most commonly occurs perinatally in commercial pig holdings (Watt 1978).

PCMV can be recovered from nasal and ocular discharge, urine, and cervical fluid (Yoon and Edington 2006). The majority of pigs shed PCMV in nasal secretions between 3 and 8 weeks of age (Plowright et al. 1976). This suggests that infection is usually acquired by contact within the infected cohort. Virus recrudescence from latent infections is possible (Edington et al. 1976c; Narita et al. 1985).

The stability of PCMV in the environment is unknown. No specific disinfectants are recommended.

Pathogenesis

The primary replication site of PCMV is the nasal mucosa and/or the lachrymal or Harderian glands. Cell-associated viremia follows primary replication 14–21 DPI in animals older than 3 weeks (Edington et al. 1976c, 1977). Shedding in nasal secretions lasts from 10 to over 30 days. Congenitally infected pigs excrete virus until death (Edington et al. 1977). Excretion in cervical fluids from pregnant sows is found concomitant with fetal deaths.

The site of secondary viral replication varies with age. In nursery or growing pigs, PCMV spreads to the nasal mucosal glands, Harderian and lachrymal glands, kidney tubules, and, more rarely, the epididymis and mucous glands of the esophagus. Hepatocytes and duodenal epithelium are rarely infected. It remains unclear how PCMV spreads systemically. Apparently, PCMV is able to infect cells of the monocyte–macrophage system and endothelial cells, causing renal petechiation, particularly in the subcapsular spaces (Sekiguchi et al. 2012).

In the fetus or neonate, there is predominantly infection of capillary endothelium and sinusoids of lymphoid tissues, thus resulting in systemic spread of PCMV and generalized lesions (Edington et al. 1977, 1988). This observation is important in terms of PCMV eradication from pigs bred for xenotransplantation. That is, spleens from donor animals should be examined as part of quality control procedures (Clark et al. 2003). Using microarray techniques, differential expression of genes associated with inflammation, immunosuppression, cellular and metabolic processes, networks of cytokine–cytokine receptor interactions, the TGF-β signaling pathway, the lymphocyte receptor signaling pathway, and the TNF-α signaling pathway was shown (Liu et al. 2014).

Clinical signs

Incubation period for PCMV may be 10–20 days (Edington et al. 1977). During viremia, animals become depressed and anorexic. Neonates may die without any clinical signs, whereas others exhibit shivering, sneezing, respiratory distress, poor weight gain, and rhinitis. A black discoloration may occasionally be observed around the eyes due to conjunctival discharge. The disease is generally self-limiting. In pigs older than 3 weeks, the course of disease is usually subclinical to mild, but can lead to death of the fetus or newborn pig. Affected pigs show respiratory signs, for example, sneezing, catarrhal nasal exudate and discharge, and coughing with dyspnea, and develop rhinitis or neurological disease (Yoon and Edington 2006). There is no link between PCMV infection and atrophic rhinitis (Edington et al. 1976b). Embryonic death and infertility may occur (Edington et al. 1977, 1988; L'Ecuyer et al. 1972; Yoon et al. 1996).

PCMV has been associated with the porcine respiratory disease complex (PRDC) in pigs of different ages (Orr et al. 1988). A significant correlation between PCMV and PCV2 infection was found, and it is speculated that the presence of PCMV causes exacerbation of PRDC (Hansen et al. 2010).

Although infection is usually subclinical in older pigs if uncomplicated, morbidity after congenital or neonatal infection is 100%. The mortality in a naive herd can be 10%, but it may increase to 50% in the presence of secondary bacterial or viral infections (Yoon and Edington 2006).

Lesions

Whereas epithelia are the target tissue in older pigs, generalized infection affects reticuloendothelial tissues in the fetus or neonate (Edington et al. 1976a).

Macroscopic changes are usually only seen in piglets less than 3 weeks of age with systemic PCMV infection, in which case catarrhal rhinitis, hydrothorax and hydropericardium, pulmonary and subcutaneous edema, and renal petechiation can be present. In fetal infections, stillbirths, mummification, embryonic death, and infertility are seen.

Microscopically, 8–12 µm basophilic intranuclear inclusion bodies, cytomegaly, and karyomegaly are seen in the nasal mucous glands (Figure 35.9), acinar and duct epithelium of Harderian and lachrymal glands, and renal tubular epithelium. The major sites of replication develop focal lymphoid hyperplasia (Figure 35.10).

Interstitial nephritis and random focal gliosis in the CNS with inclusion bodies can be additional findings, with a predilection for the choroid plexus, cerebellum, and olfactory lobes (Yoon and Edington 2006). In the acute fatal syndrome, most basophilic inclusions are seen in the capillary endothelium and sinusoidal cells of the lymphoid tissues. Multifocal edema or hemorrhage results from vascular damage. Mononuclear cells and macrophages with inclusions are found in the blood vessels, alveoli, and spleen. Focal hepatocellular necrosis and inclusions in glomerular capillary endothelium are further lesions.

Diagnosis

PCMV-associated diseases must be differentiated from infection with classical swine fever virus, enterovirus, parvovirus, PRRSV, PCV2, and PRV. Virus isolation or PCR-based assays for detection of viral DNA can be used for positive identification (Fryer et al. 2001; Hamel et al. 1999; Morozov et al. 2016c; Widen et al. 1999). Antemortem samples of choice are swabs with nasal secretion or scrapings and whole blood (Edington et al. 1976a; Watt et al. 1973).

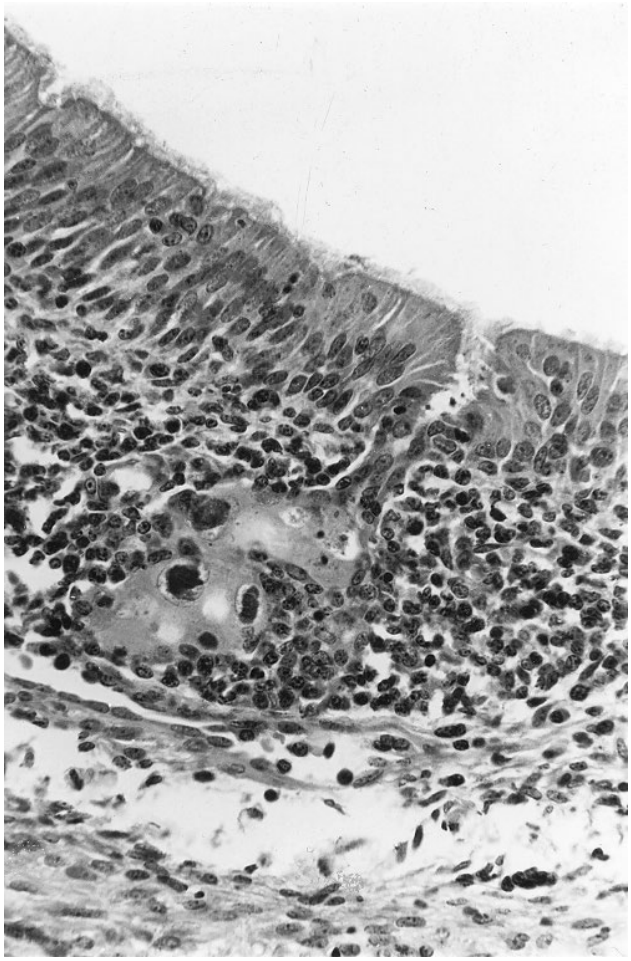


Figure 35.9 The basophilic intranuclear inclusion, translucent halo, and defined nuclear membrane are prominent in the enlarged superficial mucous gland epithelium of an animal 18 days after experimental intranasal inoculation (hematoxylin and eosin [H&E]; ×480).

Preferred postmortem samples are turbinate mucosa, lungs, pulmonary macrophages obtained by lung lavage, and kidneys. In cases of early reproductive failure, PCMV can occasionally be demonstrated in the brain, liver, and bone marrow of fetuses (Yoon and Edington 2006).

Virus isolation is possible on primary or immortalized cells. Viral antigen can be detected by immunostaining on frozen tissue sections. The combination of inclusion bodies, cytomegaly, and karyomegaly is pathognomonic (Yoon and Edington 2006). PCMV infection in a herd is confirmed by serology using serum samples from grower–finishers. ELISAs have been described and adapted to differentiate IgG and IgM responses (Tajima et al. 1994). It should be noted that no PCMV antibodies are induced by *in utero* infection. Therefore, antibody is not expected in colostrum-deprived neonatal sera.

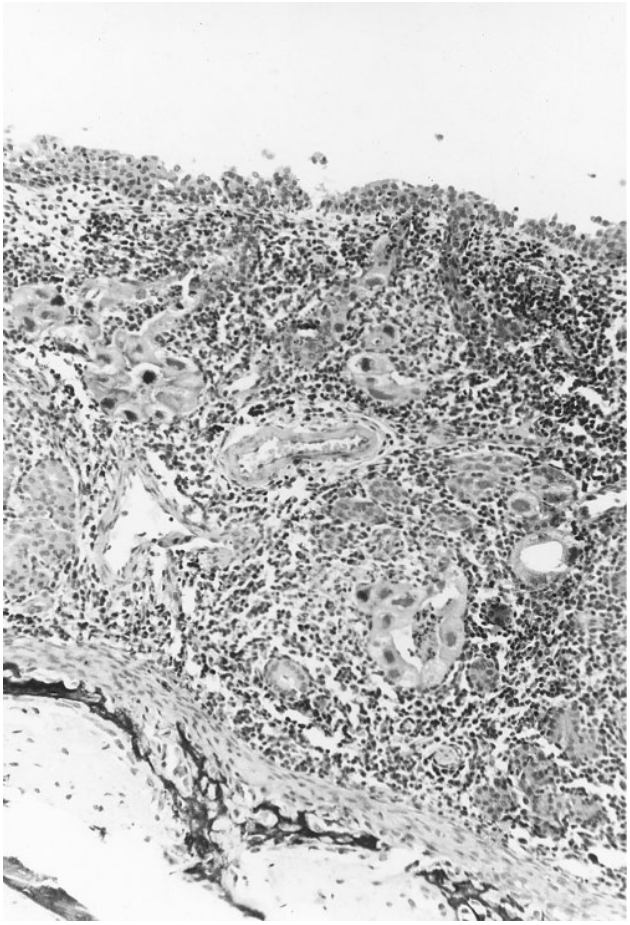


Figure 35.10 The lamina propria is heavily infiltrated with lymphocytes and plasma cells 24 days after inoculation with PCMV. Many of the acini of the mucous glands still show cytomegaly and prominent inclusion bodies (hematoxylin and eosin [H&E]; ×120).

Immunity

After experimental infection, antibodies are detected by indirect immunofluorescent antibody (IFA) tests 2–3 weeks after inoculation. IFA-detectable antibodies peak at approximately 6 weeks and remain at high levels for at least 10–11 weeks (Edington et al. 1976c, 1977). Development of serum antibody levels detectable by IFA coincides with the disappearance of viremia, but nasal excretion of virus continues for another 2–3 weeks. Piglets with congenital and neonatal infections do not seroconvert but excrete virus and develop fatal systemic infections (Edington et al. 1977). Piglets acquire maternal antibodies and these provide some protection, but virus is shed even in the presence of circulating maternal antibody in PCMV-endemic farms (Plowright et al. 1976). Maternal antibodies persist for approximately 2 months (Tajima et al. 1994).

Prevention and control

No vaccine or specific treatment for PCMV is available. Introduction of new stock into herds poses a significant risk due to reactivation of latent infections or primary infection of susceptible herds.

Porcine lymphotropic herpesviruses

Relevance

The detection by PCR of the genetic material of two porcine herpesviruses in leukocytes and lymphoid organs of healthy pigs (*Sus scrofa*) led to the discovery of PLHV-1 and PLHV-2. Sequence analysis revealed that they were the first porcine herpesviruses belonging to the subfamily *Gammaherpesvirinae* (Ehlers et al. 1999a). In 2003, a third porcine gammaherpesvirus, PLHV-3, was identified (Chmielewicz et al. 2003a).

Little is known about the pathogenic potential of the PLHVs. However, the presence of these viruses in apparently healthy pigs (Chmielewicz et al. 2003a; Ehlers et al. 1999a; Ulrich et al. 1999) raised concerns about the safety of pig-to-human xenotransplantation. These concerns were reinforced by the high worldwide prevalence of the PLHVs, the difficulties in eliminating them from pigs bred for use as tissue and organ donors (Tucker et al. 2003), and the association of PLHV-1 with a porcine lymphoproliferative disease of high mortality (Goltz et al. 2002; Huang et al. 2001).

Etiology

Approaches to characterize the PLHV genomes were hampered by the fact that propagation of these viruses in cell culture was not successful. Therefore, the genomes were amplified directly from PLHV-positive pig samples by a PCR-based genome walking technique. About 101 kb, 68 kb, and 98 kb of PLHV-1, PLHV-2, and PLHV-3, respectively, were characterized and found to harbor more than 60 genes with counterparts in other herpesviruses (Chmielewicz et al. 2003a; Goltz et al. 2002; Lindner et al. 2007). Upon comparison of genes conserved among the *Herpesviridae*, the PLHVs were then classified by the International Committee on Taxonomy of Viruses (ICTV) as members of the subfamily *Gammaherpesvirinae* and named suid gammaherpesvirus 3 (PLHV-1), suid gammaherpesvirus 4 (PLHV-2), and suid gammaherpesvirus 5 (PLHV-3) (Table 35.1; Davison et al. 2009). Close relatives of the PLHVs were also found in other suid species, that is, *Phacochoerus africanus* and *Sus barbatus* (Ehlers and Lowden 2004).

Phylogenetic analysis revealed that the PLHVs are most closely related to a group of ruminant gammaherpesviruses (Figure 35.11). Members of this group cause

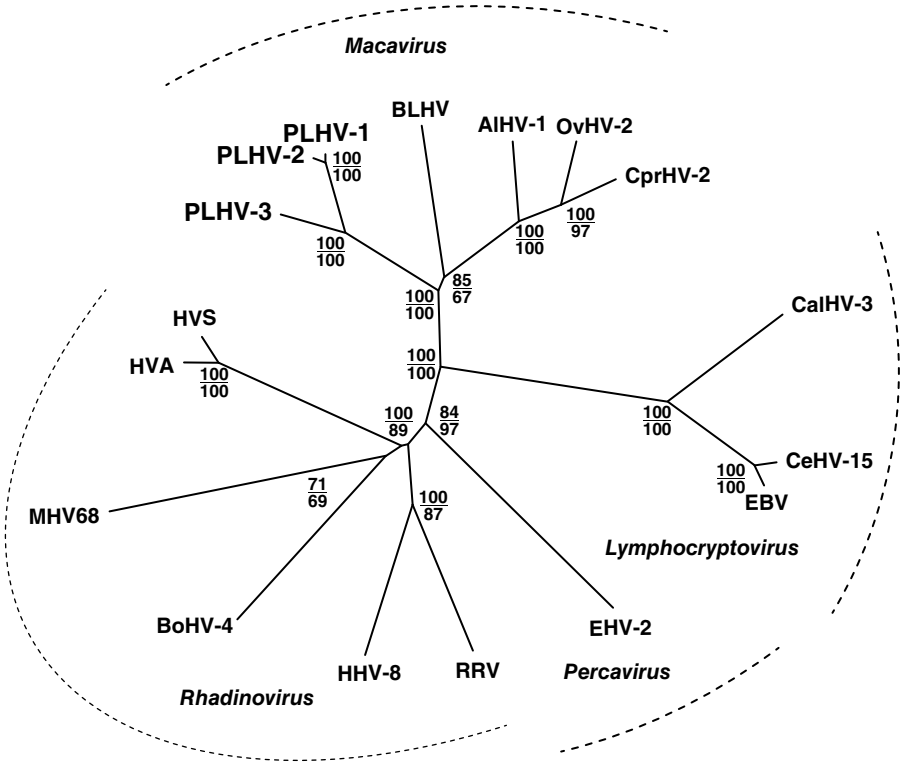


Figure 35.11 Phylogenetic tree showing the positions of the PLHVs within the herpesvirus subfamily *Gammaherpesvirinae*, genus *Macavirus*. Members of the genera *Macavirus*, *Rhadinovirus*, *Percavirus*, and *Lymphocryptovirus* were included. The tree was constructed using a multiple amino acid sequence alignment of concatenated glycoprotein B and DNA polymerase sequences. The bootstrap values derived from 100 repetitions are indicated at the branching points of the tree. The upper values were obtained with neighbor-joining analysis, and the lower values were obtained with parsimony analysis. *Source:* Adapted from Chmielewicz et al. (2003a), and reprinted by permission of the publisher.. PLHV, porcine lymphotropic herpesvirus; BLHV, bovine lymphotropic herpesvirus; AlHV-1, Alcelaphine gammaherpesvirus 1; OvHV-2, Ovine gammaherpesvirus 2; CprHV-2, Caprine gammaherpesvirus 2; CalHV-3, Callitrichine gammaherpesvirus 3; CeHV-15, Cercopithecine gammaherpesvirus 15; EBV, Epstein–Barr virus; EHV-2, Equid gammaherpesvirus 2; RRV, rhesus monkey rhadinovirus; HHV-8, Human gammaherpesvirus 8; BoHV-4, Bovine gammaherpesvirus 4; MHV68, Murine gammaherpesvirus 68; HVA, Herpesvirus ateles or Ateline gammaherpesvirus 3; HVS, *Herpesvirus saimiri* or Saimiriine gammaherpesvirus 2.

MCF in foreign hosts (AlHV-1, ovine herpesvirus 2, caprine herpesvirus 2) (Li et al. 2003; Russell et al. 2009). These viruses were all classified as members of the newly established genus *Macavirus* (Table 35.1; Davison et al. 2009).

The approximately 100 kb determined for PLHV-1 and PLHV-3 encompass the ORFs ORF03 to ORF69 (ORF nomenclature according to *Herpesvirus saimiri* [HVS]). In addition, five ORFs were identified that have counterparts only in the gammaherpesviruses AlHV-1, equine herpesvirus 2 (EHV-2), and/or EBV, but not in HVS or KSHV. These encode a viral BCL-2 protein (ORFE4/BALF1), a G-protein-coupled receptor (ORFA5/BILF1), an immediate-early transactivator (ORFA6/BZLF1), a cell fusion protein (ORFA7/BZLF2), and a cell attachment protein (ORFA8/BLLF1) (Chmielewicz et al. 2003a; Goltz et al. 2002; Lindner et al. 2007).

Due to long intragenomic repeat regions, an entire PLHV genome could not be amplified by genome walking. However, Gardella gel electrophoresis and blotting revealed the total length as slightly shorter than the genome of EBV (approximately 170 kb) (Chmielewicz et al. 2003a).

More than 20 porcine cell lines were tested by PCR for the presence of PLHV sequences. One of them, the B-cell line L23, was found to carry PLHV-3 genomes (Chmielewicz et al. 2003a). However, attempts to induce efficient lytic replication failed (B. Ehlers, unpublished data). Attempts to isolate PLHV from primary samples were not reported. Therefore, a lytic cell culture system is not available.

Public health

The PLHVs have the potential to cause a lymphoproliferative disease of high mortality in experimentally immunosuppressed pigs that resembles the posttransplantation lymphoproliferative disease (PTLD) in humans. The PLHVs are closely related to AlHV-1 and OvHV-2, which are innocuous in their natural hosts, but

cause MCF in other animals. Porcine cells, tissues, and organs offer a potential solution to the shortage of organs available for human-to-human allotransplantation (Yang and Sykes 2007), but the pathogenic potential and worldwide prevalence of the PLHVs in pigs have raised concerns regarding the consequences of pig-to-human xenotransplants in immunosuppressed humans (Ehlers et al. 1999a; Mueller and Fishman 2004).

Epidemiology

Knowledge on the epidemiology of the PLHVs is limited, but there is growing evidence that they are present in pigs and their relatives in the family *Suidae* worldwide. PLHVs were detected in domestic pigs (*S. scrofa domestica*) from Germany, Italy, France, Belgium, Denmark, Ireland, the United Kingdom, the United States, Australia, and Vietnam and in wild boar (*S. scrofa*) from Germany, the United States, and Australia (Chmielewicz et al. 2003a,b; Ehlers et al. 1999a; Garkavenko et al. 2004; Goltz et al. 2002; McMahon et al. 2006; Tucker et al. 2003; Ulrich et al. 1999; B. Ehlers, unpublished data). PLHV-1 sequences were also amplified from *S. barbatus oi*, *S. barbatus*, *S. celebensis*, and *P. africanus*. PLHV-3 sequences were identified in *Babyrousa babyrussa* and a sequence with 98% identity to the PLHV-3 DPOL gene in *S. barbatus* (Ehlers and Lowden 2004).

The prevalence of the PLHVs was determined with real-time PCR in German domestic pigs. Depending on the sample type (blood, spleen, and lungs), prevalences of 48–62% (PLHV-1), 16–41% (PLHV-2), and 54–78% (PLHV-3) were estimated. In German wild boar, PLHV-1 was detected in 1 of 19 and PLHV-2 in 18 of 19 bone marrow samples (Ulrich et al. 1999). Spleens of domestic pigs from Ireland were 74, 21, and 45% positive for PLHV-1, PLHV-2, or PLHV-3, respectively (McMahon et al. 2006). In New Zealand, 95% of domestic piglets (20 weeks) and pigs (>6 months) were PCR positive for PLHV-2. PLHV-1 was not detected and PLHV-3 was not tested (Garkavenko et al. 2004). In the United Kingdom, PLHV sequences were amplified in 80 and 67% of adult Large White pigs and adult miniature swine, respectively (Tucker et al. 2003). These data indicated a high PLHV prevalence in commercial and experimental pig herds. They further revealed a remarkably low level of intraspecies sequence variation (well below 1%) in coding regions. This indicated that the PLHVs are genetically stable and well adapted to their natural host.

Very few data allow conclusions about the mode of PLHV transmission. Cesarean-derived pigs were found to be far less frequently infected with PLHVs than conventionally reared pigs. This reduced rate may indicate that PLHVs are rarely transmitted *in utero*, but frequently postpartum (Tucker et al. 2003). Further evidence for horizontal transmission as the major route emerged by repeated analysis of piglets after birth using ELISA and PCR. These data indicated *de novo* PLHV infection by contact to the infected dam and subsequent seroconversion (Brema et al. 2008).

Pathogenesis, clinical signs, and lesions

A clinical disease associated with PLHV infection under field conditions is not known. The closely related gammaherpesviruses naturally infecting wildebeest (AlHV-1) and sheep (OvHV-2) can be horizontally transmitted to cattle or pigs and cause MCF, a lymphoproliferative disease of high mortality (Russell et al. 2009). Therefore, the PLHVs might have a similar pathogenic potential, either in foreign hosts or in their natural host. The latter may be difficult to observe since most commercial pigs are slaughtered at approximately 6 months of age.

Evidence for the pathogenicity of the PLHVs under experimental conditions came from immunosuppressed miniature swine subjected to allogeneic hematopoietic stem cell transplantation. A high incidence of PTLD was observed, B-cell proliferation occurred, and the majority of the animals died (Huang et al. 2001). In the PTLD-affected individuals, high PLHV-1 genome copy numbers were detected. In addition, transcripts of several PLHV-1 genes were demonstrated in PTLD pigs, but not in healthy pigs, strongly suggesting a causative role of PLHV-1 in PTLD (Goltz et al. 2002; Huang et al. 2001). Similar findings were reported after allogeneic spleen transplantation in miniature swine (Dor et al. 2004).

The clinical signs of experimental porcine PTLD resemble those of human PTLD associated with EBV, a human gammaherpesvirus. Lethargy, fever, anorexia, enlarged lymph nodes, and elevated numbers of leukocytes were observed. Pigs suffering from PTLD showed enlarged lymphoreticular organs, airway obstruction, and respiratory failure. Microscopically, a mixture of immunoblasts, plasmacytoid cells, and plasma cells was typical of porcine PTLD (Huang et al. 2001).

Pigs have the potential to be used as cell, tissue, and organ donors in pig-to-human xenotransplantation. The theoretical concern is that PLHVs might recombine with human herpesviruses in the human xenotransplant recipients, giving rise to recombinants with novel pathogenic properties. In addition, activation of human herpesviruses through PLHVs might occur in xenotransplant patients, leading to lytic infection and rejection of the transplanted organ (Santoni et al. 2006).

Diagnosis

Nucleic acid-based and antibody-based diagnostic assays are available for PLHV diagnosis. Three independent real-time PCR assays have been developed to quantify PLHV-1, PLHV-2, or PLHV-3 genome copy numbers in

porcine samples (Chmielewicz et al. 2003a). Single-round conventional PCR assays for the specific detection of a certain PLHV or the simultaneous detection of PLHV-1 and PLHV-2 have also been published (Chmielewicz et al. 2003a; Ehlers et al. 1999a). The very low level of PLHV intraspecies variation (<1% in coding regions) ensures the conservation of the primer binding sites used in the PCR and real-time PCR assays.

In addition, a panherpes consensus PCR has been designed for the universal detection of all mammalian and avian herpesviruses with degenerate primers (Ehlers et al. 1999b). This assay readily amplifies PLHV DNA polymerase sequences (Chmielewicz et al. 2003a; Ehlers et al. 1999a). However, many porcine specimens are infected with more than one PLHV. Due to the degenerate nature and universal binding properties of the consensus primers, sequences of only one virus are preferentially amplified from a mixture. Therefore, the simultaneous diagnosis of multiple PLHV infections in a single specimen is usually not successful using the panherpes consensus PCR.

With the PCR assays described above, the PLHVs were frequently detected in blood leukocytes, in lymphoreticular organs (spleen, lymph nodes, tonsils, bone marrow), and in the lungs (Chmielewicz et al. 2003a; Ehlers et al. 1999a; Ulrich et al. 1999). Since organ samples are not readily available from live pigs, routine PLHV diagnosis relies on testing white blood cells. This has to be taken with caution since PLHVs are more frequently detected in the spleen and lungs than in the blood (Chmielewicz et al. 2003a).

Two ELISA-based serological assays have been published, using recombinant gB of PLHV-1 as antigen, for the detection of anti-PLHV antibodies (Brema et al. 2008; Plotzki et al. 2016). The assay (Brema et al. 2008) was used for the analysis of sera from groups of pigs, differing by age and origin. Seropositivity ranged from 38% (piglets) to 90% (gilts) and 100% (breeding sows, miniature pigs, and pigs for slaughter). Compared with the percentages of PCR-positive samples in the same groups of sera (20, 80, and 0–75%, respectively), this ELISA is suitable for PLHV diagnosis (Brema et al. 2008).

Immunity

The type and efficacy of immune responses against the PLHVs are largely unknown. Test systems relying on viral cell culture are not available, and infection studies with cultured viruses are not possible at present. A group of 12 piglets was tested repeatedly after birth until the age of 156 days for the presence of anti-PLHV antibodies (Brema et al. 2008). At birth, antibodies of probable maternal origin were detected, which declined to background levels during the first 3 weeks of life. Thereafter, seroconversion due to *de novo* PLHV infection by contact with the infected dam (or already infected piglets) was observed (Figure 35.12).

In the blood, the PLHVs infect predominantly B cells. This was assessed with real-time PCR analysis of microbead-sorted B cells, T cells, and macrophages (Chmielewicz et al. 2003b). In immunosuppressed miniature swine developing PTLD, a rise of PLHV-1 genome copies and a concomitant expansion of the B cells were observed (Huang et al. 2001). In addition, transcripts of an ORF were found in the PTLD-affected pigs, which encode a protein for B-cell entry (Goltz et al. 2002).

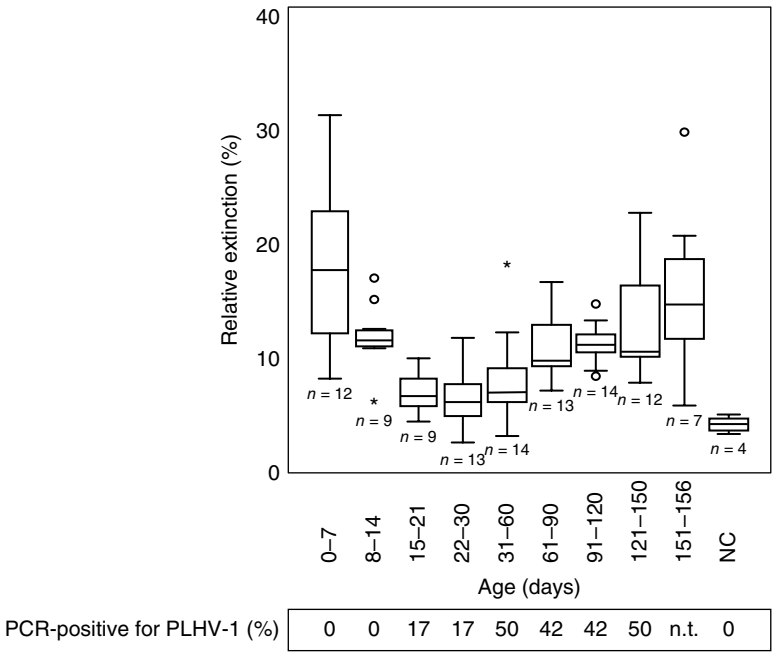


Figure 35.12 Analysis of PLHV antibody titers in piglets of different ages. Sera were obtained from newborn pigs up to 5 months of age and split into nine age groups (days 0–7, 8–14, 15–21, 22–30, 31–60, 61–90, 91–120, 121–150, and 151–156). All sera were examined for anti-PLHV antibodies by ELISA. The number of samples (*n*) in each group is shown below each box plot. Outliers (circles) and extreme values (stars) are also shown. ELISA was performed using 1.25 µg antigen (N-terminal part of PLHV-1 glycoprotein B) per well and 1 : 50 diluted swine sera. In addition, peripheral blood leukocyte samples were analyzed for PLHV-1 DNA by PCR. The percentages of PCR-positive samples are listed in the box at the bottom of the figure. *Source:* Brema et al. (2008). Reproduced with permission of John Wiley and Sons.

Prevention and control

Concerns regarding the safety of pig-to-human xenotransplantation make PLHV-free donor pigs a desirable goal. Early weaning of piglets failed to exclude PLHV (Mueller et al. 2005), but a markedly reduced prevalence of PLHVs was achieved using Cesarean-derived, barrier-reared breeding conditions (Tucker et al. 2003; Morozov et al. 2016d). These results indicated that the derivation of PLHV-free animals is a realistic objective.

Ovine herpesvirus 2 causing porcine malignant catarrhal fever

Relevance

MCF is a sporadic, systemic gammaherpesvirus infection of ungulates. It was first described as a fatal disease, primarily of cattle, characterized by high fever, copious nasal discharge, corneal opacity, generalized lymphadenopathy with lymphopenia, inflammation, necrosis of mucosal surfaces, and vasculitis. In Europe, an association with sheep was recognized as a prerequisite (Götze and Liess 1930), whereas in Africa, the source of infection was wildebeests (*Connochaetes taurinus*), which are inapparent carriers (Plowright et al. 1960). Later, the sheep-associated (SA) form was reported worldwide in a wide variety of species belonging to the subfamily *Bovinae* and the family *Cervidae* (Hüssy et al. 2000; Müller-Doblies 1998; Plowright 1990).

A naturally occurring disease similar to MCF has been described in pigs, particularly in Scandinavian countries (Løken et al. 1998). However, the first cases of MCF in pigs were reported from Italy and Germany, where single sows developed clinical signs resembling the disease in cattle (Kurtze 1950; Morselli 1901). For decades, overt disease in swine seemed to be limited to Norway, but has since occurred in Finland, Sweden, and North America (Alcaraz et al. 2009; Bratberg 1980; Gauger et al. 2010; Grytting 1974; Holmgren et al. 1983; Løken et al. 2009; Okkenhaug and Kjelvik 1995; Syrjälä et al. 2006). Other cases of porcine MCF were also reported from Switzerland (Pohlenz et al. 1974). Although the etiology had not been clarified, in most reports, contact with sheep was mentioned before the disease was noted (Albini et al. 2003a).

Porcine MCF is a very rare, poorly documented disease of swine (Alcaraz et al. 2009). The small number of reported cases of MCF is mainly based on the fact that the clinical signs can be subtle and nonspecific and diagnostic tools are not generally available (Albini et al. 2003a). Compared with other herpesviral diseases of pigs, porcine MCF does not pose a significant threat to the porcine population, even if underdiagnosed in the field.

Etiology

There are two etiologically distinct forms of MCF: (1) a wildebeest-associated form (WA-MCF), caused by AlHV-1, and (2) a sheep-associated form (SA-MCF), occurring worldwide and caused by OvHV-2 (Baxter et al. 1993; Meier-Trummer et al. 2010). Together with caprine herpesvirus 2 (CpHV-2) and the malignant catarrhal fever virus of white-tailed deer (WTD-MCFV) and based on their molecular biology, these viruses belong to the genus *Macavirus* within the subfamily *Gammaherpesvirinae* and are closely related to PLHV-1–PLHV-3 (Figure 35.11). In contrast to AlHV-1, there is no permissive cell culture system for OvHV-2.

Public health

OvHV-2 infection in humans has not been reported.

Epidemiology

Knowledge about porcine MCF caused by OvHV-2 is sparse, although a few cases with proven etiology were reported from European countries and North America (Albini et al. 2003a,b; Alcaraz et al. 2009). This may reflect the limited availability of molecular diagnostic assays and the possibility that a considerable number of cases may not be recognized due to abortive forms with full clinical recovery. However, it cannot be excluded that the low frequency of MCF cases in pigs is due to the low susceptibility of pigs.

In general, gammaherpesviruses have a narrow host range (Ackermann 2005, 2006; Meier-Trummer et al. 2010). However, OvHV-2 affects a broader range of natural hosts such as sheep, goats, cattle, bison, swine, mule deer, and, at least experimentally, rabbits and hamsters (Ackermann 2006; Albini et al. 2003b; Jacobsen et al. 2007; Li et al. 2003; Løken et al. 1998; O'Toole et al. 2007). Sheep and goats remain healthy upon infection, whereas the other susceptible hosts develop MCF (Meier-Trummer et al. 2010).

The exact mode of transmission of OvHV-2 is uncertain, but there is convincing evidence that the predominant mode is via nasal secretions (Li et al. 2004; Løken et al. 2009) by contact or aerosol, mainly from lambs under 1 year old (Russell et al. 2009). However, descriptions of outbreaks of SA-MCF in cattle on farms with no contact with sheep have also been documented (Kersting 1985). Pigs and sheep usually do not share close environments, which might also explain the infrequent presentation of MCF in pigs (Alcaraz et al. 2009). However, a case of MCF in a Vietnamese potbellied pig in a zoological garden, kept in proximity with sheep and goats, has been described recently (Lapp et al. 2015).

MCF-susceptible species are thought to be dead-end hosts that do not transmit virus, thus limiting the spread of disease during outbreaks (Russell et al. 2009). OvHV-2 transmission by the respiratory route of sheep had strongly been postulated on the basis of the detection of infectivity in nasal secretions (Taus et al. 2005).

Pathogenesis

OvHV-2 DNA can be detected in tissues of pigs with MCF, but not in healthy pigs. Simultaneous infections with OvHV-2 and other porcine gammaherpesviruses have never been observed (Albini et al. 2003a,b).

MCF in ruminants has an autoimmune-like pathology, mainly caused by the cytotoxic action of noninfected lymphocytes under the regulatory influence of a small number of infected cells. Cerebral vasculitis and perivasculitis in MCF-affected cattle and bison contain $CD8^+$ OvHV-2-infected lymphocytes in large numbers, as shown by *in situ* PCR. Therefore, the pathogenesis of MCF may be due to the direct action of virus-infected, dysregulated cytotoxic T cells at sites of lesions (Russell et al. 2009). Whether this model also applies for pigs is not yet known.

Clinical signs

The clinical signs and lesions in pigs are similar to those described for MCF in cattle. Usually, pigs of any age over 3 months can be affected (Løken et al. 1998).

No reliable data are available concerning the incubation period in pigs. Cattle can show clinical signs as soon as 9 days after exposure to sheep, but some cases occur 70 days or longer after contact. In bison exposed to sheep, the incubation period is often a month or more.

Clinically, porcine MCF is associated with a high and persistent fever (40.5–42 °C; 105–107.6 °F), anorexia, depression, weight loss, recumbency, a high pulse rate, and expiration dyspnea with loud snoring stenotic sounds. Foul-smelling nasal discharge and partially dried crusts in and around the nostrils, obstructing the upper airways, cause strained respiration. Ocular discharge, nystagmus, bilateral corneal edema, and keratoconjunctivitis often develop. Signs of CNS disease may appear, mostly as ataxia (central vestibular), hyperesthesia, tremors, and balance loss with convulsions or paralysis of hind limbs (Alcaraz et al. 2009; Lapp et al. 2015). In the cases reported from Norway, diarrhea has been occasionally seen, but all four Swiss cases had diarrhea (Løken et al. 1998). Multiple to coalescent small, slightly elevated red foci are seen in the skin. Pregnant sows may abort before death occurs (Albini et al. 2003b).

Hematological changes can include lymphopenia and a moderate neutrophilia. There can be biochemical abnormalities such as increases in urea, total bilirubin, and creatinine (Løken et al. 1998).

Incubation period and severity of clinical signs vary with the virus, the host, and other factors that are not completely understood. Evidence suggests that subclinical or clinical courses are possible, depending on whether the animals were stressed by handling. The clinical course of the disease is usually short, with death generally occurring after 2–4 days (Løken et al. 1998).

Lesions

Macroscopic lesions can be scant and not typical. Usually, pigs with MCF are in good body condition. They have cyanotic areas or petechiation in their skin. Fine crusts can cover the hyperemic skin. The lymph nodes are moderately enlarged, hyperemic, and moist on cut surfaces. The mucosa of the respiratory tract is characterized by hyperemia and covered by mucopurulent exudate. The lungs are congested and edematous; catarrhal or suppurative bronchitis and bronchopneumonia may develop. The spleen and liver can be engorged. No consistent gross lesions are reported for the alimentary tract. Kidneys are occasionally swollen and pale, and multiple cortical grayish-red foci up to 5 mm in diameter can be present. The CNS appears normal upon gross examination, although hyperemic meninges may be seen. Corneal opacity, conjunctivitis, and acral cyanosis are observed at necropsy (Albini et al. 2003b; Løken et al. 1998).

The most consistent histological finding, and the hallmark of MCF in pigs, is acute vasculitis in the CNS and other organs. This is characterized by the presence of numerous adventitial and transmural mononuclear cells and focal and segmental fibrinoid necrosis of the vessel walls in many of the tissues, including myocardium, spleen, leptomeninges and neuroparenchyma of the brain and spinal cord, skin, and kidneys. The lymphoproliferative vasculitis and associated degeneration and necrosis are most prominent in the media and adventitia of medium-sized and small arteries (panarteritis).

In the skin, perivascular and intravascular accumulations of mononuclear inflammatory cells occur in the dermis associated with subepithelial edema and focal epidermal necrosis. Lymph nodes show hyperplasia of lymphoid cells within the paracortex with scattered areas of necrosis. The alveolar septa of the lungs are thickened with increased numbers of lymphocytes and plasma cells. Perilobular and periportal accumulations of lymphoid cells are found in the liver. Multifocal lymphoplasmacytic interstitial nephritis, with necrotizing arteritis and periarteritis involving blastic lymphoid infiltrates, occurs in the kidney (Figure 35.13).

Cerebrum, cerebellum, and meninges show mild to moderate perivascular cuffs and transmural infiltrations of mononuclear cells, including some histiocytes and

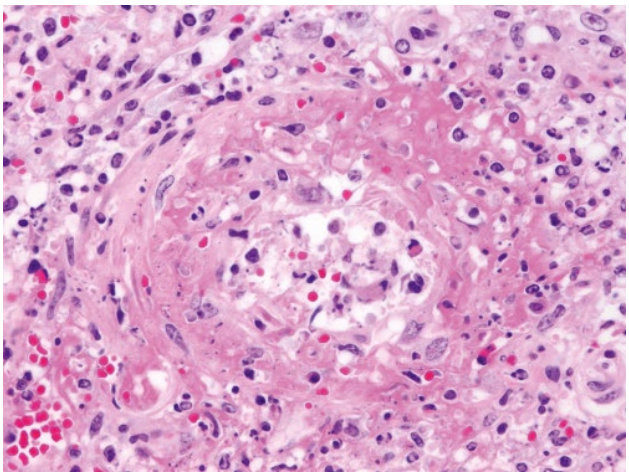


Figure 35.13 Mesentery; Vietnamese potbellied pig; medium-sized artery with lymphoplasmacytic vasculitis and transmural fibrinoid necrosis (hematoxylin and eosin [H&E]; *Source:* Courtesy Dr. Peter Wohlsein, University of Veterinary Medicine Hannover).

very few neutrophils with minimal karyorrhectic debris and plasma exudation into Virchow–Robin spaces. Ocular lesions consist of corneal edema and lymphoplasmacytic conjunctivitis. The uvea and retina are heavily infiltrated by lymphocytes, especially prominent around the blood vessels and in vessel walls (Løken et al. 1998). Lymphocytic optic neuritis can also be present. Similar moderate blastic lymphoid infiltrates might be present in the zona glomerulosa of the adrenal cortex and the intestinal submucosa (Alcaraz et al. 2009). Rarely, intranuclear inclusion bodies can be found.

Diagnosis

Differential diagnoses include Aujeszky's disease in particular, but also classical swine fever, African swine fever, porcine teschovirus infection, PCV2 infection, and rabies. Since lesions found at necropsy are not sufficiently specific for an etiological diagnosis, histopathology including molecular tests is necessary to confirm MCF. As in cattle, panarteritis is the most significant histological lesion. Consistent changes are seen in the brain. It is beneficial to microscopically investigate the epidural carotid rete mirabile around the pituitary gland for characteristic vasculitis, as in cattle. A combination of arteritis, lymphoid hyperplasia, and multifocal intraepithelial lymphocytic infiltrates is very characteristic for MCF and is essentially consistent with the classical head-and-eye form of MCF seen in ruminants (Løken et al. 1998).

Diagnosis of MCF depends on a combination of clinical signs, histopathology, and detection of virus-specific antibodies in blood or DNA in peripheral blood leukocytes or lymphoid tissue samples (Baxter et al. 1993). The OIE recognizes histopathology as the definitive diagnostic test for cattle, but laboratories have adopted other approaches, such as PCR assays that detect OvHV-2 DNA sequences. Using a mAb (15A) specific for a conserved antigen, a competitive inhibition ELISA test has been developed and refined (Li et al. 1994, 2001). A direct ELISA has been developed recently that offers a simple and inexpensive alternative. Conventional and real-time PCR assays allow sensitive confirmation of the presence of OvHV-2 in infected pigs and may also be useful for phylogenetic and epidemiological studies in natural and MCF-susceptible hosts (Albini et al. 2003a; Baxter et al. 1993; Hüssy et al. 2001; Russell et al. 2009).

Immunity

Prophylactic immunization is not available.

Prevention and control

Despite the low rate of porcine MCF in Europe and North America, it is important to discourage contact between pigs and sheep to minimize any infections.

Acknowledgment

We thank Viola Damrau for help in the preparation of this chapter.

References

Ackermann M. 2005. *Schweiz Arch Tierheilkd* 147:155–164.
Ackermann M. 2006. *Vet Microbiol* 113:211–222.
Albini S, Zimmermann W, Neff F, et al. 2003a. *Schweiz Arch Tierheilkd* 145:61–68.
Albini S, Zimmermann W, Neff F, et al. 2003b. *J Clin Microbiol* 41:900–904.
Alcaraz A, Warren A, Jackson C, et al. 2009. *J Vet Diagn Invest* 21:250–253.
Allan GM, McNulty MS, McCracken RM, et al. 1984. *Res Vet Sci* 36:235–239.
Ashworth LA, Baskerville A, Lloyd G. 1980. *Arch Virol* 63:227–237.
Aujeszky A. 1902. Über eine neue Infektionskrankheit bei Haustieren. *Zentralbl Bakteriol Orig* 32:353–357.
Banks M, Torraca LS, Greenwood AG, et al. 1999. *Vet Rec* 145:362–365.
Bartha A. 1961. Experimental reduction of virulence of Aujeszky's disease virus. *Magy Allatorvosok Lapja* 16:42–45.
Bartha A, Belak S, Benyeda J. 1969. *Acta Vet Acad Sci Hung* 19:97–99.

Baskerville A. 1971. *Res Vet Sci* 12:590–592.
Baskerville A, McFerran JB, Dow C. 1973. *Vet Bull* 43:465–480.
Baxter SI, Pow I, Bridgen A, et al. 1993. *Arch Virol* 132:145–159.
Becker CH. 1964. Zur Bedeutung der Lunge für die pathologisch anatomische Diagnose der Aujeszkyschen Krankheit des Schweines. *Monatsh Veterinarmed* 19:5–11.
Benndorf E, Hantschel H. 1963. Zum Verhalten des Aujeszkyvirus bei veschiedenen Wasserstoffkonzentrationen. *Arch Exp Vet Med* 17:1357–1362.
Beran GW. 1991. Transmission of Aujeszky's disease virus. In Proceedings of the 1st International Symposium on the Eradication of Pseudorabies (Aujeszky's) Virus. St. Paul, MN, USA, pp. 93–111.
Bitsch V, Ekildsen M. 1982. Complement-dependent neutralization of Aujeszky's disease virus by antibody. In Wittmann G, Hall SA, eds. *Current Topics in Veterinary Medicine and Animal Science*, Vol. 17. Boston, MA and The Hague: Martinus Nijhoff, pp. 41–49.
Bitsch V, Munch B. 1971. *Acta Vet Scand* 12:274–284.
Blaha T. 1989. Aujeszky's disease (pseudorabies). In Blaha T, ed. *Applied Veterinary Epidemiology*. Amsterdam: Elsevier, pp. 83–87.
Bolin CA, Bolin SR, Kluge JP, et al. 1985. *Am J Vet Res* 46:1039–1042.
Booth JC, Goodwin REW, Whittlestone P. 1967. *Res Vet Sci* 8:338–345.
Botner A. 1991. *Vet Microbiol* 29:225–235.
Bratberg B. 1980. Acute vasculitis in pigs: A porcine counterpart to malignant catarrhal fever. In Proceedings, of International Pig Veterinary Society Congres, p. 353.
Brema S, Lindner I, Goltz M, et al. 2008. *Xenotransplantation* 15:357–364.
Brockmeier SI, Lager KM, Mengeling WL. 1997. *Res Vet Sci* 62:281–285.
Brown TT, Shin KO, Fuller FJ. 1995. *Am J Vet Res* 56:587–594.
Card JP, Whealy ME, Robbins AK, et al. 1992. *J Virol* 66:3032–3041.
Cheung AK. 1989. *J Virol* 63:2908–2913.
Cheung AK. 1995. *Am J Vet Res* 56:45–50.
Chmielewicz B, Goltz M, Franz T, et al. 2003a. *Virology* 308:317–329.
Chmielewicz B, Goltz M, Lahrmann KH, et al. 2003b. *Xenotransplantation* 10:349–356.
Christensen LS. 1995. *APMIS Suppl* 48:1–48.
Christensen LS, Mousing J, Mortensen S, et al. 1990. *Vet Rec* 127:471–474.
Clark DA, Fryer JF, Tucker AW, et al. 2003. *Xenotransplantation* 10:142–148.
Collett MG, Roberts DC. 2002. *J S Afr Vet Assoc* 73:44–46.
Corn JL, Lee RM, Erickson GA, et al. 1987. *J Wildl Dis* 23:552–557.
Curanovic D, Enquist LW. 2009. *Future Virol* 4:591–603.
Davison AJ. 2010. *Vet Microbiol* 143:52–69.
Davison AJ, Eberle R, Ehlers B, et al. 2009. *Arch Virol* 154:171–177.
Deim Z, Glávits R, Biksi I, et al. 2006. *Vet Rec* 158:832–834.
Donaldson AI. 1983. *Vet Rec* 113:490–494.
Done JT. 1955. *Vet Rec* 67:525–527.
Dong B, Zarlenga DS, Ren X. 2014. *J Immunol Res* 2014:824630.
Dor FJ, Cheng J, Alt A, et al. 2004. *Xenotransplantation* 11:101–106.
Duncan JR, Ramsey EK, Switzer WP. 1965. *Am J Vet Res* 29:939–946.
Edington N, Plowright W, Watt RG. 1976a. *J Comp Pathol* 86:191–202.
Edington N, Smith IM, Plowright W, et al. 1976b. *Vet Res* 98:42–45.
Edington N, Watt RG, Plowright W. 1976c. *J Hyg (Lond)* 77:283–290.
Edington N, Watt RG, Plowright W, et al. 1977. *J Hyg (Lond)* 78:243–251.
Edington N, Wrathal AE, Done JT. 1988. *Vet Microbiol* 17:117–128.
Ehlers B, Lowden S. 2004. *J Gen Virol* 85:857–862.
Ehlers B, Ulrich S, Goltz M. 1999a. *J Gen Virol* 80:971–978.
Ehlers B, Borchers K, Grund C, et al. 1999b. *Virus Genes* 18:211–220.
Ekser B, Rigotti P, Gridelli B, et al. 2009. *Transpl Immunol* 21:87–92.
Enquist IW. 1994. *Semin Virol* 5:221–231.
Enquist LW, Husak IJ, Banfield BW, et al. 1998. *Adv Virus Res* 51:237–347.
Ezura K, Usami Y, Tajima K, et al. 1995. *J Vet Diagn Invest* 7:451–455.
Favoreel H, Nauwynck HJ, van Oostfeldt P, et al. 1997. *J Virol* 71:8254–8261.
Ficinska J, van Minnebruggen G, Nauwynck HJ, et al. 2005. *J Virol* 79:7248–7254.
Fonseca AA Jr., Camargos MF, de Oliveira AM, et al. 2010. *Vet Microbiol* 141:238–245.
Freuling CM, Müller T, Mettenleiter TC. 2017. *Vet Microbiol* 206:3–9.
Fryer JF, Griffiths PD, Fishman JA, et al. 2001. *J Clin Microbiol* 39:1155–1556.
Garkavenko O, Muzina M, Muzina Z, et al. 2004. *J Med Virol* 72:338–344.
Garkavenko O, Dieckhoff B, Wynyard S, et al. 2008. *J Med Virol* 80:2046–2052.
Gauger PC, Patterson AR, Kim WI, et al. 2010. *Swine Health Prod* 18:244–248.
Gerdts V, Jons A, Makoschey B, et al. 1997. *J Gen Virol* 78:2139–2146.
Glass CM, McLean RG, Katz JB, et al. 1994. *J Wildl Dis* 30:180–184.
Golais F, Sabo A. 1975. *Acta Virol* 19:387–392.
Goltz M, Ericsson T, Patience C, et al. 2002. *Virology* 294:383–393.
Gortazar C, Vincente J, Fierro Y, et al. 2002. *Ann N Y Acad Sci* 969:210–212.

Götze R, Liess J. 1930. Untersuchungen über das Bösartige Katarrhalfieber des Rindes. Schafe als Überträger. *Dtsch Tierarztl Wochenschr* 38:194–200.
Grytting I. 1974. Ondartet katarrfeber hos gris [Malignant catarrhal fever in pigs]. *Nor Vet Tidsskr* 86:489–490.
Gutekunst DE. 1979. *Am J Vet Res* 40:1568–1572.
Hahn EC, Fadl-Alla B, Lichtensteiger CA. 2010. *Vet Microbiol* 143:45–51.
Hall LB, Kluge JP, Evans LE, et al. 1984. *Can J Comp Med* 48:192–197.
Hamel AL, Lin L, Sachvie C, et al. 1999. *J Clin Microbiol* 37:3767–3768.
Hampl H, Ben-Porat T, Ehrlicher L, et al. 1984. *J Virol* 52:583–590.
Hansen MS, Pors SE, Jensen HE, et al. 2010. *J Comp Pathol* 143:120–131.
Herrmann S, Heppner B, Ludwig H. 1984. Pseudorabies viruses from clinical outbreaks and latent infections grouped into four major genome types. In Wittmann G, RM Gaskell, eds. *Current Topics in Veterinary Medicine and Animal Science*, Vol. 27. Boston, MA and The Hague: Martinus Nijhoff, pp. 387–401.
Holmgren N, Bjorklund NE, Persson B. 1983. Acute vasculitis among swine in Sweden. *Nord Vet Med* 35:103–106.
Huang CA, Fuchimoto Y, Gleit ZL, et al. 2001. *Blood* 97:1467–1473.
Huemer HP, Larcher C, Coe NE. 1992. *Virus Res* 23:271–280.
Hüssy D, Müller-Doblies U, Stäuber N, et al. 2000. Diagnosis and pathogenesis of the putative ovine herpesvirus 2, agent of malignant catarrhal fever. In Brocchi E, Lavaza A, eds. 5th International Congress of Veterinary Virology, Brescia, Italy, pp. 105–106.
Hüssy D, Stäuber N, Leutenegger CM, et al. 2001. *Clin Diagn Lab Immunol* 8:123–128.
Iglesias G, Trujano M. 1989. *Zentralbl Veterinarmed B* 36:57–62.
Jacobsen B, Thies K, von Altrock A, et al. 2007. *Vet Microbiol* 124:353–357.
Jakubik J. 1977. *Zentralbl Veterinarmed B* 24:765–766.
Jentzsch KD, Apostoloff EA. 1970. *Z Gesamte Hyg* 16:692–696.
Jestin A, Foulon T, Pertuiset B, et al. 1990. *Vet Microbiol* 23:317–328.
Jin L, Schnitzlein W, Scherba G. 2000. *J Virol* 74:6333–6338.
Karger A, Mettenleiter TC. 1993. *Virology* 194:654–664.
Kersting KW. 1985. *Compend Contin Educ Pract Vet* 7:663–668.
Kirkpatrick CM, Kanitz CL, McCrocklin SM. 1980. *J Wildl Dis* 16:601–614.
Kit S, Kit M. 1991. *Prog Med Virol* 38:128–166.
Kluge JP, Maré CJ. 1976. Gross and microscopic lesions of prenatal pseudorabies (Aujeszky's disease) in swine. *Proc Congr Int Pig Vet Soc* 4:G3.
Kluge JP, Maré CJ. 1978. Natural and experimental in utero infection of piglets with Aujeszky's disease (pseudorabies) virus. *Am Assoc Vet Lab Diagn* 21:15–24.
Kluge JP, Beran GW, Hill HT, et al. 1999. Pseudorabies (Aujeszky 's disease). In Straw BE, D'Allaire S, WL Mengeling, et al., eds. *Disease of Swine*, 8th ed. Ames, IA: Iowa State University Press, pp. 233–246.
Klupp BG, Hengartner CJ, Mettenleiter TC, et al. 2004. *J Virol* 78:424–440.
Koppers-Lalic D, Reits E, Ressing M, et al. 2005. *Proc Natl Acad Sci USA* 102:5144–5149.
Kretzschmar C. 1970. *Die Aujeszkysche Krankheit: Diagnostik, Epizootiologie und Bekämpfung*. Jena: Gustav Fischer Verlag, pp. 131–135.
Kritas SK, Pensaert MB, Nauwynck HJ, et al. 1999. *Vet Microbiol* 69:143–156.
Kurtze H. 1950. Übertragung des «Bösartigen Katarrhalfiebers des Rindes» auf ein Schwein. *Dtsch Tierarztl Wochenschr* 57:261.
Lapp S, Forster C, Kummrow M, et al. 2015. *Tierarztl Prax Ausg G Grosstiere Nutztiere* 43:165–168.
L'Ecuyer C, Corner AH, Randall GC. 1972. Porcine cytomegalic inclusion disease: Transplacental transmission. *Proc Congr Int Pig Vet Soc* 2:99.
Li H, Shen DT, Knowles DP, et al. 1994. *J Clin Microbiol* 32:1674–1679.
Li H, McGuire TC, Müller-Doblies UU, et al. 2001. *J Vet Diagn Invest* 13:361–364.
Li H, Wunschmann A, Keller J, et al. 2003. *J Vet Diagn Invest* 15:46–49.
Li H, Taus NS, Lewis GS, et al. 2004. *J Clin Microbiol* 42:5558–5564.
Lindner I, Ehlers B, Noack S, et al. 2007. *Virology* 357:134–148.
Lipowski A, Mokrzycka A, Pejsa, Z, 2002. *Med Weter* 58:35–39.
Liu X, Xu Z, Zhu L, et al. 2014. *PLos One* 9:e113921.
Løken T, Aleksandersen M, Reid H, et al. 1998. *Vet Rec* 143:464–467.
Løken T, Bosman AM, van Vuuren M. 2009. *J Vet Diagn Invest* 21:257–261.
Lomniczi B, Kaplan AS. 1987. *Virology* 161:181–189.
Lomniczi B, Watanabe S, Ben-Porat T, et al. 1984. *J Virol* 52:198–205.
Lukacs N, Thiel HJ, Mettenleiter TC, et al. 1985. *J Virol* 56:166–173.
Ma WJ, Lager KM, Richt JA, et al. 2008. *J Vet Diagn Invest* 20:440–447.
MacDiarmid SC. 2000. *Aust Vet J* 78:470–471.
Maes RK, Kanitz CL, Gustafson DP. 1983. *Am J Vet Res* 44:2083–2086.
Mahjoub N, Dhorne-Pollet S, Fuchs W, et al. 2015 *J Virol* 89:428–442.
Mathijs E, Vandenbussche F, Verpoest S et al. 2016 *Genome Announc* 4: pii e00440-16.
McMahon KJ, Minihan D, Campion EM, et al. 2006. *Vet Microbiol* 116:60–68.
Meier-Trummer CS, Ryf B, Ackermann M. 2010. *Vet Microbiol* 141:199–207.
Mengeling WL. 1991. *J Vet Diagn Invest* 3:133–136.

Mengeling WL, Lager KM, Volz DM, et al. 1992. *Am J Vet Res* 53:2164–2173.
Mengeling WL, Brockmeier SL, Lager KM, et al. 1997. *Vet Microbiol* 55:49–60.
Mettenleiter TC. 1996. *Vet Immunol Immunopathol* 54:221–229.
Mettenleiter TC. 2000. *Vet Res* 31:99–115.
Mettenleiter, TC. 2004. Budding events in herpesvirus morphogenesis. *Virus Res* 106: 167–180.
Mettenleiter TC, Lukács N, Rziha HJ. 1985. *J Virol* 56:307–311.
Mettenleiter TC, Keil G, Fuchs W. 2008. Molecular biology of animal herpesviruses. In Mettenleiter TC, Sobrino F, eds. *Molecular Biology of Animal Viruses*. Norfolk: Caister Academic Press, pp. 375–456.
Mettenleiter TC, Klupp BG, Granzow H. 2009. *Virus Res* 143:222–234.
Milicevic V, Radojicic S, Valcic M, et al. 2016. *BMC Vet Res* 12:134.
Miller LC, Bayles DO, Zanella EL, et al. 2016. *Bioinform Biol Insights* 9(Suppl. 2):25–36.
Morandi F, Ostanello F, Fusaro L, et al. 2010. *J Comp Pathol* 142:74–78.
Moreno A, Sozzi E, Grilli G, et al. 2015 *Vet Microbiol* 177:359–365.
Morozov VA, Ludwig S, Ludwig B et al. 2016a. *Xenotransplantation* 23:320–327.
Morozov VA, Abicht JM, Reichart B, et al. 2016b. *Ann Virol Res* 2:1018.
Morozov VA, Morozov AV, Denner J. 2016c. *Arch Virol* 161:1159–1168.
Morozov VA, Plotzki, E, Rotem A, et al. 2016d. *Xenotransplantation* 23:490–496.
Morselli R. 1901. La febbre catarrhale maligna dei bovini è contagiosa? *G R Soc Vet* 1901:813–815.
Mueller NJ, Fishman JA. 2004. *Xenotransplantation* 11:486–490.
Mueller NJ, Barth RN, Yamamoto S, et al. 2002. *J Virol* 76:4734–4740.
Mueller NJ, Kuwaki K, Knosalla C, et al. 2005. *Xenotransplantation* 12:59–62.
Müller T, Conraths FJ, Hahn EC. 2000. *Infect Dis Rev* 2:27–34.
Müller T, Teuffert J, Zellmer R, et al. 2001. *Am J Vet Res* 62:252–258.
Müller T, Bätza HJ, Schlüter H, et al. 2003. *J Vet Med B Infect Dis Vet Public Health* 50:207–213.
Müller T, Teuffert J, Staubach C, et al. 2005. *J Vet Med B Infect Dis Vet Public Health* 52:432–436.
Müller T, Klupp BG, Freuling C, et al. 2010. *Epidemiol Infect* 12:1–11.
Müller T, Hahn EC, Tottewitz F, et al. 2011. *Arch Virol* 156:1691–1705.
Müller-Doblies U. 1998. Untersuchungen zur Diagnostik, Epidemiologie und Ätiologie des Bösartigen Katarrhalfiebers beim Rind in der Schweiz. Inaugural dissertation, University of Zurich.
Narita M, Haritani M, Moriwaki M. 1984a. *Nippon Juigaku Zasshi* 46:119–122.
Narita M, Kuto M, Fukush OA, et al. 1984b. *Vet Pathol* 21:450–452.
Narita M, Shimizu M, Kawanuru H, et al. 1985. *Am J Vet Res* 46:1506–1510.
Nauwynck H. 1997. *Vet Microbiol* 55:3–11.
O'Toole D, Taus NS, Montgomery DL, et al. 2007. *Vet Pathol* 44:655–662.
Ober BT, Teufel B, Wiesmüller KH, et al. 2000. *J Virol* 74:1752–1760.
Office International des Épizooties (OIE). 2009. Chapter 8.2: Aujeszky's disease. In Terrestrial Animal Health Code, Vol. 2, 18th ed., online. Paris: OIE.
Okkenhaug H, Kjelvik O. 1995. Malignant catarrhal fever in pigs: Diagnosis, clinical findings and occurrence, and reports of two outbreaks (Ondartet katarrfeber hos gris). *Nor Vet Tidsskr* 107:199–203.
Onno M, Jestin A, Wicolas JC. 1988. Rapid diagnosis of Aujeszky's disease in fattened pigs by direct immunoperoxidase labelling in nasal cells. *Vet Med Rev* 59:152–156.
Onyekaba C, Bueon L, King P, et al. 1987. *Comp Immunol Microbiol Infect Dis* 10:163–166.
Orr JP, Althouse E, Dulac GC, et al. 1988. *Can Vet J* 29:45–50.
Pejsak ZK, Truszczynski T. 2006. Aujeszky's disease (pseudorabies). In Straw BE, Zimmerman J, D'Allaire S, et al., eds. *Diseases of Swine*, 9th ed. Ames, IA: Blackwell Publishing Company, pp. 419–433.
Pensaert MB, Kluge JP. 1989. Pseudorabies virus (Aujeszky's disease). In Pensaert MB, ed. *Virus Infections of Porcines*. New York: Elsevier, pp. 39–64.
Platt KB, Mare CJ, Hinz PN. 1980. *Arch Virol* 63:107–114.
Plotzki E, Wolf-van Buerck L, Knauf Y et al. 2015. *Virus Res* 204:95–102.
Plotzki E, Keller M, Ehhers B, et al. 2016. *J Virol Methods* 233:72–77.
Plowright W. 1990. Malignant catarrhal fever virus. In Dinter Z, Morein B, eds. *Virus Infections of Ruminants*. New York: Elsevier, pp. 123–150.
Plowright W, Ferris RD, Scott GR. 1960. *Nature* 188:1167–1169.
Plowright W, Edington N, Watt RG. 1976. *J Hyg (Lond)* 75:125–135.
Pohlenz J, Bertschinger HU, Koch W. 1974. A malignant catarrhal fever-like syndrome in sows. In Proceedings, of International Pig Veterinary Society Congress, pp. V15–1–V15–3.
Pomeranz L, Reynolds AE, Hengartner CJ. 2006. *Microbiol Mol Biol Rev* 69:462–500.
Priola S, Gustafson DP, Wagner EK, et al. 1990. *J Virol* 64:4755–4760.
Quint W, Gielkens A, Van Oirschot J, et al. 1987. *J Gen Virol* 68:523–534.
Reiner G, Melchinger E, Kramarova M, et al. 2002. *J Gen Virol* 83:167–172.

Rodák L, Smid B, Valicek L, et al. 1987. *Vet Microbiol* 13:121–133.
Rodgers SJ, Karges SL, Saliki JT. 1996. *J Vet Diagn Invest* 8:168–171.
Romero CH, Meade PN, Shultz JE, et al. 2001. *J Wildl Dis* 37:289–296.
Romero CH, Meade PN, Homer BL, et al. 2003. *J Wildl Dis* 39:567–575.
Rupasinghe V, Iwatsuki-Horimoto K, Sugii S, et al. 2001. *J Vet Med Sci* 63:609–618.
Russell GC, Stewart JP, Haig DM. 2009. *Vet J* 179:324–335.
Rziha HJ, Mettenleiter TC, Ohlinger V, et al. 1986. *Virology* 155:600–613.
Santoni F, Lindner I, Caselli E, et al. 2006. *Xenotransplantation* 13:308–317.
Schang LM, Osorio FA. 1993. *Rev Sci Tech* 12:505–521.
Schang LM, Kutish GF, Osorio FA. 1994. *J Virol* 68:8470–8476.
Schmidt J, Gerdts V, Beyer J, et al. 2001. *J Virol* 75:10054–10064.
Schoenbaum MA, Zimmermann JJ, Beran GW, et al. 1990. *Am J Vet Res* 51:331–333.
Schoenbaum MA, Freund JD, Beran GW. 1991. *J Am Vet Med Assoc* 198:1393–1397.
Schulze C, Hlinak A, Wohlsein P, et al. 2010. *Berl Munch Tierarztl Wochenschr* 123:359–364.
Sekiguchi M, Shibahara T, Miyazaki A, et al. 2012. *J Virol Methods* 179:272–275.
Sozzi E, Moreno A, Lelli D, et al. 2014 *Transbound Emerg Dis* 61:334–340.
Steinrigl A, Revilla-Fernandez S, Koldziejek J, et al. 2012 *Vet Microbiol* 157:276–284.
Sun Y, Luo Y, Wang CH, et al. 2016. *Vet Microbiol* 183:119–124.
Syrjälä P, Saarinen H, Laine T, et al. 2006. *Vet Rec* 159:406–409.
Szpara JL, Tafur YR, Parsons L, et al. 2011 *PLoS Pathog* 7:10.
Tahir RA, Goyal SM. 1995. *J Vet Diagn Invest* 7:173–176.
Tajima T, Kawamura H. 1998. *J Vet Med Sci* 60:107–109.
Tajima T, Hironao T, Kajikawa T, et al. 1994. *J Vet Med Sci* 56:189–190.
Taus NS, Traul DL, Oaks JL, et al. 2005. *J Gen Virol* 86:575–579.
Tenhagen BA, Bollwahn W, Seidler MJ. 1995. *Dtsch Tierarztl Wochenschr* 102:86–90.
Tielen MJ, van Exsel AC, Brus DH, et al. 1981. *Tijdschr Diergeneeskd* 106:739–747.
Todd D, Hull J, McNair J. 1987. *Arch Virol* 96:215–224.
Toma B. 1982. Serological diagnosis of Aujeszky's disease using enzyme-linked immunosorbent assay (ELISA). In Wittmann G, Hall SA, eds. *Current Topics in Veterinary Medicine and Animal Science*, Vol. 17. The Hague: Martinus Nijhoff, pp. 65–74.
Tombácz D, Tóth JS, Petrovszki P, et al. 2009. *BMC Genomics* 10:491.
Tucker AW, Galbraith D, McEwan P, et al. 1999. *Transplant Proc* 31:915.
Tucker AW, McNeilly F, Meehan B, et al. 2003. *Xenotransplantation* 10:343–348.
Ulrich S, Goltz M, Ehlers B. 1999. *J Gen Virol* 80:3199–3205.
Valicek L, Smid B. 1979. *Zentralbl Veterinarmed B* 26:371–381.
van de Walle G, Favoreel HW, Nauwynck HJ, et al. 2003. *J Gen Virol* 84:939–948.
van Oirschot JT. 1999. *J Biotechnol* 73:195–205.
van Oirschot JT, Rziha HJ, Moonen PJ, et al. 1986. *J Gen Virol* 67:1179–1182.
van Rijn PA, Wellenberg GJ, Hakze-van der Honing R, et al. 2004. *J Virol Methods* 120:151–160.
Vannier P. 1988. The control programme of Aujeszky's disease in France: Main results and difficulties. In van Oirschot JT, ed. *Vaccination and Control of Aujeszky's Disease*. Boston, MA: Kluwer Academic Publishers, pp. 215–226.
Watson WA. 1986. *Rev Sci Tech* 5:363–378.
Watt RG. 1978. *Res Vet Sci* 24:147–153.
Watt RG, Plowright W, Sabo A, et al. 1973. *Res Vet Sci* 14:119–121.
Weigel RM, Lehman JR, Herr L, et al. 1995. *Am J Vet Res* 56:1155–1162.
Wernike K, Beer M, Freuling CM, et al. 2014 *J Virol Methods* 209:110–115.
Whitteker JL, Dudani AK, Tackaberry ES. 2008. *Transplantation* 86:155–162.
Widen F, Goltz M, Wittenbrink N, et al. 2001. *Virus Genes* 23:339–346.
Widen BF, Lowings JP, Belak S, et al. 1999. *Epidemiol Infect* 123:177–180.
Wittmann G. 1991. *Comp Immunol Microbiol Infect Dis* 14:165–173.
Wittmann G, Rziha HJ. 1989. Aujeszky's disease (pseudorabies) in pigs. In Wittmann G, ed. *Herpesvirus Diseases of Cattle, Horses and Pigs*. Boston, MA: Kluwer Academic Publishers, pp. 230–325.
Wynyard S, Nathu D, Garkavenko O et al. 2014. *Xenotransplantation* 21:309–323.
Yang YG, Sykes M. 2007. *Nat Rev Immunol* 7:519–531.
Yoon KJ, Edington N. 2006. Porcine cytomegalovirus. In Straw BE, Zimmerman J, D'Allaire S, et al., eds. *Diseases of Swine*, 9th ed. Ames, IA: Blackwell Publishing Company, pp. 323–329.
Yoon KJ, Henry SC, Zimmerman JJ, et al. 1996. *Vet Med* 91:779–784.
Yu T, Chen F, Ku X, et al. 2016. *Virus Genes* 52:474–483.
Yu X, Zhou Z, Hu D, et al. 2014. *Emerg Infect Dis* 20:102–104.
Zuckermann FA, Zsal L, Mettenleiter TC, et al. 1990. *J Virol* 64:802–812.

36

Influenza Viruses

Kristien Van Reeth and Amy L. Vincent

Relevance

The first reports of influenza-like disease in pigs occurred concurrently in the United States and Europe in conjunction with the 1918 human influenza pandemic. Contemporary genetic analyses confirmed that the early H1N1 swine viruses, progenitors of the "classical" H1N1 lineage of swine influenza viruses, and the human viruses of 1918 were closely related, with both derived from a wholly avian ancestral virus (Taubenberger and Palese 2006). One anecdotal report suggested that the ecobiological association between human and swine influenza began long before 1918 (Morens and Taubenberger 2014; Worobey et al. 2014).

Influenza viruses of genus (type) A, B, C, and D have been identified. Influenza A virus (IAV) is infectious for a wide range of host species, including birds, mammals, and bats. Influenza B virus mainly causes respiratory disease in humans, although it can also infect seals and pigs (Ran et al. 2015). Influenza C virus is infectious for humans and pigs (Guo et al. 1983). Influenza D virus (initially called influenza C-like virus) was first isolated from a diseased pig in 2011 (Hause et al. 2013), but this virus primarily affects cattle and is not known to infect or cause illness in humans. Among the four genera of influenza viruses, only IAVs are of routine clinical significance in swine. Therefore, the remainder of this chapter is focused on IAVs.

IAV is a major cause of acute respiratory disease outbreaks in pigs (Loeffen et al. 1999; Terebuh et al. 2010), but infections are frequently subclinical. The epidemiology of influenza A virus in swine (IAV-S) encompasses a complex interplay of viruses of human, avian, and swine evolutionary origins. Conversely, pigs are postulated to play important roles as intermediate hosts in the reassortment and/or adaptation events leading to development of influenza viruses of pandemic potential for people (Subbarao et al. 2006; Webster et al. 1992). On the other hand, it is now clear that human-to-swine transmission of influenza virus is far more frequent than swine-to-human and is central in globally seeding swine populations with new viral diversity (Nelson and Vincent 2015).

Genetic reassortment of human, avian, and/or swine IAVs is extremely common in pigs, and the resulting viruses have fundamentally altered the epidemiology of influenza in pigs in many parts of the world. Most recently, the 2009 pandemic H1N1 (H1N1pdm09) virus arose as the result of reassortment between North American and Eurasian lineages of IAV-S (Garten et al. 2009; Smith et al. 2009). H1N1pdm09 then infected swine populations worldwide via humans. Not only was this virus antigenically distinct from previously known viruses circulating in swine, but it has now reassorted extensively with almost all of the regionally established swine IAVs, leading to a further expansion of novel IAV-S genotypes and an increasingly complex epidemiological picture.

Etiology

Influenza viruses are members of the family *Orthomyxoviridae* that includes seven genera: *Influenzavirus A*, *Influenzavirus B*, *Influenzavirus C*, *Influenzavirus D*, *Isavirus*, *Quaranjavirus*, and *Thogotovirus*. Influenza viruses are polymorphic, enveloped viruses approximately 80–120 nm in diameter (Figure 36.1). The lipid envelope renders the virus highly susceptible to detergents and most commonly used antiviral disinfectants.

Influenza viruses possess 7–8 separate segments of negative-sense RNA (8 segments for influenza A and influenza B viruses; 7 segments for influenza C and influenza D viruses) (Hause et al. 2014; Shaw and Palese 2013). A schematic diagram of IAV particle is shown in Figure 36.1. Eight segments of IAV encode 10–12 viral proteins (the PB1, M, and NS segments can encode more

Diseases of Swine, Tenth Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson.

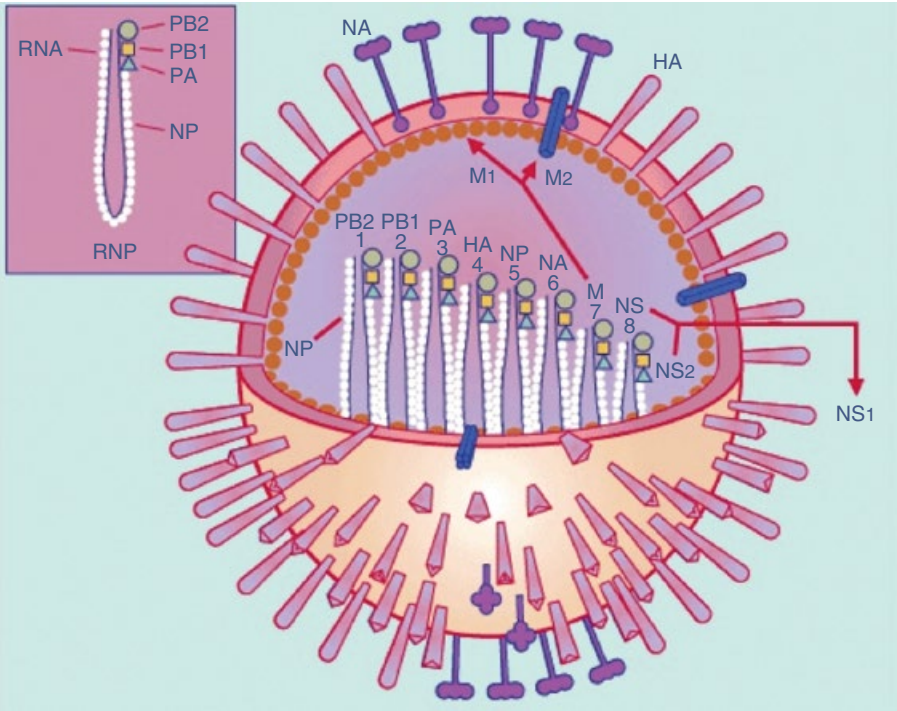


Figure 36.1 Structure of an influenza A virus. The hemagglutinin (HA), neuraminidase (NA), and part of the matrix2 (M2) protein are on the outer surface of the virion and protrude through the envelope. The viral RNA segments are coated with nucleoprotein (NP) and bound by a complex of three polymerases (PB2, PB1, PA). The eight segments are arranged according to their length, which puts the HA gene at place 4 and the NA gene at place 6. NS1 is the single nonstructural protein and is not part of the viral particle. *Source:* Wright and Webster (2001).

than one protein). The segmented nature of the influenza genome allows two viruses (within the same genera) that coinfect a single host to exchange RNA segments during viral replication, a process known as genetic "reassortment." Reassortment between members of different genera has not been reported (Shaw and Palese 2013).

IAVs are named using the following convention: A/species of origin/location of isolation/isolate number/year of isolation (subtype) (e.g. A/Swine/Wisconsin/125/97[H1N1]). If no species is designated, it is, by default, a human isolate. A detailed review of influenza virus structure and genetics can be found elsewhere (Shaw and Palese 2013).

IAV subtypes are defined by the hemagglutinin (HA or H) and neuraminidase (NA or N) spike-like glycoproteins that project from the surface of the viral envelope. There are 16 HAs and 9 NAs that are conventionally distinguished antigenically and genetically, but 2 additional HAs (H17–H18) and NAs (N10–N11) have recently been identified in bats (Tong et al. 2013; Wu et al. 2014). The combination of HA and NA in a virus defines its subtype (e.g. H1N1, H1N2, H3N2). The HA viral glycoprotein mediates attachment to sialic acid-containing receptors on the host cell and is the principal target for the induction of neutralizing antibodies. At the molecular level, sialic acids are attached to galactose residues by alpha2,3 or alpha2,6 linkages. In binding tests, human influenza viruses prefer to bind to 2,6-linked ("human-type") receptors, whereas avian viruses prefer 2,3-linked ("avian-type") receptors. Sialic acid binding is also responsible for the agglutination of red blood cells, and this property is used in diagnostic applications (hemagglutination (HA) and hemagglutination inhibition (HI) assays – see below). The NA viral glycoprotein enables virus release from the infected cell by breaking the bonds between sialic acids and adjacent sugar residues.

Depending on the strain of virus and species of bird, IAVs in waterfowl can infect and be shed from the respiratory and/or the enteric tract (Webster et al. 1978). Enteric infection leads to virus excretion in feces, which can play a role in avian influenza virus outbreaks (Rohani et al. 2009) and possibly in avian-to-swine transmission (Karasin et al. 2000b, 2004; Ma et al. 2007). Although interspecies IAV transmission occurs, there is a strong species barrier (Landolt and Olsen 2007; Neumann and Kawaoka 2006; Van Reeth 2013), particularly between birds and mammals. Thus, only a very restricted number of HA subtypes have become endemic in mammals (e.g. H1, H2, and H3 in humans; H1 and H3 in pigs; H3 and H8 in horses). In contrast, 16 HA and 9 NA subtypes have been found in IAVs in wild waterfowl, the global reservoir of influenza viruses (Hinshaw et al. 1980; Webby and Webster 2001; Webster et al. 1992).

Both subtype and genotype are important in understanding the epidemiology and evolution of IAVs. Genotyping is done by sequencing each viral RNA segment and then conducting a phylogenetic analysis to generate an 8-gene "constellation." These analyses define the evolutionary lineages (host species and geographic region) from which each viral gene originated. A phylogenetic lineage refers to a group of viruses that share a common genetic origin for a particular gene. It is possible for a virus to possess genes from distinct lineages, a reflection of the different origins of individual genes. Genotyping has been an important tool in recent years for understanding the origins and continued evolution of influenza in pigs.

Public health

Human infections with swine IAVs of various subtypes, lineages, and genotypes have been reported (Freidl et al. 2014; Myers et al. 2007; Shinde et al. 2009; Van Reeth 2007), particularly with H3N2, H1N2, and H1N1 viruses of the North American triple-reassortant lineages (Bowman et al. 2014; Choi et al. 2015; Jhung et al. 2013). The number of documented infections is small compared with the number of people in contact with pigs worldwide. However, limited studies of IAV-S antibodies in humans suggest that IAV-S infections may be widespread and undiagnosed (Gray et al. 2007; Myers et al. 2006, 2007; Olsen et al. 2002; Terebuh et al. 2010). Granted, the interpretation of such studies is compromised by the technical limitations of differentiating antibodies against swine versus human influenza viruses by serology (Sikkema et al. 2016). This is especially true at the current time with the circulation of the H1N1pdm09 virus in people and repeated human-to-swine transmission of this virus (Nelson et al. 2015c). In addition, most H1 and H3 swine IAVs are derived from viruses that once circulated in the human population (Nelson et al. 2015a,b). With the exception of the swine influenza outbreak at Fort Dix, New Jersey, in 1976 (Top and Russell 1977), evidence for person-to-person spread of swine-lineage viruses is scarce (Jhung et al. 2013; Myers et al. 2007), although the pandemic of 2009 differs in this regard.

Pandemic strains of IAV must possess at least three properties: able to infect humans, able to spread person to person, and able to evade immunity in the human population. Such viruses may develop through *in toto* transmission from an animal host (e.g. emergence of a wholly avian virus to create the 1918 pandemic strain [Taubenberger and Palese 2006]) or through genetic reassortment between a virus with an immunologically unique HA and another virus(es), ideally one already adapted to human infection. The "Asian flu" virus of the 1957 pandemic and the "Hong Kong" virus of the 1968 pandemic both arose via the latter mechanism, with reassortment between a novel avian virus (providing new HA, PB1 [and NA in 1957] genes) and the previously circulating seasonal human IAV (Kawaoka et al. 1989; Webby and Webster 2001; Webster et al. 1992). The H1N1pdm09 virus likely arose through a combination of these two scenarios from a unique "intercontinental" reassortment between swine IAVs of North American and Eurasian lineages (Figure 36.2) (Mena et al. 2016; Smith et al. 2009), followed by *in toto* transmission from pigs to humans, human-to-human transmission, and global spread by humans (Chowell et al. 2011; Dawood et al. 2009; Garten et al. 2009; Mena et al. 2016). Evidence that this reassortment event occurred in pigs and the reassorted precursor virus circulated in pigs in Mexico during the years prior to its reemergence as a human pandemic strain has been reported (Mena et al. 2016).

All eight influenza genomic segments contribute to host range (i.e. "multigenic" species specificity) (Landolt and Olsen 2007; Neumann and Kawaoka 2006; Van Reeth 2013), but the HA is particularly important because of its role in receptor binding and the presumed differences in viral receptors between different hosts (Ito 2000; Landolt and Olsen 2007; Matrosovich et al. 2000; Nicholls et al. 2008).

The human-type receptor is found throughout the upper and lower respiratory tract of humans, as well as pigs, whereas the avian-type receptor is almost exclusively found in the lungs of both species (Nelli et al. 2010; Nicholls et al. 2007; Shinya et al. 2006; Van Poucke et al. 2010). The scarcity of avian-type receptors in the nose and trachea of humans is believed to hamper transmission of avian viruses to and between humans; the same may also apply to pigs.

However, receptor recognition is complicated, and the implications for host range are not fully appreciated (Bateman et al. 2008, 2010; Byrd-Leotis et al. 2014; Gambaryan et al. 2005; Nicholls et al. 2008; Sorrell et al. 2011; van Riel et al. 2010). For instance, virus binding to a given sialic acid may not lead to productive infection of cells (Bateman et al. 2008, 2010), and additional receptors were recently discovered in pigs (Byrd-Leotis et al. 2014). In addition, both types of sialic acids are also expressed in humans and some land-based poultry species (Gambaryan et al. 2002; Landolt and Olsen 2007; Pillai et al. 2010; Wan and Perez 2006; Webby and Webster 2001), so they are not unique to pigs. Nonetheless, pigs remain the focus, in particular because of the genetic origins of the H1N1pdm09 virus.

Epidemiology

Susceptible species

In addition to domestic pigs and humans, IAV-S can infect wild boar (Saliki et al. 1998), domestic turkeys (Choi et al. 2004; Hinshaw et al. 1983; Ludwig et al. 1994;

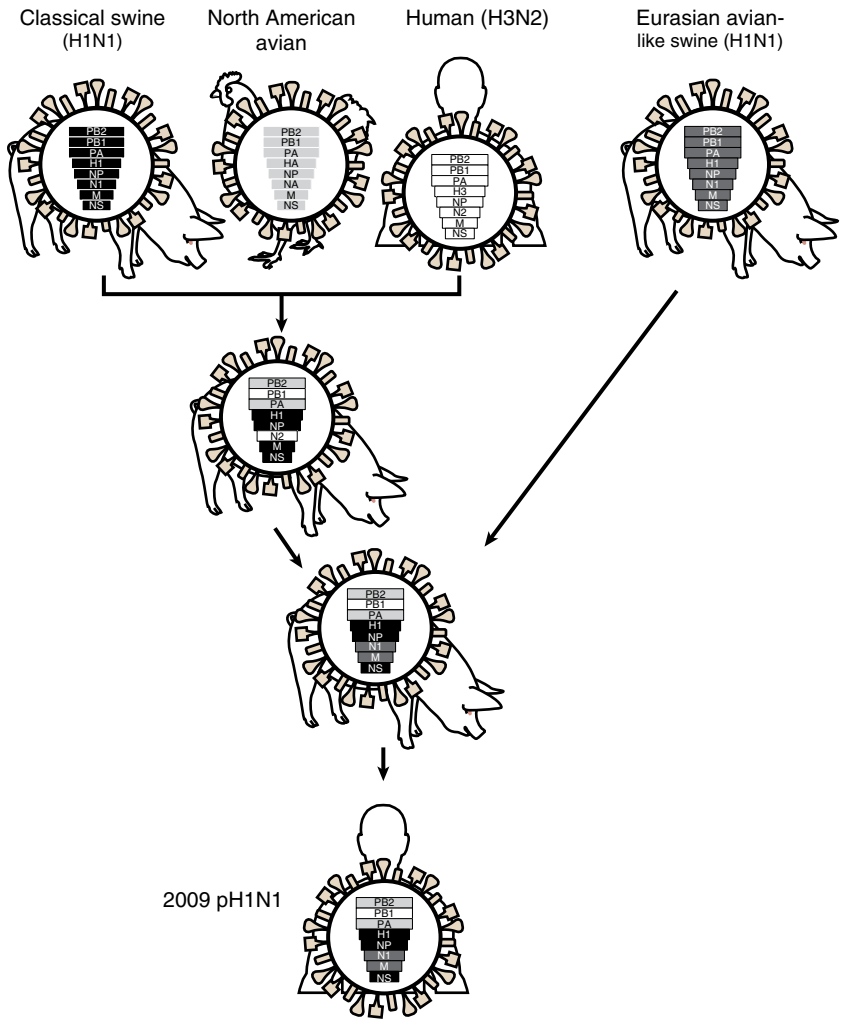


Figure 36.2 Supposed origin of the 2009 pH1N1 influenza virus. The genes encoding the NA and M proteins seem to be derived from Eurasian avian-like swine H1N1 IAVs. The remaining six genes are most closely related to those of trH1N2 swine IAVs with a mix of human, swine, and avian virus genes, which are widespread in swine in the United States. These two viruses likely reassorted in a pig or another animal species some 10 years ago. *Source:* Adapted from Neumann et al. (2009). Reproduced with permission of Springer Nature.

Olsen et al. 2006; Suarez et al. 2002; Tang et al. 2005; Wood et al. 1997; Wright et al. 1992), and, rarely, free-ranging waterfowl (Olsen et al. 2003).

Although naturally acquired infections of pigs with avian influenza viruses have been documented in many parts of the world, only the European avian-like swine H1N1 virus became swine adapted (Brown et al. 1997; Donatelli et al. 1991; Kyriakis et al. 2011; Scholtissek et al. 1983; Van Reeth et al. 2008). Other avian influenza virus subtypes have been sporadically detected in pigs in North America or Asia, including avian H1N1, H2N3, H3N2, H3N3, H4N6, H4N8, H5N1, H5N2, and especially H9N2 viruses (Brown 2000; Choi et al. 2005; Cong et al. 2008; Guan et al. 1996; Karasin et al. 2000b, 2004; Kida et al. 1988; Lee et al. 2009; Ma et al. 2007; Peiris et al. 2001; Shi et al. 2008; Su et al. 2012; Takano et al. 2009; Yu et al. 2008a). In some instances, these viruses spread between pigs on a limited scale, but they have not formed a stable lineage in swine (Choi et al. 2005; Jung et al. 2007; Nidom et al. 2010; Santhia et al. 2009). This is consistent with experimental studies showing that avian influenza viruses are restricted in their replication in pigs and transmission between pigs (Balzli et al. 2016; Choi et al. 2005; De Vleeschauwer et al. 2009a,b; Lee et al. 2009; Lipatov et al. 2008; Manzoor et al. 2009).

There is evidence that avian influenza viruses must mutate or reassort with swine-adapted viruses to replicate efficiently in pigs (Abente et al. 2016; Mancera Gracia et al. 2017; Qiao et al. 2012), but no natural reassortant viruses have been able to maintain themselves in swine (Bi et al. 2010; Cong et al. 2007, 2010; Lee et al. 2009; Shi et al. 2008).

Sequencing has shown that many swine lineages contain gene segment contributions from human seasonal viruses (Nelson et al. 2015c). Numerous reports demonstrated that human viruses were transmitted to swine and remained undetected for decades in under-sampled swine populations. Introductions of seasonal H3N2 and H1N1 from humans to pigs at different points in time, in different geographic regions, and with reassortment and evolution within swine added marked heterogeneity to the genetic lineages of IAV-S currently circulating

globally. H1N1, H1N2, and H3N2 viruses maintained in swine populations worldwide are primarily reassortants with a mix of human- and swine-adapted genes, with distinct genetic properties of the HA based on the year of introduction from human to swine (or avian to swine in the case of the Eurasian avian H1N1 lineage). This allows for discrimination of IAV-S isolates from different regions, as well as discrimination of swine-adapted viruses from contemporary human seasonal influenza strains. A global nomenclature system along with an automated Web-based tool was generated to facilitate the identification and classification of swine H1 lineages (Anderson et al. 2016).

Once established in swine, antigenic drift in a given HA lineage is slower in swine than in humans. That is, swine viruses retained more antigenic cross-reactivity to precursor human viruses compared with contemporary human seasonal viruses (de Jong et al. 1999, 2007; Kyriakis et al. 2011). However, antigenic analyses of various IAV-S lineages and comparisons to human-lineage precursors revealed a tremendous amount of antigenic diversity in IAV-S overall due to the regular human-to-swine transmission events that seeded multiple endemic lineages, as well as the geographic separation of swine populations (Lewis et al. 2016). Thus, swine can act as reservoirs for older human HAs. Several studies have shown a lack of protective serum antibody titers against endemic swine IAVs in people born after the circulation of the respective human precursor viruses (Bangaru et al. 2016; Bravo-Vasquez et al. 2017; Hoschler et al. 2013; Qiu et al. 2015b). Swine IAVs, therefore, pose a threat for reintroduction into the human population, once immunity has waned sufficiently to allow widespread transmission.

Transmission

IAV-S can be detected throughout the year (Hinshaw et al. 1978; Kaplan et al. 2015; Kyriakis et al. 2011; Olsen et al. 2000; Van Reeth and Pensaert 1994), with seasonal disease peaks. Influenza viruses are most likely to be introduced into herds with the movement of animals. The primary route of virus transmission is direct contact with infectious oronasal secretions, with virus titers of up to 1×10^7 infectious particles/mL at the peak of shedding in nasal secretions (De Vleeschauwer et al. 2009a,b; Landolt et al. 2003; Larsen et al. 2000; Van Reeth et al. 2003a). Additionally, aerosol detection of IAV-S during naturally occurring outbreaks in swine barns demonstrated significant amounts of virus in the air. Thus, aerosolized virus is also a source of infection, an exposure risk to humans during outbreaks (Neira et al. 2016), and may contribute to IAV-S infections on farms with high biosecurity. In some cases, viruses will disappear from finishing herds, especially with all-in/all-out management, only to be reintroduced later. In breeding herds, virus was more frequently detected in replacement gilts and suckling piglets compared with sows (Diaz et al. 2015). On farrow-to-finish farms, the virus may persist in the growing pig populations due to the continuous introduction of susceptible young pigs with declining levels of maternal immunity (Loeffen et al. 2009).

Geographic distribution

2009 Pandemic H1N1 (H1N1pdm09) virus in pigs

The distribution of subtypes and genotypes of endemic IAV-S varies widely among geographic regions. The exception is H1N1pdm09, which is widely distributed – largely through repeated reintroduction from humans to swine (Forgie et al. 2011; Hofshagen et al. 2009; Howden et al. 2009; Pareda et al. 2010; Vijaykrishna et al. 2010; Welsh et al. 2010). Although the H1N1pdm09 virus was coined "swine flu," there is no evidence to suggest that infection in pigs contributed to widespread human transmission or maintenance as a human seasonal strain. Although H1N1pdm09 has only been maintained in pigs in its entirety on a limited basis, reassortment with regional endemic subtypes and lineages has been widespread, with various genes incorporated (Charoenvisal et al. 2013; Kanehira et al. 2014; Kim et al. 2014; Kitikoon et al. 2013b; Lange et al. 2013; Liang et al. 2014; Nelson et al. 2012a, b, c; Pascua et al. 2013a, b; Poonsuk et al. 2013; Vijaykrishna et al. 2011; Watson et al. 2015). The impact of H1N1pdm09 introductions to pigs on the evolution of endemic swine lineages in various regions is discussed below.

IAV-S in North America

The United States, Canada, and Mexico share North American lineages, albeit with regional differences in the relative predominance and genetic variation within clades. Reports are limited, but it appears that pigs in Mexico have viruses with an ancestral origin that overlaps with some of the North American swine lineages (Anderson et al. 2016; Lopez-Robles et al. 2014; Mena et al. 2016), as well as a potentially unique human seasonal lineage H3N2 (Nelson et al. 2015a).

Classical H1N1 lineage viruses were the dominant cause of influenza among pigs in North America from 1930 through the 1990s (Chambers et al. 1991; Hinshaw et al. 1978; Olsen et al. 2000). In 1998, triple-reassortant (tr) H3N2 viruses were identified with HA, NA, and PB1 genes of human influenza virus lineage; M, NP, and NS genes of classical swine H1N1 virus lineage; and PA and PB2 genes of North American avian virus lineage (Karasin et al. 2000c; Zhou et al. 1999). The PB2, PB1, PA, NP, M, and NS genes were referred to as the triple-reassortant internal gene (TRIG) cassette (Vincent et al. 2008b). TRIG-containing H3N2 IAV-S strains spread

widely throughout the North American swine population and evolved into the contemporary phylogenetic clade IV (Olsen et al. 2006; Richt et al. 2003; Webby et al. 2000, 2004). These viruses also reached parts of Asia. The trH3N2 and the existing classical swine H1N1 viruses reassorted to produce trH1N2 (Choi et al. 2002a,b; Karasin et al. 2000a, 2002) and trH1N1 (Subbarao et al. 2006).

Two additional genotypes of trH1 virus emerged in the early 2000s containing the TRIG cassette, but the H1 and N1 or N2 genes derived from two seasonal human influenza virus introductions, resulting in delta1 and delta2 clades (Vincent et al. 2009b). For clarity, the H1 HA genes among US swine viruses were designated to 5 phylogenetic clades before 2009: alpha, beta, and gamma of classical H1 origin and delta1 and delta2 of human seasonal H1 origin (Vincent et al. 2009b,c).

H1N1pdm09 further expanded the diversity of IAV-S strains by contributing the internal genes to preexisting endemic subtypes in multiple combinations via reassortment (Ducatez et al. 2011; Kitikoon et al. 2013b; Liu et al. 2012), with the matrix gene found in a majority of endemic IAV-S strains in 2012 (Anderson et al. 2013; Rajao et al. 2016). The resulting reassortant viruses are currently undergoing rapid genetic evolution, most notably in surface glycoprotein HA genes (Kitikoon et al. 2013a; Rajao et al. 2016).

It remains to be seen if all of the expanded HA genetic clades will sustain transmission and evolution, but the biological consequences of the genetic diversity are recognized by antigenic drift (Feng et al. 2013; Lewis et al. 2014). In 2012, a virus with newly introduced human seasonal H3 and N2 genes in combination with H1N1pdm09 internal genes was first isolated in the United States, followed by infrequent detections of reassorted humanlike H3N1 and H3N2 isolates with a mix of TRIG and pdm09 internal genes (Rajao et al. 2015). Since 2014, detections of the humanlike H3N2 continued to rise, and it now appears to be an established clade in the United States (Rajao et al. 2016).

IAV-S in South America

Very few publically accessible IAV-S sequences from swine isolates are available from South America. Argentina reported distinct human-lineage viruses of H1N1, H1N2, and H3N2 subtypes (Cappuccio et al. 2011; Pereda et al. 2011), along with a reassorted human H3N2 with internal genes from the H1N1pdm09 virus (Dibarbora et al. 2013). In Brazil, an H1N2 with H1 and N2 genes of human seasonal origin and internal genes (M, NP, PB1, PB2, and PA) from H1N1pdm09 were detected (Biondo et al. 2014), followed by an additional lineage of humanlike H1N2 and H3N2 reassortants with the H1N1pdm09 internal genes (Nelson et al. 2015b). Chile has also reported two human seasonal lineage H1 paired with N1 or N2 and one H3N2 that are each distinct from those found in other South American countries (Nelson et al. 2015a).

IAV-S in Europe

Three IAV-S subtypes have been circulating in European swine for decades. Avian-like H1N1 viruses (H1avN1) were introduced from wild ducks to pigs in 1979 (Pensaert et al. 1981; Scholtissek et al. 1983) and have remained one of the major lineages on the European mainland. The European swine H3N2 viruses were derived from descendants of the 1968 "Hong Kong" pandemic human virus, but they have acquired the internal gene cassette from the H1avN1 virus through reassortment (Campitelli et al. 1997; Castrucci et al. 1993; de Jong et al. 1999; Haesebrouck et al. 1985). In the mid-1990s, H1N2 viruses became established in European pigs. The dominant H1N2 viruses (H1huN2) retained the genotype of these reassortant H3N2 viruses, but they acquired the H1 gene from a human seasonal H1N1 virus from the mid-1980s (Brown et al. 1998; Marozin et al. 2002; Schrader and Suss 2003; Van Reeth et al. 2004). Thus, these three subtypes share common internal genes, but they have clearly distinguishable HAs.

As occurred elsewhere, the introduction of H1N1pdm09 in European swine led to an increase in second-generation reassortants between the pandemic virus and the established endemic lineages (Watson et al. 2015). Among the most frequently reported genotypes were H1huN2 and H1pdmN2, with internal genes from H1N1pdm09. Between 2009 and 2013 the European Surveillance Network for Influenza in Pigs identified 23 different genotypes in 14 different countries (Watson et al. 2015). Regardless, the six genotypes mentioned above were the most frequent and accounted for 77% of the detections (Simon et al. 2014; Watson et al. 2015).

However, large differences were found between countries, and the emergence of H1N1pdm09 appears to have changed the ratios between various lineages in several countries as compared to the pre-2009 situation (Kyriakis et al. 2011). Most remarkable was the contrast between mainland Europe, where H1avN1-based genotypes were prevailing, and the United Kingdom, with a predominance of H1N1pdm09-based genotypes (Simon et al. 2014; Watson et al. 2015). While H1avN1 was still reported at a high prevalence in all European mainland countries, H3N2 and H1huN2 had an inverse relationship with each other. During recent years H3N2 was virtually absent in France, Poland, the United Kingdom, and Denmark. A reassortant H1avN2 virus accounted for 70% of the swine IAVs in Denmark, but was found at much lower frequencies in only a few other countries (Simon et al. 2014).

IAV-S in Asia

The epidemiology of IAV-S in Asia differs among countries, with reporting generally limited to China, South Korea, Japan, Thailand, Taiwan, and Vietnam. Since the 1970s, H3N2 viruses were repeatedly transmitted from people to pigs, and variants of the Hong Kong/68 pandemic virus co-circulate in pigs with several contemporary humanlike H3N2 viruses (Choi et al. 2013; Kida et al. 1988; Nerome et al. 1995; Peiris et al. 2001; Sun et al. 2009; Yu et al. 2007, 2008b). Human seasonal H1-related viruses are less widespread in Asian swine and had not been reported beyond China (Lu et al. 2010; Sun et al. 2009; Yu et al. 2007, 2009b) until recent reports in Vietnam (Takemae et al. 2016).

Prior to 2009, co-circulating viruses in China included H1N2 viruses carrying a classical H1 and an NA of contemporary human origin, multiple lineages of human-origin H3N2, Eurasian avian-like H1N1 viruses (Guan et al. 1996; Shortridge et al. 1977), and North American TRIG viruses (Vijaykrishna et al. 2011). South Korea also imported North American TRIG-containing H1 and H3 viruses (Lee et al. 2008), and reassortant viruses with Eurasian avian-like H1 (Pascua et al. 2013a) have been reported. Various H3N2 reassortant viruses were also reported throughout Asia, and some have a common ancestor with European or North American virus lineages (Takemae et al. 2008; Yu et al. 2008b). Thai swine H1N1 viruses clustered as either classical H1 or Eurasian H1 swine lineages with specific genotype reassortment patterns (Takemae et al. 2008). H3N2 viruses with human-lineage HA and NA genes of either European swine descent or a more recent human seasonal lineage were detected, with a mixture of the internal genes derived from Eurasian and classical swine lineages. H1N2 viruses containing combinations of genes from the endemic H1N1 viruses and humanlike H3N2 are also present (Takemae et al. 2008).

The H1N1pdm09 was also introduced to swine in Asia and reassorted with the endemic subtypes in China (Vijaykrishna et al. 2010), Japan (Matsuu et al. 2012), and Thailand (Kitikoon et al. 2011). Similar to other regions, the contemporary Thai H1N1, H1N2, and H3N2 viruses incorporated various internal gene segments from H1N1pdm09 (Charoenvisal et al. 2013; Poonsuk et al. 2013) as in other Asian countries (Lu et al. 2010; Vijaykrishna et al. 2010; Xu et al. 2011; Yu et al. 2008b, 2009a), resulting in multiple, very complex reassortant viruses.

Pathogenesis

The pathogenesis of influenza in pigs is well studied and very similar to that in humans (De Vleeschauwer et al. 2009a; Khatri et al. 2010; Van Reeth et al. 1998). Influenza is an acute infection of the respiratory tract. Virus replication is limited to epithelial cells of the upper and lower respiratory tract of pigs – the nasal mucosa, ethmoid, trachea, and lungs – and virus excretion and transmission occur exclusively via the respiratory route. Infectious virus can thus be isolated from the tissues mentioned, as well as from tonsils and lymph nodes in the respiratory tract, bronchoalveolar lavage (BAL) fluid, and nasal, tonsillar, or oropharyngeal swabs (Brown et al. 1993; De Vleeschauwer et al. 2009a,b; Heinen et al. 2001b; Khatri et al. 2010; Landolt et al. 2003; Richt et al. 2003; Vincent et al. 2009a). In most experimental studies, virus can be isolated from 1 day post inoculation onward and becomes undetectable after approximately 7 days. IAV-S has a preference for the lower over the upper respiratory tract (De Vleeschauwer et al. 2009a; Khatri et al. 2010). This is shown by virus titration and by immunohistochemical studies that reveal massive numbers of viral antigen-positive cells in bronchial, bronchiolar, and alveolar epithelia, compared with fewer positive cells in the nasal mucosa (Figure 36.3). Viral nucleic acid or antigen has also been found in alveolar macrophages, but there is no proof for a productive infection of these cells (Brookes et al. 2010; Jung et al. 2002; Weingartl et al. 2010). IAV-S is unlikely to spread beyond the respiratory tract. The brain stem is the single extra-respiratory tissue from which low amounts of virus are occasionally isolated (De Vleeschauwer et al. 2009a). Only one study describes a low titered and transient viremia (Brown et al. 1993). In a few studies, feces, intestines, or spleen occasionally tested positive by PCR, but virus-positive cells have

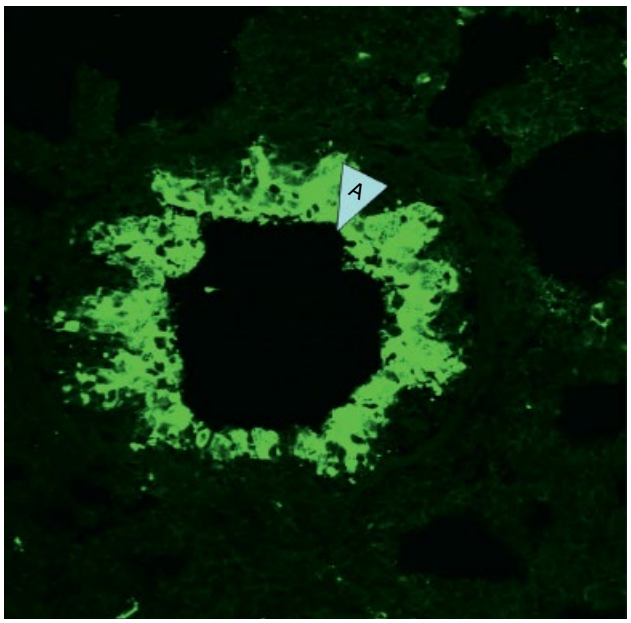


Figure 36.3 Immunofluorescence staining of influenza A virus antigen-positive cells in the lungs of a pig. In smaller bronchioli (A), up to 100% of the epithelial lining may be infected.

never been demonstrated outside the respiratory tract (Brookes et al. 2010; De Vleeschauwer et al. 2009a). Pig infection studies with the H1N1pdm09 virus confirmed the absence of virus in pork and muscle tissue, so IAV in swine is not a food safety or pork trade concern (Brookes et al. 2010; Vincent et al. 2009a).

Infection with IAV-S is easily reproduced by experimental inoculation of influenza-naïve pigs via intranasal (IN), aerosol, or intratracheal (IT) routes, but the kinetics of virus replication in the respiratory tract and the severity of lung inflammation and disease are markedly dependent upon the strain, inoculation route, and dose (Hemmink et al. 2016; Pomorska-Mol et al. 2014a). IT inoculation produces the characteristic infiltration of the lungs with neutrophils and the typical lower respiratory tract disease. Virus titers in lung may exceed 1×10^8 infectious particles/g of tissue. Infection also produces high fever (≥40.5 °C) and lethargy (De Vleeschauwer et al. 2009a; Van Reeth et al. 1998, 2002). Less invasive methods – IN inoculation or IT inoculation of a lower virus dose – result in slower kinetics of viral replication in the lungs, milder lung inflammation, and less specific clinical signs, mainly nasal discharge, sneezing, and low to moderate fevers, or subclinical infection (Brown et al. 1993; Larsen et al. 2000; Richt et al. 2003). Cytokines that are produced by the host during the acute stage of infection appear to be associated with the difference between subclinical infection and disease. Titers of interferon-alpha and interferon-gamma, tumor necrosis factor-alpha, and the interleukins 1, 6, and 12 in BAL fluids are considerably higher after IT inoculation than after IN inoculation. Experimental studies support the notion that a heavy viral load in the lungs is required to induce high levels of these cytokines, which in turn induce acute-phase proteins, and the typical lung inflammation and disease (Barbé et al. 2011; Kim et al. 2009; Pomorska-Mol et al. 2014b; Van Reeth et al. 1998, 2002). Factors that reduce the extent of virus replication in pigs in the field – partial active or passive immunity and sanitary measures to reduce infection pressure – are likely to reduce the severity of illness. Many cytokines, however, also have antiviral and immunostimulating effects and thus may contribute to clearance of influenza viruses.

Experimental infection studies have not yielded convincing evidence for distinct differences in the pathogenesis or virulence between IAV-S lineages or strains, rather a spectrum of similar clinical signs. The H1N1pdm09 virus has a similar pathogenic course in pigs as the previously established swine IAVs (Brookes et al. 2010; Hemmink et al. 2016; Lange et al. 2009; Vincent et al. 2009a; Weingartl et al. 2010). Although some studies describe more extensive lung lesions, one must consider the impact of factors that may vary between studies such as the inoculation dose and route of inoculation, as well as differences in replication competency between viruses and biological variation between pigs. In most comparative studies, lesion differences are not dramatic (Henningson et al. 2015; Janke 2014; Landolt et al. 2003; Richt et al. 2003; Sreta et al. 2009; Vincent et al. 2006, 2009b). On the other hand, researchers have demonstrated differing lung lesions, virus shedding patterns, and innate and adaptive immune responses following aerosol, IN, and contact infection with a single H1N1pdm09 isolate (Hemmink et al. 2016). One study has reported differences in transmission efficiency between North American H3N2 reassortant genotypes with H1N1pdm09 internal genes (Ma et al. 2015). In comparison to swine-adapted strains, infection studies with avian or human influenza viruses typically result in a mild or subclinical infection, consistent with low to moderate virus titers in the respiratory tract (Choi et al. 2005; De Vleeschauwer et al. 2009a; Landolt et al. 2003; Lipatov et al. 2008).

Clinical signs

Infections with any of the endemic swine IAVs are clinically similar, and all can produce acute respiratory episodes (Karasin et al. 2000a–c, 2002; Loeffen et al. 1999; Zhou et al. 1999). Experimental studies have failed to demonstrate differences in clinical signs or lesions among IAV-S strains (see above).

Typical swine flu outbreaks are characterized by high fever (40.5–41.5 °C), anorexia, inactivity, huddling, reluctance to rise, tachypnea, and, after a few days, coughing. Labored abdominal breathing and dyspnea are most typical. The onset of the disease is sudden, after an incubation period of 1–3 days. Morbidity is high (up to 100%), but mortality is low (usually less than 1%) in uncomplicated infections. Generally, rapid recovery begins 5–7 days after onset. Acute outbreaks of clinically typical IAV-S are generally limited to fully susceptible, seronegative pigs, either unprotected nursery pigs or older pigs (Loeffen et al. 1999).

In addition to immune status, other factors also affect the clinical outcome of IAV-S infection, including age, infection pressure, climatic conditions, housing, and concurrent infections. Secondary bacterial infections with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Mycoplasma hyopneumoniae*, *Haemophilus parasuis*, or *Streptococcus suis* type 2 may enhance the severity of clinical disease. Other respiratory viruses, such as porcine respiratory coronavirus (PRCV) and porcine reproductive and respiratory syndrome virus (PRRSV), frequently infect pigs around the same age as IAV-S (Van Reeth and Pensaert 1994). Of these pathogens, PRRSV, *M. hyopneumoniae*, and IAV-S are most frequently detected in 10- to 22-week-old pigs in association with the "porcine respiratory disease complex

(PRDC)" (Thacker et al. 2001). Dual-infection studies combining IAV-S infection with *M. hyopneumoniae*, *Bordetella bronchiseptica*, PRCV, or PRRSV showed more severe disease in dual infections as compared with IAV-S alone (Brockmeier et al. 2002; Deblanc et al. 2012, 2013; Kowalczyk et al. 2014). However, consistently reproducing clinical signs caused by combinations of infectious agents is challenging, and some studies failed to show enhancement of disease. Thus, the role of IAV-S in PRDC remains elusive.

Subsequent to an influenza outbreak in a herd, producers and veterinarians sometimes report reduced reproductive performance – increased infertility, abortion, small weak litters, and stillbirths. However, little data exist to suggest that influenza viruses infect the reproductive tract of pigs or directly induce reproductive disease (Kwit et al. 2014, 2015).

Lesions

Like clinical signs, lung lesions can be mild or unremarkable. When present, the gross lesions found in uncomplicated influenza are mainly those of a viral pneumonia and limited to the apical and cardiac lobes of the lung. The percent of lung tissue with grossly visible consolidation varies greatly within and between experimental infection studies, but >50% of the lung may be affected by 4–5 days post inoculation (Janke 2014; Khatri et al. 2010; Landolt et al. 2003; Richt et al. 2003). Generally, there is a sharp line of demarcation between the affected and normal lung tissues, and the involved areas will be purple and firm. Some interlobular edema may be evident, airways may be filled with blood-tinged fibrinous exudates, and the associated bronchial and mediastinal lymph nodes are usually enlarged. In naturally occurring influenza, these lesions may be complicated or masked by concurrent, especially bacterial, infections.

Microscopically, the hallmarks of IAV include necrosis of lung epithelia, desquamation/denudation of the bronchial epithelial cell layer, and airways obstructed with necrotic epithelial and inflammatory cells, mainly neutrophils (Haesebrouck et al. 1985; Haesebrouck and Pensaert 1986; Janke 2014) (Figure 36.4). Neutrophils may account for up to 50% of the cell population in BAL fluids collected 24 hours after IT inoculation, while macrophages are the dominant cells in uninfected healthy pigs (Barbé et al. 2011; Khatri et al. 2010; Van Reeth et al. 1998). The neutrophils not only cause obstruction of the airways but also contribute to lung damage by release of their enzymes. After a few days, there is peribronchial and perivascular infiltration of lymphocytes (Landolt et al. 2003; Richt et al. 2003). Similar pathologic lesions have been observed in clinically typical IAV-S outbreaks in the field (Loeffen et al. 1999).

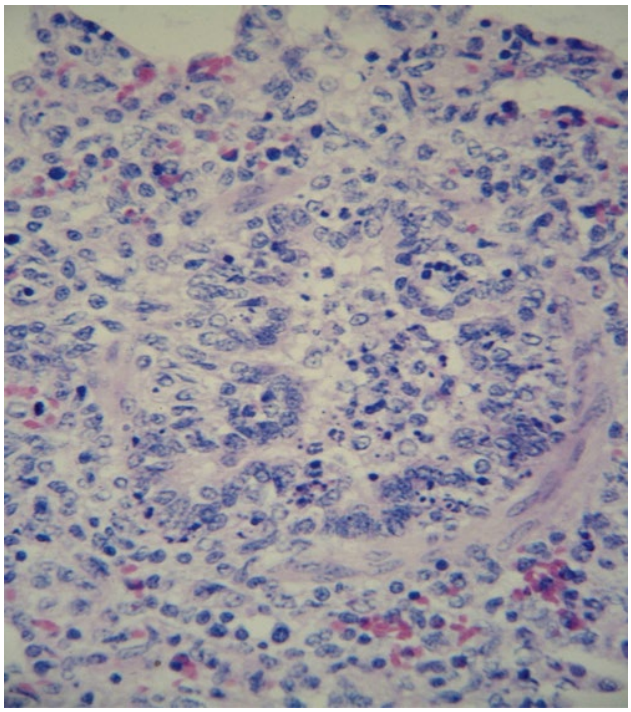


Figure 36.4 Small bronchiole with desquamation of epithelial cells after experimental infection of a pig with swine influenza A virus. The lumen is completely obstructed by desquamated epithelial cells and neutrophils.

"Vaccine-associated enhanced respiratory disease" (VAERD) has been described in pigs vaccinated with experimental whole virus inactivated vaccine and challenged with heterologous strains of the same IAV-S subtype (Vincent et al. 2008a, 2012). VAERD is characterized by more gross and microscopic pathology in vaccinated pigs versus unvaccinated controls following challenge. It appears to be restricted to pairs of viruses of the same HA subtype, which have drifted sufficiently so as to no longer stimulate cross-reacting HI or virus neutralization (VN) antibodies (Khurana et al. 2013).

Diagnosis

There are no pathognomonic signs, and swine influenza must be differentiated from a variety of respiratory diseases of swine with similar clinical and pathologic presentation. A diagnosis is only possible through isolation of virus, through detection of viral proteins or nucleic acid, or by demonstration of virus-specific antibodies. Diagnostic techniques for IAV-S have been described in detail (Swenson et al. 2015). The sensitivity of these approaches will be dependent on the specialized reagents used in the assay(s) and their "degree of match" to circulating field strains.

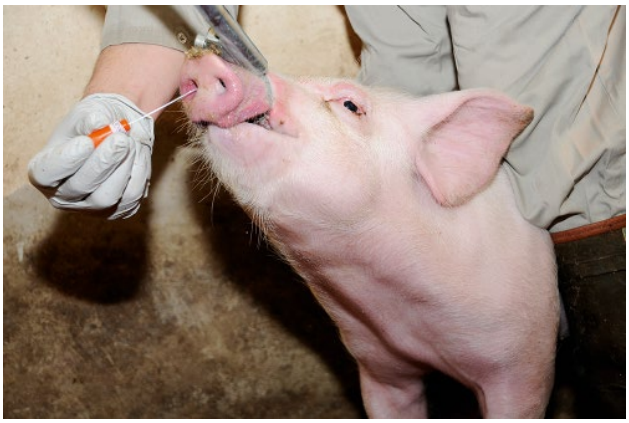


Figure 36.5 Collection of nasal swabs for the detection of influenza virus. Restrain the pig with the head upward and then insert the swab into the nasal cavity in a dorsal-medial direction. Use a circular motion to gently collect as much nasal secretion as possible.

IAV for detection by isolation or RT-PCR is most likely to be found in nasal and pharyngeal secretions during the febrile period of illness. Samples should be collected on polyester (e.g. Dacron®), not cotton, swabs (Figure 36.5). Swabs should be suspended in a suitable transport medium, such as cell culture medium or phosphate-buffered saline at neutral pH, and kept cold. If the samples for virus isolation can be tested within 48 hours after collection, they should be maintained at refrigerator temperature (4 °C). If samples are held longer, storage at −70 °C is recommended because IAV-S is not stable at −20 °C. Virus may also be isolated/detected from the trachea or lung tissues of pigs that die or are euthanized during the acute stage of the disease. The tissue should be held under the same conditions as swab material until ready for culture. Oral fluids are increasingly used for screening populations for IAV-S and other swine pathogens (Detmer et al. 2011; Goodell et al. 2013; Panyasing et al. 2014). Although isolation and sequencing rates are lower for oral fluids than for other specimens, RT-PCRs work reasonably well. Madin–Darby canine kidney (MDCK) is the most commonly used cell line for IAV-S virus isolation, although embryonated chicken eggs and other primary cells or continuous cell lines can be used (Zhang and Gauger 2014).

Historically, IAV HA and NA subtypes were determined by HI and neuraminidase inhibition (NI) tests using HA and NA subtype-specific antibodies. Currently, molecular methods based on detecting gene signatures unique to different subtypes (e.g. RT-PCR, gene sequencing, microarrays, whole genome sequencing) are generally used to detect and characterize IAVs (Ducatez et al. 2011; Heil et al. 2010).

RT-PCR assays (traditional and real-time technologies) provide highly analytically sensitive and specific detection of viral nucleic acid extracted from clinical sample preparations. Well-validated methods are at least equivalent to virus isolation (Landolt et al. 2005), with greater speed, reduced cost, and scalability as inherent advantages. However, due to increased analytical sensitivity in these assays, "weak" positive samples may contain degraded, rather than infectious, virus and not yield a virus isolate.

RT-PCR assays can be classified into two broad types. The first allow generic detection of any IAV and are applicable to all swine IAVs, but do not provide information on the virus subtype. These assays usually offer high levels of analytical sensitivity and specificity and are most suitable for initial screening of clinical samples (Spackman et al. 2002). The second are subtype-specific assays designed to detect specific HA subtypes and possibly to discriminate strains within the same HA subtype (e.g. classical, avian-like swine, or H1N1pdm09 viruses). These are usually of slightly lower analytical sensitivity and less useful for primary screening of samples from clinical cases than the pan-influenza A assays. The occurrence of H1N1pdm09 virus in pigs has required that any method be applicable to both endemic IAV-S and this virus (Hiromoto et al. 2010; Lorusso et al. 2010; Slomka et al. 2010).

Other methods for detecting virus or viral antigen can be applied to fresh, non-autolyzed tissues of the respiratory tract, including the lung and trachea. These include direct or indirect immunofluorescence techniques (Onno et al. 1990) and immunohistochemical detection in fixed tissue (Swenson et al. 2001; Vincent et al. 1997). In addition, commercial enzyme immunoassay membrane tests to rapidly detect influenza antigens in nasal swabs can be used without laboratory facilities (Nava et al. 2013). Although easy to perform, these tests generally lack sufficient analytical sensitivity to reliably detect virus shed from the nasal cavity and do not differentiate between virus subtypes.

Serological tests are used to demonstrate the presence of influenza-specific antibodies. Diagnosis of acute IAV-S infection by serology requires the use of paired acute and convalescent (3–4-week interval) serum samples. Serology is most useful to determine the immune status of a herd, levels of maternally derived antibody in the young piglets and their kinetics, post vaccination antibody titers and for pre-movement testing of pigs.

The HI test remains the most common test for specific anti-IAV-S antibody detection. A number of ELISA assays for IAV-S are commercially available. Broadly, these can be separated into two groups. The first group of assays detect antibody to a highly conserved core antigen of influenza A, such as the nucleoprotein. These tests generally have good diagnostic sensitivity (Ciacci-Zanella et al. 2010) and are useful as a screening assay to determine herd status, but they do not differentiate between virus subtypes. The second group of ELISAs detect subtype-specific antibodies. These ELISA assays generally offer lower diagnostic sensitivity than the HI

test (Barbé et al. 2009; Leuwerke et al. 2008) but may have application in studies where status to a specific virus subtype/strain is required. VN assays show similar performance characteristics to the HI test (Leuwerke et al. 2008; Van Reeth et al. 2006), but they are more appropriate for use in specialized laboratories.

Interpretation of serologic data is complicated by the concurrent circulation of different virus subtypes and gene lineages. This is especially problematical with co-circulation of strains within the same subtype. For example, H1 viruses possess variable cross-reactivity in tests that detect antibodies to the HA, such as HI and VN, as well as subtype-specific ELISAs (Barbé et al. 2009; Leuwerke et al. 2008). The emergence of the H1N1pdm09 virus in pigs has further complicated interpretation through a broad spectrum and sometimes unpredictable range of cross-reacting antibody responses with endemic strains in HI and VN tests (Dürrwald et al. 2010; Kyriakis et al. 2010b; Vincent et al. 2010b). Therefore, where H1N1 is endemic, infection with the H1N1pdm09 virus may not be diagnosed by serological tests alone (Kyriakis et al. 2010b).

Immunity

The adaptive immune response to IAV-S infection includes both humoral and cell-mediated immunity (CMI). Antibody responses mainly develop to the HA, NA, M, and NP proteins (Shaw and Palese 2013). Only antibodies to the globular head region of the HA can block attachment of the virus to host cell receptors and neutralize viral infectivity. These antibodies can be measured in VN or HI assays. Antibodies against the NA primarily act after infection has been initiated by limiting virus release from infected cells. Antibodies to other proteins also cannot prevent the initiation of infection, but they can mediate killing of infected cells by antibody-dependent mechanisms.

T cells are more broadly directed against epitopes on all internal and surface proteins. CD4+ or helper T cells facilitate the antibody and CMI responses, whereas CD8+ T cells differentiate into cytotoxic T lymphocytes (CTLs) and kill virus-infected cells directly, thereby helping in clearing virus from the lungs. Thus, the CTLs are the key players of the CMI response, but studies in pigs have mainly measured T helper cell activity because it is technically difficult to quantify CTLs in outbred animal species. Similarly, most studies have focused on the antibody response in serum, while mucosal antibodies in the respiratory tract are most important for protection.

The immune response to IAV-S infection is rapid and efficient with complete elimination of the virus from the respiratory tract within 7 days post inoculation. T-cell responses have been detected from 7 days post inoculation onward (Heinen et al. 2001a, b; Khatri et al. 2010; Larsen et al. 2000). HI antibodies in serum can be detected by 7–10 days post inoculation and peak by 2–3 weeks post inoculation (Heinen et al. 2000; Larsen et al. 2000). Antibody titers remain high for several weeks before beginning to decline by 8–10 weeks post inoculation (Van Reeth et al. 2004, 2006).

As would be expected, IgM and later IgG are the dominant isotypes in serum, whereas IgA is the main isotype in nasal washes (Heinen et al. 2000; Larsen et al. 2000). Larsen et al. (2000) demonstrated antibody-secreting cells in nasal mucosal tissue, which proved that antibodies are locally produced in the respiratory tract of pigs. Antibodies at the lung level are, at least in part, transudated from serum, as suggested by the dominance of virus-specific IgG in BAL samples of pigs (Heinen et al. 2000). However, substantial IgA levels have also been found in lung lavage fluids of IAV-infected pigs (Kitikoon et al. 2006; Larsen et al. 2000), and local antibody production in the lung parenchyma cannot be excluded.

After a primary IAV-S infection, there is solid protection against reinfection with the same or a similar virus strain (De Vleeschauwer et al. 2011; Larsen et al. 2000; Van Reeth et al. 2003a). Based on findings in humans, it is assumed that this immunity may last for several years, but its exact duration has not been studied in the pig. HA-specific VN antibodies are likely the primary mediators of this "homologous" immunity. Protection studies to prove the role of other immune mechanisms in the pig are still lacking.

Because of the concurrent circulation of different subtypes and lineages of influenza viruses, pigs are typically exposed to antigenically different IAV-S strains during their lifetime. There is no serologic cross-reaction between H1 and H3 subtype IAV-S strains in the HI test, nor in some cases between viruses within H1 and H3 lineages. Still, several experimental infection studies have shown cross-protection between influenza viruses in the absence of cross-reactive serum HI antibodies. There is evidence for complete or partial cross-protection between the three European H1 IAV-S lineages, between North American alpha- and gamma-cluster H1 IAVs, and between European and North American H1N1 as well as H3N2 IAV-S lineages (Busquets et al. 2010; De Vleeschauwer et al. 2011; Qiu et al. 2014, 2015a; Van Reeth et al. 2003a; Vincent et al. 2008a). The immune mediators of this "broad" protection need further study but may include a combination of mucosal IgA antibodies, CMI responses, and cross-reactive VN and/or NI antibodies. Cross-protection between H1 and H3 IAV-S lineages, in contrast, is minimal (Kappes et al. 2012; Qiu et al. 2015a).

The immune response after intramuscular administration of killed IAV-S vaccine is fundamentally different from live virus infection. Vaccination immunity relies largely on inducing high titers of serum HI and VN antibodies to the HA of the vaccine strain(s). In contrast, mucosal antibodies or CD8+ T cells are not efficiently induced by inactivated virus vaccines.

Maternally derived serum IgG antibodies to IAV-S can protect young pigs against antigenically related viruses, but they will also interfere with the development of an active antibody response to vaccination or infection. Maternal IAV-S antibody levels in newborn pigs reflect the dam's immune status. Thereafter, passive IAV-S antibody declines in the pig over a period of 4–14 weeks (Loeffen et al. 2003). Research showed that pigs with maternal antibodies were not completely protected from nasal virus shedding upon challenge, but some pigs showed protection against clinical disease (Loeffen et al. 2003; Renshaw 1975).

Prevention and control

Vaccination is the primary means of preventing influenza in pigs. Most commercial IAV-S vaccines are inactivated whole virus vaccines with an adjuvant. Primary vaccination consists of two intramuscular injections 2–4 weeks apart, with twice-yearly booster vaccinations recommended for sows. Routine pre-farrow booster vaccination of sows results in higher and longer-lasting maternal IAV-S antibody levels – which may protect pigs against clinical disease through the nursery phase. Vaccination of feeder pigs is less commonly performed and may be difficult to combine with vaccination of sows because prolonged passive immunity may interfere with effective vaccination of piglets. However, this strategy may be beneficial in herds where influenza is a problem in growers/finishers.

Inactivated whole virus vaccines are intended to induce serum antibodies to the viral HA. NI antibodies have also been detected in the serum of vaccinated pigs, and they may play a secondary role in protection (Duerrwald et al. 2013; Sandbulte et al. 2016). Experimental vaccination-challenge studies have shown a strong inverse correlation between virus titers in the lungs post challenge and pre-challenge serum HI or VN antibody titers to the challenge virus. Depending on the virus-specific antibody titer, virus replication will either be completely blocked or reduced, perhaps sufficiently so as to prevent disease (Van Reeth et al. 2017b). Inactivated vaccines are thus designed to protect individual pigs against the clinical effects of IAV-S by reducing lung virus titers. Although beneficial effects on nasal virus excretion or IAV-S transmission have been found in some studies, the reduction of virus titers in the lungs is almost invariably greater than that in the upper airways (Van Reeth et al. 2017b). This is consistent with the lack of mucosal IgA antibodies after vaccination with inactivated vaccines and with a more efficient transudation of serum antibodies in the lungs than in the nose.

Because HA antibodies are subtype and strain specific, traditional inactivated IAV-S vaccine strains should match the circulating strains. In keeping with the different virus lineages in Europe versus the United States, the vaccines for each region are produced locally and contain entirely different strains. Unlike for human influenza vaccines, there is no formal system for recommending IAV-S vaccine strains. Even within a continent, there is no standardization of vaccine strains, and the antigenic mass and adjuvants can also differ between different commercial products. In Europe, a trivalent vaccine containing H1N1, H3N2, and H1N2 strains and a Carbomer adjuvant is currently the major vaccine on the market. Bivalent vaccines containing H1N1 and H3N2 strains from the 1970s and 1980s combined with oil- or aluminum hydroxide-based adjuvants are also used in Spain and Italy. In North America, two of the four commercial vaccines contain swine IAV strains from multiple H1 and/or H3 clades. A monovalent vaccine for the H1N1pdm09 influenza virus is only available in North America and not in Europe. Custom-made, inactivated autogenous vaccines are also used in the United States (reviewed by Sandbulte et al. (2015)). IAV-S vaccines are not available in many countries, including China. In general, the commercial products available in North America are also available in South America, whereas locally produced vaccines based on local strains are often used in Japan and South Korea (Van Reeth et al. 2017b).

The efficacy of inactivated IAV-S vaccines is confounded by the current diversity in IAV-S strains. It is highly unlikely that one strain per subtype would be efficacious globally. In addition, a single H1 or H3 vaccine strain will frequently fail to cross-protect against all of the co-circulating virus lineages within a given region, and multiple within-subtype strains seem to be required. For example, European bivalent H1N1/H3N2 vaccines failed to induce cross-reactive HI antibodies or protection against the European H1N2 virus lineage (Van Reeth et al. 2003b). In the United States, polyvalent commercial vaccines containing cluster IV H3N2 offered better protection against a drifted contemporary cluster IV strain than an older commercial vaccine containing only cluster I H3N2 (Loving et al. 2013). In 2009–2010, the existing European and North American vaccines were shown to induce partial serological cross-reaction and cross-protection against the novel H1N1pdm09 virus, but superior protection was obtained with specific monovalent vaccines (Dürrwald et al. 2010; Kyriakis et al. 2010b; Vincent et al. 2010a). The genetic and antigenic proximity between vaccine and field strains is often used to infer vaccine efficacy, but sequence analyses or antigenic data are unreliable predictors. Factors like the immunogenicity of vaccine strains, the antigenic mass, and the adjuvant are also determinants of vaccine potency and cross-reactivity. In particular, the oil-based adjuvants used in many IAV-S vaccines likely account for the much broader protection afforded by these vaccines compared with that observed with unadjuvanted influenza vaccines for humans. These

additional factors could explain why some commercial vaccines in Europe have shown the ability to provide protection against mismatched H1N1 and H3N2 swine IAVs isolated over the course of many years (De Vleeschauwer et al. 2015; Heinen et al. 2001b; Kyriakis et al. 2010a; Van Reeth et al. 2001). The HA of the challenge strains used in these studies showed only 78–93% and 84–92% amino acid homology with that of H1 and H3 vaccine strains, respectively. Similarly, Canadian researchers have shown significant cross-cluster protection against a 2008 alpha-cluster H1N1 IAV-S with a trivalent commercial vaccine containing gamma- and delta2-cluster H1N1 strains only. Despite a common ancestral HA, the alpha- and gamma-cluster viruses shared only 87.2% nucleic acid sequence homology. Though cross-protection challenge data remain the ultimate test, one of the most relevant ways to evaluate the suitability of vaccine strains is to test vaccine-induced antisera for reactivity against field isolates in HI or VN assays.

IN administration of live attenuated influenza virus (LAIV) vaccines is the preferred method for inducing respiratory mucosal antibodies for preventing or reducing IAV-S transmission in the population. Stably attenuated vaccines have been developed by reverse genetics technology (Van Reeth et al. 2017b), and the first of its kind for swine became available in 2018 in the United States. The fully licensed commercial bivalent H3N2 and H1N1 LAIV is based on prior work with an H3N2 containing a truncated NS-1 protein, shown to be attenuated in pigs (Solórzano et al. 2005). In vaccination-challenge studies, LAIV vaccines protect against antigenic variants of the vaccine strain, but multivalent formulations will be required to protect against a variety of swine H1 and H3 IAVs. In contrast to most label recommendations for inactivated vaccines for swine, the label for the newly licensed LAIV is for healthy pigs 1 day of age or older, and marketed to protect growing pigs. Maternal antibody interference was demonstrated to be less pronounced with LAIV, thus it may be used in suckling pigs prior to weaning. Although the impact may be less dramatic than is the case for killed vaccines, the presence of matching maternal antibodies reduces the efficacy and therefore the effects of preexisting active immunity, and establishing other correlates of protection rather than serum HI titers for LAIV vaccines requires further study.

Most IAV-S vaccine is used in gilts and sows that were previously infected with one or more swine IAVs. In one study, pigs primed by IN inoculation with live contemporary European swine IAV(s) were injected with a single dose of commercial inactivated vaccine based on older H1N1 and H3N2 strains. Unlike infection or vaccination alone, the prime-boost approach resulted in a dramatic increase in HI antibody titers to all virus strains used for immunization and broader anti-H1 antibody responses (Van Reeth et al. 2006). This may mean that a close antigenic match between killed vaccine and circulating strains is less important in pigs primed by infection with field strains.

These data are in line with studies in humans in which it was shown that vaccination or infection with a given influenza virus strain stimulates an antibody response against that particular strain but also against all strains of that subtype to which the individual has previously been exposed (Fonville et al. 2014).

In further support of this concept, a recent study using inactivated H3N2 IAV-S vaccines showed that priming with one strain and then boosting pigs with an antigenically diverse strain elicited antibodies and protection against both strains, unlike the traditional approach of using identical strains for primary and booster vaccinations (Van Reeth et al. 2017a). The heterologous prime-boost strategy also induced antibodies to H3 clades that were not included in the vaccine. This approach could increase vaccine efficacy in the current era of increasing IAV-S diversity.

Recombinant protein vaccines, viral vector vaccines, and DNA vaccines have been tested experimentally, with largely disappointing results. One new generation IAV-S vaccine is currently licensed. In the United States, an RNA "replicon" particle vaccine expressing the HA of a North American cluster IV H3N2 IAV-S and farm strain-specific replicon particle vaccines are available (see Van Reeth and Ma 2013; Van Reeth et al. 2017b).

Acknowledgments

The authors have adapted their chapter from that written for the 10th edition and would like to thank Christopher Olsen and Ian Brown for their contribution to the previous edition.

References

Abente EJ, Kitikoon P, Lager KM, et al. 2016. *J Gen Virol* 98:18–30.

Anderson TK, Nelson MI, Kitikoon P, et al. 2013. *Influenza Other Respir Viruses* 7 (Suppl 4):42–51.

Anderson TK, Macken CA, Lewis NS, et al. 2016. *mSphere* 1:e00275-16.

Balzli C, Lager K, Vincent A, et al. 2016. *Influenza Other Respir Viruses* 10:346–352.

Bangaru S, Nieusma T, Kose N, et al. 2016. *JCI Insight* 1:e86673.
Barbé F, Labarque G, Pensaert M, et al. 2009. *J Vet Diagn Invest* 21:88–96.
Barbé F, Atanasova K, Van Reeth K. 2011. *Vet J* 187:48–53.
Bateman A, Busch MG, Karasin AI, et al. 2008. *J Virol* 82:8204–8209.
Bateman AC, Karamanska R, Busch MG, et al. 2010. *J Biol Chem* 44:34016–34026.
Bi Y, Fu G, Chen J, et al. 2010. *Emerg Infect Dis* 16:1162–1164.
Biondo N, Schaefer R, Gava D, et al. 2014. *Vet Microbiol* 168:34–40.
Bowman AS, Nelson SW, Page SL, et al. 2014. *Emerg Infect Dis* 20:1472–1480.
Bravo-Vasquez N, Karlsson EA, Jimenez-Bluhm P, et al. 2017. *Emerg Infect Dis* 23:241–251.
Brockmeier S, Halbur P, Thacker E. 2002. Porcine respiratory disease complex. In Brogden KA, Guthmiller JM, eds. *Polymicrobial Diseases*. Washington, DC: ASM Press, pp. 231–258.
Brookes SM, Núñez A, Choudhury B, et al. 2010. *PLoS One* 5:e9068.
Brown IH. 2000. *Vet Microbiol* 74:29–46.
Brown IH, Done SH, Spencer YI, et al. 1993. *Vet Rec* 132:598–602.
Brown IH, Ludwig S, Olsen CW, et al. 1997. *J Gen Virol* 78:553–562.
Brown IH, Harris PA, McCauley JW, et al. 1998. *J Gen Virol* 79:2947–2955.
Busquets N, Segalés J, Córdoba L, et al. 2010. *Vet Res* 41:74.
Byrd-Leotis L, Liu R, Bradley KC, et al. 2014. *Proc Natl Acad Sci U S A* 111:E2241–2250.
Campitelli L, Donatelli I, Foni E. 1997. *Virology* 232:310–318.
Cappuccio JA, Pena L, Dibarbora M, et al. 2011. *J Gen Virol* 92:2871–2878.
Castrucci MR, Donatelli I, Sidoli L, et al. 1993. *Virology* 193:503–506.
Chambers TM, Hinshaw VS, Kawaoka Y, et al. 1991. *Arch Virol* 116:261–265.
Charoenvisal N, Keawcharoen J, Sreta D, et al. 2013. *Virus Genes* 47:75–85.
Choi YK, Goyal SM, Farnham MW, et al. 2002a. *Virus Res* 87:173–179.
Choi YK, Goyal SM, Joo HS. 2002b. *Arch Virol* 147:1209–1220.
Choi YK, Lee JH, Erickson G, et al. 2004. *Emerg Infect Dis* 10:2156–2160.
Choi YK, Nguyen TD, Ozaki H, et al. 2005. *J Virol* 79:10821–10825.
Choi YK, Pascua PN, Song MS. 2013. *Curr Top Microbiol Immunol* 370:147–172.
Choi MJ, Morin CA, Scheftel J, et al. 2015. *Zoonoses Public Health* 62:326–330.
Chowell G, Echevarria-Zuno S, Viboud C, et al. 2011. *PLoS Med* 8:e1000436.
Ciacci-Zanella JR, Vincent AL, Prickett JR, et al. 2010. *J Vet Diagn Invest* 22:3–9.
Cong YL, Pu J, Liu QF, et al. 2007. *J Gen Virol* 88:2035–2041.
Cong YL, Wang CF, Yan CM, et al. 2008. *Virus Genes* 36:461–469.
Cong Y, Wang G, Guan Z, et al. 2010. *PLoS One* 5:e12591.
Dawood FS, Jain S, Finelli L, et al. 2009. *N Engl J Med* 360:2605–2615.
De Vleeschauwer A, Atanasova K, Van Borm S, et al. 2009a. *PLoS One* 4:e6662.
De Vleeschauwer A, Van Poucke S, Braeckmans D, et al. 2009b. *J Inf Dis* 200:1884–1892.
De Vleeschauwer A, Van Poucke S, Karasin A, et al. 2011. *Influenza Other Respir Viruses* 5:115–122.
De Vleeschauwer A, Qiu Y, Van Reeth K. 2015. *Vaccine* 33:2360–2366.
Deblanc C, Gorin S, Queguiner S, et al. 2012. *Vet Microbiol* 157:96–105.
Deblanc C, Robert F, Pinard T, et al. 2013. *Vet Microbiol* 162:643–651.
Detmer SE, Patnayak DP, Jiang Y, et al. 2011. *J Vet Diagn Invest* 23:241–247.
Diaz A, Perez A, Sreevatsan S, et al. 2015. *PLoS One* 10:e0129213.
Dibarbora M, Cappuccio J, Olivera V, et al. 2013. *Influenza Other Respir Viruses* 7 (Suppl 4):10–15.
Donatelli I, Campitelli L, Castrucci MR, et al. 1991. *J Med Virol* 34:248–257.
Ducatez MF, Hause B, Stigger-Rosser E, et al. 2011. *Emerg Infect Dis* 17:1624–1629.
Duerrwald R, Schlegel M, Bauer K, et al. 2013. *PLoS One* 8:e61597.
Dürrwald R, Krumbholz A, Baumgarte S, et al. 2010. *Emerg Infect Dis* 16:1029–1030.
Feng Z, Gomez J, Bowman AS, et al. 2013. *J Virol* 87:7655–7667.
Fonville JM, Wilks SH, James SL, et al. 2014. *Science* 346:996–1000.
Forgie SE, Keenliside J, Wilkinson C, et al. 2011. *Clin Infect Dis* 52:10–18.
Freidl GS, Meijer A, de Bruin E, et al. 2014. *Eurosurveillance* 19:8–26.
Gambaryan A, Webster R, Matrosovich M. 2002. *Arch Virol* 147:1197–1208.
Gambaryan A, Karasin AI, Tuzikov A, et al. 2005. *Virus Res* 114:15–22.
Garten RJ, Davis CT, Russell CA, et al. 2009. *Science* 325:197–201.
Goodell CK, Goodell CK, Prickett J, et al. 2013. *Vet Microbiol* 166:450–460.
Gray GC, McCarthy T, Capuano AW, et al. 2007. *Emerg Infect Dis* 13:1871–1878.

Guan Y, Shortridge KF, Krauss S, et al. 1996. *J Virol* 70:8041–8046.
Guo YJ, Jin FG, Wang P, et al. 1983. *J Gen Virol* 64(Pt 1):177–182.
Haesebrouck F, Pensaert M. 1986. *Vet Microbiol* 11:239–249.
Haesebrouck F, Biront P, Pensaert MR, et al. 1985. *Am J Vet Res* 46:1926–1928.
Hause BM, Ducatez M, Collin EA, et al. 2013. *PLoS Pathog* 9:e1003176.
Hause BM, Collin EA, Liu R, et al. 2014. *MBio* 5:e00031-14.
Heil GL, McCarthy T, Yoon KJ, et al. 2010. *Influenza Other Respir Viruses* 4:411–416.
Heinen PP, van Nieuwstadt AP, Pol JM, et al. 2000. *Viral Immunol* 13:237–247.
Heinen PP, de Boer-Luijtze EA, Bianchi AT. 2001a. *J Gen Virol* 82:2697–2707.
Heinen PP, van Nieuwstadt AP, de Boer-Luijtze EA, et al. 2001b. *Vet Immunol Immunopathol* 82:39–56.
Hemmink JD, Morgan SB, Aramouni M, et al. 2016. *Vet Res* 47:103.
Henningson JN, Rajao DS, Kitikoon P, et al. 2015. *Vet Microbiol* 176:40–49.
Hinshaw VS, Bean WJ, Webster RG, et al. 1978. *Virology* 84:51–62.
Hinshaw VS, Webster RG, Turner B. 1980. *Can J Microbiol* 26:622–629.
Hinshaw VS, Webster RG, Bean WJ, et al. 1983. *Science* 220:206–208.
Hiromoto Y, Uchida Y, Takemae N, et al. 2010. *J Virol Methods* 170:169–172.
Hofshagen M, Gjerset B, Er C, et al. 2009. *Euro Surveill* 14:19406.
Hoschler K, Thompson C, Casas I, et al. 2013. *Euro Surveill* 18:20578.
Howden KJ, Brockhoff EJ, Caya FD, et al. 2009. *Can Vet J* 50:1153–1161.
Ito T. 2000. *Microbiol Immunol* 44:423–430.
Janke BH. 2014. *Vet Pathol* 51:410–426.
Jhung MA, Epperson S, Biggerstaff M, et al. 2013. *Clin Infect Dis* 57:1703–1712.
de Jong JC, van Nieuwstadt AP, Kimman TG, et al. 1999. *Vaccine* 17:1321–1328.
de Jong JC, Smith DJ, Lapedes AS, et al. 2007. *J Virol* 81:4315–4322.
Jung T, Choi C, Chae C. 2002. *Vet Pathol* 39:10–16.
Jung K, Song DS, Kang BK, et al. 2007. *Prev Vet Med* 79:294–303.
Kanehira K, Takemae N, Uchida Y, et al. 2014. *Microbiol Immunol* 58:327–341.
Kaplan BS, Debeauchamp J, Stigger-Rosser E, et al. 2015. *Emerg Infect Dis* 21:1834–1836.
Kappes MA, Sandbulte MR, Platt R, et al. 2012. *Vaccine* 30:280–288.
Karasin AI, Anderson GA, Olsen CW. 2000a. *J Clin Microbiol* 38:2453–2456.
Karasin AI, Brown IH, Carman S, et al. 2000b. *J Virol* 74:9322–9327.
Karasin AI, Schutten MM, Cooper LA, et al. 2000c. *Virus Res* 68:71–85.
Karasin AI, Landgraf JG, Swenson SL, et al. 2002. *J Clin Microbiol* 40:1073–1079.
Karasin AI, West K, Carman S, et al. 2004. *J Clin Microbiol* 42:4349–4354.
Kawaoka Y, Krauss S, Webster RG. 1989. *J Virol* 63:4603–4608.
Khatri M, Dwivedi V, Krakowka S, et al. 2010. *J Virol* 84:11210–11218.
Khurana S, Loving CL, Manischewitz J, et al. 2013. *Sci Transl Med* 5:200ra114.
Kida H, Shortridge KF, Webster RG. 1988. *Virology* 162:160–166.
Kim B, Ahn KK, Ha Y, et al. 2009. *J Vet Med Sci* 71:611–616.
Kim SH, Roh IS, Lee KK, et al. 2014. *Virus Genes* 48:193–198.
Kitikoon P, Nilubol D, Erickson BJ, et al. 2006. *Vet Immunol Immunopathol* 112:117–128.
Kitikoon P, Sreta D, Na Ayudhya SN, et al. 2011. *Virus Genes* 43:1–5.
Kitikoon P, Gauger PC, Anderson TK, et al. 2013a. *Influenza Other Respir Viruses* 7 (Suppl 4):32–41.
Kitikoon P, Nelson MI, Killian ML, et al. 2013b. *J Gen Virol* 94:1236–1241.
Kowalczyk A, Pomorska-Mol M, Kwit K, et al. 2014. *Vet Microbiol* 170:206–212.
Kwit K, Pomorska-Mol M, Markowska-Daniel I. 2014. *BMC Vet Res* 10:123.
Kwit K, Pomorska-Mol M, Markowska-Daniel I. 2015. *Arch Virol* 160:2415–2425.
Kyriakis CS, Gramer MR, Barbé F, et al. 2010a. *Vet Microbiol* 144:67–74.
Kyriakis CS, Olsen CW, Carman S, et al. 2010b. *Emerg Infect Dis* 16:96–99.
Kyriakis CS, Brown IH, Foni E, et al. 2011. *Zoonoses Public Health* 58:93–101.
Landolt GA, Olsen CW. 2007. *Anim Health Res Rev* 8:1–21.
Landolt GA, Karasin AI, Phillips L, et al. 2003. *J Clin Microbiol* 41:1936–1941.
Landolt GA, Karasin AI, Hofer C, et al. 2005. *Am J Vet Res* 66:119–124.
Lange E, Kalthoff D, Blohm U, et al. 2009. *J Gen Virol* 90:2119–2123.
Lange J, Groth M, Schlegel M, et al. 2013. *Vet Microbiol* 167:345–356.
Larsen DL, Karasin A, Zuckermann F, et al. 2000. *Vet Microbiol* 74:117–131.
Lee CS, Kang BK, Kim HK, et al. 2008. *Virus Genes* 37:168–176.

Lee JH, Pascua PN, Song MS, et al. 2009. *J Virol* 83:4205–4215.
Leuwerke B, Kitikoon P, Evans R, et al. 2008. *J Vet Diagn Invest* 20:426–432.
Lewis NS, Anderson TK, Kitikoon P, et al. 2014. *J Virol* 88: 4752–4763.
Lewis NS, Russell CA, Langat P, et al. 2016. *Elife* 5:e12217.
Liang H, Lam TT, Fan X, et al. 2014. *J Virol* 88:10864–10874.
Lipatov AS, Kwon YK, Sarmento LV, et al. 2008. *PLoS Pathog* 4:e1000102.
Liu Q, Ma J, Liu H, et al. 2012. *Arch Virol* 157:555–562.
Loeffen WLA, Kamp EM, Stockhofe-Zurwieden N, et al. 1999. *Vet Rec* 145:123–129.
Loeffen WL, Heinen PP, Bianchi AT, et al. 2003. *Vet Immunol Immunopathol* 92:23–35.
Loeffen WLA, Hunneman WA, Quak J, et al. 2009. *Vet Microbiol* 137:45–50.
Lopez-Robles G, Montalvo-Corral M, Burgara-Estrella A, et al. 2014. *Vet Microbiol* 172:323–328.
Lorusso A, Faaberg KS, Killian ML, et al. 2010. *J Virol Methods* 164:83–87.
Loving CL, Lager KM, Vincent AL, et al. 2013. *J Virol* 87:9895–9903.
Lu L, Yin Y, Sun Z, et al. 2010. *J Clin Virol* 49:186–191.
Ludwig S, Haustein A, Kaleta EF, et al. 1994. *Virology* 202:281–286.
Ma W, Vincent AL, Gramer MR, et al. 2007. *Proc Natl Acad Sci USA.* 104:20949–20954.
Ma J, Shen H, Liu Q, et al. 2015. *J Virol* 89:2831–2841.
Mancera Gracia JC, Van den Hoecke S, Richt JA, et al. 2017. *Sci Rep* 7:1323.
Manzoor R, Sakoda Y, Nomura N, et al. 2009. *J Virol* 83:1572–1578.
Marozin S, Gregory V, Cameron K, et al. 2002. *J Gen Virol* 83:735–745.
Matrosovich M, Tuzikov A, Bovin N, et al. 2000. *J Virol* 74:8502–8512.
Matsuu A, Uchida Y, Takemae N, et al. 2012. *Microbiol Immunol* 56:792–803.
Mena I, Nelson MI, Quezada-Monroy F, et al. 2016. *Elife* 5: pii e16777.
Morens DM, Taubenberger JK. 2014. *Lancet Infect Dis* 14:169–72.
Myers KP, Olsen CW, Setterquist SF, et al. 2006. *Clin Infect Dis* 42:14–20.
Myers K, Olsen CW, Gray GC. 2007. *Clin Infect Dis* 44:1084–1088.
Nava GM, Merino R, Jarquin R, et al. 2013. *Vet Rec* 173:424–425.
Neira V, Rabinowitz P, Rendahl A, et al. 2016. *PLoS One* 11:e0146616.
Nelli RK, Kuchipudi SV, White GA, et al. 2010. *BMC Vet Res* 6:4.
Nelson MI, Vincent AL. 2015. *Trends Microbiol* 23:142–153.
Nelson MI, Detmer SE, Wentworth DE, et al. 2012a. *J Gen Virol* 93:2584–2589.
Nelson MI, Gramer MR, Vincent AL, et al. 2012b. *J Gen Virol* 93:2195–2203.
Nelson MI, Vincent AL, Kitikoon P, et al. 2012c. *J Virol* 86:8872–8878.
Nelson M, Culhane MR, Rovira A, et al. 2015a. Novel human-like influenza a viruses circulate in swine in Mexico and Chile. *PLoS Curr* 7.
Nelson MI, Schaefer R, Gava D, et al. 2015b. *Emerg Infect Dis* 21:1339–1347.
Nelson MI, Stratton J, Killian ML, et al. 2015c. *J Virol* 89:6218–6226.
Nerome K, Kanegae Y, Shortridge KF, et al. 1995. *J Gen Virol* 76:613–624.
Neumann G, Kawaoka Y. 2006. *Emerg Infect Dis* 12:881–886.
Neumann G, Takeshi N, Kawaoka Y. 2009. *Nature* 459:931–939.
Nicholls JM, Bourne AJ, Chen H, et al. 2007. *Respir Res* 8:73.
Nicholls JM, Chan RW, Russell RJ, et al. 2008. *Trends Microbiol* 16:149–157.
Nidom CA, Takano R, Yamada S, et al. 2010. *Emerg Infect Dis* 16:1515–1523.
Olsen CW, Carey S, Hinshaw L, et al. 2000. *Arch Virol* 145:1399–1419.
Olsen CW, Brammer L, Easterday BC, et al. 2002. *Emerg Infect Dis* 8:814–819.
Olsen CW, Karasin A, Erickson G. 2003. *Virus Res* 93:115–121.
Olsen CW, Karasin AI, Carman S, et al. 2006. *Emerg Infect Dis* 12:1132–1135.
Onno M, Jestin A, Nannier P, Kaiser C. 1990. *Vet Quart* 12:251–254.
Panyasing Y, Goodell C, Kittawornrat A, et al. 2014. *Transbound Emerg Dis* 63:e328–e338.
Pareda A, Cappuccio J, Quioga MA, et al. 2010. *Emerg Infect Dis* 16:304–307.
Pascua PN, Lim GJ, Kwon HI, et al. 2013a. *Genome Announc* 1:e00552-13.
Pascua PN, Lim GJ, Kwon HI, et al. 2013b. *Influenza Other Respir Viruses* 7: 1283–1291.
Peiris JSM, Guan Y. Markwell D, et al. 2001. *J Virol* 75:9679–9686.
Pensaert M, Ottis K, Vandeputte J, et al. 1981. *Bull World Health Org* 59:75–78.
Pereda A, Rimondi A, Cappuccio J, et al. 2011. *Influenza Other Respir Viruses* 5:409–412.
Pillai SP, Pantin-Jackwood M, Yassine HM, et al. 2010. *Avian Dis* 54:522–526.
Pomorska-Mol M, Kwit K, Markowska-Daniel I, et al. 2014a. *Res Vet Sci* 97:412–421.
Pomorska-Mol M, Kwit K, Pejsak Z, et al. 2014b. *Influenza Other Respir Viruses* 8:228–234.

Poonsuk S, Sangthong P, Petcharat N, et al. 2013. *Vet Microbiol* 167:314–326.
Qiao C, Liu Q, Bawa B, et al. 2012. *J Gen Virol* 93:2337–2345.
Qiu Y, Mancera Gracia JC, Li Y, et al. 2014. Prior infection of pigs with European H3N2 SIV confers partial protection against North American swine-origin H3N2v influenza virus. In Abstracts of the 5th ESWI Influenza Conference, Riga, Latvia (SPB4P094).
Qiu Y, De Hert K, Van Reeth K. 2015a. *Vet Res* 46:105.
Qiu Y, Muller CP, Van Reeth K. 2015b. *Euro Surveill* 20:25–33.
Rajao DS, Gauger PC, Anderson TK, et al. 2015. *J Virol* 89:11213–11222.
Rajao DS, Walia R, Campbell B, et al. 2016. *J Virol* 91:e01763–16.
Ran Z, Shen H, Lang Y, et al. 2015. *J Virol* 89:4818–4826.
Van Reeth K, Ma WJ. 2013. Swine influenza virus vaccines – to change, or not to change: That's the question. In Richt J, Webby R, eds. *Current Topics in Microbiology and Immunology*, Vol. 370. Berlin and Heidelberg: Springer-Verlag, pp. 173–200.
Renshaw HW. 1975. *Am J Vet Res* 36:5–13.
Richt JA, Lager KM, Janke BH, et al. 2003. *J Clin Microbiol* 41:3198–3205.
Rohani P, Breban R, Stallknecht DE, et al. 2009. *Proc Natl Acad Sci USA* 106:10365–10369.
Saliki JT, Rodger SJ, Eskew G. 1998. *J Wildlife Dis* 34:834–838.
Sandbulte MR, Spickler AR, Zaabel PK, et al. 2015. *Vaccines (Basel)* 3:22–73.
Sandbulte MR, Gauger PC, Kitikoon P, et al. 2016. *Vaccine* 34:3773–3779.
Santhia K, Ramy A, Jayaningsih P, et al. 2009. *Influenza Other Respir Viruses* 3:81–89.
Scholtissek C, Burger H, Bachmann PA, et al. 1983. *Virology* 129:521–523.
Schrader C, Suss J. 2003. *Intervirology* 46:66–70.
Shaw ML, Palese P. 2013. Orthomyxoviridae. In Knipe DM, Howley PM, eds. *Fields Virology*, 6th ed. Alphen aan den Rijn: Wolters Kluwer; Lippincott Williams and Wilkins, pp. 1151–1185.
Shi WF, Gibbs MJ, Zhang YZ, et al. 2008. *Arch Virol* 153:211–217.
Shinde V, Bridges CB, Uyeki TM, et al. 2009. *N Eng J Med* 360:2616–2615.
Shinya K, Ebina M, Yamada S, et al. 2006. *Nature* 440:435–436.
Shortridge KF, Webster RG, Butterfield WK, et al. 1977. *Science* 196:1454–1455.
Sikkema RS, Freidl GS, De Bruin E, et al. 2016. *Euro Surveill* 21:30388.
Simon G, Larsen LE, Durrwald R, et al. 2014. *PLoS One* 9:e115815.
Slomka MJ, Densham AL, Coward VJ, et al. 2010. *Influenza Other Respir Viruses* 4:277–293.
Smith GJD, Vijaykrishna D, Bahl J, et al. 2009. *Nature* 459:1122–1125.
Solórzano A, Webby RJ, Lager KM, et al. 2005. *J Virol* 79:7535–7543.
Sorrell EM, Schrauwen EJ, Linster M, et al. 2011. *Curr Opin Virol* 1:635–642.
Spackman E, Senne DA, Myers TJ, et al. 2002. *J Clin Microbiol* 40:3256–3260.
Sreta D, Kedkovid R, Tuamsang S, et al. 2009. *Virol J* 6:34.
Su S, Qi WB, Chen JD, et al. 2012. *J Virol* 86:9542.
Suarez DL, Woolcock PR, Bermudez AJ, et al. 2002. *Avian Dis* 46:111–121.
Subbarao K, Murphy BR, Fauci AS. 2006. *Immunity* 24:5–9.
Sun L, Zhang G, Shu Y, et al. 2009. *J Clin Virol* 44:141–144.
Swenson SL, Vincent LL, Lute BM, et al. 2001. *J Vet Diagn Invest* 13:36–42.
Swenson SL, Foni E, Saito T, Brown I. 2015 Chapter 2.8.7: Influenza A virus of swine. In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2016. World Organisation for Animal Health (OIE), pp. 1–14. (Adopted by the OIE in 2015).
Takano R, Nidom CA, Kiso M, et al. 2009. *Arch Virol* 154:677–681.
Takemae N, Parchariyanon S, Damrongwatanapokin S, et al. 2008. *Influenza Other Respir Viruses* 2:181–189.
Takemae N, Harada M, Nguyen PT, et al. 2016. *J Virol* 91:e01490-16.
Tang Y, Lee CW, Zhang Y, et al. 2005. *Avian Dis* 49:207–213.
Taubenberger JK, Palese P. 2006. The origin and virulence of the 1918 'Spanish' influenza virus. In Kawaoka Y, ed. *Influenza Virology Current Topics*. Norfolk: Caister Academic Press, pp. 299–321.
Terebuh P, Olsen CW, Wright J, et al. 2010. *Influenza Other Respir Viruses* 6:387–396.
Thacker EL, Thacker BJ, Janke BH. 2001. *J Clin Microbiol* 39:2525–2530.
Tong S, Zhu X, Li Y, et al. 2013. *PLoS Pathog* 9:e1003657.
Top FH, Russell PK. 1977. *J Infect Dis* 136:S376–S380.
Van Poucke SGM, Nicholls JM, Nauwynck HJ, et al. 2010. *BMC Virology J* 7:38.
Van Reeth K. 2007. *Vet Res* 38:243–260.
Van Reeth K. 2013. Influenza pandemics: Does the greater threat come from pigs, not birds? *Pig J* 69:17–24.
Van Reeth K, Pensaert M. 1994. *Vet Rec* 135:594–597.
Van Reeth K, Nauwynck HJ, Pensaert MB. 1998. *J Infect Dis* 177:1076–1079.
Van Reeth K, Labarque G, De Clercq S, et al. 2001. *Vaccine* 19:4479–4486.
Van Reeth K, Van Gucht S, Pensaert M. 2002. *Viral Immunol* 15:583–594.
Van Reeth K, Gregory V, Hay A, et al. 2003a. *Vaccine* 21:1375–1381.
Van Reeth K, Van Gucht S, Pensaert M. 2003b. *Vet Rec* 153:9–13.

Van Reeth K, Brown I, Essen S, et al. 2004. *Virus Res* 103:115–124.
Van Reeth K, Labarque G, Pensaert M. 2006. *Viral Immunol* 19:373–382.
Van Reeth K, Brown IH, Dürrwald R, et al. 2008. *Influenza Other Respir Viruses* 2:99–105.
Van Reeth K, Mancera Gracia JC, Trus I, et al. 2017a. *NPJ Vaccines* 2:11.
Van Reeth K, Vincent L, Lager M. 2017b. Vaccines and vaccination for swine influenza: differing situations in Europe and the USA. In Swayne DE, ed. *Animal Influenza*, 2nd ed. Ames, IA: Wiley Publishing, pp. 480–501.
Van Riel D, Munster VJ, de Wit E, et al. 2010. *Am J Pathol* 171:1215–1223.
Vijaykrishna D, Poon LL, Zhu HC, et al. 2010. *Science* 328:1529.
Vijaykrishna D, Smith GJ, Pybus OG, et al. 2011. *Nature* 473:519–522.
Vincent LL, Janke BH, Paul PS, et al. 1997. *J Vet Diagn Invest* 2:191–205.
Vincent AL, Lager KM, Ma W, et al. 2006. *Vet Microbiol* 118:212–222.
Vincent AL, Lager KM, Janke BH, et al. 2008a. *Vet Microbiol* 126:310–323.
Vincent AL, Ma W, Lager KM, et al. 2008b. *Adv Virus Res* 72:127–154.
Vincent AL, Lager KM, Harland M, et al. 2009a. *PLoS One* 4:e8367.
Vincent AL, Ma W, Lager KM, et al. 2009b. *Virus Genes* 39:176–185.
Vincent AL, Swenson SL, Lager KM, et al. 2009c. *Vet Microbiol* 137:51–59.
Vincent AL, Ciacci-Zanella JR, Lorusso A, et al. 2010a. *Vaccine* 28:2782–2787.
Vincent AL, Lager KM, Faaberg KS, et al. 2010b. *Influenza Other Respir Viruses* 4:53–60.
Vincent AL, Ma W, Lager KM, et al. 2012. *J Virol* 86:10597–10605.
Wan H, Perez DR. 2006. *Virology* 346:278–286.
Watson SJ, Langat P, Reid SM, et al. 2015. *J Virol* 89:9920–9931.
Webby RJ, Webster RG. 2001. *Philos Trans R Soc Lond B Biol Sci* 356:1817–28.
Webby RJ, Swenson S, Krauss SL, et al. 2000. *J Virol* 74:8243–8251.
Webby RJ, Rossow K, Erickson G, et al. 2004. *Virus Res* 103:67–73.
Webster RG, Yakhno M, Hinshaw VS, et al. 1978. *Virology* 84:268–278.
Webster RG, Bean WJ, Gorman OT, et al. 1992. *Microbiol Rev* 56:152–179.
Weingartl HM, Berhane Y, Hisanaga T, et al. 2010. *J Virol* 84:2245–2256.
Welsh MD, Baird PM, Guelbenzu-Gonzalo MP, et al. 2010. *Vet Rec* 166:642–645.
Wood GW, Banks J, Brown IH, et al. 1997. *Avian Pathol* 26:347–355.
Worobey M, Han GZ, Rambaut A. 2014. *Proc Natl Acad Sci U S A* 111:8107–8112.
Wright PF, Webster RG. 2001. Orthomyxoviruses. In Knipe DM, Howley PM, Griffin DE, et al. (editors). *Field's Virology*, 4th ed. Philadelphia: Lippincott-Raven Publishers, pp. 1533–1579.
Wright SM, Kawaoka Y, Sharp GB, et al. 1992. *Am J Epidemiol* 136:488–497.
Wu Y, Tefsen B, Shi Y, Gao GF. 2014. *Trends Microbiol* 22:183–191.
Xu M, Huang Y, Chen J, et al. 2011. *Vet Microbiol* 147:403–409.
Yu H, Zhang GH, Hua RH, et al. 2007. *Biochem Biophys Res Commun* 356:91–96.
Yu H, Hua RH, Wei TC, et al. 2008a. *Vet Mic* 131:82–92.
Yu H, Hua RH, Zhang Q, et al. 2008b. *J Clin Microbiol* 46:1067–1075.
Yu H, Zhang PC, Zhou YJ, et al. 2009a. *Biochem Biophys Res Commun* 386:278–283.
Yu H, Zhou YJ, Li GX, et al. 2009b. *Virus Res* 140:85–90.
Zhang J, Gauger PC. 2014. *Methods Mol Biol* 1161:265–276.
Zhou NN, Senne DA, Landgraf JS, et al. 1999. *J Virol* 73:8851–8856.

37

Paramyxoviruses

Peter D. Kirkland, Alberto Stephano, and Hana M. Weingartl

Overview

The family *Paramyxoviridae* contains viral pathogens of international significance in most animal species and humans. Prior to the Menangle and Nipah virus outbreaks, there were no major paramyxovirus pathogens of pigs, the single possible exception being the porcine rubulavirus that causes blue eye (BE) disease (blue eye paramyxovirus [BEP]). However, BEP is confined to Mexico. During a 3-year period commencing in 1997, several new paramyxoviruses were identified, and two (Menangle and Nipah viruses) were found to be serious pathogens in both pigs and humans.

The family *Paramyxoviridae* encompasses a group of large (150–400 nm in diameter) pleomorphic viruses. The genome consists of a long single strand of RNA within a herringbone-like nucleocapsid. A lipid envelope that usually contains an outer fringe of surface projections or "spikes" surrounds the nucleocapsid.

Previously the family *Paramyxoviridae* included two subfamilies: *Paramyxovirinae* and *Pneumovirinae*. However, these two subfamilies were removed in the International Committee on Taxonomy of Viruses report released in 2016 (https://talk.ictvonline.org/taxonomy), and two families, *Paramyxoviridae* and *Pneumoviridae*, were proposed. There are seven genera in the family *Paramyxoviridae*: *Aquaparamyxovirus*, *Avulavirus*, *Ferlavirus*, *Henipavirus*, *Morbillivirus*, *Respirovirus*, and *Rubulavirus*. Also there are major pathogens of animals and humans in each of these genera: Newcastle disease virus of poultry in the genus *Avulavirus*, canine distemper and human measles viruses in the genus *Morbillivirus*, several parainfluenza viruses of animals and humans in the genus *Respirovirus*, and human mumps virus, BEP, and Menangle virus in the genus *Rubulavirus*. The genus *Henipavirus* was created for two of the most recently recognized and closely related paramyxoviruses, Hendra and Nipah, reflecting their morphological and genetic differences from other paramyxoviruses.

Because of their tissue tropisms, there are broad similarities in the diseases caused by paramyxoviruses. Typically, pathogenic paramyxoviruses are associated with diseases of the central nervous system (CNS) (canine distemper and Newcastle disease) and respiratory system (parainfluenza infections, Hendra, Nipah, and Newcastle disease). Some, especially Menangle virus and BEP, are also important reproductive pathogens.

The paramyxoviruses that cause significant disease in pigs are BEP, Menangle virus, and Nipah virus. There have been occasional reports of other paramyxoviruses associated with respiratory and CNS disease in pigs in Japan (Sasahara et al. 1954), Canada (Greig et al. 1971), Israel (Lipkind et al. 1986), and the United States (Janke et al. 2001; Qiao et al. 2010), but none has proven to be of significance. Recently porcine parainfluenza virus 1 (PPIV-1) has been identified in pigs in Hong Kong, China, and the United States and appears to be widespread (Lau et al. 2013; Palinski et al. 2016). Hendra virus is not known to have infected pigs naturally, but experimental infections caused clinical signs in pigs similar to Nipah virus (Li et al. 2010; Pickering et al. 2016). This chapter will provide an overview of BEP, Menangle, and Nipah viruses and the diseases they cause in pigs. The recently identified PPIV-1 will be briefly discussed.

Porcine rubulavirus (blue eye disease)

Relevance

BE is a disease of swine caused by infection with BEP (Stephano et al. 1988b) or porcine rubulavirus, also known as La Piedad-Michoacan virus (LPMV). BE was first reported in 1980 in central Mexico, with numerous outbreaks of encephalitis and corneal opacity in piglets (Stephano et al. 1982). A hemagglutinating

Diseases of Swine, Tenth Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson.

virus was isolated, characterized, and identified as a serologically distinct member of the family *Paramyxoviridae* (Stephano and Gay 1983, 1984, 1985a; Stephano et al. 1986b).

The first reported outbreak of BE was on a commercial farm with 2500 sows in La Piedad, Michoacán, Mexico (Stephano et al. 1982). Thereafter, BE was recognized as an important pathogen in central Mexico, with serological evidence of BEP in at least 16 Mexican states (Stephano et al. 1988b). The disease is still seen only in central Mexico and has never been reported outside of Mexico.

Etiology

Extensive molecular characterization of BEP (Berg et al. 1991, 1992; Sundqvist et al. 1990, 1992), combined with its morphology and biological properties, support its placement in the genus *Rubulavirus*.

BEP particles are similar to other paramyxoviruses, measuring 135–148 nm by 257–360 nm (Figure 37.1). The virion is pleomorphic, but usually more or less spherical. No filamentous forms have been observed. Nucleocapsids from disrupted virus particles have a diameter of 20 nm and a length of 1000–1630 nm (Figure 37.2) or more (Stephano and Gay 1985a).

In the laboratory, BEP replicates and produces cytopathology in a wide range of cell cultures from many different animal species, including both continuous cell lines and primary cell cultures. Changes consist of individual rounded cells, cytoplasmic vacuoles, and syncytium formation. Some cells also contain viral inclusion bodies (Moreno-Lopez et al. 1986; Stephano and Gay 1985a; Stephano et al. 1986a). The chick embryo also supports BEP replication.

BEP agglutinates erythrocytes from a wide range of animal species, as well as humans. Spontaneous elution occurs at 37 °C after 30–60 minutes. Hemadsorption of chicken erythrocytes has also been described (Stephano and Gay 1985a; Stephano et al. 1986b).

BEP is not known to share any antigens with other paramyxoviruses (Stephano et al. 1986b), but comparison of genome organization indicates that BEP is closely related to Mapuera virus from bats (Wang et al. 2007). Antigenic differences have been observed between different isolates of BEP (Sánchez-Betancourt et al. 2012).

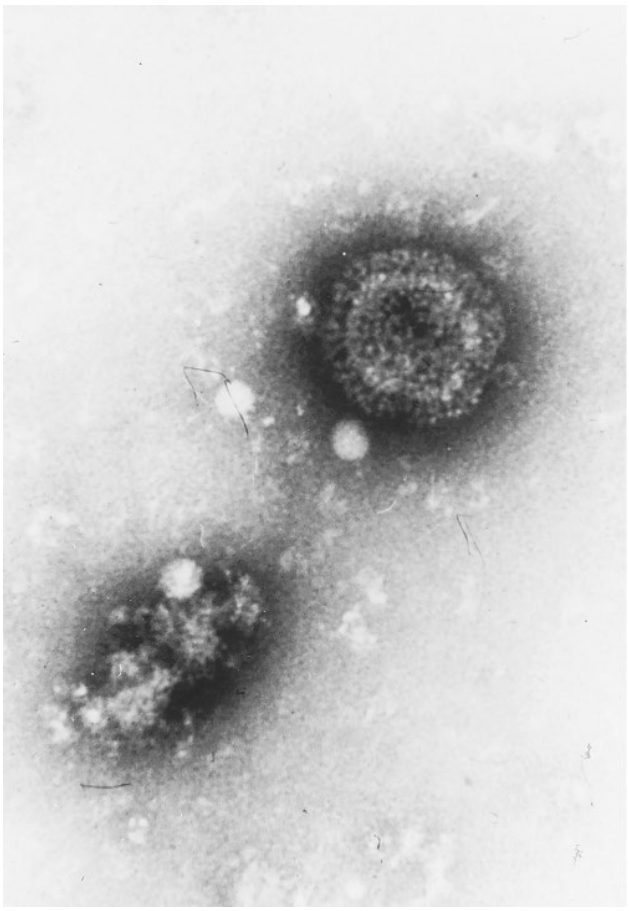


Figure 37.1 BE paramyxovirus particles showing surface projections by negative stain electron microscopy (×108,200).

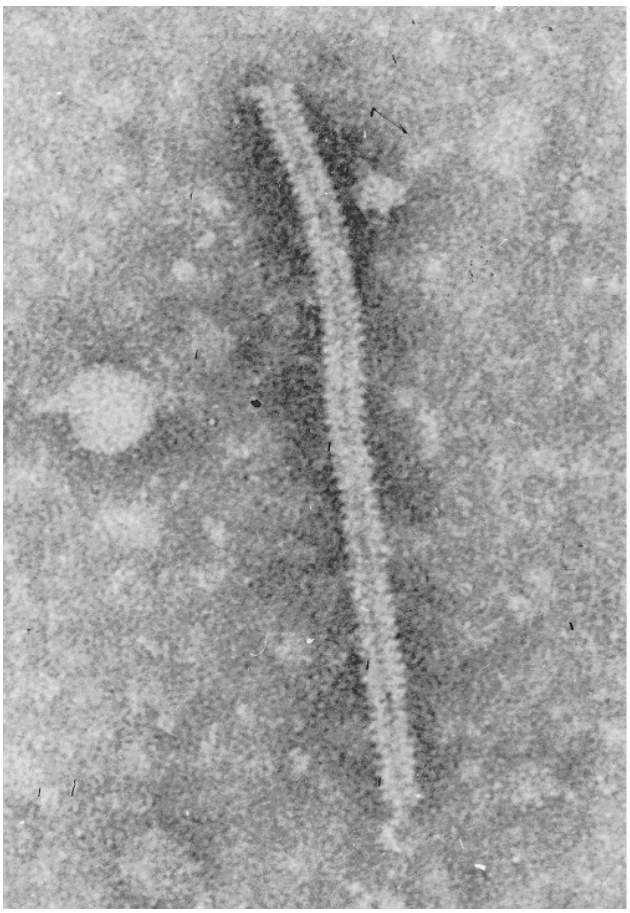


Figure 37.2 Fraction of a nucleocapsid from a disrupted BEP (negative stain electron microscopy; ×203,000).

Public health

Unlike some of the other paramyxoviruses, BEP does not have any public health significance and is not infectious for humans.

Epidemiology

Pigs are the only species known to be clinically affected by BEP following natural exposure. Experimentally, BEP affects mice, rats, and chick embryos. Rabbits, dogs, cats, and peccaries do not show clinical signs; rabbits, cats, and peccaries produce antibodies (Stephano and Gay 1985a; Stephano et al. 1988a).

Subclinically infected pigs from affected farms are the primary source of BEP. The virus is apparently transmitted by nose-to-nose contact between infected and susceptible pigs. Thus, BE was reproduced in susceptible pigs placed in contact with experimentally infected pigs as long as 19 days after inoculation.

The virus is mainly disseminated in nasal secretions and urine. Transmission through semen is possible, and virus can be recovered from semen, testis, epididymis, prostate, seminal vesicles, and bulbourethral glands 10–45 days after inoculation (Solís et al. 2007). The virus may also be disseminated by fomites (e.g. people and vehicles) and possibly by bats (Wang et al. 2007), birds, and wind. Other sources of infection have not been demonstrated.

The disease is self-limiting in closed herds. Sentinel pigs introduced into a farm 6–12 months after an outbreak did not seroconvert. Further, positive animals moved to seronegative herds did not spread the virus (Stephano and Gay 1986b; Stephano et al. 1986a). However, the disease can recur if a susceptible group of pigs is introduced into a farm and large farms with a continuous system of production may have cases periodically. Although there was evidence of persistence of BEP RNA in the brain, lung, lymph nodes, pancreas, and epididymis up to 57 and 277 days in experimentally infected pigs, neither infectious virus nor viral antigen was detected, even after immunosuppression (Cuevas et al. 2009; Wiman et al. 1998).

Seemingly, genetically and antigenically distinct strains of BEP can circulate simultaneously in swine populations. Three genetic variants of BEP were identified in central Mexico, and a new generation of viruses has apparently begun to emerge (Cuevas-Romero et al. 2016). Cross-neutralization studies suggested that the hemagglutinin–neuraminidase (HN) protein was not the only antigenic determinant participating in the antigenic changes detected among BEP strains (Sánchez-Betancourt et al. 2012).

Pathogenesis

It is believed that BEP infection is acquired by inhalation. Experimentally, intratracheal or intranasal exposures are effective routes of infection. The initial site of BEP replication is the nasal mucosa and tonsils. Thereafter, BEP spreads quickly to the brain through the olfactory and trigeminal nerves and subsequently to other organs by viremia. Dissemination by viremia is indicated by the appearance of interstitial pneumonia and isolation of BEP from blood and various organs in either naturally or experimentally infected pigs (e.g. brain, lung, tonsil, liver, turbinate, spleen, kidney, mesenteric lymph node, heart, pancreas, ovary, etc.) (Allan et al. 1996; Stephano and Gay 1983; Stephano et al. 1988b). Variation in the viral neuraminidase is associated with differences in virulence among BEP isolates (Sánchez-Betancourt et al. 2008).

CNS manifestations occur early in the disease. Nervous signs have been induced in 1-day-old piglets 20–66 hours after inoculation, weaned pigs (21–50 days old) developed a nervous syndrome at 11 days post inoculation (DPI), and pregnant sows or gilts developed reproductive failure when inoculated during pregnancy after the virus crossed the placenta and reached the fetuses.

Corneal opacity may occasionally be observed in these cases. The cause of the corneal opacity is unknown, but anterior uveitis is commonly observed microscopically in the cornea (Stephano and Gay 1986b). The opacity usually occurs late in the course of the disease and is thought to be due to an immunological reaction similar to that observed in dogs after adenovirus-induced hepatitis.

Infection of pregnant sows and gilts results in reproductive failure due to embryonic mortality. BEP virus can cross the placenta. In the first one-third of gestation, affected sows usually return to estrus. When infection occurs later in gestation, the result is stillbirths and fetal mummification. Abortion can also occur (Hernández-Jáuregui et al. 2004; Stephano and Gay 1984).

Intranasal inoculation of young boars results in inflammation and edema of the testis and epididymis within 15 days of exposure. By 30 days, there is necrosis of the seminiferous tubules and rupture of the epithelial wall of the epididymis with leakage of spermatozoa from the lumen, leading to abscess formation. Boars sacrificed 80 days after infection showed fibrosis and granuloma formation in the epididymis, as well as testicular atrophy (Ramirez et al. 1995).

Clinical signs

An outbreak of BE may start in any area of a pig farm, but is usually first observed in the farrowing house, with CNS signs and high piglet mortality. At about the same time, corneal opacity may be observed in some weaned or fattening pigs (Stephano and Gay 1985a, 1986a; Stephano et al. 1988a). Mortality increases rapidly and then declines within a short time. Once the initial outbreak

is over, no new clinical cases appear, unless susceptible pigs are introduced into the population. Clinical signs are variable and depend primarily on the age of the pig. However, corneal opacity, the sign that gives the disease its name, frequently occurs in pigs of all ages without other signs and resolves spontaneously.

During an outbreak, about 20% of the litters farrowed are affected. In these litters, piglet morbidity is 20–50%, and mortality in affected piglets is about 90%. In the first cases observed, piglets usually died within 48 hours of the appearance of clinical signs, but in later cases, death occurred after 4–6 days. During an initial outbreak, deaths occur for 2–9 weeks, depending on the management system. Most of the sows suckling affected litters are clinically normal. Some show moderate anorexia 1 or 2 days before the appearance of clinical signs in piglets. Corneal opacity has also been observed in the farrowing house during outbreaks.

Piglets 2–15 days old are the most susceptible and the onset of clinical signs is sudden. Healthy piglets may suddenly become prostrate, generally in lateral recumbency, or show nervous signs. However, the disease usually runs a course that starts with fever, rough hair coat, and an arched back, sometimes accompanied by constipation or diarrhea. These signs are followed by progressive nervous signs, including ataxia, weakness, rigidity (mainly of the hind legs), muscle tremor, and abnormal posture, such as a sitting position. Anorexia does not occur as long as piglets can still walk. Some piglets are hyperexcitable and may squeal and show paddling movements when handled. Other signs include lethargy, with some involuntary movements, dilated pupils, apparent blindness, and, occasionally, nystagmus. Some piglets suffer from conjunctivitis, with swollen eyelids and lacrimation. Often the eyelids are closed and adherent with exudate. In up to 10% of affected piglets, either unilateral or bilateral corneal opacity is present.

Pigs older than 30 days show moderate and transient clinical signs such as anorexia, fever, sneezing, and coughing. Nervous signs are less common and less obvious but, when present, consist of listlessness, ataxia, circling, and, rarely, swaying of the head. Unilateral or bilateral corneal opacity and conjunctivitis continue to appear on the farm for another month without other signs. Fewer than 2% of pigs older than 30 days are affected, and the mortality is generally low. Outbreaks with 20% mortality and severe CNS manifestations have been observed in 15–45 kg pigs. Corneal opacity was present in up to 30% of these pigs (Stephano and Gay 1985b).

In pregnant sows, reproductive failure lasting 2–11 months (usually 4 months) is observed. Reproductive signs during outbreaks include an increase in the number of animals returning to estrus, a reduction in farrowing rate, and an increase in the weaning-to-service interval and nonproductive sow days. The rate of stillbirths and mummified fetuses also increases, and, consequently, there is a reduction in the number of pigs born alive. Later, there is also a reduction in the total number of pigs born. Abortion is not a cardinal feature, but has sometimes been observed during an acute outbreak. Gilts and other adult pigs occasionally develop corneal opacity.

Boars, like other adult animals, generally do not show clinical signs, but mild anorexia and corneal opacity have been reported. Semen evaluation demonstrated that about 30% of boars in herds infected with BEP showed temporary or permanent infertility, with a decrease in concentration, an increase in abnormalities, and a decrease in motility and viability of spermatozoa. In some boars there is azoospermia, and the ejaculate becomes clear and resembles coconut water. Some boars develop swollen testicles. The testis and epididymis become turgid with marked edema. Later, some develop a granular texture and most atrophy (generally unilateral) or become soft and flabby with, or without, granular epididymitis. Boars with severe lesions lose libido (Campos and Carbajal 1989; Stephano et al. 1990).

Differences in clinical signs became evident a few years after the virus was discovered. In 1980, piglets were primarily affected. Mortality and CNS disorders in pigs older than 30 days were uncommon. In 1983, severe outbreaks of encephalitis with high mortality in pigs weighing 15–45 kg were observed on badly managed farms, always with concomitant viral and bacterial diseases (Stephano and Gay 1985b, 1986a). Also in 1983, reproductive failure in sows and transient infertility in boars were identified (Stephano and Gay 1984, 1985a). In 1988, severe orchitis, epididymitis, and testicular atrophy in boars became evident (Campos and Carbajal 1989; Stephano et al. 1990).

Lesions

There are no specific gross changes in cases of BE. In piglets, a mild pneumonia is frequently observed at the ventral tips of the cranial lung lobes. There is mild distension of the stomach with milk, distension of the urinary bladder, and a slight accumulation of peritoneal fluid with fibrin. The brain is often congested and there is an increase in the quantity of cerebrospinal fluid. Conjunctivitis, chemosis, and varying degrees of corneal opacity (Figure 37.3), usually unilateral, are observed. Vesicle formation, ulcers, and keratoconus have been observed in the cornea, as well as exudate in the anterior chamber. Pericardial and renal hemorrhages are occasionally observed (Stephano and Gay 1985a, 1986b).

Boars develop swollen testicles and epididymes. These changes are frequently unilateral. Orchitis, epididymitis, and,

Figure 37.3 Corneal opacity in a 7-day-old piglet.

later, atrophy of the testicle, with or without granulomatous formation, in the epididymis are observed. Hemorrhages are occasionally observed in the tunica albuginea, epididymis, or testis (Campos and Carbajal 1989; Ramirez et al. 1995; Stephano et al. 1990).

The main histological changes are seen in the brain and spinal cord. These reflect a nonsuppurative encephalomyelitis affecting mainly the gray matter of the thalamus, midbrain, and cerebral cortex and include a multifocal and diffuse gliosis, perivascular cuffing with lymphocytes, plasma cells, and reticular cells, neuronal necrosis, neuronophagia, meningitis, and choroiditis (Ramirez and Stephano 1982). Intracytoplasmic inclusion bodies are found in neurons (Stephano and Gay 1986b; Stephano et al. 1988a).

The lungs have localized areas of interstitial pneumonia characterized by thickened septa with mononuclear cell infiltration.

Changes in the eye are mainly corneal opacity, characterized by corneal edema and anterior uveitis. Neutrophils, macrophages, or mononuclear cells infiltrate the iridocorneal endothelium, corneoscleral angle, and cornea (Stephano and Gay 1986b; Stephano et al. 1988a).

In boars, the affected testes show degeneration and necrosis of the germinal epithelium. The interstitial tissue shows Leydig cell hyperplasia, mononuclear cell infiltration, hyalinization of the vascular wall, and fibrosis. The epididymis shows vesicle formation, loss of epithelial cilia, rupture of the epithelial wall, presence of spermatozoa in the intertubular space, and severe infiltration of inflammatory mononuclear cells with macrophage phagocytosis of fragmented sperm. Fibrosis and spermatic granuloma are organized.

Diagnosis

Clinical signs such as encephalitis, corneal opacity, and reproductive failure in the sow and orchitis and epididymitis in the boar are consistent with a diagnosis of BE. Histological lesions, such as nonsuppurative encephalitis, anterior uveitis, keratitis, orchitis, and epididymitis, provide additional diagnostic evidence. The presence of intracytoplasmic inclusions in neurons and corneal epithelium in conjunction with these clinical signs and histological findings provides strong support for a diagnosis of BE.

Other causes of encephalitis and reproductive disease must also be considered, e.g. pseudorabies (Aujeszky's disease) virus and porcine reproductive and respiratory syndrome virus (PRRSV). Only BEP produces corneal opacity along with orchitis and epididymitis in boars (Campos and Carbajal 1989; Stephano and Gay 1985b; Stephano et al. 1988a, 1990).

Paired serum samples (15 days apart) are recommended for serological confirmation by hemagglutination inhibition (HI), virus neutralization (VN), or ELISA. HI is the most frequently used test, but false-positive titers up to 1/16 have been detected when chicken erythrocytes were used or when the antigen was grown in chicken embryos (Ramirez et al. 1996). Therefore, bovine erythrocytes are recommended. Naturally infected pigs develop antibodies that usually persist for life.

The brain is the best tissue for virus isolation and antigen detection, but the lung and tonsil are also suitable (Stephano et al. 1988a). PK-15 cells or primary pig kidney cells are preferred for virus isolation. Virus replication induces syncytium formation. Direct immunofluorescence and polymerase chain reaction (PCR) have been used to detect virus in tissue sections and monolayers (Cuevas et al. 2009; Stephano and Gay 1985a; Stephano et al. 1988a). Quantitative real-time polymerase chain reaction (qRT-PCR) assays are more sensitive and detect either the phosphoprotein gene or the nucleoprotein gene across all strains of BEP (Rivera-Benitez et al. 2013).

Prevention and control

As with most viral diseases of swine, there is no specific treatment. Pigs with corneal opacity frequently recover spontaneously, whereas pigs with central nervous signs generally die. Antimicrobial therapy is commonly used to treat and prevent secondary infections. At present, there are two commercial inactivated virus vaccines approved for use in pregnant sows, gilts, boars, and piglets. However, some data indicate that a monovalent vaccine will not completely protect against the different antigenic subtypes (Escobar-Lopez et al. 2012).

Herd health programs are the most reliable method of preventing the introduction of BEP into a farm. Replacements should be selected from a healthy herd and quarantined prior to introduction. Standard biosecurity measures provide insurance against infection (e.g. perimeter fencing, separate load-out areas, changing rooms and showers, wildlife control [birds, rats, and mice], prompt waste removal and disposal of dead pigs, control of the movement of personnel, visitors, and vehicles). Serological screening of replacement animals is recommended.

Elimination of BEP from infected herds has been accomplished by management practices (e.g. herd closure, cleaning, and disinfecting, all-in/all-out production, elimination of clinically affected animals, and disposal of dead pigs). Serological testing of the population and sentinel animals (BEP seronegative pigs) should be used to confirm the elimination of BEP (Stephano et al. 1986b).

Menangle virus

Relevance

Menangle virus was first identified during a disease outbreak in New South Wales, Australia, in 1997 and was subsequently eradicated from the affected farms. The virus causes reproductive disease and congenital defects in pigs, occasionally causes moderately severe disease in humans, and has fruit bats (*Pteropus* sp., flying foxes) as a reservoir host. Although there are no records of the occurrence of disease in any other country, bats in Papua New Guinea are known to carry the virus (Breed et al. 2010).

Etiology

Like BEP, Menangle virus is a member of the genus *Rubulavirus* within the family *Paramyxoviridae* (Bowden et al. 2001). Other well-known viruses in this genus are human rubulavirus 2 (formerly human parainfluenza virus type 2), human rubulavirus 4 (formerly human parainfluenza virus type 4), and human mumps virus.

Menangle virus has typical paramyxovirus morphology. Virions are pleomorphic with both spherical and elongated forms that range in size from 100 to 350 nm. Virions possess a single layer of surface spikes approximately 17 nm in length. Ruptured particles reveal long herringbone-shaped nucleocapsids of approximately 19 nm in diameter (Philbey et al. 1998).

Menangle virus induces pronounced cytopathology in cell culture, including prominent vacuolation of cells and the development of large syncytia. The virus replicates and produces cytopathology in a wide range of cell types from many animal species, including birds and fish. There is no evidence of hemadsorption or hemagglutinating activity (Philbey et al. 1998). Menangle virus is not related antigenically to any other paramyxovirus.

Public health

In contrast to Nipah virus, Menangle virus does not appear to be highly infectious for humans. However, care should be taken when working with potentially infected pigs or suspect reproductive specimens. While only 2 out of more than 30 humans directly exposed to infected pigs were infected with the virus, both experienced a severe febrile illness associated with a macular rash, followed by prolonged debility (Chant et al. 1998). There was no evidence of infection in a large number of other people, including veterinarians, abattoir workers, and laboratory workers, who had less direct and less protracted contact with potentially infective material. Transmission to humans may require the contamination of cuts and abrasions with infectious body fluids or tissues or possibly splashing of material onto the conjunctivae (Chant et al. 1998).

Epidemiology

Studies of archival and newly collected sera suggested that Menangle virus was not highly contagious among the pigs on the affected farm (Kirkland et al. 2001). This was deduced by the relatively slow spread of infection in a building that contained pens of sows (i.e. it took several weeks for all of the sows to become infected). Nevertheless, the virus was widely dispersed through the pig population on the affected farm. About 6 months after the estimated time of entry of the virus to the farm, a high proportion (>90%) of sera collected from pigs of all ages contained high levels of virus-neutralizing (VN) antibody. Positive VN antibody titers ranged from 1/16 to 1/4096 and remained high for at least 2 years after infection. In contrast, all samples collected prior to the estimated time of entry of the virus into the pig population were negative. All serum samples collected at the two grower–fattening farms were positive (Kirkland et al. 2001). Testing of 1114 swine sera from other pig farms throughout Australia indicated that infection was confined to the affected pig farm and the two associated grow-out facilities.

Following the initial spread of the infection through the herd, the virus was maintained by infection of young pigs at about 10–12 weeks of age (i.e. as they lost the protection provided by maternally derived antibodies). In a large pig population, the constant availability of susceptible animals was sufficient to ensure persistence of the virus. In smaller pig herds, such persistence would be much less likely. Almost all selected replacement breeding

pigs on the farm had been exposed to the virus and were seropositive before mating at around 28–30 weeks of age, preventing further reproductive failure.

It appeared that close contact between pigs was required for spread of infection and that the virus did not survive in the environment for long. Susceptible sentinel pigs moved into an uncleaned area occupied 3 days previously by infected pigs did not become infected.

It is believed that flying foxes are a reservoir of Menangle virus (Kirkland et al. 2001; Philbey et al. 1998). During the summer–autumn period, when the virus was thought to have entered the pig farm, there was a large breeding colony of gray-headed fruit bats (*Pteropus poliocephalus*), as well as little red fruit bats (*P. scapulatus*), roosting within 200 m of the affected pig farm. Sera collected from gray-headed fruit bats in this colony had VN antibodies to Menangle virus. A more extensive study of sera collected from several species of fruit bats in various locations in Australia found that approximately one-third were seropositive, with VN titers ranging from 1/16 to 1/256. Positive samples were found in gray-headed fruit bats, black fruit bats (*P. alecto*), and spectacled fruit bats (*P. conspicillatus*), but not in little red fruit bats. These results indicated that Menangle virus was endemic in the fruit bat population and preceded the infection in pigs. Subsequently, seropositive fruit bats have been detected in Papua New Guinea (Breed et al. 2010), and Menangle virus has been isolated from the urine of fruit bats (Barr et al. 2012).

Except for spread to humans, there is no evidence that this virus has spread naturally to other animal species. Samples collected from rodents, birds, cattle, sheep, cats, and a dog in the vicinity of the affected pig farm were all seronegative.

Pathogenesis

The route of transmission of Menangle virus and the mechanism of spread are not known, although fecal–oral or urinary–oral transmission is suspected (Love et al. 2001). Experimental transmission studies in weaned pigs have shown that virus is shed in both feces and urine (Bowden et al. 2012). Virus was detected longer in urine than in any other source. Infection in pigs appears to be of short duration (10–14 days) (Bowden et al. 2012) and results in strong immunity. Virus was not detected in surviving piglets born during the outbreak, suggesting that persistent infection is unlikely. There is also strong circumstantial evidence that adult pigs do not become persistently infected.

The principal cause of reproductive loss associated with Menangle virus appears to be *in utero* infection, often resulting in fetal death. In many sows, there was early death of the whole litter, resulting in a delayed return to estrus or sometimes a state of pseudopregnancy.

Figure 37.4 Litter of piglets affected by Menangle virus.

At parturition, affected litters sometimes contained piglets of varying size and with a range of abnormalities. Some piglets were mummified and were of different gestational ages, some piglets were stillborn and had congenital malformations, and there were a few normal piglets (Figure 37.4). These findings indicated that as with parvovirus, transplacental infection of a few fetuses can occur early in gestation followed by progressive spread of the virus from fetus to fetus within the uterus. The teratogenic defects observed are the direct result of virus replication and cell destruction in rapidly developing fetal tissues.

Clinical signs

To date, there has only been one known disease outbreak due to Menangle virus in pigs (Love et al. 2001; Philbey et al. 1998). In 1997, over a 5-month period (mid-April to early September), sows in a 3000-sow, intensive farrow-to-finish pig farm near Sydney, New South Wales, Australia, experienced severe reproductive failure (Love et al. 2001). There was a marked increase in the incidence of mummified fetuses and stillborn piglets. After

a period, some of the stillborn piglets were born with severe malformations. Sows in all four breeding units on the farm were affected. There were some weeks when the farrowing rate decreased from an expected 82% to as low as 38%. Many sows showed delayed returns to estrus at approximately 28 days after mating, while others remained in a state of pseudopregnancy until more than 60 days post mating. The disease occurred sequentially in all four breeding units at the pig farm, affecting the progeny of sows of all parities. In the weeks of low farrowing rates, up to 45% of sows farrowed litters with reduced numbers of live piglets and an increase in the proportion of mummified and stillborn piglets, some of which had congenital deformities.

Individual litters contained mummified fetuses of varying size, ranging upward in gestational age from 30 days, together with stillborn piglets (some with malformations) and a few normal piglets (Figure 37.4). Teratogenic defects including arthrogryposis, brachygnathia, and kyphosis were frequently seen in stillborn piglets, and there were occasional cases of artiodactyla (Love et al. 2001). The cranium of some piglets was slightly domed.

Although the virus was also detected on two associated growing farms, there were no breeding animals held on these farms, and no clinical disease was recognized. Virus had apparently spread to these farms (separated from the main farm and each other by several hundred kilometers) when young growing pigs were moved. There were no clinical signs evident in growing pigs of any age, and the only clinical signs in sows on the main farm were those associated with reproductive failure. No clinical signs were observed in weaner aged pigs after experimental infection (Bowden et al. 2012). It is not known whether Menangle virus can be spread in the semen of acutely infected boars.

Following the isolation of Menangle virus, two seropositive workers were identified (Chant et al. 1998). During subsequent medical investigations, it was found that both had experienced a severe febrile illness with headache. Extensive testing failed to identify any other possible cause of the illness, and it was concluded that the disease was due to Menangle virus infection (Chant et al. 1998). Both workers recovered fully after a prolonged period of convalescence.

Lesions

Affected litters usually consist of a mixture of mummified fetuses, autolyzed and fresh stillborn piglets, and a few normal live piglets (Love et al. 2001; Philbey et al. 1998). Congenital defects, including arthrogryposis, brachygnathia, kyphosis, and, occasionally, artiodactyla, are only seen in dead piglets. Affected stillborn piglets frequently have slight to severe degeneration of the brain and spinal cord (Figure 37.5). Gross defects ranging from

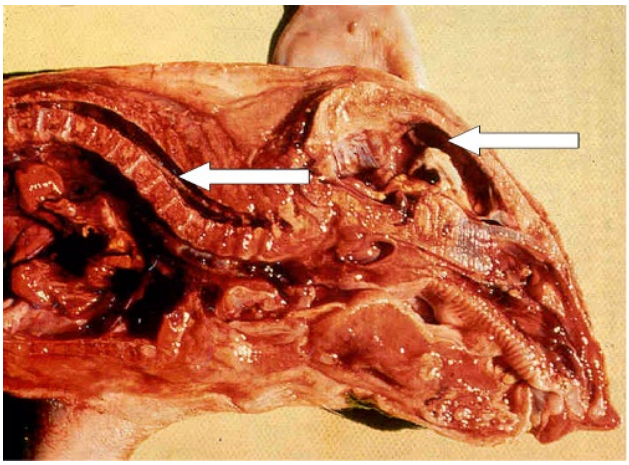


Figure 37.5 CNS abnormalities due to *in utero* Menangle virus infection.

porencephaly to hydranencephaly are most common in the cerebrum. Occasionally, there may be fibrinous body cavity effusions and pulmonary hypoplasia.

Histological changes are most marked in the CNS (Love et al. 2001; Philbey et al. 1998). There is extensive degeneration and necrosis of gray and white matter of the brain and spinal cord associated with infiltrations of macrophages and other inflammatory cells. Intranuclear and intracytoplasmic inclusion bodies may be observed in neurons of the cerebrum and spinal cord. These bodies are eosinophilic to amphophilic and consist of aggregates of nucleocapsids. Nonsuppurative multifocal meningitis, myocarditis, and, occasionally, hepatitis may be present in some cases.

Diagnosis

Menangle virus has only been detected in the 1997 outbreak; thus most pig populations should be fully susceptible. A marked reduction in normal live piglets at farrowing and a number of stillborn piglets with teratogenic defects is suggestive of Menangle virus infection. The most rapid method of excluding Menangle virus infection is to test affected sows for the presence of specific antibody to the virus or to test tissues from affected piglets in a qRT-PCR assay.

At farrowing, litters with mummified fetuses of varying sizes together with stillborn piglets are indicative of *in utero* viral infection. By far the most common cause of similar losses is porcine parvovirus, but a variety of other viral infections, including encephalomyocarditis virus, pestiviruses (e.g. classical swine fever virus and Bungowannah virus), pseudorabies (Aujeszky's disease) virus, Japanese encephalitis virus, porcine reproductive and respiratory syndrome (PRRS) virus, and porcine rubulavirus (BEP), may cause significant fetal death. A feature that distinguishes Menangle virus infection from all but Japanese encephalitis virus infection is the presence of

congenital malformations in piglets. However, it should be noted that these are only evident in approximately one-third of affected litters. In addition, many of these other viral infections cause disease in both piglets and adults. BEP is the only other paramyxovirus to cause significant fetal loss as a presenting sign but differs from Menangle virus in that neurological and other signs are usually observed in young piglets and the virus can be readily distinguished by its ability to agglutinate erythrocytes from mammals and birds (Moreno-Lopez et al. 1986). Reproductive disease is not a major feature of infection with Nipah virus.

For laboratory diagnosis, fetal specimens should be collected for virus detection by either PCR or virus isolation in cell culture, serology, and pathology. Virus can be isolated from a number of organs from stillborn piglets, especially the brain, lung, and myocardium. A wide range of cell cultures supports replication of Menangle virus, but baby hamster kidney cells (BHK-21) have been used for the isolation of the virus from field specimens. Three to five passages may be necessary before suggestive cytopathology is observed. As the virus does not hemagglutinate, identification will depend on electron microscopy, neutralization of an isolate with specific antiserum, or testing by a Menangle virus specific qRT-PCR assay (Bowden et al. 2012). This qRT-PCR assay has also been used for the direct detection of Menangle virus in tissues, swabs of mucosal surfaces, and various excretions. Specific antibodies may be detected in body cavity fluids of some stillborn piglets.

Prevention and control

There is no specific treatment for Menangle virus. By the time clinical signs are observed, the virus is likely to be widely disseminated in the affected population, negating measures to limit spread.

Fruit bats (Megachiroptera) are the primary source of infection for pig populations. Megachiroptera are not found in North America, but are present in Africa, the Middle East, southern Asia, Australia, and many Pacific Islands. It is not known whether small bats (Microchiroptera) are susceptible to Menangle virus, but it is important to restrict direct and indirect contact between pigs and bats to prevent introduction of this virus to pigs. Fruit bats do not normally enter pig farm buildings, but they defecate and urinate during flight over and around buildings and occasionally inadvertently drop their young in flight. Cover all outside areas (e.g. outside walkways) to prevent contamination of the facilities and infection of the herd. Flowering trees and fruiting trees should not be grown in the immediate vicinity of pig farm buildings, as these may attract fruit bat activity.

In an outbreak of reproductive disease, the infection will probably have already spread through the entire population of a pig farm by the time the first affected litters are farrowed. In small piggeries, there would be insufficient numbers of susceptible animals available to maintain a cycle of infection, as there is no carrier state and, unlike parvovirus, environmental survival of this virus is poor. In large piggeries, infection may become endemic, with the infection being maintained in groups of pigs as they lose their maternally derived protection. In such a situation, it is important to maximize the opportunity for infection of all selected replacement breeding stock prior to mating.

The eradication of Menangle virus from an endemically infected pig population can be achieved by moving all the age groups in which infection is active (e.g. pigs between 10 and 16 weeks of age) to another site (Love et al. 2001). If affected facilities are cleaned, vacated for a few weeks, and then restocked with unexposed pigs or pigs known to be immune to the virus, the cycle of endemic infection in the herd should be broken and the virus eliminated naturally.

Nipah virus

Relevance

Nipah virus is a zoonotic virus that first caused a major epidemic in pigs in 1998–1999 in a relatively small geographic area in Southeast Asia, but has since been detected in other countries in Asia. In this instance, it is believed that the virus jumped from a wildlife reservoir to domestic pigs and then spread to humans and to other domestic animals including cats, dogs, and horses. The virus was not contagious in cats or dogs, but was associated with a high case fatality rate. Nipah virus poses a continuing threat in countries where pteropid bats are present.

Etiology

Nipah virus and the related Hendra virus are negative-stranded RNA viruses in the family *Paramyxoviridae*, genus *Henipavirus* (Chua et al. 2000). Two genotypes of Nipah virus have been identified to date: genotype Malaysia and genotype Bangladesh (Lo et al. 2012). Nipah is a large pleomorphic virus similar to most paramyxoviruses. Virus particles vary in size, but their average diameter is 500 nm. Surface spikes on the envelope are approximately 10 nm in length. The typical long "herringbone- shaped" nucleocapsids have a diameter of approximately 21 nm and an average length of 1.67 µm.

Nipah virus replicates readily in several continuous cell lines, especially Vero and BHK-21, and produces

cytopathic effects (CPE) in 3–4 days. In Vero cells, virus replication induces large syncytia in which the cell nuclei are arranged around the periphery of the multinucleated cell (Daniels et al. 2002). Antigenically, Nipah virus is closely related to Hendra virus, and diagnostic reagents for Hendra virus have been utilized to assist Nipah virus investigations.

Public health

Nipah virus presents a serious threat to public health. Although the first recognized outbreak of Nipah virus was confined to a relatively small geographical area, it resulted in numerous human infections and deaths in pig farmers and other people who had close contact with pigs, including abattoir workers in a neighboring country. Later, human disease outbreaks without the known involvement of pigs occurred in India and Bangladesh and in 2014 in the Philippines, where horses were identified as the source of human infection (Ching et al. 2015; Luby and Gurley 2012). If there is a risk of an outbreak, it is essential to prevent the movement of all animals and minimize human contact with potentially infected animals.

Epidemiology

There is strong evidence that fruit bats mainly from the genus *Pteropus* are a reservoir of Nipah virus (Luby and Gurley 2012). The disease outbreak observed in Malaysia is assumed to be the result of the virus "jumping" from bats into farmed domestic pigs (Chua et al. 2000). Observations made during the outbreak in 1998–1999 in Malaysia indicated that Nipah virus was highly contagious among swine (Mohd Nor et al. 2000). Direct, and possibly airborne, exposure to secretions from infected animals was the presumed mode of transmission of Nipah virus among pigs. This was supported by detection of the virus in the epithelium of the upper and lower respiratory tract, as well as airways and oral/nasal swabs. The pattern of human infection suggested spread via sputum or large droplets rather than by fine aerosol. Coughing, a frequent clinical sign in infected pigs, or loud squealing would facilitate such a mode of transmission. Contact with infected animals and subcutaneous, oral, and nasal routes of inoculation may result in infection (Hooper et al. 2001; Middleton et al. 2002; Weingartl et al. 2006).

During the outbreak in Malaysia, Nipah virus infected other domestic animals. Large numbers of dogs died on infected farms, and clinically affected dogs were identified during investigations of outbreaks on swine farms (Chua et al. 2000; Daniels et al. 2000). Nipah virus was isolated from the kidney of a clinically affected dog (Chua et al. 2000), which suggests that urinary excretion by domestic carnivores may be a mode of spread, but there was no evidence of lateral transmission between dogs (Asiah et al. 2001). Cats were also reported by farmers to have been affected. Under experimental conditions, cats were susceptible to infection and excreted Nipah virus in urine (Muniandy 2001).

In Malaysia, the movement of infected pigs was the primary means by which Nipah virus spread between farms, between states, and internationally to Singapore, where abattoir workers processing Malaysian pigs became infected (Mohd Nor et al. 2000; Paton et al. 1999). The outbreak probably originated from a point source (Lye et al. 2001), implying that the virus moved from its wildlife reservoir on only one occasion.

Human infection was associated with close contact with infected pigs (Parashar et al. 2000). Feeding or handling pigs, assisting with farrowing, treatment, and removal of sick or dead pigs were most likely to result in infection. Living on an infected pig farm was not a significant risk factor.

Later outbreaks in Bangladesh and India had higher case fatality rates than the first outbreak in Malaysia and Singapore, and human-to-human transmission of the virus was reported. Significantly, drinking date palm sap, apparently contaminated by flying foxes, was associated with some of the index cases, while contact with pigs was not reported (Luby and Gurley 2012).

The more recent outbreak of Nipah virus in the Philippines was presumed to be a result of Nipah virus transmission from bats to horses and from sick horses to humans. Horse slaughter and consumption of horse meat were considered to be the main mode of transmission to humans, and human-to-human transmission occurred in 30% of cases. Furthermore, cats that were fed meat from affected horses were also fatally infected (Ching et al. 2015).

Pathogenesis

Pathogenesis studies with Nipah virus in swine have been limited to pigs 4–10 weeks of age due to the constraints of working under Biosafety Level 4 (BSL-4) conditions. Nipah virus primarily targets three systems: vascular, nervous, and lymphoreticular. Upon oronasal inoculation, Nipah virus infects a number of cell types present in the oronasal mucosa: epithelial cells, cells of the immune system, and, presumably, extracellular peripheral nerve endings of the cranial nerves, leading to direct invasion of the brain in some animals (Weingartl et al. 2005). Endothelial cells of small blood and lymph vessels are an early characteristic target for Nipah virus. Infection of endothelial cells and cells of the immune system leads to viremia and subsequent spread of Nipah virus, as the virus appears to prefer endothelial cells of specific organs and tissues (Meisner et al. 2009). Infection

of endothelial cells results in vasculitis and recruitment of immune cells that can be also infected (Berhane et al. 2008). The virus productively infects monocytes, NK cells, and CD8+ T lymphocytes (Stachowiak and Weingartl 2012), and viral antigen was detected in macrophages and dendritic cells. In addition, lymphocyte necrosis and depletion were observed, especially in lymph nodes. Reduction of specific populations of immune cells may be a contributing factor in secondary infections (Berhane et al. 2008). In addition, infiltration of infected immune cells into parenchyma, as well as direct infection of cells adjacent to the endothelial cells, further increases the virus load in tissues. Thus, Nipah virus can alternatively infect the CNS by crossing the blood–brain barrier (Weingartl et al. 2005).

Infection of the lower respiratory tract most likely occurs by viremia and the associated vascular damage and infiltration of infected mononuclear cells. However direct infection of epithelial cells due to inhalation of the virus, or spread of the virus along the respiratory epithelium from the upper respiratory tract, or a combination of all routes cannot be excluded.

Clinical signs

Nipah virus differs from most paramyxoviruses in that it causes a severe, often fatal disease in a number of species. Disease and death in people may be the first indication of an outbreak. Nipah virus infection of humans presents as an encephalitis and/or severe respiratory disease. A full clinical description of Nipah virus-induced disease in humans has been provided elsewhere (Chua et al. 1999; Goh et al. 2000; Hossain et al. 2008).

Nipah virus infection in pigs may be subclinical or result in acute febrile disease with respiratory and/or CNS signs, for which reason it was initially termed "porcine respiratory and encephalitis syndrome." Clinical signs of Nipah virus infection in pigs vary by age (Mohd Nor et al. 2000) and vary in severity from self-limiting to occasionally fatal. There are no pathognomonic clinical signs of Nipah virus infection in pigs, although a "barking" cough is considered characteristic.

In field outbreaks, an acute febrile illness was described in weaner and grower pigs, with temperatures of ≥40 °C (104 °F). Respiratory signs ranging from increased or forced respiration to a harsh, paroxysmal nonproductive cough (a loud barking cough) or open-mouth breathing were prominent, especially if animals were forced to move. Neurological signs such as muscle fasciculation, rear leg weakness, and varying degrees of spastic paresis and uncoordinated gait when driven and hurried were observed. Clinical signs may progress to lateral recumbency accompanied by thrashing of the limbs or tetanic spasms. Mortality in this age group is low (<5%). Animals that die may show blood-tinged discharge from the nose. However, infection is frequently subclinical.

Acute death was occasionally observed in sows and boars, either with no prior clinical signs or within 24 hours of onset of clinical disease. However, sudden death in sows and boars is considered an unusual outcome of Nipah virus infection. A bloody nasal discharge was frequently apparent after death. Neurological signs were frequently observed, and included head pressing, agitation displayed as biting the bars of the pen, tetanic spasms or seizures, and an apparent pharyngeal muscle paralysis resulting in the inability to swallow, frothy salivation, and drooping of the tongue. Abortions were also reported.

Suckling pigs showed open-mouth breathing, leg weakness with muscle tremors, and neurological twitches. Mortality was high in this age group, but whether from primary disease or because of disease in the sow was not clearly established.

Under experimental conditions, the majority of infected piglets did not develop apparent disease, although they showed a transient increase in rectal temperatures between 3 and 6 DPI. Some developed mild respiratory signs, and about 20% of piglets that were inoculated nasally or subcutaneously developed CNS signs requiring euthanasia (Berhane et al. 2008; Middleton et al. 2002; Pickering et al. 2016; Weingartl et al. 2005, 2006). However, clinical signs observed after the first week post inoculation were suspected to be partially due to secondary infection, and bacteria, including *Enterococcus faecalis*, *Streptococcus suis*, and *Staphylococcus hyicus*, were isolated from piglets with different signs (Berhane et al. 2008; Middleton et al. 2002).

Lesions

The most common clinical sign in pigs is respiratory disease characterized by severe coughing. However, there is no pathognomonic gross pathology, and concurrent pulmonary disease from other causes may be present. Macroscopic lesions were consistently observed in the lungs and meninges of pigs infected with Nipah virus, both naturally and experimentally. Mild to severe pulmonary consolidation was also observed in subclinical cases, as well as distended interlobular septa on cut surfaces. Occasionally, dark depressed lobules were notable, mostly in the diaphragmatic lobes. In the Malaysian outbreak, bronchi and trachea of pigs were frequently filled with exudate or frothy fluid sometimes tinged with blood. Enlarged lymph nodes, most frequently bronchial, submandibular, and mesenteric, were also observed. In cases with neurological disease, the meninges were congested and edematous. In experimentally infected animals, Nipah virus-related gross pathological lesions resolved by approximately 3 weeks post inoculation

(Berhane et al. 2008; Hooper et al. 2001; Middleton et al. 2002; Weingartl et al. 2005).

Microscopically, the presence of multinucleated alveolar macrophages and syncytial cells in respiratory epithelium may indicate Nipah virus infection. Infrequently, syncytia may be found in the endothelium of small blood and lymphoid vessels (Figure 37.6), mainly in the lung, spleen, and lymph nodes. In the absence of fresh tissue, this suspicion could be confirmed by immunohistochemistry (IHC). Syncytia in the endothelium stain especially strongly for Nipah virus antigen.

Other microscopic lesions in the lungs include interstitial pneumonia with peribronchiolar, peribronchial, and perivascular infiltration of mononuclear cells and vasculitis often with fibrinoid necrosis. In some instances, alveolitis with infected macrophages, cellular debris (also in bronchioles), and proteinaceous fluid has been observed. Viral antigen has been detected in the endothelial cells, in the smooth muscle cells of the tunica media, and in macrophages and bronchiolar and, less frequently, alveolar epithelial cells (Berhane et al. 2008; Hooper et al. 2001; Middleton et al. 2002; Tanimura et al. 2004; Weingartl et al. 2005).

In cases with neurological disease, a nonsuppurative meningitis or meningoencephalitis was observed more frequently than encephalitis. Prominent perivascular cuffing was observed in the meninges and sometimes also in the brain. Virus antigen was detected in neurons and glial cells, endothelial and smooth muscle cells of the tunica media of blood vessels, infiltrating mononuclear cells, ependyma, choroid plexus, and the meninges (Hooper et al. 2001; Middleton et al. 2002; Weingartl et al. 2005).

Important pathological changes occur in the lymphoid organs and tissues. Besides the presence of syncytia in endothelial cells of blood and lymphoid vessels and vasculitis, necrosis of lymphocytes and their depletion from the lymph nodes are another important feature of Nipah virus infection in swine. Nipah virus antigen is detected mainly in endothelial cells, multinucleated giant cells, and dendritic cells (Berhane et al. 2008; Hooper et al. 2001; Middleton et al. 2002; Weingartl et al. 2005, 2006) (Figure 37.7). Other organs, such as the kidney (Middleton et al. 2002; Tanimura et al. 2004), can also be affected, most likely due to vascular damage, but the extent and frequency of lesions are much less significant than those described for the lung, brain, lymph nodes, and spleen.

Diagnosis

Nipah virus may be suspected if a clinical picture consistent with Nipah virus disease occurs on a pig farm in an area where there is opportunity for contact with pteropid bats. The list of differential diagnoses should include those that cause sudden death in boars and/or sows; reproductive failure characterized by abortion; respiratory disease in any age group characterized by severe coughing; and neurological disease characterized by tremors, muscle fasciculation, and agonal thrashing of the limbs or tetanic spasms in lateral recumbency.

Nipah virus does not produce pathognomonic clinical signs, and clinical signs vary by the age and reproductive status of the animals affected. Thus, the differential diagnosis may vary with the age and class of pigs affected.

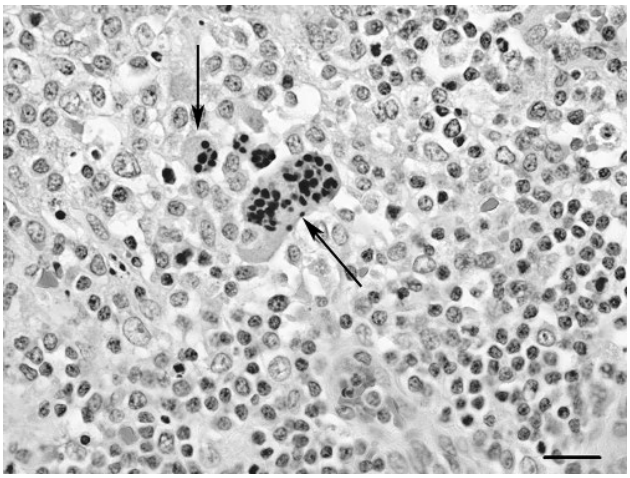


Figure 37.6 Submandibular lymph node of a pig experimentally infected with Nipah virus showing multinucleated syncytial cells with pyknotic nuclei (arrows) (bar = 20 µm, hematoxylin and eosin [H&E]). *Source:* Photo courtesy of Dr. Carissa Emburry-Hyatt.

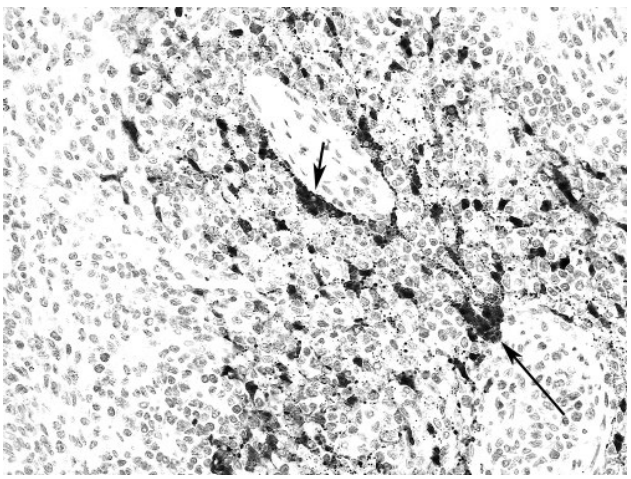


Figure 37.7 Immunohistochemical staining of submandibular lymph nodes revealed abundant Nipah virus antigen (black) within lymphocytes, reticulodendritic cells, and multinucleated syncytial cells (arrows). *Source:* Photo courtesy of Dr. Carissa Emburry-Hyatt.

In addition, secondary infections with other microorganisms can contribute to a wide range of nonspecific clinical signs. Animals coinfected with other pathogens, such as classical swine fever virus and opportunistic bacteria, are likely to display clinical signs most compatible with the coinfectants (Berhane et al. 2008). This may partially explain how it was possible for Nipah virus to be present, but unidentified, in pigs in Malaysia since 1996, as indicated in retrospective studies of archival specimens (Chua et al. 2000).

Nipah virus is a high-risk (BSL-4) zoonotic agent, and extreme care must be taken during sample collection in cases suspected to involve Nipah virus. Antemortem and post mortem sampling has to be conducted while wearing personal protective equipment, including respiratory protection, in a manner that will exclude human contact with body fluids from affected animals. Nasal and oral specimens are most effective for virus detection from live animals and serum for antibody detection. Nipah virus is detectable in oral and nasal swabs at 2 DPI, prior to the onset of clinical signs, and shedding can last up to 3 weeks post infection. Antibody against Nipah virus becomes detectable in all animals 10 DPI. From post mortem samples, trigeminal ganglion, olfactory bulb, cerebrospinal fluid, lung-associated lymph nodes, lungs, and spleen may be the most useful for virus detection by RT-PCR or virus isolation (Berhane et al. 2008; Daniels 2001; Middleton et al. 2002; Weingartl et al. 2005).

Nipah virus is a high-risk zoonotic agent, and diagnostic procedures that do not amplify the virus and minimize the handling of infectious material are preferred. Detection of viral RNA by reverse transcription polymerase chain reaction (RT-PCR) is rapid, usually more sensitive than virus isolation, and offers greater biosafety for the operator. Real-time RT-PCR (qRT-PCR) assays specific for Hendra virus or Nipah virus have been described (Guillaume et al. 2004; Li et al. 2010; Pickering et al. 2016) and allow the reliable detection of viral RNA in a wide range of tissues and serum.

Demonstration of viral antigens in formalin-fixed post mortem samples is also a rapid and safe option for confirmation of a diagnosis, albeit less sensitive than real-time RT-PCR. Nipah virus antigens have been demonstrated in formalin-fixed tissues, especially the lung and upper airways, meninges, spleen, olfactory bulb, trigeminal ganglion, lymph nodes, and kidney (Daniels 2001; Middleton et al. 2002; Weingartl et al. 2005). It is likely that a retrospective confirmation could be achieved with formalin-fixed tissues (Chua et al. 1999; Hooper and Williamson 2000).

When virus isolates are required, either for confirmation of a diagnosis or for additional research, procedures should be conducted in a BSL-4 laboratory. Vero cells are preferred for virus isolation. Cytopathology characterized by the formation of large syncytia may be observed in 2–3 days, but several passages of at least 5 days are usually conducted before declaring an isolation attempt unsuccessful.

ELISA is the preferred method for antibody detection because of the availability of recombinant reagents (e.g. NiV N or GP protein) and high throughput capacity of ELISAs (Pickering et al. 2016). Prior to testing, serum samples should be heated at 60 °C for 1 hour to eliminate the possibility that samples might contain infectious virus. Any ELISA reactors should be confirmed by VN, either by CPE reduction or by microtiter plaque reduction neutralization assay (Daniels 2001; Weingartl et al. 2006), again under BSL-4 conditions.

Specimen collection and diagnostic assays available for Nipah and Hendra viruses are also reviewed elsewhere (Daniels and Narasiman 2008).

Immunity

Neutralizing antibodies provide protection against Nipah virus disease in pigs (Weingartl et al. 2006) and appear 7–10 DPI, reaching maximum titers (approximately 1/1280) by 14–16 DPI (Berhane et al. 2008; Middleton et al. 2002). Full protection against Nipah virus challenge, however, requires both the development of neutralizing antibodies and cell-mediated immune response (Pickering et al. 2016). There is currently no evidence that persistent infections occur, although the possibility cannot be excluded.

Prevention and control

Nipah virus is a dangerous zoonotic agent and treatment of affected animals should not be considered. In countries where pteropid bats may be a reservoir of Nipah virus, pig farms should be managed to ensure that infection cannot reach the population (Choo 2001; Daniels 2001). That is, farms should be devoid of fruit trees and other vegetation that might attract the bats to the proximity of the animal housing. Strict biosecurity should be in place to preclude the importation of infected animals. Herd replacements should be isolated and quarantined prior to introduction.

Control measures in cases of confirmed Nipah virus infection will reflect its extreme hazard as a zoonotic agent. It is essential to prevent spread of infection among domestic animals and to preclude the possibility of infection of humans. Rapid eradication is the recommended response. This was achieved in Malaysia by quarantine of infected premises and the culling of all susceptible animals on those premises. It is essential that quarantine and associated movement controls be enforced during this period of culling (Mangat 2001).

Porcine parainfluenza virus 1

Relevance

PPIV-1 was identified in pigs in 2013 in Hong Kong, China, with sequences most closely related to human parainfluenza virus 1 and Sendai virus (Lau et al. 2013). PPIV-1 is widespread in the United States and likely elsewhere (Palinski et al. 2016). Field and experimental studies have demonstrated PPIV-1 replication and shedding in pigs, but clinical signs and pathological changes produced by PPIV-1 infection appear to be minimal (Palinski et al. 2016; Gauger et al. 2016; Sun et al. 2013; Welch et al. 2018).

Etiology

PPIV-1 (species name *Porcine respirovirus 1*) is a member of the genus *Respirovirus* in the family *Paramyxoviridae*. PPIV-1 has a single-stranded, negative-sense linear RNA genome approximately 15 kb in length, and the viral genome consists of six genes (3′-N-P-M-F-HN-L-5′), which encode the nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin–neuraminidase (HN), and large (L) proteins, respectively (Lau et al. 2013; Palinski et al. 2016; Park et al. 2017a).

PPIV-1 can be propagated in a rhesus monkey kidney epithelial cell line (LLC-MK2) in the presence of trypsin and produces CPE, including syncytia formation (Park et al. 2017b).

Public health

There are no reports of PPIV-1 infections in humans. However, PPIV-1 is genetically similar to human parainfluenza 1, and the possibility of human infection should be a consideration until human susceptibility or resistance to PPIV-1 is clearly established.

Epidemiology

PPIV-1 was first detected by PCR in 12 of 386 nasopharyngeal swabs (3.1%) and 2 of 303 rectal swabs (0.7%) collected from slaughtered pigs in Hong Kong between September 2008 and June 2012 (Lau et al. 2013). Subsequently, Sun et al. (2013) detected a virus with over 92.5% nucleotide identity to the Hong Kong PPIV-1 in piglets exhibiting influenza-like respiratory signs on farms in Iowa, Illinois, Oklahoma, and North Carolina in the United States. Subsequently, Palinski et al. (2016) detected PPIV-1 not only in piglets with moderate cough, sneezing, and serous nasal discharge on commercial farms in Oklahoma and Illinois but also in pigs with no clinical signs on a farm in Nebraska. PPIV-1 PCR testing of 279 lung, oral fluid, and nasal swab samples produced 17 (6.1%) positive samples (Palinski et al. 2016).

Gauger et al. (2016) reported that PPIV-1 PCR testing of 1,589 diagnostic submission samples collected in the spring to fall of 2016 from 27 US states produced 542 (34.1%) positive samples. PPIV-1 was detected most often in nursery pigs, followed by grow–finish pigs, and was uncommon in suckling and adult pigs (Gauger et al. 2016). The US PPIV-1 strains had 97–98% nucleotide identity to each other and 90–96% nucleotide identity to the Chinese PPIV-1 at the whole genome level (Palinski et al. 2016; Park et al. 2017a). Cumulatively, the data suggest that PPIV-1 is widespread.

PPIV-1 is assumed to be transmitted mainly via aerosols. Under experimental conditions, naïve pigs placed 0.6 m (2 ft) from PPIV-1-inoculated pigs became infected (Welch et al. 2018).

Pathogenesis, clinical signs, lesions, and immunity

There are few reports on PPIV-1 pathogenesis, clinical signs, lesions, or immunity. Influenza-like signs such as moderate cough, minor sneezing, and serous nasal discharge were noted in the initial cases in which PPIV-1 was detected by PCR (Palinski et al. 2016; Sun et al. 2013). However, the contribution of PPIV-1 to the observed clinical signs is uncertain because other viruses including porcine astrovirus 4, PCV2, porcine kobuvirus, and/or porcine hemagglutinating encephalomyelitis virus were detected concomitantly with PPIV-1 in some cases (Palinski et al. 2016).

Palinski et al. (2016) randomly selected 11 weaned pigs (18–19 days of age) from a farm naturally infected with PPIV-1 and placed them in an animal research unit for follow-up investigation. At day 0, 6 of 11 pigs (55%) were PPIV-1 positive by PCR, and 3 additional pigs became PPIV-1 PCR positive during the 2-week observation period. Virus was detected in nasal secretions for 2–10 days by PCR, but no clinical signs were observed. Histopathological examination revealed only atelectasis in the lung tissues in 4 of 8 pigs. *In situ* hybridization (ISH) revealed PPIV-1 in turbinate respiratory epithelial cells and the trachea of only 1 pig.

Welch et al. (2018) conducted experimental inoculation studies using a PPIV-1 cell culture isolate in PPIV-1 seronegative conventional pigs (3 weeks of age) and Cesarean-derived colostrum-deprived (CDCD) pigs. In 30 conventional pigs inoculated intranasally and intratracheally, PPIV-1 was detected in nasal swabs from 29/30 pigs by PCR at 1 DPI, and all pigs were PPIV-1 PCR positive from DPI 2 to 9. Viral shedding significantly decreased by 14 DPI but was detectable in some pigs through 21 DPI. Naïve conventional pigs placed 0.6 m

(2 ft) from PPIV-1-inoculated pigs on DPI 2 were PPIV-1 PCR positive 1 day later. The same pattern of viral shedding was seen in 10 CDCD pigs receiving the same dose of virus by the same inoculation routes as the conventional pigs.

Although PPIV-1 shedding was demonstrated under experimental conditions, no clinical signs were observed in either conventional or CDCD pigs, with the exception of sporadic mild coughing after 9 DPI in the conventional pigs.

Macroscopic lung lesions were minimal in PPIV-1-inoculated pigs, and histopathology lung lesion scores were not significantly different between the PPIV-1-inoculated pigs and control pigs. PPIV-1 antigen was detected in abundance by IHC in the epithelium of pulmonary bronchi and bronchioles, tracheal epithelium, and the turbinate epithelium when examined at 5 DPI. Preliminary data showed that pigs seroconverted at 21 DPI by serum VN test and indirect fluorescent antibody assay.

Diagnosis

PPIV-1 infection must be differentiated from agents that produce respiratory signs, such as influenza virus, PRRSV, PCV2, and others. A PPIV-1-specific real-time RT-PCR has been developed (Lau et al. 2013; Palinski et al. 2016), and nucleotide sequencing and comparative analyses can be used for further characterization.

PPIV-1 can be isolated on LLC-MK2 cells (Park et al. 2017b). Appropriate specimens for PPIV-1 PCR and virus isolation include lung, nasal swab, and oral fluid samples. Experimental ISH, IHC, and serological assays have been developed for PPIV-1; however, the diagnostic performance of these assays is not well established.

Prevention and control

The clinical significance of PPIV-1 infection is not fully understood, and no specific prevention and control measures have been developed for PPIV-1.

References

Allan GM, McNeilly F, Walker I, et al. 1996. *J Vet Diagn Invest* 8:405–413.

Asiah NM, Mills JN, Ong BL, et al. 2001. Epidemiological investigation on Nipah virus infection in peridomestic animals in peninsula Malaysia and future plans. In Report of the regional seminar on Nipah virus infection. Tokyo, Office International des Epizooties Representation for Asia and the Pacific, pp. 47–50.

Barr JA, Smith C, Marsh GA, et al. 2012. *J Gen Virol* 93:2590–2594

Berg M, Sundqvist A, Moreno-Lopez J, et al. 1991. *J Gen Virol* 72:1045–1050.

Berg M, Hjertner B, Moreno-Lopez J, et al. 1992. *J Gen Virol* 73:1195–1200.

Berhane Y, Weingartl HM, Lopez J, et al. 2008. *Transbound Emerg Dis* 55:165–174.

Bowden TR, Westenberg M, Wang LF, et al. 2001. *Virology* 283:358–373.

Bowden TR, Bingham J, Harper JA, et al. 2012. *J Gen Virol* 93:1007–1016.

Breed AC, Yu M, Barr JA, et al. 2010. *Emerg Infect Dis* 16:1997–1999.

Campos HR, Carbajal SM. 1989. Trastornos reproductivos de los sementales de una granja porcina de ciclo completo ante un brote de ojo azul. Memorias del XXIV Congreso de la Asociación Mexicana de Veterinarios Especialista en Cerdos (AMVEC), pp. 62–64.

Chant K, Chan R, Smith M, et al. 1998. *Emerg Infect Dis* 4:273–275.

Ching PKG, de los Reyes VC, Sucaldito MN, et al. 2015. *Emerg Infect Dis* 21:328–331.

Choo PY. 2001. Pig industry perspectives on herd health monitoring and biosecurity in Malaysia. In Report of the regional seminar on Nipah virus infection. Tokyo, Office International des Epizooties Representation for Asia and the Pacific, pp. 90–93.

Chua KB, Goh KJ, Wong KT, et al. 1999. *Lancet* 354:1257–1259.

Chua KB, Bellini WJ, Rota PA, et al. 2000. *Science* 288:1432–1435.

Cuevas JS, Rodríguez-Ropón A, Kennedy S, et al. 2009. *Vet Immunol Immunopathol* 127:148–152.

Cuevas-Romero S, Rivera-Benıtez JF, Blomstrom AL, et al. 2016. *Virus Genes* 52:81–90.

Daniels PW. 2001. Nipah virus preparedness – aspects for a veterinary plan. In Report of the regional seminar on Nipah virus infection. Tokyo, Office International des Epizooties Representation for Asia and the Pacific, pp. 84–89.

Daniels P, Narasiman M. 2008. Chapter 2.9.6: Hendra and Nipah viruses. In: OIE Manual of Standards for Diagnostic Tests and Vaccines: Sixth Edition, pp. 1227–1238. Paris, France: World Organisation for Animal Health (OIE).

Daniels PW, Aziz J, Ksiazek TG, et al. 2000. Nipah virus: developing a regional approach. In Comprehensive Reports on Technical Items Presented to the International Committee or Regional Commissions, Edition 1999. Office International des Epizooties, Paris, pp. 207–217.

Daniels PW, Ong BL, Aziz J. 2002. Nipah virus diagnosis and control in swine herds. In Morilla A, Yoon KJ, Zimmerman J, eds. *Trends in Emerging Viral Infections of Swine*. Ames, IA: Iowa State Press, pp. 111–116.

Escobar-Lopez AC, Rivera-Benitez1 JF, Castillo-Juarez H, et al. 2012. *Transbound Emerg Dis* 59:416–420.
Gauger PC, Lin S, Bade S, et al. 2016. Porcine parainfluenza virus type 1 (PPIV-1) disease, diagnostics, and clinical significance. In Proceedings of the ISU Swine Disease Conference, Ames, pp. 106–114.
Goh KJ, Tan CT, Chew NK, et al. 2000. *N Engl J Med* 342:1229–1235.
Greig AS, Johnson CM, Bouillant AMP. 1971. *Res Vet Sci* 12:305–307.
Guillaume V, Lefeuvre A, Faure C, et al. 2004. *J Virol Methods* 120:229–237.
Hernández-Jáuregui P, Ramírez-Mendoza H, Mercado-García C, et al. 2004. *J Comp Pathol* 130:1–6.
Hooper PT, Williamson MM. 2000. *Vet Clin North Am Equine Pract* 16:597–603.
Hooper P, Zaki S, Daniels P, et al. 2001. *Microbes Infect* 3:315–322.
Hossain MJ, Gurley ES, Montgomery JM, et al. 2008. *Clin Infect Dis* 46:977–984.
Janke BH, Paul PS, Landgraf JG, et al. 2001. *J Vet Diagn Invest* 13:428–433.
Kirkland PD, Love RJ, Philbey AW, et al. 2001. *Aust Vet J* 79:199–206.
Lau SK, Woo PC, Wu Y, et al. 2013. *J Gen Virol* 94:2184–2190.
Li M, Embury-Hyatt C, Weingartl HM. 2010. *Vet Res* 41:33.
Lipkind M, Shoham D, Shihmanter E. 1986. *J Gen Virol* 67:427–439.
Lo MK, Lowe L, Hummel KB, et al. 2012. *Emerg Infect Dis* 18:248–255.
Love RJ, Philbey AW, Kirkland PD, et al. 2001. *Aust Vet J* 79:192–198.
Luby SP, Gurley ES. 2012. *Curr Top Microbiol Immunol* 359:25–40.
Lye MS, Ong F, Parashar UD, et al. 2001. Report on the epidemiological studies conducted during the Nipah virus outbreak in Malaysia in 1999. In Report of the regional seminar on Nipah virus infection. Tokyo, Office International des Epizooties Representation for Asia and the Pacific, pp. 31–37.
Mangat AA. 2001. Management of Nipah virus outbreaks. In Report on the regional seminar on Nipah virus infection, Tokyo, OIE Representation for Asia and the Pacific, pp. 51–53.
Meisner A, Neufeld J, Weingartl H. 2009. *Thromb Haemost* 102:1014–1023.
Middleton DJ, Westbury HA, Morrissy CJ, et al. 2002. *J Comp Pathol* 126:124–126.
Mohd Nor MN, Gan CH, Ong BL. 2000. *Rev Sci Tech* 19:160–165.
Moreno-Lopez J, Correa-Giron P, Martinez A, et al. 1986. *Arch Virol* 91:221–231.
Muniandy N. 2001. Serological screening using ELISA for IgG and IgM. In Report of the regional seminar on Nipah virus infection. Tokyo, Office International des Epizooties Representation for Asia and the Pacific, pp. 73–76.
Palinski RM, Chen Z, Henningson JN, et al. 2016. *J Gen Virol* 97:281–286.
Parashar UD, Sunn LM, Ong F, et al. 2000. *J Infect Dis* 181:1755–1759.
Park JY, Welch M, Harmon KM, et al. 2017a. *Genome Announc* 5:e01139-17.
Park JY, Welch M, Harmon KM, et al. 2017b. Isolation and characterization of a porcine parainfluenza virus-1 associated with respiratory disease in weaned pigs. Conference Research Workers in Animal Disease (Abstract P098).
Paton NI, Leo YS, Zaki SR, et al. 1999. *Lancet* 354:1253–1256.
Philbey AW, Kirkland PD, Ross AD, et al. 1998. *Emerg Infect Dis* 4:269–271.
Pickering BS, Hardham JM, Smith G, et al. 2016. *Vaccine* 34:4777–4786.
Qiao D, Janke BH, Elankumaran S. 2010. *J Virol* 84:686–694.
Ramirez TCA, Stephano AH. 1982. Histological central nervous system lesions produced by an haemagglutinating virus in naturally infected piglets. *Proc Congr Int Pig Vet Soc* 7:154.
Ramirez MH, Reyes LJ, Kennedy S, et al. 1995. Studies on the pathogenesis of the pig paramyxovirus of the blue eye disease on the epididymis and testis. Memorias del XX Reunión de la Academia de la Investigación en Biología y Reproducción (México), pp. 211–214.
Ramirez MH, Carreon NR, Mercado GC, et al. 1996. Hemoaglutinación e inhibición de la hemoaglutinación del paramixovirus porcino a traves de la modificación de algunas variables que participan en la prueba. *Veterinaria (México)* 27:257–259.
Rivera-Benitez JF, Garcıa-Contreras AC, Reyes-Leyva J, et al. 2013. *Arch Virol.* 158:1849–1856.
Sánchez-Betancourt JI, Santos-López G, Alonso R, et al. 2008. *Res Vet Sci* 85:359–367.
Sánchez-Betancourt JI, Trujillo ME, Mendoza SE, et al. 2012. *Can J Vet Res*, 2012: 76: 33–37.
Sasahara J, Hayashi S, Kumagai T, et al. 1954. On a swine virus disease newly discovered in Japan. 1. Isolation of the virus. 2. Some properties of the virus. *Virus* 4:131–139.
Solís M, Ramírez-Mendoza H, Mercado C, et al. 2007. *Res Vet Sci* 83:403–409.
Stachowiak B, Weingartl HM. 2012. *PLoS ONE* 7:e30855.
Stephano AH, Gay GM. 1983. El syndrome del ojo azul. Estudio experimental. Memorias de la Reunión de Investigación Pecuaria en México, pp. 523–528.
Stephano AH, Gay GM. 1984. Experimental studies of a new viral syndrome in pigs called "blue eye" characterized by encephalitis and corneal opacity. *Proc Congr Int Pig Vet Soc* 8:71.

Stephano AH, Gay GM. 1985a. El syndrome del ojo Azul en cerdos I. *Síntesis Porcina (México)* 4(5):42–49.

Stephano AH, Gay GM. 1985b. El syndrome del ojo azul en granjas engordadoras. Memorias del IXX Congreso de la Asociación Mexicana de Veterinarios Especialista en Cerdos (AMVEC), pp. 71–74.

Stephano AH, Gay GM. 1986a. El syndrome del ojo azul. Una nueva enfermedad en cerdos asociada a un paramyxovirus. *Veterinaria (México)* 17:120–122.

Stephano AH, Gay GM. 1986b. Encefalitis, falla reproductiva y opacidad de la cornea, ojo Azul. *Síntesis Porcina (México)* 5(12):26–39.

Stephano AH, Gay GM, Ramirez TC, et al. 1982. An outbreak of encephalitis in piglets produced by a hemagglutinating virus. *Proc Congr Int Pig Vet Soc* 7:153.

Stephano AH, Doporto JM, Gay M. 1986a. Estudio epidemiologico en dos granjas afectadas por el ojo Azul. *Proc Congr Int Pig Vet Soc* 9:456.

Stephano AH, Gay M, Kresse J. 1986b. Properties of a paramyxovirus associated to a new syndrome (blue eye) characterized by encephalitis, reproductive failure and corneal opacity. *Proc Congr Int Pig Vet Soc* 9:455.

Stephano AH, Fuentes RM, Hernandez JP, et al. 1988a. Encefalitis y opacidad de la cornea en cerdos destetados, inoculados experimentalmente con paramyxovirus de ojo azul. Memorias del XXIII Congreso de la Asociación Mexicana de Veterinarios Especialista en Cerdos (AMVEC), pp. 90–92.

Stephano AH, Gay GM, Ramirez TC. 1988b. *Vet Rec* 122:6–10.

Stephano AH, Hernandez D, Perez C, et al. 1990. Boar infertility and testicle atrophy associated with blue eye paramyxovirus infection. *Proc Congr Int Pig Vet Soc* 11:211.

Sun D, Stevenson GW, Madson D, et al. 2013. Identification and characterization of novel parainfluenza virus type 1-like virus in pigs with influenza-like respiratory disease. Conference Research Workers in Animal Disease Abstract 143.

Sundqvist A, Berg M, Hernandez-Jauregui P, et al. 1990. *J Gen Virol* 71:609–613.

Sundqvist A, Berg M, Moreno-Lopez J, et al. 1992. *Arch Virol* 122:331–340.

Tanimura N, Imada T, Kashiwazaki Y, et al. 2004. *J Comp Pathol* 131:199–206.

Wang LF, Hansson E, Yu M, et al. 2007. *Arch Virol* 152:1259–1271.

Weingartl H, Czub S, Copps J, et al. 2005. *J Virol* 79:7528–7534.

Weingartl HM, Berhane Y, Caswell JL, et al. 2006. *J Virol* 80:7929–7938.

Welch M, Park J, Harmon K, et al. 2018. Pathogenesis of a porcine parainfluenza virus-1 isolate (USA/MN25890NS/2016) in conventional and CDCD piglets. Proceedings of the Annual Meeting American Association Swine Veterinarians, pp. 46–50.

Wiman AC, Hjertner B, Linne T, et al. 1998. *J Neurovirol* 4:545–552.

38

Parvoviruses

Uwe Truyen and André Felipe Streck

Relevance

Porcine parvovirus (PPV) was first detected in 1965 by Anton Mayr and coworkers in Munich, Germany, as a contaminant of a porcine primary cell line used for the propagation of classical swine fever virus (Mahnel 1965; Mayr et al. 1968). To differentiate this historical virus from newly identified PPVs, it is designated "PPV1" in the remainder of this chapter. Infection with PPV1 causes reproductive losses in swine characterized by stillbirths, mummification, embryonic death, and infertility (SMEDI). The affected sow does not typically show clinical signs, and virus transmission to the fetuses only occurs if she is seronegative. PPV1 is probably the most important cause of reproductive failure in pigs worldwide, but it took several years to link PPV1 to endemic reproductive disorders and to show that it was globally distributed (Cartwright and Huck 1967; Joo et al. 1976a; Mengeling and Cutlip 1976).

More recently, PPVs 2, 3, 4, 5, 6, and 7 were identified in swine in various countries (Cheung et al. 2010; Cságola et al. 2012; Cui et al. 2017; Hijikata et al. 2001; Lau et al. 2008; Palinski et al. 2016; Streck et al. 2013; Xiao et al. 2013b). PPVs 2–7 may be detected even in the absence of PPV1 (Cui et al. 2017). Current work shows the highest prevalence in growing–finishing pigs and the lowest in pigs <9 weeks of age, with PPV2 the most frequently detected (Cui et al. 2017; Opriessnig et al. 2014). The role of these viruses in swine health has not been defined, that is, no association with clinical signs has been established. Further, the prevalence of these viruses in clinically healthy animals raises the possibility that they are commensals (Streck et al. 2015a).

Etiology

The family *Parvoviridae* consists of two subfamilies: *Parvovirinae* and *Densovirinae*. Viruses in the subfamily *Parvovirinae* mainly infect vertebrate hosts, whereas viruses in the subfamily *Densovirinae* mainly infect arthropods. PPVs 1–7 and porcine bocaviruses belong to the subfamily *Parvovirinae* (Table 38.1). Porcine bocaviruses have not been shown to be pathogenic in pigs and are not discussed in this chapter.

Like the closely related parvoviruses of carnivores, canine parvovirus and feline panleukopenia virus, the PPV virion is about 28 nm in diameter and consists of 60 copies of the structural protein VP1/VP2, about 90% of which are VP2 and 10% VP1 molecules. The capsid structure is characterized by a simple T = 1 icosahedral symmetry (Simpson et al. 2002).

The PPV genome is a single-stranded DNA molecule of about 5000 bases. Like all parvoviruses, complex palindromic hairpin structures located at each terminus are required for DNA replication. The genome encodes four proteins transcribed from two promoters. "Alternative splicing" extends the coding capacity of the small genome. Two nonstructural (NS) proteins, NS1 and NS2, operate in the replication of the virus, particularly for DNA replication. Two structural proteins (VP1 and VP2) are transcribed and translated from the parvovirus genome. The smaller protein (VP2) is produced by splicing from the same RNA template as the larger protein (VP1). Thus, the entire VP2 sequence is present in the VP1 sequence, but the latter has a unique amino terminus of about 120 amino acids (see Cotmore and Tattersall [2006] for a review of parvovirus genome organization and gene expression). Some VP molecules are posttranslationally trimmed by proteases to create the minor protein VP3.

Sequence analyses of recent isolates suggested active evolution of PPV1. Specifically, sequence alignments and phylogenetic studies of the capsid protein gene (VP1) revealed a new cluster of viruses characterized by specific nucleotide and amino acid changes (represented by the predominant strain 27a) (Zimmermann et al. 2006) (Figure 38.1). Preliminary data indicated that these "new" viruses (here called *27a-like* viruses) were spreading through European pig populations and perhaps worldwide. The appearance of the 27a-like viruses could be important because changes in the capsid protein influence the

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 38.1 Porcine parvoviruses and porcine bocaviruses (Family *Parvoviridae*, subfamily *Parvovirinae*) with common name, taxonomic species and genus, and presence or absence of clinical signs.

Common name	Species	Genus	Clinical signs
Porcine parvovirus (1)	*Ungulate protoparvovirus 1*	*Protoparvovirus*	Present
Porcine parvovirus 2	Ungulate tetraparvovirus 3	*Tetraparvovirus*	Mild or absent
Porcine parvovirus 3, or porcine hokovirus, or PARV4-like	*Ungulate tetraparvovirus 2*	*Tetraparvovirus*	Mild or absent
Porcine parvovirus 4	*Ungulate copiparvovirus 2*	*Copiparvovirus*	Mild or absent
Porcine parvovirus 5	?	*Copiparvovirus*	Mild or absent
Porcine parvovirus 6	?	*Copiparvovirus*	Mild or absent
Porcine parvovirus 7	?	*Chapparvovirus* (proposed)	Mild or absent
Porcine bocaviruses 1, 2, and A6	*Ungulate bocaparvovirus 2*	*Bocaparvovirus*	Mild or absent
Porcine bocavirus SX	*Ungulate bocaparvovirus 3*	*Bocaparvovirus*	Mild or absent
Porcine bocavirus H18	*Ungulate bocaparvovirus 4*	*Bocaparvovirus*	Mild or absent
Porcine bocavirus 3, 4	*Ungulate bocaparvovirus 5*	*Bocaparvovirus*	Mild or absent

Source: Adapted from Streck et al. (2015). Reproduced with permission of Elsevier.

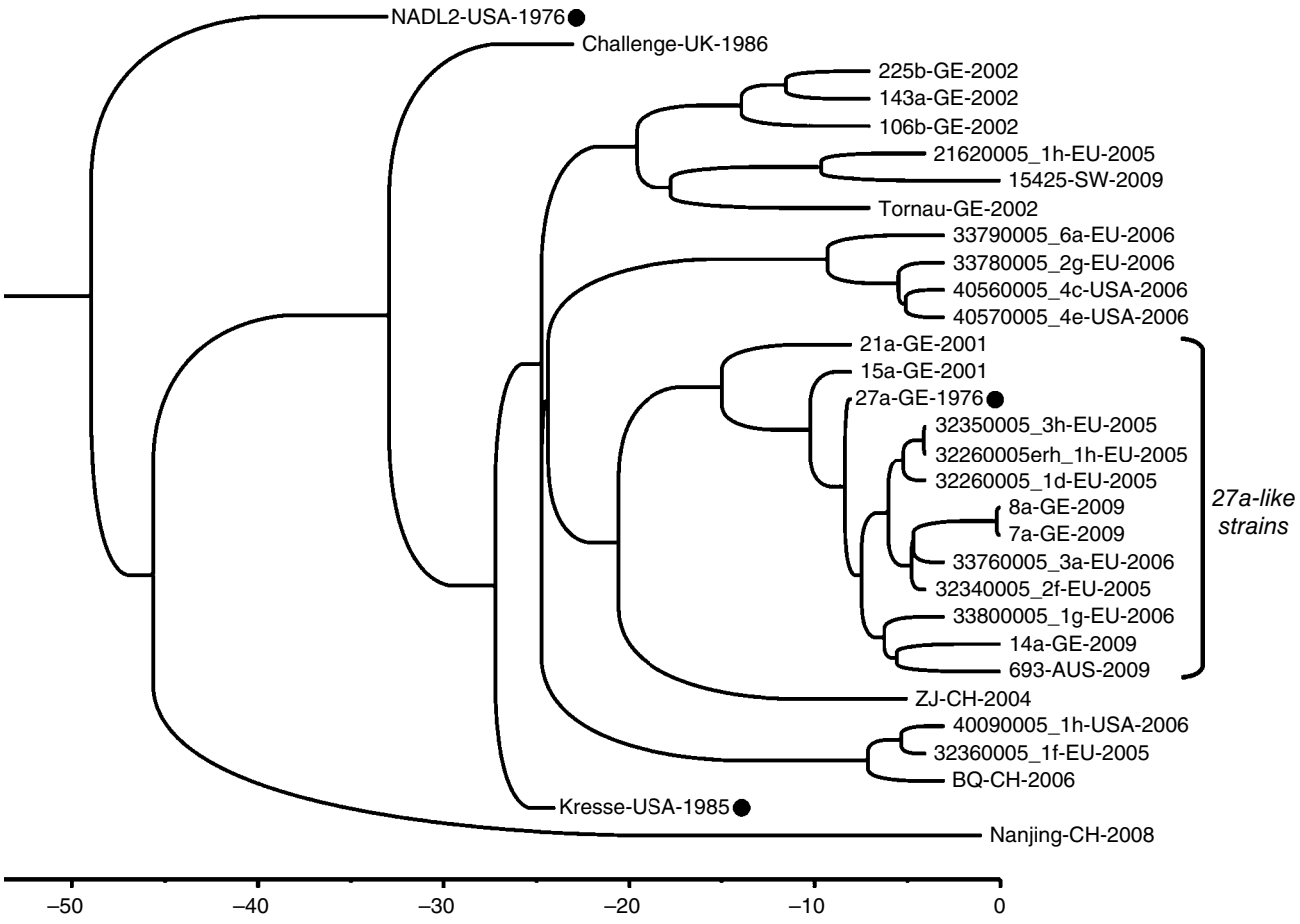


Figure 38.1 Phylogenetic trees based on Bayesian inference analysis of PPV1 for the VP1 complete gene. The scale axis indicates the distance in years. The identification name of each sequence is followed by the location and year of isolation. AUS, Austria; CH, China; EU, Europe (no precise location available); GE, Germany; SW, Switzerland. The reference strains NADL-2, Kresse, and 27a indicated by black dots. *Source:* Adapted from Streck et al. (2011).

antigenic properties of the virus. There is only one serotype for PPV1, and all PPV1 isolates show a high degree of cross-reactivity in various serological tests, for example, virus neutralization or hemagglutination inhibition (HI) assays. However, differences in cross-neutralization of some 27a-like viruses have been demonstrated using sera raised against "classic" PPV1 strains (Zeeuw et al. 2007).

Based on genomic sequences, the phylogeny of PPV1 isolates suggests a rather geographic clustering and a broad distribution of the 27a-like viruses (Cadar et al. 2012; Streck et al. 2011). These viruses are characterized by specific sequences in the capsid protein gene, resulting in specific amino acid changes at positions 414, 419, and 436, predominantly located in the protein loops (Streck et al. 2015). Because PPV1 protein loops are generally located on the capsid surface, these amino acid substitutions may influence receptor binding and/or antigenicity. Based on studies done using animal models and on observations in vaccinated herds, it appears that the 27a-like viruses do not cause the outbreaks in PPV-vaccinated sows that would be expected if there were true immune escape. To understand the importance of these mutations, a series of mutants containing amino acid substitutions of the 27a-like viruses were constructed. The mutants (containing 27a mutations) had relatively higher replication efficiency when compared to the classical NADL-2 or Kresse strains. This has possibly contributed to the predominance of the 27a-like viruses.

Public health

There is no evidence that PPVs are infectious for humans or play any role in public health.

Epidemiology

PPV1 is endemic in pigs in most of the world. Although antibody responses must be interpreted cautiously because of the extensive use of inactivated vaccines, serological surveys in various parts of the world showed that PPV1 antibodies were present in 70–100% of the herds (Foni and Gualandi 1989; Nash 1990; Oravainen et al. 2005; Robinson et al. 1985). PPV1 HI antibody titers ≥512 (a typical field infection titer) were found in approximately 40% of animals in vaccinated herds (Oravainen et al. 2005).

PPV2 was first detected in Myanmar (Hijikata et al. 2001) and then reported in multiple countries such as China, Hungary, the United States, Germany, Thailand, Japan, South Korea, and Poland (Cságola et al. 2012; Cui et al. 2017; Lee et al. 2017; Saekhow and Ikeda 2015; Saekhow et al. 2014; Streck et al. 2013; Wang et al. 2010; Xiao et al. 2013). PPV3, also known as porcine hokovirus or PARV4-like virus, has been identified in many places, including Hong Kong, Romania, Germany, the United States, China, and Poland (Adlhoch et al. 2010; Cadar et al. 2011; Cheung et al. 2010; Cui et al. 2017; Lau et al. 2008; Zhong et al. 2016). PPV4 was initially detected from pigs in the United States (Cheung et al. 2010) and then detected in China, Hungary, Romania, Poland, and elsewhere (Cadar et al. 2013; Cui et al. 2017; Cságola et al. 2012; Huang et al. 2010). PPV5 was first identified in pigs in the United States (Xiao et al. 2013) and subsequently in China and Poland (Cui et al. 2017; Wu et al. 2014). PPV6 was first reported in pigs in China in 2014 and then detected in the United States and Poland (Cui et al. 2017; Ni et al. 2014; Schirtzinger et al. 2015). PPV7 has been reported in the United States and China (Palinski et al. 2016; Xing et al. 2018).

Although PPVs 2–7 are commonly detected in pigs, their clinical significance and basic epidemiology, including transmission routes, pathogenesis, clinical signs, and lesions, have not been established. Therefore, the remainder of this discussion reflects information based on studies of PPV1.

PPV1 readily replicates in susceptible pigs, although clinical signs (reproductive losses) only occur in pregnant females. The virus is shed in feces and other secretions from acutely infected pigs, and the epidemiology of PPV1 is shaped by the capacity of the virus to resist inactivation in the environment. That is, PPV1 can remain infectious for months in the environment and contaminated tools and may therefore be a constant source of new infections.

PPV1 can be transported between herds via fomites, for example, the clothes, boots, and equipment. Likewise, rodents functioning as mechanical vectors have reportedly introduced the virus into herds. The virus can also be introduced into populations by infected boars. Whether PPV1 is shed in the semen of infected boars or whether PPV1 in semen represents environmental contamination is unresolved. Regardless, there are numerous reports on PPV1 in the semen of naturally infected boars (Cartwright and Huck 1967; Ruckerbauer et al. 1978).

PPV1 is resistant to inactivation by ethanol (70%) and quaternary ammonium (0.05%), as well as low concentrations of sodium hypochlorite (2,500 ppm) and peracetic acid (0.2%), but is readily inactivated by aldehyde-based disinfectants and higher concentrations of sodium hypochlorite (25,000 ppm) and hydrogen peroxide (7.5%). The virus is relatively heat stable and may resist dry (but not moist) heat at 90 °C (194 °F) (Eterpi et al. 2009). For efficient disinfection an efficacy-tested disinfectant based on peracetic acid, aldehydes, or sodium hypochlorite (the latter only at concentrations above 2500 ppm) should be used.

The introduction of PPV1 into a herd does not cause immediate problems if a sufficient proportion of the

sows are immune through vaccination or natural exposure. However, the virus is able to replicate even in vaccinated pigs (Foerster et al. 2016; Jóźwik et al. 2009). This was demonstrated by a sharp rise in antibody titers in vaccinated sows of the same magnitude as in unvaccinated controls and by active shedding of the challenge virus from vaccinated sows. Thus, virus circulation within a population cannot be completely prevented by vaccination.

Pathogenesis

The pathogenesis of PPV1 reflects the ability of the virus to infect the fetus (Mengeling et al. 2000). However, it is not clear how PPV1 actually crosses the porcine transplacental barrier. As in other viruses, PPV1 could reach the fetus in one of three ways: in body fluids, such as blood or lymph; by progressive replication through continuous placental cell layers; or in cells, such as macrophages or lymphocytes (Mengeling et al. 2000).

After primary replication in lymphoid tissues, PPV1 is distributed systemically via cell-free viremia (Brown et al. 1980; Paul et al. 1980). However, the swine epitheliochorial placenta is composed of six tissue layers that completely separate maternal from fetal blood circulation, and placental cells are so closely connected that they do not allow the passage of even small molecules, for example, antibodies. Placental cells are not susceptible to PPV1 infection, and PPV1 has not been demonstrated in placental tissues, so it is not likely that the virus can cross the barrier through progressive replication (Mengeling et al. 1978). Therefore, it is most probable that the virus reaches the fetus via virus-infected immune cells. Studies have reported both the presence of the virus in lymphoid tissues from pigs (Lucas et al. 1974; Mengeling et al. 2000) and fetal lymphocytes in the circulatory system of pregnant sows (Rudek and Kwiatkowska 1983). Virus replication in macrophages has not been observed, but phagocytized PPV1 remains infectious for an extended period of time (Paul et al. 1979).

Once in the fetus, PPV1 encounters an environment conducive to virus replication because of the high mitotic index of most tissues of the developing fetus. The virus can be detected in many tissues and organs, suggesting that there is no specific tissue tropism (Wilhelm et al. 2005).

PPV1 enters cells through a series of interactions that culminate in the release of viral genetic material into a cell compartment in which replication can occur (Harbison et al. 2008). The entry mechanisms of PPV are unclear, but include clathrin-mediated endocytosis, or macropinocytosis, followed by transportation through the endosomal pathway (Boisvert et al. 2010). Endosomal trafficking and acidification are essential for PPV to enter in the nucleus (Boisvert et al. 2010), resulting in reversible modifications of the capsid that allow the virus to escape from the endosome (Farr et al. 2005; Vihinen-Ranta et al. 2002). In this process, the phospholipase A2 motif (PLA2) must be externalized from the capsid. This motif activity is essential for breaking the vesicular membranes, resulting in the formation of pores (Girod et al. 2002). After the virus arrives in the nucleus, PPV replicates using the cells own replication mechanism. The virus replicates in cells in replication phase (S) using the cellular DNA polymerase for DNA replication. This explains the requirement for cells with a higher replication index (Rhode 1973). The PPV1 replication decreased mitochondrial membrane potential. The subsequent oxidative damage also leads to the release of cellular toxic proteins such as cytochrome c from the mitochondria to the cytosol (Zhao et al. 2016), subsequently triggering apoptosis and causing cell death and tissue damage in viral diseases.

Factors affecting the severity of disease

Several PPV1 biotypes with marked differences in pathogenicity are recognized (Choi et al. 1987; Kresse et al. 1985; Mengeling and Cutlip 1975; Mengeling et al. 1984). Some PPV1s are completely nonpathogenic and do not cause disease even if experimentally inoculated into a fetus; others can cause disease even in immunocompetent fetuses, that is, after day 70 of gestation (see Table 38.2).

The genetic basis of pathogenicity has not been resolved, but the structural protein VP1/VP2 appears to play a major role. Presumably, pathogenicity or virulence

Table 38.2 Viremia, transplacental transmission, and death caused by distinct porcine parvovirus 1 (PPV1) strains.

Virus isolate	Viremia after oral inoculation	Fetal death after intrauterine inoculation	Transplacental transmission after oral inoculation	Death of immunocompetent fetuses
NADL-2	–	+	–	–
NADL-8	+	+	+	–
Kresse	+	+	+	+
KBSH	–	–	–	–

is determined (at least in part) by tissue tropism (Bergeron et al. 1996). *In vitro* studies using recombinant viruses derived from pathogenic (Kresse strain) and nonpathogenic (NADL-2 strain) PPV1s identified "allotropic determinants" and showed that single amino acids in the capsid protein affected an isolate's capacity to replicate in certain cell lines. Comparisons between Kresse and NADL-2 genomes showed that the noncoding regions were nearly identical. All differences found in the NS region were silent, while six of the eight differences identified in the structural genes (VP1/VP2) altered the coding sequence. Among the VP2 amino acids, five changes were consistent with comparisons in field isolates (I-215-T, D-378-G, H-383-Q, S-436-P, and R-565-K) and three of these (D-378-G, H-383-Q, and S-436-P) were considered responsible for differences in tissue tropism (Bergeron et al. 1996). Vasudevacharya and Compans (1992) showed that just two changes were sufficient to extend the host range of a PPV1 variant to canine cells *in vitro*. One of the mutations occurred in the NS gene and the other in the capsid gene (Vasudevacharya and Compans 1992).

In addition to tissue tropism, the absence of duplication of a 127 nucleotide sequence directly downstream of the VP1 gene was associated with virulence. That is, all but one of the virulent field isolates examined lacked this repeat (Bergeron et al. 1996; Soares et al. 2003; Zimmermann et al. 2006).

The pathogenicity of PPV1 is also influenced by the presence of other viruses. In particular, the recognition of low-level PPV1 contamination in the inoculum used in experiments that reproduced post weaning multisystemic wasting syndrome (PMWS) in gnotobiotic pigs (Ellis et al. 1999) led to the recognition that PPV1 in combination with porcine circovirus type 2 (PCV2) can increase the severity of the PMWS lesions (Kennedy et al. 2000). However, PPV1 coinfection is not a necessary requirement for the development of PMWS (Ellis et al. 2004).

Clinical signs

PPV1 is widespread among swine, but the frequency of reproductive losses is difficult to estimate because evidence of infection may appear weeks after infection, that is, an increase in the return-to-estrus index or the observation of affected litters. Likewise, diagnostic tests on fetal tissues often produce false-negative results, possibly due to the autolyzed state of fetal tissues and the high antibody titers in the sows.

Maternal reproductive failure is the major and only well-established clinical sign of PPV1 infection. Reproductive losses are typically low in vaccinated herds, but PPV1 can cause devastating abortion storms in unvaccinated herds or in situations in which the vaccine was administered incorrectly. Diarrhea and skin lesions, with PPV1 and PPV-like structures in diarrheic feces and isolation of PPV1 from "vesicle-like" skin lesions, have been described (Brown et al. 1980; Dea et al. 1985; Duhamel et al. 1991). These reports represent rare findings in which the etiological role of the virus remains to be fully established.

Even under experimental conditions, gilts and boars infected with PPV1 remain clinically healthy, except for reproductive losses in seronegative gilts or sows (Mengeling and Cutlip 1976; Mengeling and Paul 1981; Thacker et al. 1987; Zeeuw et al. 2007). A moderate and transient lymphopenia may be observed 5–10 days post inoculation, regardless of gender or age (Joo et al. 1976a; Mengeling and Cutlip 1976; Zeeuw et al. 2007).

The early phases of PPV1 infection and the incubation period are not well defined. Apparently, the virus first replicates in the tonsils and oral/nasal cavities. After 1–3 days, the virus reaches the lymphatic system and causes a cell-free viremia. Transplacental transmission and subsequent embryo/fetal infection occur around 15 days after inoculation of susceptible gestating females with PPV1 (Brown et al. 1980; Mengeling et al. 1978; Paul et al. 1980).

Reproductive clinical signs correlate to the stage of gestation at which infection occurs (Figure 38.2). At the beginning of gestation, the conceptus is protected by the zona pellucida and is not susceptible to infection. Thereafter and until approximately day 35 of gestation, PPV1 infection results in embryonic death and maternal resorption of fetal tissues. About gestation day 35, fetal organogenesis is essentially complete, and ossification of the fetal skeleton begins. PPV1 infection after this time typically results in fetal death followed by mummification. At or about day 70 of gestation, the fetus is able to mount an effective immune response and eliminate the virus. After day 70, fetal infection is subclinical, and the piglet is born with anti-PPV antibodies (Bachmann et al. 1975; Joo et al. 1977; Lenghaus et al. 1978; Mengeling et al. 2000).

Lesions

Experimental PPV1 inoculation in boars, gilts, and sows does not produce gross lesions (Bachmann et al. 1975; Lenghaus et al. 1978; Mengeling and Cutlip 1976; Thacker et al. 1987). Embryonic death followed by resorption of fluids and soft tissues is the most common sequel to PPV1 infection. Gross lesions in fetuses include a variable degree of stunting before other external changes are evident. Occasionally, blood vessels on the body surface become prominent due to congestion and leakage of blood into connective tissues. Congestion,

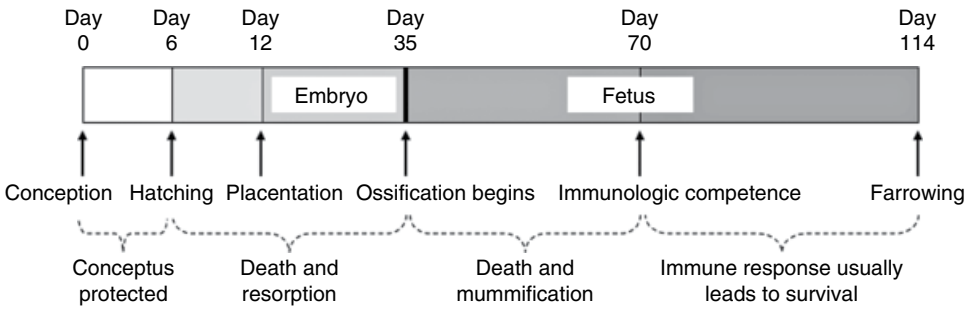


Figure 38.2 Consequence of PPV1 infection by day of gestation. *Source:* Adapted from Mengeling et al. (2000). Reproduced with permission of Elsevier.

edema, hemorrhage with accumulation of serosanguinous fluids in body cavities, and hemorrhagic discoloration, which becomes progressively darker after death and dehydration (mummification), are typical signs of PPV infection (Figure 38.3). The placenta can be dehydrated and brown to gray in color and the extra-fetal fluid volume reduced (Joo et al. 1977; Lenghaus et al. 1978). After fetuses become immunocompetent, no macroscopic changes are observed post infection (Bachmann et al. 1975).

PPV1 has also been associated with cutaneous lesions in piglets. Kresse et al. (1985) associated PPV1 with an epidemic disease in piglets characterized by slit-like erosions and vesicle-like lesions involving the oral cavity and snout. Whitaker et al. (1990) associated PPV1 with a necrotic and exudative dermatitis in piglets. However, experimental cutaneous inoculation of this virus into piglets produced no lesions, which led to the conclusion that PPV1 may only predispose piglets to secondary skin disease (Lager and Mengeling 1994).

Microscopic lesions have been observed in tissues of gilts necropsied after their fetuses were infected by transuterine inoculation of the virus. Seronegative gilts infected at 70 days of gestation and necropsied at 12 and 21 days post inoculation had focal accumulation of mononuclear cells adjacent to the endometrium and in deeper layers of the lamina propria. There was also marked perivascular cuffing of plasma cells and lymphocytes in the brains, spinal cord, and choroid of the eye (Hogg et al. 1977). When fetuses were inoculated at time points earlier in gestation (35, 50, and 60 days) and the dams necropsied 7 and 11 days later, the lesions were similar. However, at that time uterine lesions were more severe and included extensive cuffing of mononuclear cells around myometrial and endometrial vessels (Lenghaus et al. 1978). Only focal accumulations of lymphocytes were detected in the uteri of gilts that were seropositive when their fetuses were inoculated (Cutlip and Mengeling 1975).

Histopathological changes in the fetus tend to be widespread, and the major microscopic lesions represent necrosis of cells in developing organ systems (Joo et al. 1977; Lenghaus et al. 1978). Hemorrhages are present in subcutaneous tissues and muscle masses. Necrosis and

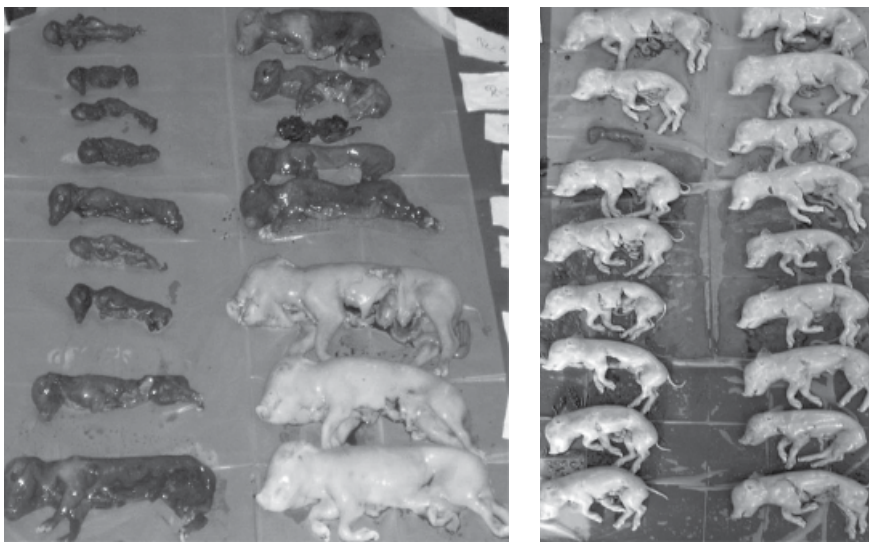


Figure 38.3 Litters of inoculated pregnant sows on day 90 of gestation displaying distinct levels of lesions. The sows were exposed to different PPV1 isolates (27a and NADL-2) on day 40 of gestation. The fetuses of each litter are placed according to their position in the uterus with the most cervical-positioned fetuses at the top. *Source:* Zeeuw et al. 2007.

mineralization are common in the lungs, kidneys, and skeletal muscle, particularly extensive in the liver and heart (Lenghaus et al. 1978) (Figure 38.4). After the fetuses become immunocompetent, microscopic lesions are primarily endometrial hypertrophy and mononuclear cell infiltration (Hogg et al. 1977; Joo et al. 1977). Meningoencephalitis characterized by perivascular cuffing with proliferating adventitial cells, histiocytes, and a few plasma cells in the gray and white matter of the cerebrum and leptomeninges were also seen in PPV1-infected live fetuses delivered late in gestation or in stillborn piglets (Hogg et al. 1977; Joo et al. 1977; Narita et al. 1975).

PPV1 has also been associated with a nonsuppurative myocarditis in piglets characterized by single foci of mild to moderate infiltration of mononuclear cells and hemorrhages between the cardiac myocytes (Bolt et al. 1997).

In boars, experimental intratesticular inoculation with PPV1 generated acute degeneration of the seminiferous epithelium, with formation and sloughing of multinucleated cells. Microscopic lesions were not observed after intramuscular inoculation (Thacker et al. 1987).

Diagnosis

Consider PPV1 when reproductive consequences compatible with PPV1 infection are observed, for example, an increase in the return-to-estrus index or delays in parturition with increased numbers of mummified fetuses and smaller litters, especially in first- or second-parity females. A mix of normal pigs and mummified fetuses that died at different development stages in the same litter is a strong indication of PPV1 infection. PPV1 infection does not normally cause abortions and does not cause clinical signs in adults (Mengeling 1978; Mengeling and Cutlip 1975). Given this clinical picture, the differential diagnosis should also include pseudorabies (Aujeszky's disease), brucellosis, leptospirosis, porcine reproductive and respiratory syndrome (PRRS), toxoplasmosis, nonspecific bacterial uterine infection, and others.

Laboratory submissions for the confirmation of PPV1 infection should include mummified fetuses and fetal remains. Detection of viral antigen in fetal tissues by immunofluorescence (IF) is a reliable procedure for the diagnosis of PPV1 (Mengeling 1978; Mengeling and Cutlip 1975). Alternatively, paired serum samples from gilts and sows can be used to document PPV1 infection. However, serum should be collected at the time of reproductive failure and a second sample 2–4 weeks later. Serum or fluids from fetuses, stillborn piglets, or umbilical cord serum before ingestion of colostrum ingestion can also be tested for PPV-specific antibodies.

PPV1 agglutinates erythrocytes of a variety of animal species, including rat, monkey, chicken, guinea pig, and human (blood group 0) (Siegl 1976). The virus grows readily in renal or testicular cells. Primary cell cultures present a higher risk of contamination with adventitious agents and are composed of cells with a lower index of division. Therefore, continuous cell lines (ESK, PK-15, SK6, ST, STE, and SPEV) are typically used for virus

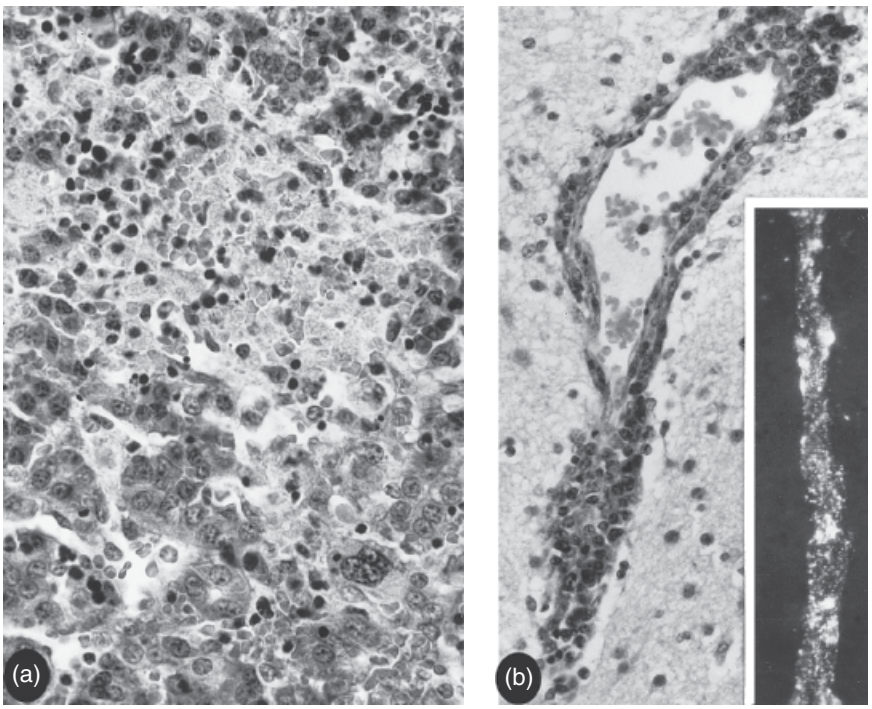


Figure 38.4 Tissues of PPV1-infected fetuses of gilts experimentally infected oronasally. (a) Necrotic focus in the liver of a live fetus of a gilt infected on day 40 of gestation and killed 42 days later; the fetus had numerous macroscopic lesions (hematoxylin and eosin [H&E]; ×400). (b) Perivascular cuffing with mononuclear cells in the cerebrum of a live fetus, littermate of (a); the fetus had no macroscopic lesions (H&E; ×320). Inset: viral antigen associated with endothelium of the cerebral vessel of a fetus of a gilt infected on day 46 of gestation and killed 25 days later (IF microscopy; ×312.5). Photographs (a) and (b) courtesy of T. T. Brown, Jr, National Animal Disease Center, Ames, IA.

propagation and titration (Mengeling 1972; Zimmermann et al. 2006). Cytopathic effects of PPV1 in cell cultures include intranuclear inclusions, pyknotic nucleus, granulations, irregular shape, slow replication, and subsequent cell death (Cartwright et al. 1969; Mengeling 1972). IF microscopy may be used to confirm PPV1 infection of cell cultures and to titrate virus (Johnson 1973; Mengeling 1978) (Figure 38.5). Alternatively, since PPV1 produces a viral hemagglutinin, the virus may also be titrated based on the hemagglutinating activity of PPV1 for erythrocytes of certain species (Joo et al. 1976b; Siegl 1976). Infectious PPV1 is slowly, but progressively, lost after fetal death (Mengeling and Cutlip 1975). Therefore, the likelihood of successful recovery of virus will depend on the condition of fetal tissues at the time of collection, but attempted virus isolation from autolyzed tissues is unproductive.

For routine diagnostics, polymerase chain reaction (PCR) is the most useful technique for the detection of PPV1 in fetal tissues, semen, and other samples. Numerous PCR protocols (and PCR-based, as qPCR and RPA) have been described (Chen et al. 2009; Gradil et al. 1994; Miao et al. 2009; Molitor et al. 1991; Prikhod'ko et al. 2003; Soares et al. 1999; Streck et al. 2015; Wilhelm et al. 2006; Yang et al. 2016), including multiplex PCRs (Cao et al. 2005; Huang et al. 2004; Kim and Chae 2003) mostly for the concurrent detection of PPV1 and PCV2.

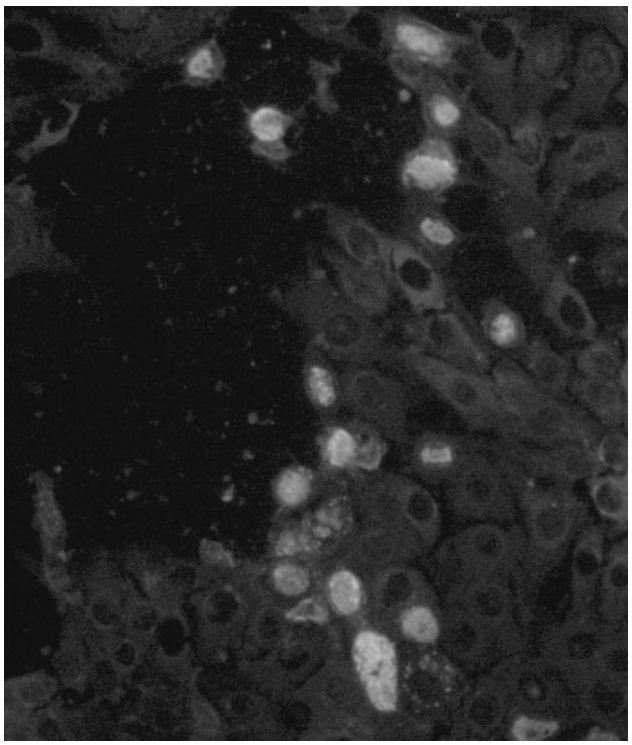


Figure 38.5 Indirect immunofluorescence of PK-15 cells infected with PPV1. Positive nuclear fluorescence is seen 5 days post infection (×400).

These methods are considered to possess higher diagnostic sensitivity and specificity than hemagglutination and are better suited for the detection of PPV1 in autolyzed tissues.

Serology may be useful for the diagnosis of PPV1 when fetal tissues are not available; however, the normally high prevalence of PPV1 in populations and the time lag between infection and the observation of reproductive losses often present challenges to the interpretation of results. For these reasons, paired serum samples should be evaluated in the context of changes in antibody titers between the two samples. Since the virus cannot cross the placental barrier, antibody-positive fluids or sera from fetuses, and pigs prior to consumption of colostrum, are indicative of an intrauterine infection.

The HI assay is commonly used for detection and quantification of PPV1-specific serum antibodies. Importantly, HI results may be affected by incubation temperature and the source of erythrocytes. Serum to be assayed in the HI test is usually pretreated by heat inactivation (56 °C [133 °F] for 30 minutes) followed by adsorption with erythrocytes (to remove nonspecific hemagglutinins) and kaolin (to remove or reduce nonspecific inhibitors of hemagglutination) (Mengeling 1972; Morimoto et al. 1972).

The enzyme-linked immunosorbent assay (ELISA) format is a preferable alternative to HI because it can be standardized and automated for high-throughput testing. Furthermore, it does not require pretreatment of serum before testing (Hohdatsu et al. 1988; Westenbrink et al. 1989). Differential ELISAs can distinguish vaccinated animals from animals infected with PPV1 (Madsen et al. 1997; Qing et al. 2006). Inactivated vaccines only elicit antibodies against the structural proteins (VP1/VP2), whereas differential ELISAs detect antibodies produced against the NS proteins that are expressed during virus replication in infected pigs.

Detection of PPVs 2–7 relies on molecular assays, such as PCR. Virus isolation of PPVs 2–7 in cell culture is difficult, and virus-specific reagents, such as monoclonal antibodies, are not widely available. Likewise assays for the detection of antibodies against PPVs 2–7 are not available, although some assay development has been conducted (Cságola et al. 2016).

Immunity

Piglets from seropositive dams are protected by colostral antibodies consumed in the first day of life. Antibodies against PPV1 are 10 times more concentrated in the colostrum relative to serum. Piglet starts to produce its own antibody in the second week of life. In most pigs, maternal antibody levels decrease steadily and reach non-detectable titers after around 20 weeks of age. In

some cases however, they may persist for up to 9 months and may interfere with the ability of young gilts to respond to vaccination.

Active immunity after field infection or vaccination with PPV1 develops within a few days. Antibodies are detected by HI or virus neutralization tests as early as 6 days post infection. There is a marked difference between antibody titers induced by commercial inactivated vaccines and infection with field virus. By HI, vaccine antibody titers are typically ≤500 or lower, whereas the infection titers regularly exceed 1 : 2000. The persistence of antibodies has been described for 4 months to 4 years for PPV1 (Johnson et al. 1976; Joo and Johnson 1977). Antibodies may prevent clinical disease, but infection and subsequent shedding of field virus occurs (Foerster et al. 2016; Jóźwik et al. 2009). Cellular immunity has also been described, and the proliferation of virus-specific $CD4^+$ and $CD8^+$ T cells proliferating after PPV antigen contact has been demonstrated (Ladekjaer-Mikkelsen and Nielsen 2002). Comparisons of immunity and/or immune responses of pigs to PPV1 versus PPVs 2–7 are not available.

Prevention and control

PPV1 is prevalent in the pig population and highly stable in the environment. These factors make it difficult to establish and maintain breeding populations free of the virus. A more practical goal in commercial herds is to maintain herd immunity against PPV1. Historically, swine producers used various approaches to infect gilts with PPV1 before the first breeding, for example, intentional infection of gilts to PPV1 by exposure to virus-contaminated tissues from affected litters. Approaches of this type are both unreliable and dangerous because they can result in the dissemination of other pathogens in the population, for example, classical swine fever virus. More preferable and reliable is regular vaccination of breeding females against PPV1 (no commercial vaccines against PPVs 2–7 are available at present).

Most commercial PPV1 vaccines are based on chemical-inactivated (formalin, beta-propiolactone, or binary ethyleneimine) tissue culture-derived virus adjuvanted with mineral oil or aluminum hydroxide. These vaccines induce antibody titers sufficient to prevent disease, but not infection (Jóźwik et al. 2009; Foerster et al. 2016). In controlled studies, antibody titers stimulated by inactivated vaccines were detected for 4–13 months after vaccination (Joo and Johnson 1977; Vannier et al. 1986). Therefore, regular revaccination of breeding sows at 4–6-month intervals may be necessary to maintain protective immunity in sows.

Modified live virus (MLV) vaccines for PPV1 have also been developed. Vaccination with MLVs induces a long-lasting immune response, with viremia and shedding of the vaccine virus occurring for a short time post-vaccination. There are few reports on MLVs, and most are based on NADL-2 virus as the vaccine virus (Paul and Mengeling 1980, 1984). Parenteral transmission was more effective than oral administration, and the quantity of virus administered was related to subsequent virus shedding and antibody titers.

In all cases (for inactivated or MLV), transplacental transmission of PPV1 was prevented. Limited experimental infections of pregnant sows with the PPV1 reference strain Impfstoffwerke Dessau-Tornau (IDT), strain Stendal, strain NADL-2, and the field isolates 27a and 143 revealed that vaccination prevented reproductive disorders and a very strong humoral immune response (Jóźwik et al. 2009; Foerster et al. 2016; Zeeuw et al. 2007).

Several subunit vaccines have been described for PPV1, with most based on expression of the viral VP2 protein in a baculovirus system. They provided protection comparable with inactivated full-virus vaccines (Antonis et al. 2006).

Review of the current vaccination strategy against PPV1 infection is warranted. The use of inactivated full-virus vaccines for protection against the closely related canine and feline parvoviruses is no longer common, and inactivated whole virus vaccines have largely been replaced by MLVs. The few remaining licensed inactivated vaccines are used for special purposes, for example, vaccination of exotic zoo felids. MLVs in carnivores induce a long-lasting immune response that provides protection for several years. In swine, the appearance of "new" genotypes or antigenic types of PPV1 needs to be closely watched. New PPV1 vaccines that induce longer-lasting immunity and protect against all of the prevalent virus strains circulating in pig populations are needed.

References

Adlhoch C, Kaiser M, Ellerbrok H, et al. 2010. *Virol J* 7:171.

Antonis AF, Bruschke CJ, Rueda P, et al. 2006. *Vaccine* 24:5481–9540.

Bachmann PA, Sheffy BE, Vaughan JT. 1975. *Infect Immun* 12:455–469.

Bergeron J, Hébert B, Tijssen P. 1996. *J Virol* 70:2508–2515.

Boisvert M, Fernndes S, Tijssen P. 2010. *J Virol* 84:7782–7792.

Bolt DM, Hitni H, Mtiller E, et al. 1997. *J Comp Pathol* 117:107–118.

Brown TT Jr., Paul PS, Mengeling WL. 1980. *Am J Vet Res* 41:1221–1224.
Cadar D, Csagola A, Lorincz M, et al. 2011. *Arch Virol* 156:2233–2239.
Cadar D, Dán Á, Tombácz K, et al. 2012. *Infect Genet Evol* 12:1163–1171.
Cadar D, Csagola A, Kiss T, et al. 2013. *Mol Phylogenet Evol* 66:243–253.
Cao S, Chen H, Zhao J, et al. 2005. *Vet Res Commun* 29:263–269.
Cartwright SF, Huck RA. 1967. *Vet Rec* 81:196–197.
Cartwright SF, Lucas M, Huck RA. 1969. *J Comp Pathol* 79:371–377.
Chen HY, Li XK, Cui BA, et al. 2009. *J Virol Methods* 156:84–88.
Cheung AK, Wu G, Wang D, et al. 2010. *Arch Virol* 155:801–806.
Choi CS, Molitor TW, Joo HS, et al. 1987. *Vet Microbiol* 15:19–29.
Cotmore SF, Tattersall P. 2006. Structure and organization of the viral genome. In Kerr J, Cotmre SF, Bloom ME, et al., eds. *Parvoviruses*. London: Hodder Arnold, pp. 72–94.
Cságola A, Lörincz M, Cadar D, et al. 2012. *Arch Virol* 157:1003–1010.
Cságola A, Zádori Z, Mészáros I, et al. 2016. *PLoS One* 11:e0151036.
Cui J, Biernacka K, Fan J, et al. 2017. *Transbound Emerg Dis* 64:1945–1952.
Cutlip RC, Mengeling WL. 1975. *Am J Vet Res* 36:1751–1754.
Dea S, Elazhary MASY, Martineau GP, et al. 1985. *Can J Comp Med* 49:343–345.
Duhamel GE, Bargar TW, Schmitt BJ, et al. 1991. *J Vet Diagn Invest* 3:96–98.
Ellis JA, Krakowka S, Lairmore MD, et al. 1999. *J Vet Diagn Invest* 11:3–14.
Ellis J, Clark E, Haines D, et al. 2004. *Vet Microbiol* 98:159–163.
Eterpi M, McDonnell G, Thomas V. 2009. *J Hosp Infect* 73:64–70.
Farr GA, Zhang L, Tattersall P. 2005. *Proc Natl Acad Sci U S A* 102:17148–17153.
Foerster T, Streck AF, Speck S, et al. 2016. *J Gen Virol* 97:1–6.
Foni E, Gualandi GL. 1989. A serological survey of swine parvovirus infection in Italy. *Microbiologica* 12:241–245.
Girod A, Wobus CE, Zádori Z, et al. 2002. *J Gen Virol* 83:973–978.
Gradil CM, Harding MJ, Lewis K. 1994. *Am J Vet Res* 55:344–347.
Harbison CE, Chiorini JA, Parrish CR. 2008. *Trends Microbiol* 16:208–214.
Hijikata M, Abe K, Win KM, et al. 2001. *Jpn J Infect Dis* 54:244–245.
Hogg GG, Lenghaus C, Forman AJ. 1977. *J Comp Pathol* 87:539–549.
Hohdatsu T, Baba K, Ide S, et al. 1988. *Vet Microbiol* 17:11–19.
Huang C, Hung JJ, Wu CY, et al. 2004. *Vet Microbiol* 101:209–214.
Huang L, Zhai SL, Cheung AK, et al. 2010. *Virol J* 7:333.
Johnson RH. 1973. *Aust Vet J* 49:157–159.
Johnson RH, Donaldson-Wood CR, Joo HS, et al. 1976. *Aust Vet J* 52:80–84.
Joo HS, Johnson RH. 1977. *Aust Vet J* 53:550–552.
Joo HS, Donaldson-Wood CD, Johnson RH. 1976a. *Arch Virol* 51:123–129.
Joo HS, Donaldson-Wood CR, Johnson RH. 1976b. *Aust Vet J* 52:51–52.
Joo HS, Donaldson-Wood CD, Johnson RH, et al. 1977. *J Comp Pathol* 87:383–391.
Jóźwik A, Manteufel J, Selbitz HJ, et al. 2009. *J Gen Virol* 90:2437–2441.
Kennedy S, Moffett D, Mcneilly F, et al. 2000. *J Comp Pathol* 122:9–24.
Kim J, Chae C. 2003. *Can J Vet Res* 67:133–137.
Kresse JI, Taylor WD, Stewart WW, et al. 1985. *Vet Microbiol* 10:525–531.
Ladekjaer-Mikkelsen AS, Nielsen J. 2002. *Viral Immunol* 15:373–384.
Lager KM, Mengeling WL. 1994. *J Vet Diagn Invest* 6:357–359.
Lau SKP, Woo PCY, Tse H, et al. 2008. *J Gen Virol* 89:1840–1848.
Lee JY, Kim EJ, Cho IS, et al. 2017. *Genome Announc* 5:e01738-16.
Lenghaus C, Forman AJ, Hale CJ. 1978. *Aust Vet J* 54:418–421.
Lucas MH, Cartwright SF, Wrathall AE. 1974. *J Comp Pathol* 84:347–350.
Madsen ES, Madsen KG, Nielsen J, et al. 1997. *Vet Microbiol* 54:1–16.
Mahnel H. May, 1965. Virus-like particles from hog cholera-infected tissue cultures and demonstrated in the electron microscope. In Proceeding of the FAO/OIE International Meeting on Hog Cholera and African Swine Fever, Rome, Italy.
Mayr A, Bachmann PA, Siegl G, et al. 1968. *Arch Gesamte Virusforsch* 25:38–51.
Mengeling WL. 1972. *Am J Vet Res* 33:2239–2248.
Mengeling WL. 1978. *J Am Vet Med Assoc* 172:1291–1294.
Mengeling WL, Cutlip RC. 1975. *Am J Vet Res* 36:1173–1177.
Mengeling WL Cutlip RC. 1976. *Am J Vet Res* 37:1393–1400.
Mengeling WL, Paul PS. 1981. *Am J Vet Res* 42:2074–2076.
Mengeling WL, Cutlip RC, Barnett D. 1978. Porcine parvovirus: Pathogenesis, prevalence, and prophylaxis.

In Proceedings of the International Pig Veterinary Society Congress, p. 5, KA 15.
Mengeling WL, Pejsak Z, Paul PS. 1984. *Am J Vet Res* 45:2403–2407.
Mengeling WL, Lager KM, Vorwald AC. 2000. *Anim Reprod Sci* 60–61:199–200.
Miao LF, Zhang CF, Chen CM, et al. 2009. *Vet Microbiol* 138:145–149.
Molitor TW, Oraveerakul K, Zhang QQ, et al. 1991. *J Virol Methods* 32:201–211.
Morimoto T, Ito Y, Tanaka Y, et al. 1972. *Natl Inst Anim Health Q (Tokyo)* 12:137–144.
Narita M, Inui S, Kawakami Y, et al. 1975. *Natl Inst Anim Health Q (Tokyo)* 15:24–28.
Nash WA. 1990. *Vet Rec* 126:175–176.
Ni J, Qiao C, Han X, et al. 2014. *Virol J* 11:203.
Opriessnig T, Xiao CT, Gerber PF, et al. 2014. *Vet Microbiol* 173:9–16.
Oravainen J, Heinonen M, Tast A, et al. 2005. *Reprod Domest Anim* 40:57–61.
Palinski RM, Mitra N, Hause BM. 2016. *Virus Genes* 52:564–567.
Paul PS, Mengeling WL. 1980. *Am J Vet Res* 41:2007–2011.
Paul PS and Mengeling WL. 1984. *Am J Vet Res* 45:2481–2485.
Paul PS, Mengeling WL, Brown TT. 1979. *Infect Immun* 25:1003–1007.
Paul PS, Mengeling WL, Brown TT Jr 1980. *Am J Vet Res* 41:1368–1371.
Prikhod'ko GG, Reyes H, Vasilyeva I, et al. 2003. *J Virol Methods* 111:13–19.
Qing L, Lv J, Li H, et al. 2006. *Vet Res Commun* 30:175–190.
Rhode SL. 1973. *J Virol* 4:856–861.
Robinson BT, Cartwright SF, Danson DL. 1985. *Vet Rec* 117:611–612.
Ruckerbauer GM, Dulac GC, Boulanger P. 1978. *Can J Comp Med Vet Sci* 42:278–285.
Rudek Z, Kwiatkowska L. 1983. *Cytogenet Cell Genet* 36:580–583.
Saekhow P, Ikeda H. 2015. *Microbiol Immunol* 59:82–88.
Saekhow P, Mawatari T, Ikeda H. 2014. *Microbiol Immunol* 58:382–387.
Schirtzinger EE, Suddith AW, Hause BM, et al. 2015. *Virol J* 12:170.
Siegl G. 1976. The parvoviruses. In Gard S, Hallauer C, eds. *Virology Monographs 15*. New York: Springer-Verlag Wien, pp. 47–52.
Simpson AA, Hébert B, Sullivan GM, et al. 2002. *J Mol Biol* 315:1189–1198.
Soares RM, Durigon EL, Bersano JG, et al. 1999. *J Virol Methods* 78:191–198.
Soares RM, Cortez A, Heinemann MB, et al. 2003. *J Gen Virol* 84:1505–1515.
Streck AF, Bonatto SL, Homeier T. et al. 2011. *J Gen Virol* 92:2628–2636.
Streck AF, Homeier T, Foerster T. et al. 2013. *Arch Virol* 158:1173–1180.
Streck AF, Canal CW, Truyen U. 2015a. *Infect Genet Evol* 36:300–306.
Streck AF, Hergemöller F, Rüster D, et al. 2015b. *J Virol Methods* 218:46–50.
Thacker BJ, Joo HS, Winkelman NL, et al. 1987. *Am J Vet Res* 48:763–766.
Vannier P, Brun A, Chappuis G, et al. 1986. *Ann Rech Vet* 17:425–432.
Vasudevacharya J, Compans RW. 1992. *Virology* 187:515–524.
Vihinen-Ranta M, Wang D, Weichert WS, et al. 2002. *J Virol* 76:1884–1891.
Wang F, Wei Y, Zhu C, et al. 2010. *Virus Genes* 41:305–308.
Westenbrink F, Veldhuis MA, Brinkhof JMA. 1989. *J Virol Methods* 23:169–178.
Whitaker HK, Neu SM, Pace LW. 1990. *J Vet Diagn Invest* 2:244–246.
Wilhelm S, Zeeuw EJL, Selbitz HJ, et al. 2005. *J Vet Med B Infect Dis Vet Public Health* 52:323–326.
Wilhelm S, Zimmermann P, Selbitz HJ, et al. 2006. *J Virol Methods* 134:257–260.
Wu R, Wen Y, Huang X, et al. 2014. *Arch Virol* 159:1533–1536.
Xiao CT, Gerber PF, Giménez-Lirola LG, et al. 2013a. *Vet Microbiol* 161:325–330.
Xiao CT, Giménez-Lirola LG, Jiang YH, et al. 2013b. *PLoS One* 8:e65312.
Xing X, Zhou H, Tong L, et al. 2018. *Arch Virol* 163:209–213.
Yang Y, Qin X, Zhang W, et al. 2016. *Mol Cell Probes* 30:300–305.
Zeeuw EJL, Leinecker N, Herwig V, et al. 2007. *J Gen Virol* 88:420–427.
Zhao X, Xiang H, Bai X, et al. 2016. *Virol J* 13:26.
Zhong H, Li X, Zhao Z, et al. 2016. *Genome Announc* 4:e00036-16.
Zimmermann P, Ritzmann M, Selbitz HJ, et al. 2006. *J Gen Virol* 87:295–301.

39

Pestiviruses

Peter D. Kirkland, Marie-Frédérique Le Potier, and Deborah Finlaison

Overview

The pestiviruses are small (approximately 40–60 nm), enveloped, roughly spherical, positive-sense, single-stranded RNA viruses in the genus *Pestivirus* of the family *Flaviviridae* (Becher et al. 1999). Currently the genus *Pestivirus* includes four formally recognized species: classical swine fever virus (CSFV), bovine viral diarrhea viruses 1 and 2 (BVDV-1 and BVDV-2), and border disease virus (BDV).

Molecular characterization suggests that there are other species in the genus *Pestivirus*. These include the "pestivirus of giraffe" (Becher et al. 2003; Harasawa et al. 2000), the "HoBi" and related viruses isolated from fetal bovine serum and cattle (Schirrmeier et al. 2004), a virus isolated from pronghorn antelope in the United States (Vilcek et al. 2005), Bungowannah virus identified in an outbreak of disease in pigs in Australia (Kirkland et al. 2007), and, most recently, an "atypical porcine pestivirus" (APPV) identified in multiple countries (Beer et al. 2017; de Groof et al. 2016; Hause et al. 2015; Mosena et al. 2018; Munoz-Gonzalez et al. 2017; Postel et al. 2016a; Zhang et al. 2017). Another novel pestivirus, provisionally called "Linda" (lateral shaking inducing neurodegenerative agent) virus, was identified in Austria in 2015 from piglets with congenital tremor (Lamp et al. 2017). Interspecies transmission of the pestiviruses is well known, and the possibility that pigs could be infected with any of the other pestiviruses cannot be excluded.

The new taxonomic species names have been proposed for the genus *Pestivirus* (Smith et al. 2017). The four current species are proposed to be renamed as *Pestivirus A* (formerly *Bovine viral diarrhea virus 1*), *Pestivirus B* (*Bovine viral diarrhea virus 2*), *Pestivirus C* (*Classical swine fever virus*), and *Pestivirus D* (*Border disease virus*). In addition, seven new species (and example isolates) are proposed: *Pestivirus* E (pronghorn pestivirus), *Pestivirus F* (Bungowannah virus), *Pestivirus G* (giraffe pestivirus), *Pestivirus H* (Hobi-like pestivirus), *Pestivirus I* (Aydin-like pestivirus), *Pestivirus J* (rat pestivirus), and *Pestivirus K* (APPV).

The most important pestivirus of pigs is CSFV. Infection of pigs with BVDV or BDV can result in disease, especially reproductive loss, but without major clinical impact. Bungowannah virus is a significant pathogen, but the known geographical distribution of Bungowannah virus is extremely limited, having only been found in two herds in Australia. Although APPV has only been identified recently, it is probably widespread in the global pig population.

The majority of pestiviruses are non-cytopathogenic in cell culture, but some CSFV strains and some BVDV isolates from cases of mucosal disease are cytopathogenic *in vitro* (Gallei et al. 2008).

The pestivirus genome is 12.5–16.5 kb in size and encodes a single polyprotein (Meyers et al. 1989) encompassing all of the structural proteins (C, E^{rns}, E1, and E2) and the nonstructural proteins (N^{pro}, p7, NS2/3, NS4A, NS4B, NS5A, and NS5B) as follows: NH_2-(N^{pro}-C-E^{rns}-E1-E2-p7-NS2/3-NS4A-NS4B-NS5A-NS5B)-COOH. The single polyprotein is co- and posttranslationally converted to mature proteins by a combination of virus and host cell proteases. The structure and function of some envelope proteins have been studied in detail. Even if the nonstructural proteins are better characterized, studies are still needed to understand their role in RNA replication and virion morphogenesis (Ji et al. 2015; Tautz et al. 2015). Little is known about mechanisms of viral RNA replication, packaging, or assembly of viral particles. Virions are released from the host cell by exocytosis, usually without morphological cell damage, the exception being the cytopathogenic strains that are relatively uncommon in nature and arise from mutations of the predominant non-cytopathogenic population.

As enveloped viruses, the pestiviruses are very susceptible to treatment with detergents and lipid solvents. Although they are relatively susceptible to the effects of high or low pH and/or temperature above 60 °C (140 °F),

Diseases of Swine, Tenth Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson.

inactivation can be variable among viruses and markedly influenced by their environment. A protein-rich medium usually enhances the stability of pestiviruses (Edwards 2000).

Public health

There is no evidence of human infection with pestivirus, and they are not of any significance for public health or food safety.

Classical swine fever

Relevance

Classical swine fever (CSF), formerly known as "hog cholera," is a highly contagious viral disease of worldwide importance and one of the World Organization for Animal Health (OIE)-listed diseases. Clinical outbreaks suggestive of CSF were reported in the early nineteenth century (Fuchs 1968; Kernkamp 1961; USDA 1889), and CSF was recognized as viral in nature in 1903 (Wise 1981). Wild and domestic pigs are the only natural reservoirs of CSFV. CSFV is endemic in parts of Eastern Europe, Southeast Asia, Central America, and South America. Although eradicated from domestic pigs and wild boars in Western Europe, CSFV remains endemic in some populations of wild boar in Eastern Europe, and farms in these areas are at risk of reinfection.

Etiology

CSFV is antigenically and genetically diverse but relatively stable for an RNA virus. This has been demonstrated experimentally by serial passages in both cell culture and pigs (Vanderhallen et al. 1999) and, in the field, by a study of the isolates collected over 4 years following an outbreak in wild boars (Goller et al. 2016; Simon et al. 2013). Recombination between strains may be possible (He et al. 2007). Antigenic variability among CSFV isolates has been characterized using monoclonal antibodies with 21 antigenic virus types defined on the basis of two panels of monoclonal antibodies directed against E2 and E^{rns} glycoproteins (Kosmidou et al. 1995).

Genetic characterization of new CSFV isolates has become standardized in terms of the genomic fragment sequenced, the algorithms used in constructing phylogenetic trees, and the classification of the genetic groups. Three regions of the viral genome are usually evaluated: the 3′ end of the polymerase gene (NS5B), 150 nucleotides of the 5′ nontranslated region (NTR), and 190 nucleotides of the gene encoding E2. Genetic typing is most commonly based on the E2 glycoprotein because abundant sequence data are available.

CSFV is divided into three major genetic groups (Lowings et al. 1996), each with three or four subgroups (1.1, 1.2, 1.3; 2.1, 2.2, 2.3; 3.1, 3.2, 3.3, 3.4) (Paton et al. 2000). Phylogenetic analyses have demonstrated a link between genotype and geographic origin (Greiser-Wilke et al. 2000). Group 1 isolates are present in South America (Pereda et al. 2005) and Russia (Vlasova et al. 2003). Most viruses belonging to group 2 were isolated from outbreaks in Western, Central, or Eastern Europe (Blome et al. 2010) and some Asian countries (Blacksell et al. 2004; Kamakawa et al. 2006; Pan et al. 2005). More recently, group 2 was reported in Colombia, South America (Garrido Haro et al. 2018). Group 3 viruses are apparently confined to Asia (Parchariyanon et al. 2000). An overview of the global distribution of genotypes is depicted in Beer et al. 2015. The EU Community Reference Laboratory for CSF in Hanover, Germany, has developed a Web-accessible database of sequences from isolates throughout the world. This database is a useful tool for identifying possible viral sources for outbreaks occurring in previously uninfected areas (Postel et al. 2016b).

Epidemiology

Domestic pig populations in Australia, New Zealand, North America, and Western Europe are free of CSF (Beer et al. 2015). In South America, Chile and Uruguay have been declared CSF-free. Argentina, free of outbreaks since 1999, stopped vaccination in April 2004 (Vargas Terán et al. 2004). Extensive areas of Central and South America continue to control the disease by vaccination (Morilla and Carvajal 2002). CSF remains endemic in Asia (Luo et al. 2014; Roychoudhury et al. 2014), and although the situation in Africa is not well defined, the disease has been identified in Madagascar and South Africa (Sandvik et al. 2005).

The reemergence of CSF is always a risk and several areas previously free of CSF have had incursions in recent years. For example, CSF reemerged in Cuba in 1993 after an absence of more than 20 years. In spite of a campaign of total depopulation carried out in the Caribbean, new infections have occurred, leading the authorities to turn to vaccination as the method of control (Frias-Lepoureau 2002; Pereda et al. 2005).

Under natural circumstances, the primary routes of transmission are oronasal by direct or indirect contact with infected wild or domestic pigs or oral by ingestion of contaminated foodstuffs (Edwards 2000; Fritzemeier et al. 2000; Weesendorp et al. 2011). In finishing units and in areas with small pig farms, transport and introduction of infected pigs accounts for the majority of outbreaks and for the spread of the disease (Ribbens et al. 2004).

Airborne spread of CSFV has been demonstrated under experimental conditions (Weesendorp et al. 2009)

and could play an important role under field conditions facilitating the rapid spread of the virus, at least within a compartment (Weesendorp et al. 2014).

The possibility of CSFV transmission by semen was considered during the epidemic in the Netherlands (Hennecken et al. 2000). Infected boars can shed CSFV in semen, and it is assumed that the virus might be transmitted by artificial insemination (de Smit et al. 1999; Floegel et al. 2000).

Transmission of CSFV by rodents, dogs, and cats has been shown experimentally to be unlikely, even as mechanical vectors (Dewulf et al. 2001a). Thus, euthanasia of pets during an outbreak cannot be justified, as long as they do not leave the infected farm.

Indirect transmission via people can occur when biosecurity is deficient, for example, visitors entering the premises without changing into clothing and boots supplied by the farm (Elbers et al. 2001). However, it may be assumed that if basic hygiene measures are taken, the risk of transmitting the virus in this fashion is very low. Vehicles (trucks, trailers, cars) can carry virus-contaminated feces and urine over long distances. Depending on the temperature and duration of the transport, CSFV could survive for some hours or days in urine or feces, therefore presenting a risk of indirect contamination (Weesendorp et al. 2008).

A quantitative approach to understanding CSFV spread among animals and herds is an area of active interest. One objective is to identify the biological and population factors that affect the rate of transmission (Klinkenberg et al. 2002). Experimentally, it was shown that the virulence of the strain could influence the dynamics of virus spread (Durand et al. 2009). Pigs infected with highly virulent strains shed significantly more virus in all their secretions and excretions over the entire infectious period than pigs infected with moderately or lowly virulent strains. Exceptions were the pigs that developed the chronic form of infection after inoculation with a moderately virulent strain. During the entire infectious period, they excreted the largest amounts of virus, because they excreted virus continuously and for a long duration. This study highlighted the crucial role chronically infected pigs may play in the transmission of CSFV. Furthermore, it demonstrated the importance of discriminating between strains and the clinical appearance of infection when using excretion data for modeling (Weesendorp et al. 2011).

Mathematical models that predict the course of an epidemic could provide guidance for decision-making to control an outbreak. Such models have been created and tested with data from the epidemic in the Netherlands (Horst et al. 1999) and in Belgium (Mintiens et al. 2003). More recently, the results of the assessment of traditional control measures, such as preemptive culling and movement restrictions, have shown that movement restrictions have more impact than preemptive culling on the success of control (Thulke et al. 2011).

The survival and inactivation of CSFV has been reviewed (Edwards 2000). Typical of enveloped viruses, CSFV is inactivated by organic solvents (ether or chloroform) and by detergents. Sodium hydroxide (2%) is still considered the most suitable for disinfection of contaminated premises.

In spite of the fact that CSFV is an enveloped virus, CSFV survives for prolonged periods under certain conditions (i.e. cool, moist, protein-rich conditions, such as those found in meat), but in liquid manure CSFV can survive for 2 weeks at 20 °C (68 °F) and more than 6 weeks at 4 °C (39 °F). CSFV is relatively stable over pH ranging from 5 to 10. The rate of inactivation under pH 5 is dependent on the temperature. Thermal and pH stability may vary by strain, but inactivation of the virus is primarily dependent on the medium. For example, CSFV in cell culture loses its infectivity after 10 minutes at 60 °C (140 °F) but survives for up to 30 minutes at 68 °C (154 °F) in defibrinated blood. The inactivation of CSFV in feces and urine from pigs intranasally inoculated with a highly or moderately virulent CSFV strain was inversely related to the storage temperature. Average half-life values were 2–4 days at 5 °C (41 °F) and 1–3 hours at 30 °C (86 °F). Significant differences were observed in survival between virus strains in feces, but not in urine (Weesendorp et al. 2008). For these reasons, it is difficult to give guidelines for the survival of CSFV in the environment.

Pathogenesis

Transmission of CSFV is most commonly oronasal, with primary virus replication in the tonsils. From the tonsils, it spreads to the regional lymph nodes and then via the peripheral blood to bone marrow, visceral lymph nodes, and lymphoid structures associated with the small intestine and spleen. The spread of the virus within the pig is usually complete in less than 6 days.

Within the pig, CSFV replicates in monocyte–macrophage cells and vascular endothelial cells. CSFV is immunosuppressive and neutralizing antibodies may not appear until 2–3 weeks after infection. Leukopenia, in particular lymphopenia, is a classic early event (Susa et al. 1992). CSF leukopenia affects leukocyte subpopulations unequally, with B lymphocytes, helper T cells, and cytotoxic T cells the most affected. Depletion of lymphocyte subpopulations occurs shortly before virus can be detected in serum by reverse transcription polymerase chain reaction (RT-PCR).

The severity of the changes in bone marrow and circulating leukocytes suggest that effects of the virus on uninfected cells are induced indirectly (e.g. by a soluble

factor or by cell-to-cell contact) and are not a direct effect of the virus or viral protein(s). Research has shown that the glycoprotein E^{rns} at high concentration induces apoptosis in lymphocytes *in vitro* (Bruschke et al. 1997). However, exposure to supernatant from infected cells did not induce apoptosis in target cells. Although the process is still not fully understood, such a mechanism could also account for the delay in cellular and humoral immune responses (Summerfield et al. 2001).

In cell culture, most CSFV strains grow without producing cytopathic effect and without inducing interferon-alpha (IFN-alpha) secretion by infected cells. Indeed, CSFV infection causes cells to acquire a greater capacity to resist apoptosis (Ruggli et al. 2003). These observations provide evidence that CSFV interferes with cellular antiviral activity and suggests the possibility that the lesions seen in pigs have an immunopathological basis.

Interactions between CSFV and the monocyte–macrophage system result in the release of mediator molecules that promote the progression of the disease. Changes in hemostatic balance are thought to be caused by proinflammatory and antiviral factors that mediate the thrombocytopenia and hemorrhage characteristics of CSFV infection (Knoetig et al. 1999). The production of inflammatory cytokines by infected endothelial cells could play a role in immunosuppression and facilitate virus dissemination by attracting monocytic cells (Bensaude et al. 2004). A study further argued the role of cytokines in CSFV pathogeny. Levels of the antiviral cytokine IFN-alpha and the cytokine IL-12 coordinating innate and adaptive immune responses were enhanced when the anti-inflammatory cytokine TGF-β1 was reduced after CSFV infection (Renson et al. 2014). It has been recognized that CSFV can replicate in dendritic cells, and it is possible that these highly mobile cells disseminate CSFV to various sites in the body, especially lymphoid tissues (Jamin et al. 2008). Of themselves, interactions between CSFV-infected dendritic cells and lymphocytes are not sufficient to induce lymphocyte depletion without other interactions within the environment of the lymphoid follicles (Carrasco et al. 2004; Jamin et al. 2008).

The expression of differences in virulence between strains is a result of the CSFV–host interactions. The evasion of the host's innate immune responses delays the onset of acquired immunity and produces the resultant pathogenic effects. Results from a comparative microarray analysis strongly suggest that CSFV subverts the interferon response, leading to bystanding killing of lymphocytes and lymphopenia, the severity of which might be due to the host's loss of control of interferon production (Renson et al. 2010).

Clinical signs

In the acute form of CSF, the initial clinical signs include anorexia, lethargy, conjunctivitis, respiratory signs, and constipation followed by diarrhea (Cariolet et al. 2008; Floegel-Niesmann et al. 2009). In the chronic form, the same clinical signs are observed, but the pigs survive for 2–3 months before dying. Nonspecific signs (e.g. intermittent hyperthermia, chronic enteritis, and wasting) may also be seen.

Historically, peracute, acute, chronic, or prenatal forms of CSF were attributed to distinct levels of virus virulence. However, virus strain virulence is difficult to define because clinical signs also depend on pig age, breed, health status, and immune status (Depner et al. 1997; Floegel-Niesmann et al. 2009; Moennig et al. 2003).

Since the early 1980s, diagnosis of CSF based on clinical signs has been problematic and resulted in the belated recognition of CSF outbreaks, thereby giving time for the virus to spread (Durand et al. 2009). CSF is one of several diseases characterized by cutaneous hyperemia or cyanosis and nonspecific clinical signs. Particularly when CSFV strains of lower virulence are involved, it may be difficult to differentiate CSF from African swine fever (ASF), porcine reproductive and respiratory syndrome (PRRS), post weaning dermatitis and nephropathy syndrome (PDNS), salmonella, or cumarin poisoning. The one constant sign in CSF is hyperthermia usually >40 °C (>140 °F), with piglets often piled in a corner. Clinical signs are more marked in piglets than adults, in which hyperthermia may be lower (39.5 °C, 103 °F).

CSFV is able to cross the placenta and infect fetuses at any stage of pregnancy. Depending on the strain and the time of gestation, infection can cause abortion and stillbirths. However, infection at 50–70 days of gestation can lead to the birth of persistently viremic piglets. Such piglets initially appear clinically normal but subsequently begin to waste or develop congenital tremors (Vannier et al. 1981). This course of infection has been described as "late-onset CSF" (Van Oirschot and Terpstra 1977). Similar to BVDV in ruminants, these animals shed high levels of virus for several months and are important reservoirs of CSFV.

Depending on the virulence of the strain of virus and the host response, infected pigs can show clinical signs within 3–6 days of exposure to the virus and die rapidly, recover, or develop chronic disease, which is invariably fatal. Animals may start to shed virus within a few days of infection and before the onset of clinical signs. With less virulent strains, the time to the onset of disease can be as long as 13–19 days (Durand et al. 2009). However, due to the nonspecific nature of clinical signs, especially with strains of moderate or low virulence, the virus may remain undetected in a herd for 4–8 weeks, which increases the risk of further dissemination.

Lesions

CSF lesions vary in severity and distribution, depending on the course of the disease. In acute forms, the pathological picture is often hemorrhagic. Leukopenia, thrombocytopenia, petechiae, and ecchymoses in the skin, lymph nodes, larynx, bladder, kidney (Figure 39.1), and ileocecal junction are often described. Multifocal infarction of the margin of the spleen is characteristic of CSF, but is not always present (Figure 39.2). Swollen or hemorrhagic lymph nodes or tonsils are common (Figure 39.3). In chronic forms, button ulcers in the cecum or large intestine may be present (Figure 39.4), as well as a generalized depletion of lymphoid tissues. Hemorrhagic and inflammatory lesions are less common, or even absent, despite the degeneration of endothelial cells. Congenital CSF can result in abortion, fetal mummification, stillbirths, and congenital malformations, such as central dysmyelinogenesis, cerebellar hypoplasia, microencephaly, and pulmonary hypoplasia (van der Molen and van Oirschot 1981).

Floegel-Niesmann et al. (2009) compared the clinical signs and lesions produced by six field strains isolated during the last decade from domestic pigs or wild boars in Europe to a reference strain (Alfort 187). Comparing lesions in skin, subcutis and serosae, tonsil, spleen, kidney, lymph nodes, ileum and rectum, brain, and respiratory system, they found that lymph nodes were the tissues most severely affected by all isolates, followed by necrotic lesions in the ileum and hyperemia of the blood vessels of the brain. Thus, these tissues were the most reliable for diagnosis of CSF. Infarction of the spleen and necrotic lesions of the tonsil, although commonly described in the earlier literature, were infrequent. Likewise, respiratory signs were absent or mild.

Figure 39.1 Kidney showing numerous petechial hemorrhages. *Source:* Courtesy of W. C. Stewart.

Figure 39.3 Peripheral hemorrhage of the mandibular lymph node. *Source:* Courtesy of W. C. Stewart.

Figure 39.2 Infarction of the spleen. *Source:* Courtesy of L. D. Miller.

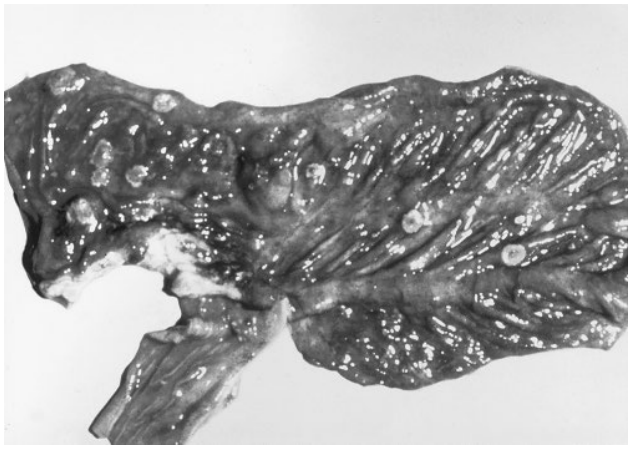


Figure 39.4 Button ulcers in the cecum and colon. *Source:* Courtesy of L. D. Miller.

Diagnosis

Response to CSF epidemics in Europe has shown that early recognition of CSF and prompt elimination of CSFV-infected animals is the key to control. The longer CSF remains undetected, the greater the opportunity for the virus to spread. It should be recognized that farmers and veterinarians detected 75% of the recent CSFV epidemics based on clinical observations. The need to establish a standardized protocol for evaluating herds for CSF has been recognized (Elbers et al. 2002; Floegel-Niesmann et al. 2009; Mittelholzer et al. 2000). However, the list of clinical criteria cannot be overly complex if it is to be used in the field. Average daily gain and feed consumption are two quantifiable measures that may be of use (Cariolet et al. 2008). Body temperature may also be useful, since hyperthermia is consistently associated with CSF and appears before, or concurrently, with the first clinical signs.

Because CSF has no pathognomonic clinical signs, laboratory diagnosis is always required. Since CSFV, BVDV, and BDV share common antigens, it is of the utmost importance to discriminate among these. A variety of methods for the detection of CSFV, components of the virion (antigens or nucleic acid), or specific antibodies against viral antigens are available. While pan-pestivirus diagnostic assays are useful for screening specimens, positive results must be confirmed with CSFV-specific assays. Real-time reverse transcription polymerase chain reaction (rRT-PCR) assays are now widely used to detect nucleic acid from pestiviruses, and both pan-reactive and CSFV-specific assays are available. Monoclonal antibodies that distinguish between pestiviruses are used in a variety of techniques to specifically identify the virus (e.g. virus isolation [VI], fluorescent antibody test [FAT], or enzyme-linked immunosorbent assay [ELISA] tests).

It is not feasible to perform all available diagnostic assays during an outbreak. Therefore, it is important to perform the assay(s) most appropriate to the situation and intended purpose. Since the key to controlling an outbreak is prevention of virus spread among farms, the assay of choice in terms of diagnostic sensitivity, diagnostic specificity, and speed is rRT-PCR. However, since the period of viremia is short, antibody detection assays are also useful, especially in herds where clinical signs have been present for more than 2 weeks (Greiser-Wilke et al. 2007).

Detection of CSFV

Depending on the virulence of the strain, the tests, and the specimens, virus can be detected within 24 hours after infection (Liu et al. 2011a). Virus may be isolated from whole blood collected in heparin or EDTA or serum, plasma, and buffy coat. The tissues most likely to contain virus are tonsils, spleen, jejunum, ileum, ileocecal, mesenteric, and retropharyngeal lymph nodes.

Although VI is the reference method to confirm the infection, it is labor intensive, time consuming, and incompatible with the rapid response required to prevent further spread of virus in CSFV eradication programs. The purpose of VI, at least in reference laboratories, is to isolate viruses for detailed characterization and for use in vaccination studies. CSFV can be isolated on porcine kidney cell lines (PK-15 or SK6). It is critical that all cells, media, and reagents have been previously determined to be free of pestiviruses or antibodies against pestiviruses.

rRT-PCR assays are currently the preferred method for the detection of viral RNA. These assays have both high sensitivity (diagnostic and analytical) and specificity, particularly probe-based assays (Hoffmann et al. 2005, 2011; Le Potier et al. 2006). Several CSFV-specific rRT-PCR kits are commercially available (Le Dimna et al. 2008). Protocols have been specifically designed for the detection of genome of the C strain of live vaccine (Liu et al. 2011b), while others were designed to allow a simultaneous detection of CSFV and ASF virus genome (Haines et al. 2013).

A wide variety of samples are suitable for testing by rRT-PCR, but mainly whole blood samples, swabs, and tissue samples are used for the diagnosis of CSF. Besides whole blood, serum, plasma, or leukocytes can be used. Preferred tissue samples for VI are the tonsil, spleen, ileum, and lymph nodes. Kidney samples may be less suitable.

Good quality, fresh specimens are preferred, but viral RNA can still be detected in specimens in which virus is inactivated or VI is no longer possible due to bacterial contamination or autolysis (e.g. wild boar samples) (Petrov et al. 2014). rRT-PCR is not affected by the presence of antibodies, so specimens from animals of any age can be tested. Viral RNA can also be detected for a long time in certain tissues (e.g. viral RNA was detectable in tonsils of pigs that had recovered from CSFV infection for at least 9 weeks) (Blome et al. 2006).

Depending on the genomic region that is targeted for amplification, rRT-PCR can be designed to differentiate between virus species (CSFV, BVDV, and BDV) and even strains of CSFV (Zhang et al. 2012). Depending on the vaccine and the sample to be tested, rRT-PCR can be used as a genetic DIVA test. Newly developed C-strain-specific rRT-PCRs (Leifer et al. 2009) can be used to test vaccinated animals for the presence of modified live virus (MLV) vaccines, but infection with wild-type virus cannot be ruled out in the case of a positive result. Combining different PCR assays that are specific for vaccine or wild-type virus (Liu et al. 2009; Zhao et al. 2008) and partial sequencing can be used to detect or rule out wild-type virus infections (Blome et al. 2011).

The high sensitivity of rRT-PCR will support the testing of pooled samples (Depner et al. 2007; Le Dimna et al. 2008), which can significantly increase throughput. There are, however, other considerations including the time to prepare sample pools and the need to retest all individual samples in a positive pool. To avoid a loss of sensitivity, detailed knowledge of the performance characteristics of the assay and the levels of RNA likely to be detected (e.g. clinical cases vs. screening in vaccinated populations) needs to be established prior to pooling.

In general, it can be concluded with a high degree of confidence that a negative RT-PCR result indicates that the tested animal or tissue sample is not infectious to other pigs. In contrast, a positive RT-PCR result does not necessarily imply that the animal is infectious (Haegeman et al. 2006).

Antigen-capture ELISAs may be used for early diagnosis of CSFV in live pigs. Double-antibody sandwich ELISAs are based on monoclonal and/or polyclonal antibodies directed against a variety of viral proteins. Serum, buffy coat fraction, whole blood in heparin or EDTA, or tissue homogenates can be tested in these assays. The technique is relatively simple to perform, does not require tissue culture facilities, is suitable for automation, and provides results within 36 hours (Depner et al. 1995). However, it is important to recognize the diagnostic limitations of antigen-capture ELISAs. All currently available commercial assays are less sensitive than VI on cell culture (Blome et al. 2006). In addition, their diagnostic sensitivity is significantly better on blood samples from piglets compared with samples from adult pigs or samples from mild or subclinical cases (Anonymous 2002). To compensate for the lack of diagnostic sensitivity, all pigs showing pyrexia in suspect herds should be tested. These tests also have lower diagnostic specificity and false-positive reactions may occur. For these reasons, the use of antigen-capture ELISAs is only recommended on samples from animals with clinical signs or pathological lesions compatible with CSF and for screening herds suspected to have been recently infected.

Although the direct FAT on frozen sections was the method of choice for detecting viral antigen, given the high sensitivity, throughput, and rapid turnaround provided by rRT-PCR, it is unlikely that this would be the assay of choice in a future outbreak.

Detection of anti-CSFV antibodies

The virus neutralization (VN) test has been considered the reference assay for the detection of CSFV-specific antibodies. CSFV neutralizing antibody levels are determined by endpoint titration of serum. However, the VN test requires good quality serum samples and the use of a cell culture system. As the VN test is relatively time consuming and takes 3–5 days to obtain results, it is not the assay of choice for routine large-scale testing.

Because of antibody cross-reactions among pestiviruses, VN tests can be used to identify the virus with which an animal has been infected. Samples are tested in dual or multiple VN tests, and the CSFV neutralizing antibody titers are compared with neutralizing antibody titers against BVDV or BDV reference strains. A difference of fourfold or more between the endpoints of the two titrations is considered conclusive for infection by the virus species yielding the highest neutralizing antibody titer (Anonymous 2002). This method is frequently used to screen neighboring herds around an outbreak prior to lifting control measures.

ELISAs for the detection of anti-CSFV antibodies are useful for conducting epidemiological surveys and for monitoring CSFV-free areas. Competitive ELISAs are based on competition between anti-CSFV serum antibodies and a CSFV-specific monoclonal antibody directed against the viral glycoprotein E2 (gp55). Cross-reactions with antibodies against other pestiviruses are reduced in the competitive ELISA format. A baculovirus recombinant E2 protein is often used as the antigen in the system. ELISA-detectable antibodies appear 10–15 days post infection, similar to the period described for the appearance of neutralizing antibodies.

The E^{rns}-ELISAs were developed as differential companion tests for use in E2-subunit-vaccinated populations (Van Rijn et al. 1999). More recently, other E^{rns}-ELISAs have been developed to be potentially used as companion ELISAs (Aebischer et al. 2013). However there is still scope for improvement of commercial ELISAs before recommending them as a companion test for the differential diagnosis with the new marker chimeric vaccine CP7_E2Alf (Schroeder et al. 2012).

Prototype pen-side antibody detection tests have been reported and could be useful in the assessment of serological prevalence for a vaccine program (Li et al. 2012).

Immunity

Even though CSFV can induce immunosuppression, pigs that recover develop neutralizing antibodies to the virus. Neutralizing antibodies are induced by the envelope glycoprotein E2, while the envelope protein E^{rns} and the nonstructural protein NS3 induce non-neutralizing antibodies. Therefore, clearance of the virus is possible after the onset of production of neutralizing antibodies at 10–20 days after infection.

Both CSFV-specific neutralizing activity and specific killer cell activity are important for an effective immune response (Renson et al. 2014). The dual combination of cellular immunity and neutralizing antibody is optimal for providing fast and complete protection that results in sterilizing immunity. However, each component in itself has the potential to protect pigs from a lethal CSFV infection. E2-subunit vaccines protect pigs by inducing

high titers of neutralizing antibodies (Bouma et al. 1999), but experimental infection with related pestiviruses or chimeric constructs were also protective, even though they did not induce detectable neutralizing activity (Reimann et al. 2004; Voigt et al. 2007).

Pigs that have been either vaccinated or infected are resistant to subsequent virus challenge. CSFV is antigenically quite stable for an RNA virus and cross-protection exists between different genogroups and even between BVDV and CSFV (Leforban et al. 1992). Maternal antibodies can confer protective immunity to piglets for 8–12 weeks, depending on the level of neutralizing antibodies in the colostrum (Kaden and Lange 2004). However, these antibodies can also interfere with the response to vaccination (Vandeputte et al. 2001).

Prevention and control

CSF is endemic in many parts of the world and remains a disease of worldwide importance. Although some regions are free of CSFV, it is still present at the borders between free and endemic areas and in some wild boar populations (Laddomada 2000). The risk of reintroducing CSFV into free areas is still high. Producers and veterinarians are in the best position to detect CSF outbreaks in free areas, but early detection will require both vigilance and training in the recognition of clinical signs.

For purposes of international trade, free areas maintain a "no vaccination" policy against CSF. Thus, control is based on early detection and stamping out infected or suspected herds, with the implementation of concomitant quarantine measures (Anonymous 2001). However, the eradication of CSF outbreaks in Europe by stamping out has raised concerns regarding the "no vaccine" policy. This is especially true in pig-dense areas, where a variety of factors increase the risk of disease spread (Koenen et al. 1996; Mintiens et al. 2003). In certain situations, like the 1997 outbreak in the Netherlands, movement restrictions that prevented the movement of pigs to slaughter resulted in the unnecessary euthanasia of large numbers of animals. While the use of vaccine has economic consequences (i.e. vaccinated areas are banned from international trade for at least 1 year), emergency vaccination in the face of an outbreak is more likely to be considered in the future.

Various CSFV vaccines are available, including the well-known live "Chinese" C strain, the Thiverval strain, and newer marker vaccines that allow differentiation of field virus-infected from vaccinated animals (Blome et al. 2017). The traditional live vaccines based on MLV induce fast protection (Graham et al. 2012), even by the oral route (Renson et al. 2013), with a high level of protection against clinical disease, and neutralizing antibodies are detectable at 2 weeks post challenge (Dahle and Liess 1995). Duration of immunity is 6–10 months, regardless of the route of administration (intramuscular or oronasal) (Kaden and Lange 2001; Kaden et al. 2008). The primary drawback of MLV is that it is impossible to differentiate vaccine antibodies from field virus-induced antibodies.

An E2 recombinant protein subunit vaccine is commercially available and provides the means to differentiate infected from vaccinated animals (DIVA). The efficacy of the baculovirus-expressed E2 protein marker vaccines has been evaluated in vaccination-challenge and transmission trials, but with variable results. A single dose of vaccine prevented clinical signs and mortality due to a CSFV challenge 3 weeks after vaccination (Bouma et al. 1999), but at least 14 days were needed to obtain clinical protection (Bouma et al. 2000; Uttenthal et al. 2001). If challenged earlier, no protection against clinical disease and no reduction of virus shedding were observed (Uttenthal et al. 2001).

Evaluation of the E2 marker vaccines showed that transplacental transmission of CSFV occurred even when two doses of vaccine were administered and challenge occurred 14 days after the second dose. The two-dose vaccination protocol protected pregnant gilts from clinical disease, but did not prevent horizontal or vertical spread of the CSFV (Dewulf et al. 2001b). Thus, transplacental infection would not be prevented in most vaccinated animals under the conditions of emergency vaccination. Vaccination could not prevent the "carrier sow syndrome" and, subsequently, the late-onset form of CSF (Depner et al. 2001).

Recently, the development of CSF vaccine has focused on five strategies, mainly based on genetically engineered constructs: immunogenic CSFV peptides, DNA vaccines, viral vectors expressing CSFV proteins, chimeric pestiviruses, and trans-complemented deleted CSFV genomes (replicons) (Blome et al. 2017). The chimeric pestivirus CP7-E2Alf has been licensed after being widely assessed for its safety and potency (Gabriel et al. 2012). It has been shown to induce a high level of immunity and protection, whatever the route of inoculation (oral or intramuscular); to prevent further virus spread; and to be safe in growing pigs and pregnant sows. Maternal-derived antibodies (Eblé et al. 2014) or preexisting BVDV-1 antibodies (Drager et al. 2016) do not seem to dramatically interfere with the efficacy of the vaccine. This vaccine could be used in an emergency vaccination program to control CSF spreading either in domestic pigs or wild boar populations. If a fully validated companion kit were available, its DIVA properties could make it a very valuable tool to control an outbreak while allowing for serological surveillance.

Classical swine fever in wild boars

Wild boars are potential reservoirs of CSF, as most outbreaks in domestic pigs have been ascribed to

transmission from wild boars (52% according to Fritzemeier et al. 2000). As a consequence, oral mass vaccination of wild boar has been implemented with success since the 1990s in the European Communities for limiting virus spread and persistence within natural populations (Rossi et al. 2015). CSFV infection is seemingly harmless for wild boars, as little mortality has been reported in past outbreaks. In the presence of natural or man-made barriers, CSFV in wild boar populations can be confined to a defined area until it is eliminated (Pol et al. 2008).

Classic measures for CSFV control in wild boars involved reduced hunting to allow the virus to spread within the susceptible population and induce mortality or immunity, followed by targeted hunting of the most susceptible animals (i.e. juveniles and the young sows). In some areas where it has been difficult to eradicate CSFV using standard methods, oral vaccination using live C strain distributed in oral bait has been attempted (Kaden et al. 2000). The safety of this vaccine has been demonstrated for other wild animal species (Chenut et al. 1999).

In pigs, the oral vaccine can induce strong protective immunity in 10 days (Kaden and Lange 2001). However, recent analysis of the data from oral vaccination field campaigns has shown that three doses were required to induce immunity in the field. Due to poor access to baits, young boars did not acquire sufficient protective immunity in most field studies (Kaden et al. 2002). Vaccination seems more effective if it is used on a preventive basis and delivered at least 1 year before the virus enters the area, giving time for a high level of population immunity to be achieved. Routine vaccination around an outbreak has been proposed to prevent the spread of disease (Rossi et al. 2010).

When vaccine is used, the only method to differentiate infected from vaccinated boars is detection of the virus by DIVA rRT-PCR (Leifer et al. 2009). Therefore, it is rather difficult to decide when to stop vaccination. Antibodies to CSFV can persist for many years and interfere with surveillance after the completion of oral mass vaccination. It is therefore recommended to increase active surveillance in order to detect dead wild boar. However, as it is rare to find carcasses in nature, serological surveillance on young animals is also recommended in combination with data modeling to identify seroprevalence hot spots exceeding 5% in young animals. In these areas, targeted serological and virological surveillance should be implemented (Saubusse et al. 2016). In the future, the use of an oral DIVA vaccine that would permit serological monitoring of a vaccinated population for the spread of wild-type virus would be valuable. The chimeric pestivirus CP7_E2alf is currently the most promising live vaccine candidate for oral application.

Bungowannah virus

Relevance

In June 2003, a severe disease outbreak characterized by an increase in stillbirths, preweaning mortality, and mummified fetuses occurred on two properties in southern New South Wales, Australia. Because the cause was unknown at the time of the outbreak, the term "porcine myocarditis syndrome" (PMC) was used to describe the disease (McOrist et al. 2004). Subsequently, a previously unidentified pestivirus, named Bungowannah virus, was identified as the cause of PMC (Finlaison et al. 2009, 2010; Kirkland et al. 2007).

Bungowannah virus has only been recognized on two farms in Australia, and its origin remains obscure. It is highly pathogenic for the porcine fetus, a feature consistent with its distant genetic relatedness to CSFV. Clinically, fetal infection with Bungowannah virus could be confused with a low virulence strain of CSFV. It is of concern that this virus is not detected by diagnostic assays that were considered to react with all members of the pestivirus genus. The global significance of this pestivirus remains unclear. Bungowannah virus has not been detected outside of Australia, but only very limited surveys have been conducted (Abrahante et al. 2014).

Etiology

Bungowannah virus is genetically distinct from CSFV, but molecular characterization supports its inclusion in genus *Pestivirus*. Phylogenetic analysis and limited antigenic cross-reactivity indicate that this pestivirus is genetically quite divergent from the recognized species of pestiviruses and also distinct from the more recently recognized APPV (Hause et al. 2015; Kirkland et al. 2007, 2015a). Bungowannah virus is non-cytopathic in cell culture and replicates to the highest titers in continuous porcine kidney cell lines; it also has a capacity to replicate efficiently in a surprisingly wide range of cells from other species, including bats (Kirkland et al. 2007; Richter et al. 2014).

Epidemiology

Bungowannah virus has only been identified in two related piggeries in New South Wales, Australia. The source of the virus is not known. The virus was eradicated from one site, and while efforts were made to eradicate the virus at the second larger multiunit site in 2010, they were ultimately unsuccessful, and the virus remains endemic. Reintroduction was thought to have occurred via aerosols generated during decontamination of an infected module 0.5–1.5 km away from the Bungowannah virus-free unit (Kirkland et al. 2015b). Other piggeries

connected to the affected farms were free of infection, and there is no evidence of infection on other farms in Australia. Serological surveillance in Germany has not identified antibodies to this virus, and a survey of undiagnosed cases of abortion and respiratory disease in the upper Midwestern United States also failed to detect evidence of the virus (Abrahante et al. 2014). While PMC is readily detected in a fully susceptible population, it is possible that it could remain unrecognized in countries where CSFV is present or pigs are affected with similar clinical entities.

Under field conditions, disease caused by Bungowannah virus has only been reported in pigs (McOrist et al. 2004). Experimentally, nonpregnant sheep and cattle have also been infected, but without evidence of disease (Kirkland et al. 2015b). The significance of fetal infection in the calf before development of immunocompetence is not clear as neutralizing antibodies could be detected in precolostral sera of the animals that became infected (Kirkland et al. 2015b).

Due to the large amount of virus shed into the environment by persistently infected pigs, postnatal transmission presumably occurs following oronasal exposure (D Finlaison, personal communication). Intranasal exposure of pigs under experimental conditions readily established infection. The risk of transmission by embryos or semen has not been fully evaluated. Strategic testing of young boars for high neutralizing antibody and negative PCR results on semen prior to movement to a quarantine facility has not been associated with introduction of Bungowannah virus to uninfected sites. Bungowannah virus RNA has been detected in the semen of approximately 20% of boars at the time of initial screening, despite high serum neutralizing antibody titers. It has not been determined whether this semen would be infective for female pigs via artificial insemination. VI studies on semen have been limited, although the RNA load in some samples is sufficiently high that transmission could be expected (Kirkland et al. 2015b).

If an animal is first infected during pregnancy, there is a high risk of transplacental transmission and fetal infection. During experimental studies fetal infection occurred in the litters of 87% of sows that became infected (Kirkland et al. 2015b).

Following the experimental infection of 5- to 6-week-old pigs by the intranasal route, viral shedding was detected by rRT-PCR for 3–10 days post challenge in oropharyngeal and nasal secretions. The duration of shedding and amount of virus shed in feces and conjunctival secretions was markedly lower (Finlaison et al. 2012). Limited experimental studies suggested that transmission is poor from transiently infected animals.

Experimental inoculation of pregnant pigs (Kirkland et al. 2015b) suggested that the epidemiology of Bungowannah virus was similar to *in utero* infections with BVDV, BDV, and low virulence CSFV. High quantities of Bungowannah virus RNA were detected in placenta and fluids (fetal and/or vaginal) at farrowing, irrespective of the stage of gestation at which infection occurred. Thus, fetal fluids and placenta are an important source of environmental contamination.

All infected piglets shed high quantities of Bungowannah virus RNA at birth, regardless of whether the dam was challenged before or after the approximate age of immunocompetence in the fetal pig (70 days). The duration of postnatal virus shedding was inversely related to the stage of gestation at which infection occurred. Thus, fetuses infected in late gestation had the shortest period of shedding (1–2 weeks of age). Most pigs from sows infected at 55 or 75 days gestation had Bungowannah virus-specific antibody at birth.

Animals persistently infected with Bungowannah virus have not been identified in the field, but they were observed during experimental studies. These pigs shed high quantities of Bungowannah virus in oropharyngeal secretions, urine, and feces, and the infection was readily transmitted to naïve pigs.

Specific studies on the persistence of Bungowannah virus in the environment and its susceptibility to disinfectants have not been carried out, but its characteristics are probably similar to those of CSFV and BVDV. Successful eradication from the affected farm described above was carried out with a combination of measures including depopulation, disinfection (detergent/Virkon/sodium hypochlorite), and site biosecurity measures.

Pathogenesis

The primary route of viral entry is presumed to be oronasal, with primary replication in the tonsils, like CSFV. Although this site of replication is yet to be confirmed, virus has been detected in oropharyngeal secretions at the earliest sampling time of 3 days post inoculation and in high quantities in tonsil tissue.

Unlike CSFV, there appears to be no appreciable clinical effects following postnatal infection with Bungowannah virus, regardless of the dose of virus (Finlaison et al. 2012). Thus, disease appears to be wholly the result of *in utero* infection of the fetus with the clinical outcome and lesions dependent on the stage of gestation at which infection occurs. Direct intrauterine transmission appears likely, and during experimental studies, most litters were completely infected by 20 days post infection (Finlaison et al. 2010).

Litters were most severely affected when sows were infected at approximately 35 days of gestation, with approximately 40% of fetuses either stillborn or mummified and 70% of the piglets born alive dying before 3 weeks of age. Of the small group of animals that survived to weaning, over 80% were persistently infected (Kirkland et al. 2015b).

Infection of the sow at ~55, ~75, or ~90 days gestation resulted in 10–15% stillbirths, with the number of mummified piglets close to normal production targets. Preweaning losses were also elevated (29%) in piglets from sows infected at 90 days of pregnancy. Persistently infected animals were immunotolerant, and most failed to mount a humoral immune response (Kirkland et al. 2015b). The pig fetus appears to be first able to mount a humoral immune response to Bungowannah virus at 57–73 days of gestation (Finlaison et al. 2010).

Clinical signs

To date, there has only been one known Bungowannah virus outbreak in pigs (McOrist et al. 2004). The most significant effects of Bungowannah virus infection were the consequence of *in utero* infection. Sudden death in healthy 2- to 3-week-old pigs triggered the initial disease investigation, but the nature of the losses altered within 2–3 weeks of the commencement of the outbreak. Sudden death became less common, and increased numbers of stillborn pigs and preweaning mortalities in the early postnatal period were noted. Piglets born alive from affected litters showed greatly reduced viability. Evidence from the field outbreak suggested that litter size remained relatively unaffected. At the peak of the outbreak, preweaning mortality reached 50%, 40% of fetuses were stillborn, and 13% were mummified in some production units. In a herd consisting of approximately 30,000 sows, it is estimated that 50,000 pigs were lost from production due to PMC over a 15-month period (Finlaison et al. 2009).

Pigs persistently infected with Bungowannah virus were not identified during the outbreak of PMC, but have subsequently been produced following experimental infection of pregnant sows up to 56 days gestation (Kirkland et al. 2015b). Those resulting from sow challenge at around 35 days of gestation experienced poor viability and high mortality in the early postnatal period. The few piglets that appeared clinically normal soon after birth developed a syndrome similar to "late-onset CSF" in the weeks following weaning, becoming severely stunted with high morbidity and mortality compared with unaffected cohorts of the same age. A small proportion of pigs born following infection of the sow at approximately 55 days appeared to be persistently infected and exhibited stunting compared with age-matched cohorts but had better long-term survival. Some of these animals seroconverted and appeared to clear the infection, cease viral shedding, and grow better after this occurred. The term "chronically infected" may therefore be more appropriate for these than "persistently infected" pigs as the latter exhibit lifelong virus excretion.

Postnatal infection with Bungowannah virus is associated with few clinical effects. An incubation period of 3–5 days prior to the detection of viremia has been observed following experimental intranasal infection of weaner aged pigs. While mild transient temperature rises were recorded, no other evidence of disease was noted (Finlaison et al. 2012). Likewise, on the piggeries affected by PMC, dams of the affected piglets were clinically normal with normal feed intake and body temperature (McOrist et al. 2004). Once the virus becomes endemic in a population, if animals are infected prior to first breeding, clinical disease may not be recognized despite ongoing transmission.

Lesions

Field cases of PMC were associated with increased stillbirths (both recent and long-standing with autolysis), fetal mummification, and preweaning mortalities (McOrist et al. 2004). A range of gross lesions were observed, including subcutaneous edema spread particularly over the head and thorax of some stillborn piglets; a dilated heart; irregular areas of myocardial pallor; increased pericardial, thoracic, and abdominal fluid; and occasional fibrin tags on the thoracic and abdominal viscera. The placentas appeared normal. Up to 50% of stillborn pigs may have elevated IgG levels. During experimental studies, some piglets born to sows infected with Bungowannah virus at 35 days of pregnancy exhibited purpura that appeared to resolve over approximately 7 days, an absence of facial whiskers, and white focal lesions in the cerebrum. Gross lesions were not observed in weaner pigs at necropsy 6 weeks post inoculation following experimental infection.

Histopathological findings in affected piglets consistently included acute-to-subacute multifocal, nonsuppurative myocarditis, with myonecrosis occasionally observed. Inflammation was frequently mild and localized and myofiber destruction was minimal. A minority of cases in older neonatal piglets had early myocardial fibrosis. Occasional affected piglets had nonsuppurative interstitial pneumonia, encephalitis, hepatitis, and lymphadenitis (McOrist et al. 2004). During experimental studies acute myocarditis was observed following infection of the dam in late gestation (day 90) (Kirkland et al. 2015b).

Diagnosis

While Bungowannah virus has to date only been identified in Australia, its origin remains unknown, and it should be included within the differential diagnoses of viral pathogens causing reproductive disease in the pig. In addition, the clinical presentation has similarities to *in utero* infection with CSFV, BVDV, and BDV and with "late-onset" CSF. For laboratory diagnosis, samples should be collected for rRT-PCR, histopathology, and serology.

Rapid identification of infected fetuses is best achieved with detection of Bungowannah viral RNA by rRT-PCR (Finlaison et al. 2009). Viral RNA can be readily detected in fetal fluids, pharyngeal swabs, and tissues of infected fetuses. Virus can be isolated from a number of tissues, including lung and lymphoid tissues, although it is not routinely used for diagnostic purposes because rRT-PCR has higher sensitivity. Additionally, the virus is not cytopathic in cell culture, so immunoperoxidase staining is required to detect virus replication in cell cultures (Kirkland et al. 2007).

Serology by peroxidase-linked immunoassay (PLA) or virus neutralization (VN) test is best utilized for surveillance and monitoring purposes. For diagnostic applications, PLA may be performed on poor quality samples that may not be suitable for testing by VN. This is of particular use for fetal specimens. Approximately 50% of stillborn piglets can have elevated IgG levels, and specific antibodies can be detected in the serum and fluids from body cavities of stillborn piglets. Pericardial and thoracic fluids are usually of better quality than peritoneal fluid.

Immunity

To date, only humoral immune responses to Bungowannah virus have been studied. During experimental studies, antibody was detected from 12 days post inoculation in weaner pigs challenged intranasally (Finlaison et al. 2012) and from 17 days in fetuses infected by direct inoculation (Finlaison et al. 2010). Postnatally, the production of specific antibody is associated with clearing of the viremia and cessation of viral shedding. In contrast, although the pig fetus is immunocompetent and able to produce detectable antibodies to Bungowannah virus from around 70 days of gestation (Finlaison et al. 2010), the humoral immune response of the fetal piglet was not sufficient to clear the infection prior to birth (Kirkland et al. 2015b). Following suckling, these animals developed high antibody titers that were maintained for at least 2–3 months. It is not known how long this naturally acquired antibody persists postnatally. Maternally derived antibody waned by approximately 2 months of age in persistently infected piglets and uninfected piglets from infected dams.

Prevention and control

There is no specific treatment for PMC or vaccine against Bungowannah virus. As the origin of the virus remains unknown, only general biosecurity measures can be adopted to prevent introduction of the virus into a population. If the virus is detected early in an outbreak, measures can be taken to attempt to reduce the impact of the disease. Evidence of poor postnatal transmission suggests that the most significant spread will occur following the farrowing of affected litters or by persistently infected piglets. In the face of an outbreak, it will be important to maximize exposure of breeding animals prior to mating to reduce the duration of the outbreak and to prevent possible exposure of pregnant animals.

Bungowannah virus eradication from an endemically infected piggery has been achieved through a combination of measures including depopulation, cleaning, disinfection, site biosecurity, and repopulation with previously unexposed animals.

Bovine viral diarrhea and border disease viruses

Relevance

Pigs are susceptible to pestiviruses other than CSFV and Bungowannah virus. Cross-species transmission among artiodactyls has been reported for strains of both BVDV and BDV (Carbrey et al. 1976; Terpstra and Wensvoort 1988).

Infection with either BVDV or BDV can complicate CSFV control or eradication programs. Cross-neutralization tests and tests using monoclonal antibodies (Leforban et al. 1990a; Wensvoort 1989) suggest that BVDV may have been isolated from pigs in the past and misidentified as CSFV by tests based on polyclonal antibodies. Likewise, serum antibodies against BVDV or BDV have the potential to cross-react in assays for the detection of CSFV antibodies. Because of cross-reactions among pestiviruses, it is essential to identify the specific etiologic agent when pestivirus antibodies are detected in CSF eradication programs.

Natural infection of swine with BVDV was first reported in Australia in 1964, but BVDV was not isolated from a naturally infected pig until 1973 (Fernelius et al. 1973). The teratogenic properties of pestiviruses are well established (Terpstra and Wensvoort 1988; Vannier et al. 1988; Wensvoort and Terpstra 1988), and infection of pregnant sows with BVDV or BDV may induce a pathology resembling congenital CSF.

Although natural infection of pigs with BVDV or BDV is relatively uncommon, interspecies transmission may occur in countries where pigs are raised in close contact with ruminants. Disease is most likely to occur when pregnant sows are infected, but may only affect a small number of litters until piglets are born and further transmission occurs. If the source of virus is a contaminated vaccine, the initial impact is likely to be much greater.

Etiology

BVDV and BDV are typical pestivirus species and cannot be differentiated morphologically or structurally from

CSFV (Laude 1979). However, they can be readily differentiated using monoclonal antibodies and molecular assays targeting the viral genome.

Epidemiology

The prevalence of BVDV antibodies in the pig populations of CSFV-free countries (Australia, Ireland, Great Britain, Denmark) has been estimated at 1.6–43.5%, depending on the age of the animals and possibly on the degree of contact with cattle (Jensen 1985). In countries where CSFV is present, the situation with regard to BVDV antibodies seems to be about the same.

Cattle are the most common source of BVDV infection in pigs. In units with dairy farming, one potential source of infection is BVDV-contaminated whey or milk fed to sows (Terpstra and Wensvoort 1988). In some cases, pigs have had contact with cattle recently vaccinated with BVDV (Stewart et al. 1971). In other reports, pigs and cattle were kept in separate lots and buildings, but personnel and equipment moved freely between the different farm units (Carbrey et al. 1976).

The prolonged presence of a persistently infected litter of pigs is the most likely source of BVDV or BDV to susceptible pregnant sows (Terpstra and Wensvoort 1988; Vannier et al. 1988). Persistent BDV infection of piglets occurs when sows are infected during early pregnancy (i.e. fetuses are transplacentally infected) and piglets become immunotolerant and persistently infected (Vannier et al. 1988). The course of the infection is quite similar to that described for BVD infection in pregnant cows (Baker 1987). When a pregnant sow is infected under experimental conditions, the litter may consist of a mixture of virus-positive and antibody-positive piglets. This suggests variability in the time at which individual fetuses became infected (Edwards et al. 1995). Congenitally BDV-infected piglets appear to excrete large amounts of virus, since susceptible young animals kept in contact rapidly seroconvert and produce high antibody titers. Conversely, when piglets are infected after birth, spread of infection to in-contact animals does not occur, suggesting low or perhaps no excretion of virus (Vannier et al. 1988).

Pigs may also become infected through the use of MLV vaccines (CSF or Aujeszky's disease) or other biologicals contaminated with virus (Vannier et al. 1988; Wensvoort and Terpstra 1988). While contaminants could be of either bovine or ovine origin, bovine are far more likely to be involved as most pestiviruses found in bovine serum products are strains of BVDV.

Pathogenesis

BVDV and BDV are pathogenic for fetal pigs, but relatively nonpathogenic for pigs after birth, apart from a slight increase in body temperature and a slight leukopenia and/or thrombocytopenia in some pigs (Makoschey et al. 2002). The ability of BVDV and BDV to establish intrauterine infections in swine is well established (Stewart et al. 1980; Vannier et al. 1988; Wrathall et al. 1978). The degree of clinical disease depends upon the stage of gestation at which the infection occurred. Clinical signs are more severe if sows are infected during the first trimester of pregnancy. The most severe clinical signs and lesions in fetuses or piglets are observed when sows are infected 25–41 days post breeding (Leforban et al. 1990b; Mengeling 1988). Under experimental conditions, piglets infected *in utero* with BVDV or BDV became persistently infected and were immunotolerant. After the disappearance of maternal antibodies, no active humoral response was detected in the majority of piglets. Furthermore, the virus was isolated from piglets and was shed by some, as evidenced by infection in young animals placed in contact.

In some experimental infections of pregnant sows with BDV, the onset of the clinical signs in the piglets was delayed until 13–14 days after birth. The reason for the delayed response is unknown, but colostral antibodies ingested by piglets would presumably block the replication of the virus and/or delay disease in transplacentally infected piglets (Leforban et al. 1990b; Mengeling 1988; Vannier et al. 1988).

The pathogenicity of BVDV or BDV seems to depend on the strain used in the experiment. BDV seems to be more consistently pathogenic for fetuses, whereas variable results are obtained with BVDV viral strains. The Singer strain, adapted to replicate in porcine cells, and BVDV strain 87/6 can infect and cause mortality in porcine fetuses, whereas the NADL strain does not induce clinical disease in piglets (Edwards et al. 1995; Leforban et al. 1990b; Mengeling 1988). Dahle et al. (1993) intranasally inoculated weaned pigs with BVDV strain Osloss/2482, then 4 weeks later challenged with decreasing doses of CSFV. After CSFV challenge, the only clinical sign observed was fever in one animal, although most animals became viremic.

Clinical signs

In the field, infection of pigs with BVDV usually occurs without clinical signs. In some cases, however, natural infection of pig herds with pestiviruses other than CSF has been associated with breeding problems (e.g. poor conception rates, small litters, and a few abortions). Hyperthermia and colic spasms have also been described (Carbrey et al. 1976). In the Netherlands and France, signs compatible with congenital CSF infection were described in piglets born to sows vaccinated 4 months earlier with CSF or Aujeszky's disease (pseudorabies) vaccines contaminated with a ruminant pestivirus

(Vannier et al. 1988; Wensvoort and Terpstra 1988). Clinical signs in piglets included anemia, rough hair coats, growth retardation, wasting, congenital tremors, conjunctivitis, diarrhea, polyarthritis, petechiae in the skin, and blue ear tips (Terpstra and Wensvoort 1988).

Natural infection of sows with BDV has been reported to result in reproductive signs (e.g. repeat breeding and mummified and stillborn pigs at farrowing) (Vannier et al. 1988). A high proportion of piglets from infected sows showed eyelid edema, locomotor disorders, and, occasionally, diarrhea and arthritis. The mortality rate in affected litters at 2 days of age ranged from 30 to 70%.

Experimental inoculation of pregnant sows with BDV field strains at 30–32 days of gestation produced transplacental infection of fetuses and newborn piglets with low body weights and short body lengths (Wrathall et al. 1978). Leforban et al. (1990b) reported an increase in perinatal mortality and eyelid edema, hyperthermia, and anemia in survivors during the second week of life. Slow growth rates, respiratory signs, and diarrhea developed in pigs, some of which died by 2 months of age. Pigs without respiratory and enteric signs survived and had normal growth despite marked snout deformations, including prognathism in one individual. BDV was isolated from blood and organs of all dead piglets, but not from survivors. When 40-day-old specific-pathogen-free (SPF) pigs were placed in contact with BDV transplacentally infected piglets, they did not show clinical signs, but developed high levels of antibody to BDV that was able to completely protect them against challenge with a virulent strain of CSFV.

Lesions

When infected postnatally with BVDV or BDV, no or very mild lesions are observed in pigs. Hyperemia of the small intestine was seen in one pig 11 days after being placed in contact with calves infected with the NADL strain of BVDV (Stewart et al. 1971). A transient leukopenia was detected during the first week following experimental infection of pigs with a pig isolate of BVDV (Carbrey et al. 1976). *In utero* infection of fetuses by transplacental transmission is followed by consistent pathological disorders in fetuses or piglets. In 13 naturally occurring BVDV outbreaks in the Netherlands, chronic gastroenteritis and septicemia with hemorrhages in lymph nodes, epicardium, and kidneys were the most consistent lesions reported. Inflammation of the digestive tract was frequently characterized by catarrh, hypertrophy, or ulceration of the mucosa. Necrotic tonsillitis, icterus, polyserositis, polyarthritis, and atrophy of the thymus were also noted (Terpstra 1987). A porcine BVDV isolate administered to gilts at 42–46 days of gestation produced significant microscopic lesions in the leptomeninges and the choroid plexus of the fetus characterized by collections of lymphocytes, histiocytes, and cellular accumulation in the vascular adventitia and perivascular spaces (Stewart et al. 1980).

In the case of BDV, experimental inoculation of sows on day 34 of gestation produced cerebellar hypoplasia in 9 of 19 live-born piglets, with a small meningocele in one of the 9 (Wrathall et al. 1978). The French BDV isolate Aveyron (Chappuis et al. 1984) inoculated into sows at day 30 of gestation produced lesions in lymphoid tissues in some piglets. Marked hemorrhages in lymph nodes and other lymphoid tissues were found in stillborn fetuses or in piglets that died shortly after birth. Histological examination of lymph nodes, spleen, and tonsil revealed marked subacute inflammatory lesions characterized by accumulations of lymphocytes, plasmacytes, and eosinophilic polymorphonuclear leukocytes, numerous secondary follicles, increased populations of reticulocytes, and lymphoid hypoplasia with pyknosis and karyorrhexis. Thymus, liver, and nervous tissues were normal (Leforban et al. 1990b).

Diagnosis

BVDV or BDV may be isolated using the same methods described for CFSV and from the same tissues submitted for CSFV diagnosis (i.e. tonsils, spleen, kidney, and whole blood collected in heparin or EDTA). However, if BVDV or BDV is isolated from pigs, these viruses grow better and to a higher titer in cells of ruminant origin, and of the homologous species, rather than in porcine cells (Wensvoort et al. 1989). In CSF-free countries, BVDV and BDV must be considered in the differential diagnosis of CSFV, and all CSF suspect cases should be tested for BVDV and BDV.

Pestiviruses have some shared antigens, and serological tests for the detection of antibodies against CSFV may cross-react with antibodies to ruminant pestiviruses. The practical importance of this is that the presence of ruminant pestivirus antibodies in pig sera often causes false-positive reactions in serological surveys for CSFV. This presents problems in CSFV eradication campaigns and in epidemiological surveys for CSFV (Jensen 1985).

Immunity

There is little practical interest in establishing protection by vaccination in pigs against BVDV or BDV. Consequently, there has been little done to study the immune response of pigs to these viruses. Nevertheless, the characteristics of the immune response are considered similar to that induced in pigs by CSFV.

Prevention and control

To prevent BVDV or BDV infection in pigs, it is necessary to avoid direct or indirect contact with cattle or sheep. Natural infection with BVDV often occurs when pigs are fed with cow's milk or bovine offal, and these practices should be avoided.

Inadvertent transmission of these viruses is a risk when live virus vaccines are used because of contamination of media and/or cells used in production of the vaccine. Cells used for multiplication of master seed virus used to prepare vaccine can be contaminated by BVDV or BDV. Indeed, some batches of CSF and Aujeszky's disease vaccines were contaminated by a pestivirus (probably BDV) because secondary lamb kidney cells were used to propagate the vaccine strain virus (Vannier et al. 1988; Wensvoort and Terpstra 1988). Both bovine and non-bovine cell lines can be contaminated with pestiviruses, and all cell cultures need to be monitored carefully for their presence. The primary source of contamination of cells is usually bovine serum added to the nutrient medium. Fetal infection with BVDV is extremely common. As commercial batches of fetal bovine serum usually involve pooling of serum from many calves or fetuses, the likelihood of BVDV contamination is high (Rossi et al. 1980). Hobi virus, one of the novel pestiviruses, has also been detected in some batches of fetal bovine serum (Ståhl et al. 2007) and may also present a possible risk, although nothing is known about its capacity to infect the pig. Therefore, to avoid interspecies transfer of these viruses, the systematic testing and treatment of bovine serum and of biological products used for the preparation of vaccines is strongly recommended.

Atypical porcine pestivirus (APPV)

Relevance

In 2015 a previously unrecognized pestivirus was detected in the United States (Hause et al. 2015). Later studies showed that this virus is also present in Europe (Beer et al. 2017; de Groof et al. 2016; Munoz-Gonzalez et al. 2017; Postel et al. 2016a; Schwarz et al. 2017), China (Zhang et al. 2017), and Brazil (Mosena et al. 2018), appearing to have a global distribution. Field observations and experimental studies suggest that APPV is associated with congenital tremors (Arruda et al. 2016; Schwarz et al. 2017). Its clinical relevance remains to be defined, although present evidence suggests it to be a cause of sporadic disease.

Etiology

APPV has been shown to be genetically most closely related to pestiviruses found in bats and rats (Hause et al. 2015) and to be very distinct from other pestiviruses, including CSFV and the other genetically distant pestivirus Bungowannah virus.

Epidemiology

So far APPV genomic RNA has been detected by PCR in pig samples from multiple countries, including the United States (Hause et al. 2015), Germany (Beer et al. 2017; Postel et al. 2016a), the Netherlands (de Groof et al. 2016), Austria (Schwarz et al. 2017), Spain (Munoz-Gonzalez et al. 2017), China (Zhang et al. 2017), and Brazil (Mosena et al. 2018). Investigations of 1,460 serum samples of healthy pigs from different parts of Europe and Asia demonstrated a geographically wide distribution of APPV (Postel et al. 2017). Considerable genetic variability was detected between strains in the United States, Europe, and Asia (de Groof et al. 2016; Postel et al. 2017).

Transmission of APPV remains unclear. Experimental study suggests that *in utero* infection and transplacental transmission to fetuses can occur (Arruda et al. 2016). Interestingly, APPV has also been detected in preputial swabs and semen of boars (de Groof et al. 2016; Gatto et al. 2017; Schwarz et al. 2017) although the role of semen in the transmission of APPV is unclear.

Little is known about the persistence of APPV in the environment and its susceptibility to disinfectants, but it could be expected to share the features of most other pestiviruses.

Pathogenesis

Presently available information suggests that APPV is only a pathogen of the fetus as a result of transplacental infection. Experimental infection of sows has resulted in the birth of piglets with a neurological disease frequently described as "congenital tremors" (Arruda et al. 2016).

Clinical signs

The only clinical entity with which APPV has been associated is the congenital tremor syndrome observed in newborn piglets. No disease has been observed in weaner aged piglets or sows. Depending on severity, signs consist of intermittent head shaking ranging to continuous trembling of the whole body as a result of myoclonic contractions.

Lesions

No gross changes have been detected in affected piglets. Histologically, lesions are restricted to the central nervous system (CNS) where hypomyelination of the white matter of the spinal cord and brain stem is observed (Schwarz et al. 2017). Virus as detected by rRT-PCR is

widely distributed in most tissues, with higher concentrations in the brain stem, spinal cord, tonsils, mesenteric lymph nodes, and serum (Arruda et al. 2016; de Groof et al. 2016; Schwarz et al. 2017).

Diagnosis

APPV was first detected using "next-generation" nucleic acid sequencing technology. This provided an insight into the virus genome and supported the development of rRT-PCR assays specific for APPV. APPV-related disease can be confirmed by testing of CNS tissue, tonsils, lymph nodes, and serum by rRT-PCR assay (Arruda et al. 2016; de Groof et al. 2016; Schwarz et al. 2017). A strain of APPV has also been isolated (Beer et al. 2017), which should provide for the development of VN test. Both an indirect (Hause et al. 2015) and blocking ELISAs (Schwarz et al. 2017) have been developed using recombinant antigens and may be useful for serological screening of populations.

Immunity

Although little is currently known about the duration of immunity following postnatal infection with APPV, it is expected that natural infection will lead to long-lived, perhaps lifetime immunity in pigs. This hypothesis is supported by the apparent lack of disease and infrequent occurrence of the congenital tremor syndrome, despite the apparent widespread distribution of the virus.

Prevention and control

Until there is knowledge of the epidemiology of APPV and also its clinical significance, the need for prevention or control measures and their nature cannot be determined.

References

Abrahante JE, Zhang JW, Rossow K, et al. 2014. *Transbound Emerg Dis* 61:375–377.

Aebischer A, Müller M, Hofmann MA. 2013. *Vet Microbiol* 161:274–285.

Anonymous. 2001. Council directive 2001/89/EC ON community measures for the control of classical swine fever. *Off J Eur Communities* L316: 5–35, 1.12.2001.

Anonymous. 2002. Commission decision approving a diagnostic manual establishing diagnostic procedures, sampling methods and criteria for evaluation of the laboratory tests for the confirmation of classical swine fever. *Off J Eur Communities* L39: 71–88.

Arruda BL, Arruda PH, Magstadt DR, et al. 2016. *PLoS One* 11:e0150104.

Baker JC. 1987. *J Am Vet Med Assoc* 190:1449–1458.

Becher P, Orlich M, Kosmidou A, et al. 1999. *Virology* 262:64–71.

Becher P, Ramirez RA, Orlich M, et al. 2003. *Virology* 311:96–104.

Beer M, Goller KV, Staubach C, et al. 2015. *Anim Health Res Rev* 16:33–39.

Beer M, Wernike K, Drager C, et al. 2017. *Transbound Emerg Dis* 64:e22–e26.

Bensaude E, Turner JL, Wakeley PR, et al. 2004. *J Gen Virol* 85:1029–1037.

Blacksell SD, Khounsy S, Boyle DB, et al. 2004. *Virus Res* 104:87–92.

Blome S, Meindl-Böhmer A, Loeffen W, et al. 2006. *Rev Sci Tech* 25:1025–1038.

Blome S, Grotha I, Moennig V, et al. 2010. *Vet Microbiol* 146:276–284

Blome S, Gabriel C, Staubach C, et al. 2011. *Vet Microbiol* 153:373–376.

Blome S, Moss C, Reimann I, et al. 2017. *Vet Microbiol* 206:10–20.

Bouma A, de Smit AJ, de Kluijver EP, et al. 1999. *Vet Microbiol* 66:101–114.

Bouma A, De Smit AJ, De Jong MC, et al. 2000. *Vaccine* 18:1374–1381.

Bruschke CJ, Hulst MM, Moormann RJ, et al. 1997. *J Virol* 71:6692–6696.

Carbrey EA, Stewart WC, Kresse JI, et al. 1976. *J Am Vet Med Assoc* 169:1217–1219.

Cariolet R, Bougeard S, Rault J-C, et al. 2008. *Journées de la Recherche Porcine* 40:45–48.

Carrasco CP, Rigden RC, Vincent IE, et al. 2004. *J Gen Virol* 85:1633–1641.

Chappuis G, Brun A, Kato F, et al. 1984. *Epidemiol Santé Anim* 6:117–118.

Chenut G, Saintilan AF, Burger C, et al. 1999. *Vet Microbiol* 64:265–276.

Dahle J, Liess B. 1995. *Berl Munch Tierarztl Wochenschr* 108:20–25.

Dahle J, Schagemann G, Moennig V, et al. 1993. *Zentralbl Veterinarmed B* 40:46–54.

Depner K, Paton DJ, Cruciere C, et al. 1995. *Rev Sci Tech* 14:677–689.

Depner KR, Hinrichs U, Bickhardt K, et al. 1997. *Vet Rec* 140:506–507.

Depner KR, Bouma A, Koenen F, et al. 2001. *Vet Microbiol* 83:107–120.

Depner K, Hoffmann B, Beer M. 2007. *Vet Microbiol* 121:338–343.

Dewulf J, Laevens H, Koenen F, et al. 2001a. *Vet Rec* 149:212–213.
Dewulf J, Laevens H, Koenen F, et al. 2001b. *Vaccine* 20:86–91.
Drager C, Schroder C, Konig P, et al. 2016. *Vaccine* 34:4666–4671.
Durand B, Davila S, Cariolet R, et al. 2009. *Vet Microbiol* 135:196–204.
Eblé PL, Quak S, Geurts Y, et al. 2014. *Vet Microbiol* 174:27–38.
Edwards S. 2000. *Vet Microbiol* 73:175–181.
Edwards S, Roehe PM, Ibata G. 1995. *Br Vet J* 151: 181–187.
Elbers ARW, Stegeman JA, de Jong MCM. 2001. *Vet Rec* 149:377–382.
Elbers ARW, Bouma A, Stegeman JA. 2002. *Vet Microbiol* 85:323–332.
Fernelius AL, Amtower WC, Lambert G, et al. 1973. *Can J Comp Med* 37:13–20.
Finlaison DS, King KR, Frost MJ, et al. 2009. *Vet Microbiol* 136:259–265.
Finlaison DS, Cook RW, Srivastava M, et al. 2010. *Vet Microbiol* 144:32–40.
Finlaison DS, King KR, Gabor M, et al. 2012. *Vet Microbiol* 160:245–250.
Floegel G, Wehrend A, Depner KR, et al. 2000. *Vet Microbiol* 77:109–116.
Floegel-Niesmann G, Blome S, Gerss-Dülmer H, et al. 2009. *Vet Microbiol* 139:165–169.
Frias-Lepoureau MT. 2002. Reemergence of classical swine fever in Cuba, 1993 to 1997. In Morilla A, Yoon KJ, Zimmerman J, eds. *Trends in Emerging Viral Infections of Swine*. Ames, IA: Iowa State Press, pp. 143–147.
Fritzemeier J, Teuffert J, Greiser-Wilke I, et al. 2000. *Vet Microbiol* 77:29–41.
Fuchs F. 1968. Schweinepest. In Rohrer H, ed. *Handbuch der virusinfektionen bei Tieren*, Band III/1, Vol. 3. Jena: VEB Gustav Fischer, pp. 15–250.
Gabriel C, Blome S, Urniza A, et al. 2012. *Vaccine* 30:2928–2936.
Gallei A, Blome S, Gilgenbach S, et al. 2008. *J Virol* 82:9717–9729.
Garrido Haro AD, Barrera Valle M, Acosta A, et al. 2018. *Transbound Emerg Dis* 65(3):782–790 doi: https://doi.org/10.1111/tbed.12803.
Gatto IRH, Arruda PH, Visek CA, et al. 2017. *Transbound Emerg Dis* doi: https://doi.org/10.1111/tbed.12759.
Goller KV, Gabriel C, Le Dimna M, et al. 2016. *J Gen Virol* 97:639–645
Graham SP, Everett HE, Haines FJ, et al. 2012. *PLoS One* 7:e29310.
Greiser-Wilke I, Fritzemeier J, Koenen F, et al. 2000. *Vet Microbiol* 77:17–27.
Greiser-Wilke I, Blome S, Moennig V. 2007. *Vaccine* 25:5524–5530
de Groof A, Deijs M, Guelen L, et al. 2016. *Viruses* 8:271–283.
Haegeman A, Dewulf J, Vrancken R, et al. 2006. *J Virol Methods* 136:44–50.
Haines FJ, Hofmann MA, King DP, et al. 2013. *PLoS One* 8:e71019.
Harasawa R, Giangaspero M, Ibata G, et al. 2000. *Microbiol Immunol* 44:915–921.
Hause BM, Collin EA, Peddireddi L, et al. 2015. *J Gen Virol* 96:2994–2998.
He CQ, Ding NZ, Chen JG, et al. 2007. *Virus Res* 126:179–185.
Hennecken M, Stegeman JA, Elbers ARW, et al. 2000. *Vet Q* 22:228–233.
Hoffmann, B, Beer M, Schelp C, et al. 2005. *J Virol Methods* 130:36–44.
Hoffmann B, Blome S, Bonilauri P, et al. 2011. *J Vet Diagn Invest* 23:999–1004.
Horst HS, Dijkhuizen AA, Huirne RB, et al. 1999. *Prev Vet Med* 41:209–229.
Jamin A, Gorin S, Cariolet R, et al. 2008. *Vet Res* 39:07.
Jensen MH. 1985. *Acta Vet Scand* 26:72–80.
Ji W, Guo Z, Ding NZ, et al. 2015. *Virus Res* 197:35–47.
Kaden V, Lange B. 2001. *Vet Microbiol* 82:301–310.
Kaden V, Lange E. 2004. *Vet Microbiol* 103:115–119.
Kaden V, Lange E, Fischer U, et al. 2000. *Vet Microbiol* 73:239–252.
Kaden V, Heyne H, Kiupel H, et al. 2002. *Berl Munch Tierarztl Wochenschr* 115:179–185.
Kaden V, Lange E, Steyer H, et al. 2008. *Vet Microbiol* 130:20–27.
Kamakawa A, Thu HTV, Yamada S. 2006. *Vet Microbiol* 118:47–56.
Kernkamp H. 1961. The natural history of hog cholera, In Mainwaring GT, Sorensen DK, eds. *Symposium on Hog Cholera*. St. Paul, MN: University of Minnesota, pp. 19–28.
Kirkland PD, Frost MJ, Finlaison DS, et al. 2007. *Virus Res* 129:26–34.
Kirkland PD, Frost MJ, King KR, et al. 2015a. *Vet Microbiol* 178:252–259.
Kirkland PD, Read AJ, Frost MJ, et al. 2015b. *Anim Health Res Rev* 16:60–63.
Klinkenberg D, De Bree J, Laevens H, et al. 2002. *Epidemiol Infect* 128:293–299.
Knoetig SM, Summerfield A, Spagnuolo-Weaver M, et al. 1999. *Immunology* 97:359–366.
Koenen F, Van Caenegem G, Vermeersch JP, et al. 1996. *Vet Rec* 139:367–371.
Kosmidou A, Ahl R, Thiel HJ, et al. 1995. *Vet Microbiol* 47:111–118.
Laddomada A. 2000. *Vet Microbiol* 73:121–130.
Lamp B, Schwarz L, Hogler S, et al. 2017. *Emerg Infect Dis* 23:1176–1179.
Laude H. 1979. *Arch Virol* 62:347–352.

Le Dimna M, Vrancken R, Koenen F, et al. 2008. *J Virol Methods* 147:136–142.
Le Potier M, Le Dimna M, Kuntz-Simon G, et al. 2006. *Dev Biol (Basel)* 126:179–186.
Leforban Y, Edwards S, Ibata G, et al. 1990a. *Ann Rech Vet* 21:119–129.
Leforban Y, Vannier P, Cariolet R. 1990b. Pathogenicity of border disease and bovine viral diarrhoea viruses for pig: experimental study on the vertical and horizontal transmission of the viruses. In Proceedings of the International Pig Veterinary Society, p. 204.
Leforban Y, Vannier P, Cariolet R. 1992. *Ann Rech Vet* 23:73–82.
Leifer I, Depner K, Blome S, et al. 2009. *J Virol Methods* 158:114–122.
Li X, Wang L, Shi X, et al. 2012. *J Virol Methods* 180:32–37
Liu L, Hoffmann B, Baule C, et al. 2009. *J Virol Methods* 159:131–133.
Liu J, Fan XZ, Wang Q, et al. 2011a. *Virol J* 8:201.
Liu L, Xia H, Everett H, et al. 2011b. *J Virol Methods* 175:170–174.
Lowings P, Ibata G, Needham J, et al. 1996. *J Gen Virol* 77:1311–1321.
Luo Y, Li S, Sun Y, et al. 2014. *Vet Microbiol* 172:1–6.
Makoschey B, Liebler-Tenorio EM, Biermann YM, et al. 2002. *Dtsch Tierarztl Wochenschr* 109:225–230.
McOrist S, Thornton E, Peake A, et al. 2004. *Aust Vet J* 82:509–511.
Mengeling W. 1988. The possible role of bovine viral diarrhoea virus in maternal reproductive failure of swine. In Proceedings of the International Pig Veterinary Society, p. 228.
Meyers G, Rumenapf T, Thiel HJ. 1989. *Virol* 171:555–567.
Mintiens K, Laevens H, Dewulf J, et al. 2003. *Prev Vet Med* 60:27–36.
Mittelholzer C, Moser C, Tratschin JD, et al. 2000. *Vet Microbiol* 74:293–308.
Moennig V, Floegel-Niesmann G, Greiser-Wilke I. 2003. *Vet J* 165:11–20.
van der Molen EJ, van Oirschot JT. 1981. *Zentralbl Veterinarmed B* 28:190–204.
Morilla A, Carvajal MA. 2002. Experiences with classical swine fever vaccination in Mexico. In Morilla A, Yoon KJ, Zimmerman J, eds. *Trends in Emerging Viral Infections of Swine*. Ames, IA: Iowa State Press, pp. 159–164.
Mosena ACS, Weber MN, da Cruz RAS, et al. 2018. *Transbound Emerg Dis* 65:22–26.
Munoz-Gonzalez S, Canturri A, Perez-Simo M, et al. 2017. *Transbound Emerg Dis* 64:1645–1649.
Pan CH, Jong MH, Huang TS, et al. 2005. *Arch Virol* 150:1101–1119.
Parchariyanon S, Inui K, Damrongwatanapokin S, et al. 2000. *Dtsch Tierarztl Wochenschr* 107:236–238.
Paton DJ, McGoldrick A, Greiser-Wilke I, et al. 2000. *Vet Microbiol* 73:137–157.
Pereda AJ, Greiser-Wilke I, Schmitt B, et al. 2005. *Virus Res* 110:111–118.
Petrov A, Schotte U, Pietschmann J, et al. 2014. *Vet Microbiol* 173:360–365.
Pol F, Rossi S, Mesplede A, et al. 2008. *Vet Rec* 162:811–816.
Postel A, Hansmann F, Baechlein C, et al. 2016a. *Sci Rep* 6:27735.
Postel A, Schmeiser S, Zimmermann B, et al. 2016b. *Viruses* 8:302.
Postel A, Meyer D, Cagatay GN, et al. 2017. *Emerg Infect Dis* 23:2104–2107.
Reimann I, Depner K, Trapp S, et al. 2004. *Virol* 322:143–157.
Renson P, Blanchard Y, Le Dimna M, et al. 2010. *Vet Res* 41:1–16.
Renson P, Le Dimna M, Keranflech A, et al. 2013. *Vet Res* 44:9.
Renson P, Le Dimna M, Gabriel C, et al. 2014. *Res Vet Sci* 96:389–395.
Ribbens S, Dewulf J, Koenen F, et al. 2004. *Vet Q* 26:146–155.
Richter M, Reimann I, Schirrmeier H, et al. 2014. *J Gen Virol* 95:2216–2222.
van Rijn PA, van Gennip HGP, Moormann RJM. 1999. *Vaccine* 17:433–440.
Rossi CR, Bridgman CR, Kiesel GK. 1980. *Am J Vet Res* 41:1680–1681.
Rossi S, Pol F, Forot B, et al. 2010. *Vet Microbiol* 142:99–107.
Rossi S, Staubach C, Blome S, et al. 2015. *Front Microbiol* 6:1141.
Roychoudhury P, Sarma DK, Rajkhowa S, et al. 2014. *Transbound Emerg Dis* 61(Suppl 1):69–77.
Ruggli N, Tratschin JD, Schweizer M, et al. 2003. *J Virol* 77:7645–7654.
Sandvik T, Crooke H, Drew TW, et al. 2005. *Vet Rec* 157:267.
Saubusse T, Masson JD, Le Dimma M, et al. 2016. *Vet Res* 47:21.
Schirrmeier H, Strebelow G, Depner K, et al. 2004. *J Gen Virol* 85:3647–3652.
Schroeder S, von Rosen T, Blome S, et al. 2012. *Rev Sci Tech* 31:997–1010.
Schwarz L, Riedel C, Högler S, et al. 2017. *Vet Res* 48:1–14.
Simon G, Le Dimna M, Le Potier MF, et al. 2013. *Vet Microbiol* 166:631–638.
de Smit AJ, Bouma A, Terpstra C, et al. 1999. *Vet Microbiol* 67:239–249.
Smith DB, Meyers G, Bukh J, et al. 2017. *J Gen Virol* 98:2106–2112.
Ståhl K, Kampa J, Alenius S, et al. 2007. *Vet Res* 38:517–523.
Stewart WC, Carbrey EA, Jenney EW, et al. 1971. *J Am Vet Med Assoc* 159:1556–1563.

Stewart WC, Miller LD, Kresse JI, et al. 1980. *Am J Vet Res* 41:459–462.
Summerfield A, McNeilly F, Walker I, et al. 2001. *Vet Immunol Immunopathol* 78:3–19.
Susa M, König M, Saalmüller A, et al. 1992. *J Virol* 66:1171–1175.
Tautz N, Tews BA, Meyers G. 2015. *Adv Virus Res.* 93:47–160.
Terpstra C. 1987. *Vet Q* 9(Suppl 1):50S–60S.
Terpstra C, Wensvoort G. 1988. *Res Vet Sci* 45:137–142.
Thulke HH, Eisinger D, Beer M. 2011. *Prev Vet Med* 99:28–37.
USDA. 1889. Hog Cholera: Its history, nature and treatment as determined by the inquiries and investigations of the Bureau of Animal industry. Washington, Government Printing Office.
Uttenthal A, Le Potier MF, Romero L, et al. 2001. *Vet Microbiol* 83:85–106.
Van Oirschot JT, Terpstra C. 1977. *Vet Microbiol* 2:121–132.
Vandeputte J, Too HL, Ng FK, et al. 2001. *Am J Vet Res* 62:1805–1811.
Vanderhallen H, Mittelholzer C, Hofmann MA, et al. 1999. *Arch Virol* 144:1669–1677.
Vannier P, Plateau E, Tillon JP. 1981. *Am J Vet Res* 42:135–137.
Vannier P, Leforban Y, Carnero R, et al. 1988. *Ann Rech Vét* 19:283–290.
Vargas Terán M, Calcagno Ferrat N, Lubroth J. 2004. *Ann N Y Acad Sci* 1026:54–64.
Vilcek S, Ridpath JF, Van Campen H, et al. 2005. *Virus Res* 108:187–193.
Vlasova A, Grebennikova T, Zaberezhny A, et al. 2003. *J Vet Med B* 50:363–367.
Voigt H, Merant C, Wienhold D, et al. 2007. *Vaccine* 25:5915–5926.
Weesendorp E, Stegeman A, Loeffen WL. 2008. *Vet Microbiol* 132:249–259.
Weesendorp E, Stegeman A, Loeffen WL. 2009. *Vet Microbiol* 135:222–230.
Weesendorp E, Backer J, Stegeman A, et al. 2011. *Vet Microbiol* 147:262–273.
Weesendorp E, Backer J, Loeffen W. 2014. *Vet Microbiol* 174:353–361.
Wensvoort G. 1989. *J Gen Virol* 70:2865–2876.
Wensvoort G, Terpstra C. 1988. *Res Vet Sci* 45:143–148.
Wensvoort G, Terpstra C, de Kluijver EP, et al. 1989. *Vet Microbiol* 21:9–20.
Wise G. 1981. Hog cholera and its eradication. A review of the U.S. experience. Animal and Plant Health Inspection Service, United States Department of Agriculture, APHIS, pp. 91–55.
Wrathall AE, Bailey J, Done JT, et al. 1978. *Zentralbl Veterinarmed B* 25:62–69.
Zhang XJ, Han QY, Sun Y, et al. 2012. *Res Vet Sci* 92:512–518.
Zhang K, Wu K, Liu J, et al. 2017. *Transbound Emerg Dis* 64:1020–1023.
Zhao JJ, Cheng D, Li N, et al. 2008. *Vet Microbiol* 126:1–10.

40

Picornaviruses

Soren Alexandersen, Nick J. Knowles, Graham J. Belsham, Aldo Dekker, Charles Nfon, Zhidong Zhang, and Frank Koenen

Overview

The family *Picornaviridae* is one of five families in the order *Picornavirales*, the others being *Dicistroviridae* and *Iflaviridae* (both infecting invertebrates), *Marnaviridae* (infecting algae), and *Secoviridae* (infecting plants) (Le Gall et al. 2008). There are currently 35 genera in the *Picornaviridae*, eight of which contain viruses that infect pigs: *Aphthovirus*, *Cardiovirus*, *Enterovirus*, *Kobuvirus*, *Pasivirus*, *Sapelovirus*, *Senecavirus*, and *Teschovirus* (Table 40.1; Figure 40.1) (Knowles et al. 2012).

Picornaviruses for which genome sequence data have become available, but which have not yet been assigned to specific taxa, include porcine picornavirus Japan (PPVJ) (Naoi et al. 2016) as well as other picornaviruses of bats, cats, cattle, chickens, dogs, ducks, lemurs, seals, rabbits, rodents, wild birds, and reptiles.

Picornaviruses infect vertebrate hosts and enter host cells using receptor-mediated endocytosis or possibly, for the enteroviruses, by direct entry of RNA across the plasma membrane following changes induced in the virus particle by receptor binding. They replicate in the cytoplasm of infected cells.

Molecular biology

Picornaviruses are small, roughly spherical viruses with a protein shell (capsid) approximately 30 nm in diameter (Ehrenfeld et al. 2010). The capsid contains a single copy of positive-sense RNA approximately 7–10 kb in length with a small virus-encoded polypeptide (VPg) covalently linked to the 5′ terminus (Figure 40.2).

The picornavirus genome includes two untranslated regions (UTRs), one preceding and one following the single open reading frame (ORF) (apart from dicipiviruses that have two ORFs, one encoding the capsid polypeptides and the other the nonstructural polypeptides, which are separated by an intergenic region containing a second internal ribosome entry site (IRES) [see below]).

Various RNA elements in the long (500–1300 nucleotide) 5′ untranslated region (5′ UTR) are involved in controlling virus replication and the initiation of viral protein synthesis and include an IRES. There are five different IRES types (I–V) (Belsham 2009; Sweeney et al. 2012). These differ in their secondary structure and mechanism of action, for example, requirement for cellular factors. IRES type IV is also found in some members of the virus family *Flaviviridae*, such as hepatitis C virus and classical swine fever virus. The 3′ untranslated region (3′ UTR) is usually much shorter (approximately 35–750 nucleotides) and also contains structures involved in virus replication. It is followed by a poly(A) tail.

The ORF is translated into a polyprotein that is usually processed by one or two virus-encoded proteinases. Between two and five primary cleavages take place during translation, while others occur later to produce the various mature proteins.

The leader polypeptide (L) is not present in all genera. For example, there is no leader protein in the enteroviruses, and it is quite diverse among the other picornavirus species. In some, including aphthoviruses and erboviruses, it is a papain-like cysteine proteinase.

The P1 (or P1-2A) capsid precursor includes the three structural proteins (VP0, VP3, and VP1) that form the basic capsid subunit (protomer). Five of these subunits come together to form a pentameric unit and 12 of these can "self-assemble" to form the complete capsid. In about half of the genera, when the RNA is packaged, there is maturation cleavage within VP0 that produces VP4 (located on the inside of the capsid) and VP2.

The P2 region consists of 2A (except when 2A is part of the P1-2A precursor), 2B, and 2C. 2A is highly variable between picornavirus species, not only in primary sequence but also in number and function. In enteroviruses, 2A is a chymotrypsin-like cysteine proteinase

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 40.1 Picornaviruses that may be isolated from pigs.

Genus	Species (former name)	Serotype	Previous serotype designation
Aphthovirus	*Foot-and-mouth disease virus*	O	—
		A	—
		C	—
		Asia 1	—
		SAT 1	—
		SAT 2	—
		SAT 3	—
Cardiovirus	*Cardiovirus A* (*Encephalomyocarditis virus*)	Encephalomyocarditis virus (EMCV) 1	—
		EMCV-2	—
	Cardiovirus B	Untyped	—
Cosavirus	*Cosavirus A*	Untyped	—
Enterovirus	*Enterovirus B* (*Human enterovirus B*)	Coxsackievirus B5 (SVDV-1[a])	—
		Coxsackievirus B4 (SVDV-2[b])	—
	Enterovirus G[c] (*Porcine enterovirus B*)	Enterovirus (EV) G1	Porcine enterovirus (PEV) 9
		EV-G2	PEV-10
		EV-G3	PEV-14
		EV-G4	PEV-15
		EV-G6	PEV-16
		EV-G8	
		EV-G9	
		EV-G10	
		EV-G11	
		EV-G12	
		EV-G13	
		EV-G14	
		EV-G15	
		EV-G16	
Kobuvirus	*Aichivirus C*	Porcine kobuvirus 1	—
Pasivirus	*Pasivirus A*	Pasivirus A1	—
		Pasivirus A2	—
		Pasivirus A3	—
Parechovirus	*Parechovirus A*	HPeV-4	—
Sapelovirus	*Sapelovirus A* (*Porcine sapelovirus*[d])	Porcine sapelovirus 1	PEV-8
Senecavirus	*Senecavirus A* (*Seneca Valley virus*)	Seneca Valley virus 1	—
Teschovirus	*Teschovirus A* (*Porcine teschovirus*)	Porcine teschovirus (PTV) 1	PEV-1
		PTV-2	PEV-2
		PTV-3	PEV-3
		PTV-4	PEV-4
		PTV-5	PEV-5
		PTV-6	PEV-6
		PTV-7	PEV-7
		PTV-8	PEV-11
		PTV-9	PEV-12
		PTV-10	PEV-13
		PTV-11	—
		PTV-12	—
		PTV-13	—
Unassigned	Unassigned	Porcine picornavirus Japan	—

[a] SVDV-1 is a genetic sublineage of human coxsackievirus B5.
[b] SVDV-2 is a genetic sublineage of human coxsackievirus B4.
[c] Enteroviruses G5 and G7 have only been isolated from sheep.
[d] Formerly *Porcine enterovirus A*.

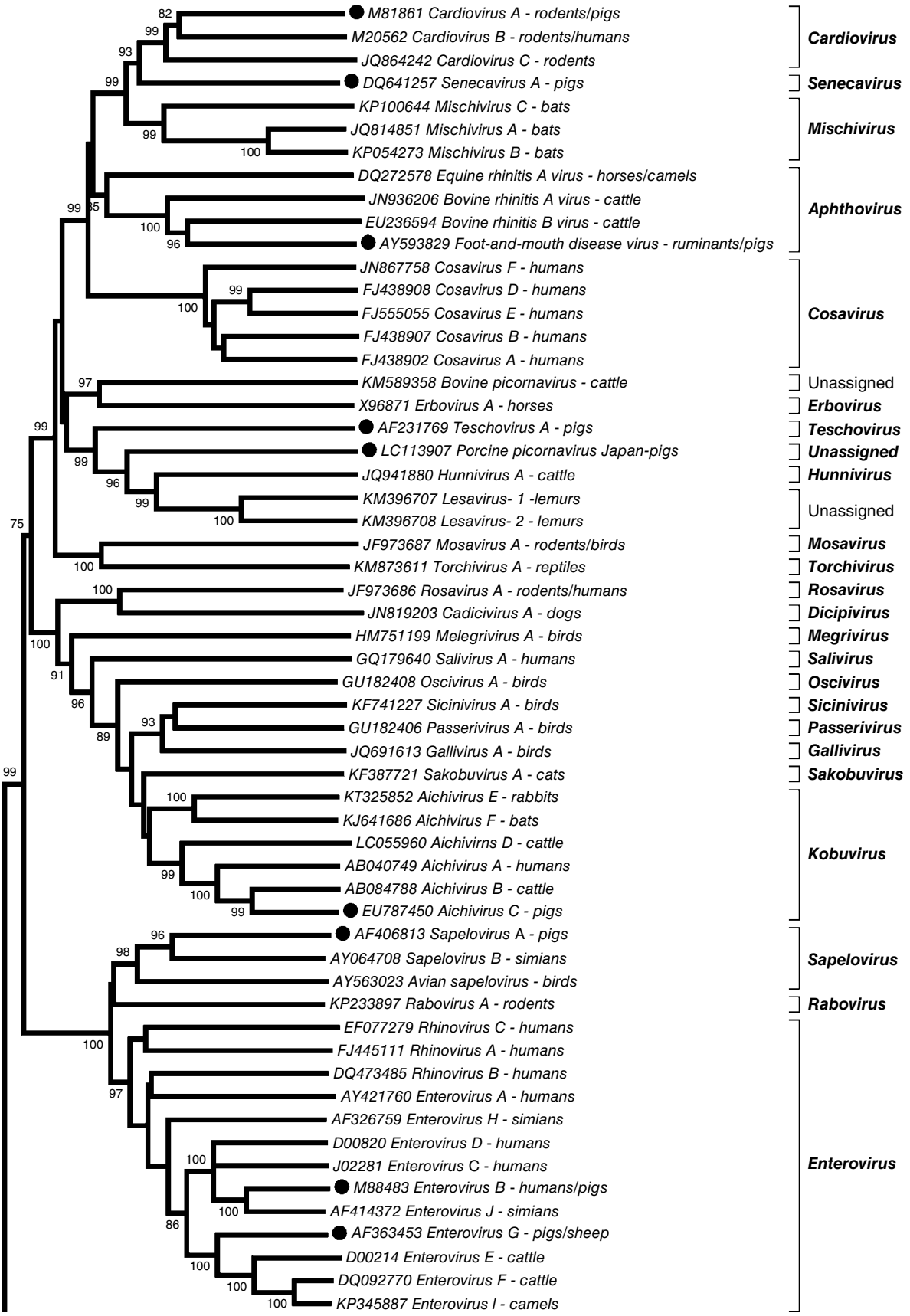


Figure 40.1 Midpoint-rooted neighbor-joining tree showing the relationships between the polymerase sequences of 86 picornaviruses. The tree was constructed with MEGA 7 (Kumar et al. 2016) using a percentage amino acid matrix. Species, rather than virus, names were used to identify each sequence, and each genus is bracketed. Picornaviruses that infect pigs are indicated by black circles.

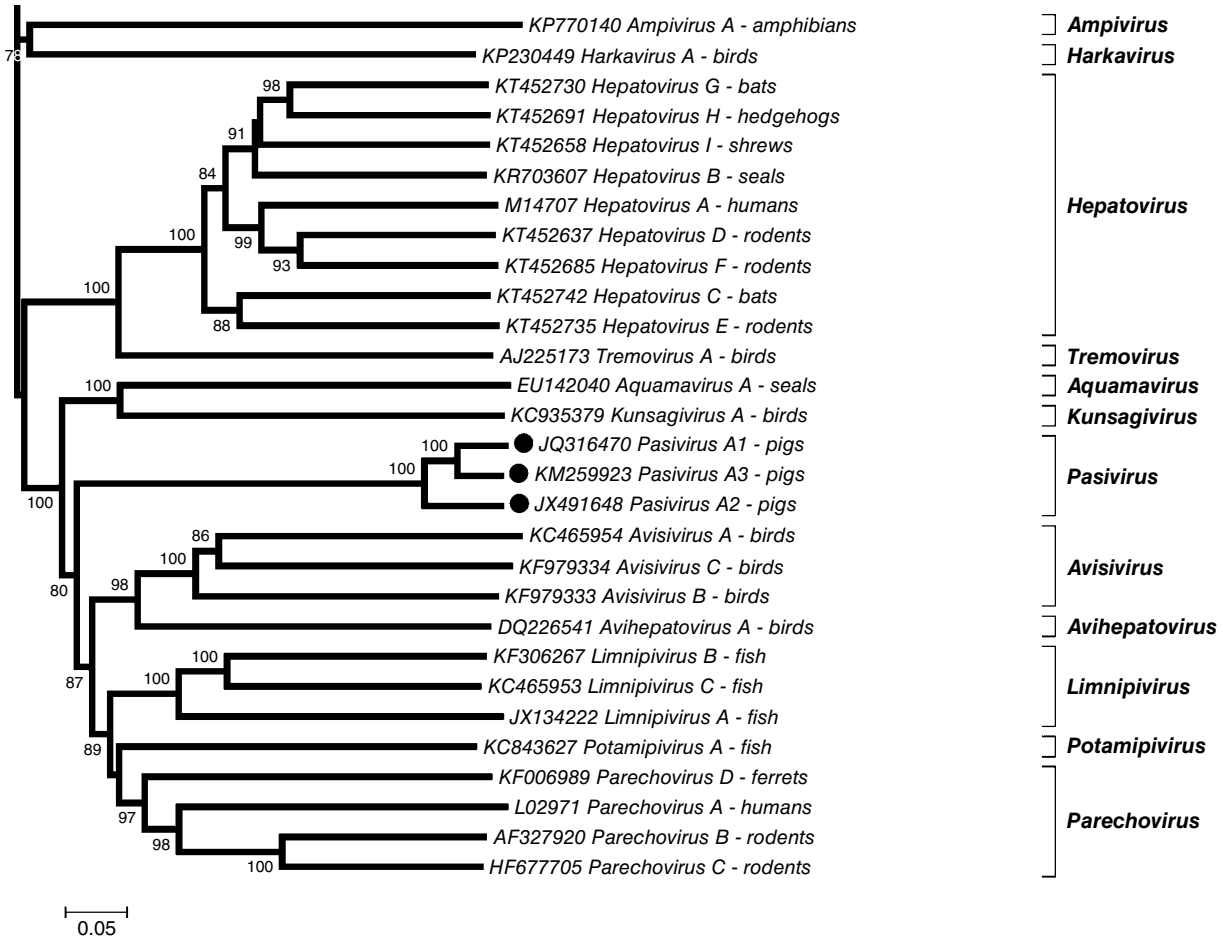


Figure 40.1 (Cont'd)

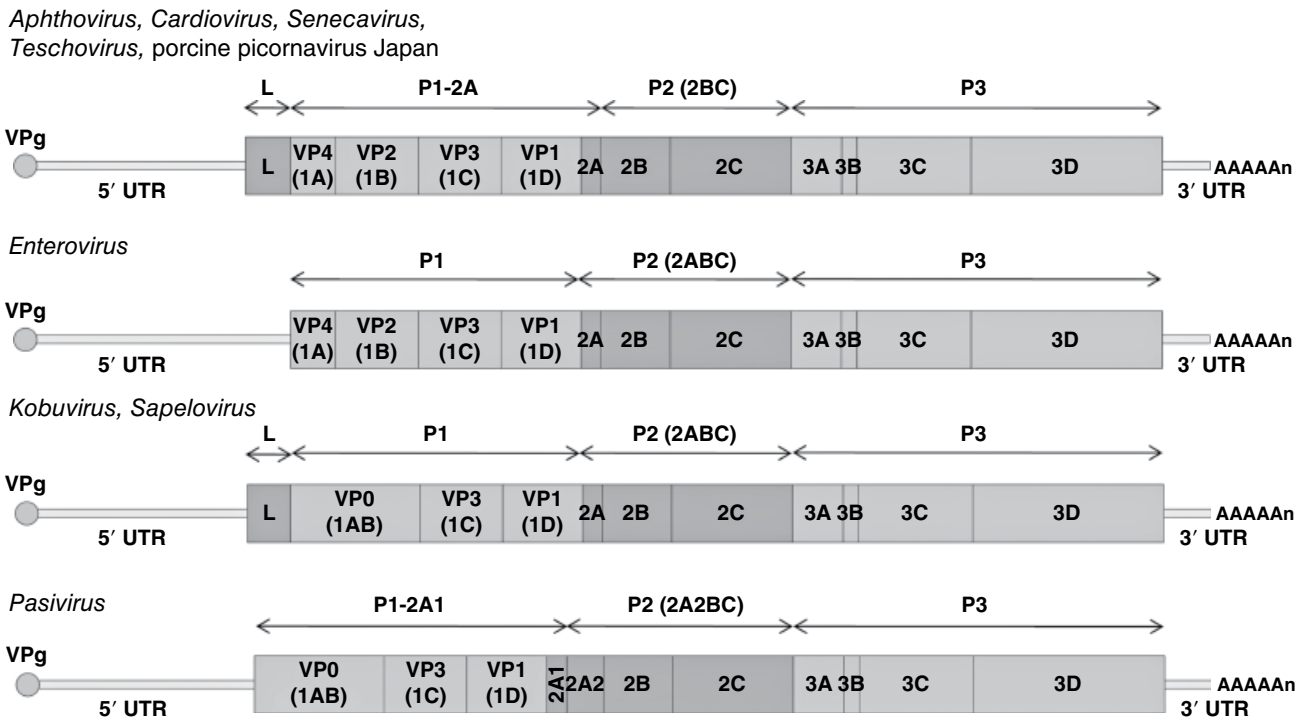


Figure 40.2 Alternative picornavirus genome organizations showing the untranslated regions, polyprotein coding region, and the location of the mature polypeptides. The presence and form of the L and 2A proteins are quite variable between genera. Foot-and-mouth disease virus differs from other aphthoviruses in having three, in tandem, 3B regions.

involved in host cell translation shutoff. In aphthoviruses, erboviruses, cardioviruses, pasiviruses, senecaviruses, and teschoviruses, it includes a short polypeptide sequence believed to mediate protein translation interruption of 2A and 2B at a specific NPG↓P motif. Therefore, in these viruses, the capsid precursor is P1-2A. In pasiviruses a second 2A of unknown function appears to be present. In kobuviruses, parechoviruses, and tremoviruses, the 2A protein belongs to the H-rev107 family of proteins. In hepatoviruses, 2A is involved in capsid assembly. In cardioviruses, the 2A protein also disrupts nucleocytoplasmic trafficking by nonproteolytic mechanisms that may contribute to the host cell translation shutoff. In addition, in some picornaviruses, multiple 2A motifs (cardioviruses, Ljungan virus) or proteins (aquamaviruses, avihepatoviruses, avisiviruses, kunsagiviruses, limnipiviruses, megriviruses and potamipiviruses) may be present. 2B, in multimeric form, can act as a viroporin. 2C belongs to the SF3 helicase family of AAA+ ATPases and has some role in RNA replication but may also contribute to the process of capsid assembly (at least for enteroviruses; see Wang et al. 2012). The FMDV 2B and 2C proteins together (or as 2BC) can also inhibit transport of proteins within the cell to the cell surface (Moffat et al. 2007); this may block the recognition of virus-infected cells by the host immune system.

The P3 region is the precursor for 3A, 3B, 3C, and 3D. 3A is a membrane-bound protein involved in viral RNA replication. 3B is a small genome-linked protein (VPg) that acts as a primer for RNA synthesis (between one and three copies are present in different picornaviruses). 3C is a chymotrypsin-like cysteine proteinase that performs the majority of the cleavages between the viral proteins. 3D is the RNA-dependent RNA polymerase. Since picornaviruses produce only 11–15 mature proteins, most are probably multifunctional. In addition, some of the processing intermediates, for example, 3CD, also have distinct functional roles.

Within species *Enterovirus G*, examples of EV-G1, EV-G2, and EV-G17 have been found that contain a porcine torovirus-like papain-like cysteine protease (PLCP) coding region inserted between 2C and 3A (Conceição-Neto et al. 2017; Knutson et al. 2017; Shang et al. 2017; Tsuchiaka et al. 2018). The inserted region maintains 3C-like cleavage sites, which would result in the production of 2C, 3A, and the PLCP polypeptides. This is unique among picornaviruses, and the PLCP protein presumably fulfills an important function. Interestingly, the PLCP regions EV-G1 and EV-G17 only share 87% nucleotide and 86% amino acid (aa) identities.

Diseases of picornaviruses

The variety of diseases caused by picornaviruses ranges from acute and sometimes fatal paralysis (e.g. poliomyelitis in humans) to mild respiratory disease (e.g. rhinoviruses in humans and equine rhinitis viruses in horses) and to inapparent infections in many hosts. The principal picornaviral diseases of pigs are acute vesicular disease caused by foot-and-mouth disease virus (FMDV), swine vesicular disease virus (SVDV), and Seneca Valley virus (SVV) (by some incorrectly referred to as Senecavirus A although this is the name of the species and not the virus); acute fatal myocarditis caused by encephalomyocarditis virus (EMCV) and FMDV; reproductive failure caused by EMCV; and teschovirus encephalomyelitis caused by porcine teschovirus (PTV).

It has been recognized in the last few years that some recently characterized picornaviruses are infectious for swine, including SVV and porcine kobuvirus (PKV). Nucleotide sequence analyses (Krumbholz et al. 2002; Zell et al. 2001) showed that the viruses initially described as "porcine enteroviruses" (PEVs) actually included a variety of different types of picornaviruses, many of which are teschoviruses (PEV-1–7 and 11–13). The group also includes 20 true enteroviruses (EV-G1 to EV-G20) belonging to the species *Enterovirus G*; 17 of them infect pigs (Table 40.1). PEV-8, formerly classified in the species *Porcine enterovirus A*, now belongs to genus *Sapelovirus* (species *Sapelovirus A*) and is known as porcine sapelovirus (PSV). SVDV-1 and SVDV-2 are also true enteroviruses, but are not included among the PEVs because they are closely related to the human coxsackievirus B5 and coxsackievirus B4, respectively.

Diagnostic reverse transcription polymerase chain reaction (RT-PCR) assays that distinguish between the different types of enteric porcine picornaviruses have been described (Krumbholz et al. 2003; Palmquist et al. 2002). An unexpected cross-reaction of specific primers designed to recognize the 5′ UTR of the teschoviruses with PSV was observed by Palmquist et al. (2002), but the products detected were of a different size. It has been shown that the teschoviruses and PSV all have a shared type of IRES structure within the 5′ UTR (Chard et al. 2006) and have some highly conserved motifs that are presumably responsible for the cross-recognition.

The clinical importance of PSV, PKV, pasivirus, PEV, and PPVJ is not well characterized, but there is some evidence that they are pathogenic for pigs, as discussed later in the chapter.

Foot-and-mouth disease virus

Relevance

Foot-and-mouth disease (FMD) is a severe, clinically acute vesicular disease of cloven-hoofed animals, including domesticated and wild swine and ruminants (Alexandersen and Mowat 2005; Thomson 1994). Because of its potential for rapid and extensive spread

within and between countries and its severe economic consequences, including its effect on trade (Leforban and Gerbier 2002), FMD is on the World Organization for Animal Health (OIE) list of diseases.

FMD has been known in Europe for many centuries and was probably one of the diseases described by the Italian physician Hieronymi Fracastorii in his work *De Contagione et Contagiosis Morbis et Eorum Curatione* in 1546 (English translation in Wright 1930). FMD spread from Europe to the Americas in the 1860s, initially to Argentina, with subsequent spread from there (Olascoaga 1984). FMDV remains endemic to many areas of the world, exceptions including North, Central, and most of South America, Australia, New Zealand, and Europe.

The scientific study of the disease and of the virus began at the end of the nineteenth century when Loeffler and Frosch demonstrated that a filterable agent could cause FMD (Brown 2003). The existence of FMDV serotypes was discovered in the 1920s (serotypes O, A, and C), followed by the recognition of Southern African territories (SAT) 1, 2, and 3 serotypes in the 1940s and serotype Asia 1 in the 1950s.

In 1947, Frenkel showed that large amounts of FMDV could be produced in cattle tongue epithelium harvested at slaughter. This discovery formed the basis of the vaccination programs initiated in Europe in the 1950s. These programs intensified in the 1960s and 1970s when continuous cell cultures made it possible to produce the large amounts of FMDV needed to produce many million doses of vaccine per year (Brown 2003; Sutmoller et al. 2003). Vaccination programs primarily focused on cattle, except when particularly aggressive strains of serotype C, and to a lesser extent serotype O, made it necessary to vaccinate swine, as occurred in the 1960s (Sutmoller et al. 2003).

Thousands of outbreaks of FMD occurred in Europe in the twentieth century, with larger epidemics every 5–10 years (Leforban and Gerbier 2002). This situation continued until 1970–1973, after which the situation improved significantly due to the availability of high-quality vaccines. FMD was eradicated from the EU, and, in 1991, prophylactic vaccination was stopped.

In North America, FMD was last reported in 1929 in the United States, in 1952 in Canada, and in 1954 in Mexico. Stamping out was used to eradicate the infection in the United States and Canada, while a combination of vaccination and stamping out was used in Mexico (Sutmoller et al. 2003). Due to significant vaccination efforts, especially under the Hemispheric Program for the Eradication of FMD (PHEFA), FMD has been controlled and/or eradicated in most countries in South America (Clavijo et al. 2017; Sutmoller et al. 2003).

Etiology

FMDV, a member of genus *Aphthovirus* in the family *Picornaviridae* (Belsham 1993), is a non-enveloped, icosahedral virus, 26–30 nm in diameter, containing a single positive-sense RNA approximately 8,300 nucleotides in length that includes the 5′ UTR, a large polyprotein coding region, and the 3′ UTR. The complete viral capsid consists of 60 copies of each of the four structural proteins (VP1–4), with many critical determinants for infection and immunity inherent in the VP1 protein. The structural proteins VP1–3 are exposed on the surface of the virus, while VP4 is located internally. FMDV comprises seven different serotypes: A, O, C, SAT1, SAT2, SAT3, and Asia 1. Notably, no FMDV serotype C has been reported since it was last detected in Kenya and Brazil in 2004 (Sangula et al. 2011).

FMDV can be propagated in continuous cell cultures, such as baby hamster kidney (BHK) cells (Mowat and Chapman 1962), a continuous porcine kidney cell line constitutively expressing bovine αVβ6 integrin (LFBKαVβ6) (LaRocco et al. 2013, 2015), fetal goat tongue cells ZZ-R127 (Brehm et al. 2009), and IB-RS-2 pig kidney cells, as well as porcine, bovine, ovine, and caprine primary cells. Kidney cells or primary bovine thyroid (BTY) cells are particularly susceptible to FMDV infection (Alexandersen et al. 2003c; Snowdon 1966). Field samples are most successfully cultured in porcine or ruminant cells, depending on the species from which the sample is derived. Thus, the usual diagnostic procedure for swine samples is to inoculate both swine and ruminant cell cultures, while samples from ruminants may either follow the same procedure or only be inoculated onto ruminant cells. FMDV can also be grown in unweaned mice (Skinner 1951).

Public health

Although historical sources report the occurrence of FMD in humans, the evidence indicates that FMDV is not zoonotic and is not a public health concern. In the vast majority of human cases, the appearance of blisters or vesicular lesions was due to other agents, including viruses such as the human enteroviruses in the *Picornaviridae* family that cause "hand, foot, and mouth disease" in humans. There have also been reports of mild clinical signs in humans after the ingestion of untreated milk or after close contact with acutely infected animals.

Proof of causality requires the isolation and identification of the agent, its successful transmission to a known susceptible species, and subsequently serological evidence in the infected individual. Most of the reports of FMD in people lack this evidence and probably did not involve FMDV. The one case in which FMDV was confirmed (Armstrong et al. 1967) involved a man that lived

on an FMD-affected farm in the United Kingdom in 1966 and consumed milk from a cow that later showed signs of FMD. Vesicular lesions developed in his mouth, on his hands, and between his toes, and FMDV serotype O was isolated. His serum, collected 30 days after infection, had high serotype O antibody titers. Thus, there was evidence that this man had been infected with FMDV. In contrast, in the UK 2001 FMD epidemic, in which 2030 outbreaks occurred among livestock, none of the 15 human suspect cases were positive for FMDV by RT-PCR (Turbitt 2001). Thus, the infection of humans with FMDV cannot be totally excluded, but it is extremely rare and in the one documented case it had only mild and transient consequences (Bauer 1997; Donaldson and Knowles 2001; Wright et al. 2010).

It follows that human infection does not have any significant role in the epidemiology of FMDV. However, people may play a significant role in passive transport of the virus from infected animals or contaminated surfaces to susceptible animals. Humans may even passively carry the virus in their respiratory tract for a day or more (Sellers et al. 1970; Sellers et al. 1971). Thus, the role of people in FMDV transmission is an important consideration in control programs.

Epidemiology

Geographic distribution

FMDV infection is endemic in large areas of Africa (mainly the SAT serotypes, but also serotypes O and A), Asia, and the Middle East (serotypes O, A, and Asia 1). Serotypes O and A were endemic in South America. However, with the exception of Venezuela and Colombia, no FMD outbreaks have been reported in the region since 2012. The last reported outbreaks in Venezuela and Colombia were in 2013 and 2017, respectively, and were caused by serotype O. Serotype C seems to have disappeared. FMD control/eradication in South America has been the result of the considerable effort expended on vaccination programs in the continent, leading to countries or regions that have achieved the official OIE status "Free of FMD" with or without vaccination.

The virus has shown an extraordinary ability to cross international boundaries and cause epidemics in previously free areas, as illustrated by the 2001 epidemic in the United Kingdom and continental Europe, as well as the outbreaks in the year 2000 in South Africa, Japan, and South Korea (Knowles et al. 2001). It has been estimated that the direct cost of the UK epidemic in 2001 was approximately $5 billion US dollars (USD) and the indirect costs from the combined losses of agricultural exports and tourist trade an additional $10 billion USD (Alexandersen et al. 2003c).

Introductions into FMDV-free countries after 2001 included Japan, with a high number of outbreaks of serotype O in 2010, and South Korea, which was able to control the 2000 and 2002 outbreaks, but experienced outbreaks of FMD in 2010 and 2014–2016. More significantly, both serotype A and serotype O appeared in South Korea within a period of a few months. Taiwan experienced an incursion of a porcinophilic strain of serotype O in 1997. Despite a massive vaccination effort and hopes of stopping vaccination in 2009, Taiwan is still not free of FMDV. Two outbreaks due to serotypes Asia 1 and O occurred in previously FMD-free Russia in 2016. These examples show that unless global eradication is achieved, FMDV will continue to spread and countries will need to be prepared.

Susceptible species

With minor exceptions, FMDV affects members of the order Artiodactyla (cloven-hoofed animals), including domestic and wild ruminants and pigs (Thomson 1994). When considering the hosts of FMDV, it is important to distinguish between species that play a significant role in the epidemiology of the disease and those that do not. The latter category would include species that only play a role under certain conditions or are only susceptible to FMDV under experimental conditions. Because they are susceptible to FMDV, these species cannot be excluded as epidemiological risks, but under field conditions, they appear to be of little or no importance (Alexandersen and Mowat 2005).

The species of greatest significance in the field include cattle, pigs, small ruminants (sheep and goats), and the water buffalo, particularly in Asia and South America. The African buffalo (*Syncerus caffer*) plays an important role as the natural maintenance host of the SAT serotypes in Africa, but other wildlife such as impala, kudu, antelopes, mountain gazelle, wild boar, and so on may also be involved in the natural epidemiology of FMDV (Thomson et al. 2003).

Species that may present some level of risk or may contribute to the transmission of virus under certain conditions include the North American bison, elk, deer, llamas, alpacas, and Bactrian camels, but not dromedary camels (Alexandersen and Mowat 2005; Alexandersen et al. 2008; Larska et al. 2008; Rhyan et al. 2008), as well as other animals of the order Artiodactyla. Interestingly, the Indian elephant, but not the African elephant, is in this category (Bengis et al. 1984; Hedger 1972; Hedger and Brooksby 1976; Howell et al. 1973; Piragino 1970). Although these species do not appear to play an important role in the wild, they may present a risk if they come into close contact with livestock, for example, when kept in zoos or under crowded conditions, such as deer farms.

A variety of other species may be infected with FMDV, but not routinely involved in the epidemiology of FMDV. However, all animals, even highly resistant animals, such

as horses and carnivores, can serve as mechanical vectors if they become contaminated with the virus and subsequently come in close contact with susceptible livestock.

The susceptibility of small mammals to infection has been extensively researched (reviewed in Alexandersen and Mowat (2005)). In brief, the coypu, water vole, mole, and the brown rat are susceptible to experimental FMDV infection, but, like other small rodents, for example, mice, are unlikely to play a role in the epidemiology of FMD because FMDV infection is usually rapidly fatal in these species. Conversely, rats appear to survive experimental infection and could migrate considerable distances. For this reason, rodent control is considered an important component of efficient FMDV outbreak control. All the same, there is nothing to suggest that rodents could maintain the disease or cause recurrence of FMDV after it is eradicated from domestic livestock.

FMDV infection in wild Australian species was mild, and clinical signs were rare, although viremia was detected and some of the animals developed antibodies. In contrast, the tree kangaroo developed tongue lesions (Snowdon 1968), and lesions were also observed in a kangaroo in a zoo in India (Bhattacharya et al. 2003). However, what role Australian wildlife species might play if FMDV were introduced into the region cannot be determined experimentally because many species that are susceptible under experimental conditions play little or no epidemiological role under field conditions.

Other species shown to be susceptible to FMDV under experimental conditions include mice, guinea pigs, rabbits, cats, dogs, mink, monkeys, snakes, birds, chickens, and embryonated eggs (Cottral and Bachrach 1968; Hyslop 1970; Skinner 1954). Interpretation of these studies must be done in the context of the experimental design. For example, in mice, susceptibility was highly dependent on age (only very young mice being highly susceptible) and the genotype of the strain of mice (Skinner 1951, 1953). Moreover, the virus had to be directly injected into the animal. Inoculation of mice did not produce vesicles, but produced infection and inflammation of the skeletal muscles (Platt 1956) or, in older mice, the pancreas (Platt 1959). Infection of the other species listed above (Henderson 1949) required multiple passage of the virus to adapt the virus to the host. Consequently, infection of these species is possible, but they are not likely to play a role in the field because infection required forced passage of high FMDV doses, leading to virus adaptation to the new vertebrate species. Often, the virus was then less fit for the original host.

Hedgehogs have long been given a special status in regard to FMDV. This is because the hedgehog is highly susceptible to experimental infection, can easily transmit the infection to other hedgehogs or livestock, and appears to excrete the virus in respiratory exhalations when infected. Although there is a report describing lesions and isolation of FMDV from hedgehogs under field conditions (McLauchlan and Henderson 1947), hedgehogs actually appear to play no role in the epidemiology of FMD. Nevertheless, during an epidemic it may be wise to exclude the access of hedgehogs to susceptible livestock.

Transmission

Under field conditions, pigs usually become infected with FMDV by direct or indirect contact with infected animals, through contaminated fomites, and, occasionally, via consumption of FMDV-contaminated products, for example, waste food. When animals are in close proximity, the movement of FMDV in aerosols and secretions from infected animals to the respiratory tract of recipient animals is probably the most common form of transmission. Intact skin provides good protection against FMDV, but abrasions or cuts in the skin permit transmission by physical contact with excretions or secretions containing infectious virus. Such skin lesions are not uncommon in swine kept on concrete floors or as a result of aggressive interactions between animals.

Transmission of virus may occur indirectly via contact with FMDV-contaminated personnel, vehicles, products, and so forth. Husbandry or disease control activities (e.g. physical handling of animals, tail or tooth clipping, vaccination, clinical examination, or collecting blood samples) increase the risk of indirect spread of the virus. Transmission has resulted from the use of contaminated instruments, medications, and FMD vaccines containing live virus before optimal inactivants were used (Beck and Strohmaier 1987).

Our understanding of the process of FMDV transmission comes primarily from experimental studies attempting to simulate natural exposure by direct or indirect contact, contaminated products or fomites, and virus aerosols. The infective dose for different routes can be calculated in such studies, but the estimates are invariably bounded by the constraints and practical considerations of performing FMDV studies under appropriate biosecurity measures (Alexandersen et al. 2003c).

Intradermal or subdermal injection of virus into the tongue, coronary bands, and heel bulbs, or application of a suspension of virus to damaged (scarified) skin, targets the highly susceptible epithelial regions (Alexandersen et al. 2003c) and simulates natural infection through damaged skin. The infectious dose by this route may be $\leq 1 \times 10^2$ tissue culture 50% infective doses ($TCID_{50}$). By comparison, a single infected animal may excrete 1×10^{10} $TCID_{50}$ or more per day at the peak of excretion, most of it in vesicular fluid, saliva, nasal fluid, and other excretions (Alexandersen et al. 2003c). Direct entry of the virus into the circulatory system by intravenous inoculation also results in infection but appears to be less

efficient and more variable than the routes targeting epithelia. Intramuscular inoculation is relatively inefficient and requires a dose of $\geq 1 \times 10^4$ $TCID_{50}$ (Burrows et al. 1981; Donaldson et al. 1984).

Several somewhat recent outbreaks of FMD have been linked to virus in contaminated human food waste subsequently fed to animals. For example, the South Africa 2000 and UK 2001 epidemics probably involved feeding unheated food waste to pigs (Alexandersen et al. 2003a; Knowles et al. 2001). The estimated infectious dose by oral exposure for pigs and ruminants is 1×10^4–10^5 and 1×10^5–10^6 $TCID_{50}$, respectively (Sellers 1971). It is conceivable that abrasions or damage to the epithelium of the buccal cavity by bone or other objects commonly present in waste food could reduce the dose required to achieve infection by the oral route.

Transmission via aerosols

Airborne transmission of FMDV is a dynamic, complex process affected by the species of animals (usually swine as the source and cattle or sheep as the recipient species), the number of animals, topography of the area, and the meteorological conditions. Airborne transmission of FMDV over significant distances becomes a significant risk when large numbers of pigs are infected because they respire large quantities of virus. Pigs aerosolize up to 1×10^6 $TCID_{50}$ per pig per day for most strains of FMDV, although up to $1 \times 10^{8.6}$ $TCID_{50}$ per pig per day has been recorded. In contrast, ruminants aerosolize less virus in their respirations (1×10^4–10^5 $TCID_{50}$ per day), but are highly susceptible to infection by inhalation. Ruminants can be infected experimentally by airborne exposure to low concentrations of FMDV (1×10^1 $TCID_{50}$), whereas pigs require airborne exposures of more than 1×10^3 $TCID_{50}$, and infection only occurs if the virus is delivered at a high concentration (Alexandersen and Donaldson 2002; Alexandersen et al. 2003b,c; Donaldson 1986; Donaldson et al. 1987, 1970). Therefore, in situations where airborne spread of FMDV occurs, it is most often from infected pigs to cattle and sheep located downwind.

Long-distance airborne spread requires atmospheric conditions that maintain the infectivity of the virus and keep the aerosol plume ("virus cloud") intact. For example, FMDV infectivity is dependent on relative humidity above 55%. Sunlight has little or no direct effect on infectivity. Stable atmospheric conditions with continuous steady or slight wind, cloud cover, and a level topography, for example, large tracts of water, tend to preserve the virus plume and increase the likelihood of airborne transmission (Alexandersen et al. 2003c; Donaldson et al. 2001; Gloster and Alexandersen 2004; Gloster et al. 2005). In contrast, air turbulence from wind, topography, trees, or buildings disperses the virus and reduces the probability of transmission.

Models to predict airborne spread of FMDV were used successfully in the United Kingdom in 1981 and 2001 and in Italy in 1993 (Alexandersen et al. 2003a; Donaldson et al. 1982; Gloster et al. 2003; Maragon et al. 1994). Current models suggest that most isolates of FMDV are unlikely to spread more than 20 km via aerosol, even under a "worst-case" scenario, that is, meteorological conditions optimal for maintaining the virus plume, the virus originating from a large population of infected pigs, and cattle located downwind of the pigs. However, specific factors can significantly affect the potential distance of airborne transmission. For example, (1) some strains of FMDV, in particular C Noville, which appears to be excreted at very high levels, may have the potential for longer spread under some conditions. (2) If the source farm contains large numbers of infected cattle or sheep excreting maximal levels of FMDV rather than pigs, the predicted distance of airborne spread would be less than 2 km (Donaldson et al. 2001). (3) Because pigs are more resistant to airborne FMDV, spread to pigs is only likely to occur at distances of 200 m or less (Alexandersen et al. 2003b,c; Donaldson et al. 2001). Thus, distances for aerosol transmission should be considered estimates, not absolutes, as some of the important parameters in the transmission model are variable or not well understood.

Aerosols are also created by splashes from infected milk and urine, by the use of high-pressure hoses to clean FMDV-contaminated animal housing and equipment, and by the process of applying infected slurry on pastures. However, the infectivity of such aerosols is probably much lower than that of aerosols exhaled by infected animals.

Duration and routes of shedding

All secretions and excretions from infected animals contain infectious virus, and some contain significant titers before the development of clinical signs. Thus, saliva, nasal and lachrymal fluid, milk, and respiratory exhalations may contain virus during the prodromal period. Urine and feces also contain virus, but to a lesser extent. It appears that feces contain only small amounts of virus (Parker 1971), but are likely to be contaminated further by desquamated lesion material, vesicular fluid, and saliva. Since preputial lesions are sometimes present, it is possible that these are the source of infectious FMDV in urine. In sheep, virus could be detected in respiratory exhalations 1–2 days before the appearance of clinical signs (Alexandersen et al. 2002b; Sellers and Parker 1969). In contrast, the peak of airborne viral excretion in cattle and pigs occurred after early generalized lesions had developed (Alexandersen et al. 2003b,c). Virus is also excreted in milk and semen (Burrows 1968; McVicar et al. 1977) from shortly before clinical signs appear and through the clinical phase, in a pattern that largely mirrors the profile of viremia. Large amounts of virus are excreted

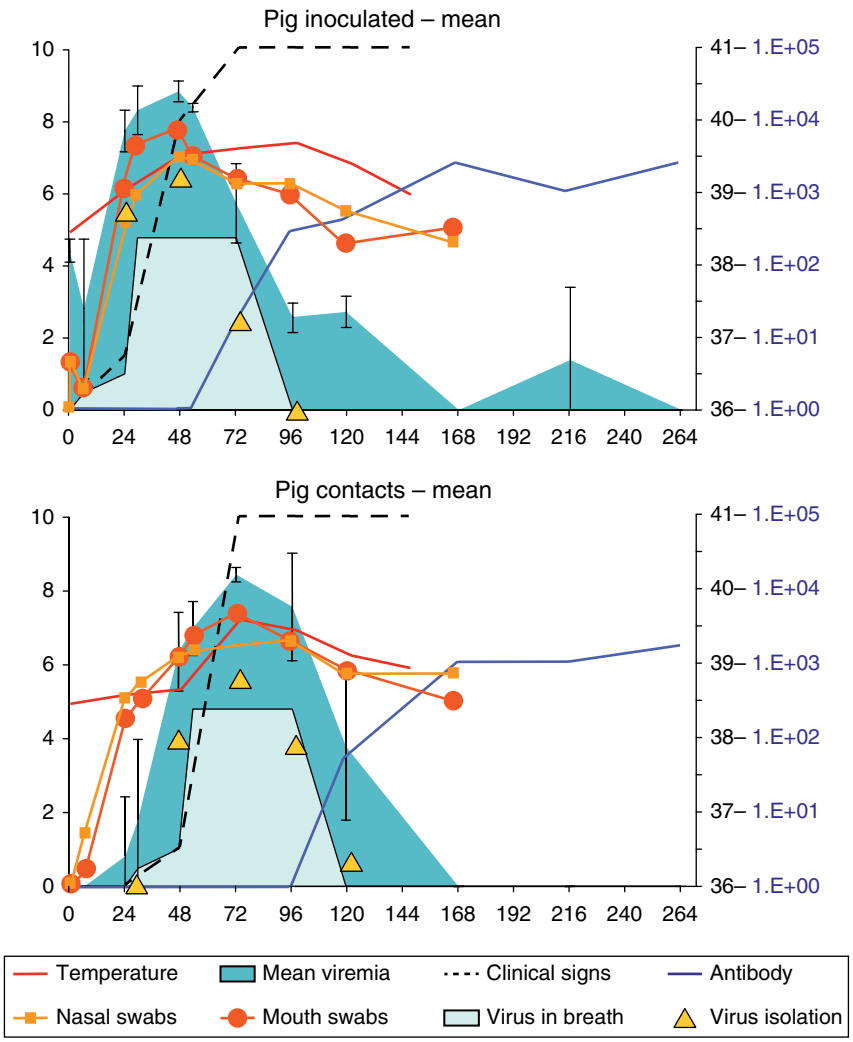


Figure 40.3 Graphs showing the time course of infection in pigs inoculated with FMDV O UKG 2001 virus (top) or kept in contact with such pigs (bottom). Time on the x-axes is given in hours. The mean levels of FMDV RNA (log_{10} genomes per milliliter) in serum samples (indicated as mean viremia) and in mouth and nasal swabs are shown together with the development of clinical signs (score of 0–6 scaled to fit on the 0–10 axis) on the left y-axes and the log_{10} ELISA antibody titer and the body temperature (°C) on the right. Virus in breath is given in log_{10} $TCID_{50}$/h, and the slopes are predicted, as only levels above approximately 2 log_{10} $TCID_{50}$/h can be measured with the methods used. Selected data on the concentration of infectious virus in serum samples, determined by virus titration in cell culture, are shown in addition. *Source:* Alexandersen et al. (2003b). Reproduced with permission from Elsevier.

in vesicular fluid, in desquamated vesicular epithelium, and in saliva (Hyslop 1965; Scott et al. 1966).

The overall pattern of viral excretion for pigs is illustrated in Figure 40.3. Airborne viral excretion coincided with the appearance of vesicular lesions and occurred within the viremic phase. Viral RNA was recovered in nasal swabs from inoculated animals soon after they developed viremia and probably reflected early production and excretion of virus. The detection of infectivity and viral RNA (Alexandersen et al. 2003b) in nasal swabs from contact animals up to 3 days before they showed signs of infection, as well as in animals after the viremic phase, probably represented background environmental virus that had been inhaled and trapped in the respiratory tract. The pattern of excretion by pigs is similar to that in cattle, but the amounts of virus and viral RNA recovered in the blood and breath are higher in pigs (Alexandersen et al. 2001, 2003b).

A sharp decline in viral excretion and load occurs around days 4–5 of clinical disease, when a significant antibody titer can be detected. However, although all secretions and excretions (other than oropharyngeal fluid in ruminants) are free of detectable infectivity at 10–14 days post infection, infectious virus already excreted during the preclinical and acute clinical phases is stable in the environment for weeks. Although tissues are also usually free from infectious virus by this time after infection, low levels of viral RNA may be found in lymph nodes and tonsils up to 4 weeks after infection (Stenfeldt et al. 2016; Zhang and Bashiruddin 2009).

Persistence in the animal

Some ruminant species exposed to FMDV become carriers (Sutmoller and Gaggero 1965; Van Bekkum et al. 1959), irrespective of whether they are fully susceptible or immune as a result of vaccination or recovery from previous infection. The percentage of ruminants that become carriers under experimental conditions is variable, but averages around 50%. The virus titer in oropharyngeal samples from carriers is usually low and declines over time. The maximum reported duration of the carrier state in ruminants is species dependent:

cattle, 3.5 years; sheep, 9 months; goats, 4 months; African buffalo, 5 years; and water buffalo, 2 months.

In contrast, pigs do not become carriers of FMDV and do not harbor the infectious virus for more than 28 days. FMDV has been found in relatively high concentrations in soft palate, tonsil, and pharynx in early infection in pigs infected by contact exposure (Alexandersen et al. 2001; Oleksiewicz et al. 2001), but there was no detectable virus and only a low residual level of viral RNA in lymph nodes and tonsils by 3–4 weeks after infection (Stenfeldt et al. 2016; Zhang and Bashiruddin 2009). Why FMDV persists in the pharyngeal region of ruminants, but not of pigs, is unknown.

Persistence in the environment

Quantitative data on the persistence of FMDV in the environment is sparse (Cottral 1969; Donaldson 1997; Sanson 1994), and results are not amenable to direct comparison because of differences in experimental design, procedures, and analyses. Typically, the kinetic curve for the decay of FMDV infectivity is biphasic: an initial steep decay curve followed by a prolonged, shallow tail. Residual virus may be remarkably resistant, especially in the presence of high concentrations of organic material.

FMDV can remain infectious in the environment for weeks, and, for any point in time, the relevant question is whether there is sufficient residual infectivity in the material or environment to initiate infection in an exposed animal. The duration of virus infectivity in the environment will depend on the matrix, the initial concentration of the virus, and the ambient conditions, for example, relative humidity, temperature, pH, and so on.

There are isolated reports of the persistence of infectious FMDV for long periods, for example, on hay and straw for at least 20 weeks and in fecal slurry for 6 months in winter (Hyslop 1970; Kindyakov 1938). More typical are reports of infectious FMDV for up to 4 weeks on cow's hair at 18–20 °C (64–68 °F), up to 14 days in dry feces, up to 39 days in urine, 3 days on soil in summer, and up to 28 days in autumn.

Most FMDV strains are only stable at lower temperatures and at pH 7–8, becoming increasingly labile at pH values outside that range (Bachrach et al. 1957; Bachrach 1968). The acidity produced in carcass meat during rigor mortis in cattle will inactivate the virus, but such acidity is variable in pig meat. Furthermore, the pH in the bone marrow, lymph nodes, certain organs, and offal does not decline during rigor mortis. Therefore, virus can be found in such material (especially if refrigerated or frozen) for a long time and may cause new outbreaks if fed to livestock as unheated waste food (Donaldson 1987).

Inactivation of virus by sunlight is indirect and occurs mainly through the effects of drying and temperature (Donaldson 1987; Donaldson and Alexandersen 2003). Drying will inactivate most, but not all, virus; hence aerosolized virus is stable at relative humidity above 55–60%. The drying of fluids or organic material containing the virus will also inactivate a large proportion of the virus, but remaining infectious virus may be more stable after drying, thereby creating a "tail" of infectivity.

Thus, the time that infectious virus will remain in the environment is difficult to predict, and restocking after an outbreak has to be done with care and only after thorough disinfection of the premises. Additional information on the stability of infectious FMDV is available elsewhere (Bachrach 1968; Bøtner and Belsham 2012; Cottral 1969; Donaldson 1987; McColl et al. 1995).

Susceptibility to disinfectants

FMDV is resistant to detergents and organic solvents, such as ether and chloroform, but can be inactivated by appropriate disinfectants and heat (Brown et al. 1963; Cunliffe et al. 1979; Dekker 1998; Fellowes 1960; Hole et al. 2017; Sellers 1968). Disinfectants that are either acidic or alkaline are highly effective, in particular alkaline disinfectants such as sodium hydroxide and sodium carbonate that also disperse organic material. The dispersing effect can be further improved by adding a small amount of detergent (not in itself an effective disinfectant of FMDV) to further increase penetration of the disinfectant and the solubilization of organic material present. Oxidizing disinfectants such as Accelerated Hydrogen Peroxide® (AHP®), sodium hypochlorite (bleach), Virkon™ S, and aldehydes, such as formaldehyde and glutaraldehyde, can be effective when applied at the correct dose and given sufficient time to work.

Pathogenesis

The pharyngeal area is the primary site of infection, except when the virus directly enters via the cornified epithelia or the circulation by damage to the intact integument. In contact- or aerosol-exposed animals, virus may be detected in the pharynx 1–3 days before viremia or clinical disease (Alexandersen et al. 2003b,c). The dorsal surface of the soft palate, the adjacent nasopharynx, and perhaps the tonsil are sites of particular significance for initial virus entry and replication. Most of the oral cavity is covered by cornified/keratinized stratified squamous epithelia, that is, a superficial layer of dead cells. In contrast, the dorsal soft palate, the roof of the pharynx, and part of the tonsil is covered by special noncornified epithelia, and therefore, live cells are on the surface of the tissue. Easily accessible to FMDV in this anatomical location, these cells provide efficient virus entry if the appropriate receptors are present.

FMDV entry into cells *in vivo* is believed to involve attachment of the viral capsid to host integrins, for example, alphaV-beta6, on the surface of target cells

(Berryman et al. 2005; Duque and Baxt 2003; Jackson et al. 2000; Monaghan et al. 2005). Little is known about the role of FMDV receptors in the context of host range, target cells, or virus persistence.

After initial replication in the pharynx, or in the skin if the virus has entered directly through damaged integument, the virus spreads to the regional lymph nodes (Henderson 1948) and into the circulation (Alexandersen et al. 2003c). Viremia usually lasts 4–5 days. Viral seeding of secondary sites is followed by multiple cycles of viral replication and spread, with the main sites of viral amplification in the cornified epithelia of the skin, tongue, and mouth. Although vesicular epithelia contain the highest concentration of virus, apparently normal skin, both hairy and hairless, also contains significant amounts (Alexandersen et al. 2001). Experimental studies suggest that lymph nodes, as well as lymphocytes and macrophages, play little or no part in FMDV replication and that any virus present in lymphoid organs is produced elsewhere, that is, the epithelia of the pharynx, mouth, and skin (Alexandersen et al. 2003c; Burrows et al. 1981; Cottral et al. 1963; Murphy et al. 2010).

Factors affecting the severity of disease

The severity of disease is affected by the virulence of the strain of FMDV involved, the inoculating dose (higher doses produce more severe clinical disease), and the physical activity of the animals (Alexandersen et al. 2003b; Murphy et al. 2010; Platt 1961; Quan et al. 2004, 2009). Little is known regarding host genetic factors associated with disease or resistance to disease, although indigenous cattle breeds in FMDV-endemic countries, for example, zebu breeds (*Bos indicus*), often exhibit less severe or no clinical signs compared to European breeds (*Bos taurus*). A difference among breeds has not been reported for pigs.

Activity associated with crowding, fighting, and damage to the skin and mucosa are more likely to lead to severe lesions. Trauma or intense physical stress increases the lateral or local spread of the virus to additional cells (Platt 1961), leading to larger foci of infected cells that, together with a physical separation of damaged tissue, appear as vesicles. The high vascularity of the coronary band, in combination with the severe local inflammatory response, may lead to cutaneous tension or stress and increased vascular permeability, both of which are likely to contribute to the development of visible vesicular lesions (Platt 1961).

While the temporal pattern of FMDV replication and the development of specific lesions are well described, relatively little is known about the pathogenesis of general acute clinical signs, for example, fever, depression/dullness, and reduced feed intake. The severity of clinical signs is not necessarily correlated with the severity of the vesicular lesions. Although FMDV, SVDV, and SVV cause very similar vesicular lesions in pigs, FMDV causes much more severe general clinical disease than SVDV or SVV. Possibly, FMDV causes a more severe proinflammatory host reaction, which is manifested as fever, general depression/dullness, reduced feed intake, occasional inability to maintain body temperature, and even mortality. These aspects of FMD, although not well understood, are probably the result of virus–host interactions extending beyond the observed acute cytopathology in virus-infected cells. Factors responsible may include cell death (releasing so-called danger signals), virus–antibody immune complex formation, complement activation, and the release of cytokines, prostaglandins, and acute-phase proteins.

Interferons alpha and beta may have a role in the host response to FMDV infection, and various FMDV isolates may differ in their ability to induce an interferon response (Chinsangaram et al. 1999; Cottral et al. 1966; Kothmann et al. 1973; McVicar et al. 1973; Seibold et al. 1964; Sellers 1963; Stenfeldt et al. 2011). For example, a large FMDV plaque size in porcine cells was associated with high virulence in pigs (Borgen and Schwobel 1964; Sellers et al. 1959). Studies on haptoglobin (Hofner et al. 1994; Stenfeldt et al. 2011) indicated that this acute-phase protein is elevated in FMDV-infected cattle when viremia and clinical signs become evident, suggesting that the inflammatory response is activated.

Clinical signs

Incubation period

The incubation period for FMDV is highly variable and depends on the virus strain, exposure dose, the route of exposure, the animal species, and the husbandry conditions (Alexandersen et al. 2002b, 2003a,b,c; Quan et al. 2004, 2009). Under experimental conditions, the mean incubation period was 3.5 days for continuous, direct cattle-to-cattle contact and 2 days for intensive sheep-to-sheep contact. Pigs were readily infected by direct and intensive pig-to-pig contact exposure and had a mean incubation period of 1–3 days, but up to 9 days, depending on the intensity of contact (Alexandersen et al. 2002b, 2003b; Quan et al. 2004, 2009). These differences confirm the close relationship between exposure dose and length of incubation: the higher the dose or the intensity of contact, the shorter the incubation period. Under field conditions, the dose of FMDV will depend on several factors, including stocking density, that is, intensive or extensive management, and how the animals are housed and handled.

The incubation period for farm-to-farm airborne spread ranges from 4 to 14 days (Sellers and Forman 1973), which is also the normal range for farm-to-farm spread by indirect contact. The incubation period for

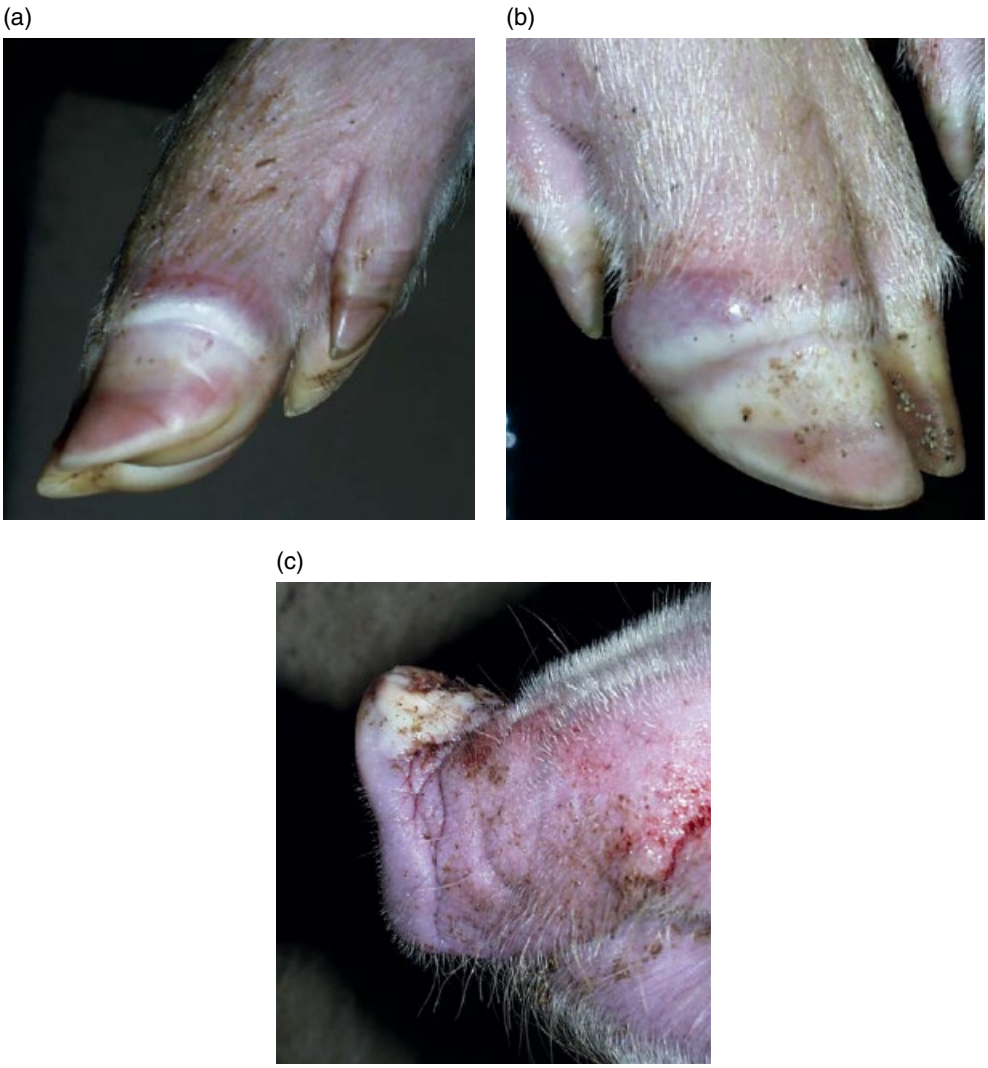


Figure 40.4 (a and b) FMD generalized lesions in pigs 3–5 days after exposure to pigs inoculated with FMDV O_1 Lausanne. Unruptured vesicles are evident along the coronary bands. (c) Lesion on the snout of a pig inoculated with FMDV O UK 2001. *Source:* Alexandersen and Mowat (2005). Reproduced with permission from Springer Nature.

farm-to-farm spread resulting from direct contact may range from 2 to 14 days (Garland and Donaldson 1990). The period for within-farm spread is generally 2–14 days, but may be as short as 24 hours, especially in pigs and under very high challenge conditions. When spread is occurring within a herd or flock, the typical incubation period is 2–6 days, although under certain conditions it may be as short as 1 day or as long as 14 days.

Clinical signs

FMD is characterized by an acute febrile reaction and the formation of vesicles in and around the mouth and on the feet (Figure 40.4). On handling, heat and local pain may be detected in the feet 1–2 days before vesicular lesions appear. Lameness or lesions may not be a consistent finding in all animals. Animals kept on soft bedding are less likely to develop severe foot lesions or show lameness.

Clinical disease is usually severe in pigs. The pain from foot lesions causes lameness, manifested by foot "flicking," a tucked-up stance, reluctance to stand or walk, and inappetence. Early signs include acute lameness, reluctance to stand, adoption of a dog-sitting posture, depression, loss of appetite, and fever. Severely affected pigs become lethargic, huddle together, and have reduced or no feed intake (Kitching and Alexandersen 2002). Fever is often variable in degree and duration; it may be as high as 42 °C (107.6 °F), but is often brief and in the 39–40 °C (102–104 °F) range, and thus close to/within the normal range for pigs. Body temperature in severely affected pigs may be below normal (Kitching and Alexandersen 2002). Consequently, body temperature in pigs can be used to support other clinical findings, but should not be used to exclude the possibility of FMDV infection.

Morbidity and mortality

Mortality in adult animals is generally low, but may be high in young animals, especially piglets, due to acute myocarditis. Although mortality is usually not significant in animals with vesicular lesions, secondary bacterial infections in vesicular lesions may lead to chronic lameness, wasting, or mortality.

FMD may cause abortion in pregnant animals, but the pathogenesis has not been established. It is possible that the fever associated with FMDV infection may be a factor, but it is also possible that the virus crosses the placenta and infects the fetus (Ryan et al. 2008a, 2007).

Stocking density may affect the expression of clinical signs at the population level. Thus, animals housed in confinement often show obvious clinical disease, while, for example, infected sheep kept under extensive conditions may show no overt signs of infection (Alexandersen et al. 2003c). Similarly, vaccines will not prevent infection but may prevent development of severe clinical disease, hence allowing the infection to continue unrecognized for some time.

Lesions

Gross lesions

Lesions often appear initially as blanched areas that subsequently develop into vesicles. They are found most consistently in and around the mouth, and on the feet, but may also be seen on the snout, teats, mammary gland, prepuce, vulva, and other sites. Lesions of the feet of swine may include the shedding of claws ("thimbling"). The accessory digits may be affected, as well as pressure points on the knees, hocks, and elbows, particularly if kept on concrete. Lactating sows may develop vesicles on the udder (Kitching and Alexandersen 2002). The expression of gross lesions may differ by virus isolate and species-specific virulence. For example, the O Taiwan 1997 strain caused severe lesions in pigs, but no cases were seen in ruminants (Dunn and Donaldson 1997).

If oral lesions are present in pigs, they are most often on the tongue, either far back on the dorsum or as tiny lesions at the tip. Vesicles on the feet of pigs are most often seen in the interdigital space, at the bulb of the heel, and along the coronary band. As in sheep and goats, oral lesions in pigs may heal without much exudate or subsequent scarring. However, soon after rupture, the base of vesiculated areas often becomes covered within a few days by a serofibrinous exudate. The regeneration of epithelia is usually well advanced within 2 weeks, although usually with a variable degree of scarring, in particular after the occurrence of severe lesions. The rupture of vesicles, especially on the feet or teats, may predispose the affected areas to secondary bacterial infections, which may complicate and prolong the healing processes.

The age of lesions can be assessed using the following criteria: development of vesicles on days 0–2, rupture of vesicles on days 1–3 (initially with fragments of epithelia attached), sharply marginated erosion (days 2–3), with the sharpness lost around day 3, serofibrinous exudation on days 4–6, and beginning of repair with a marked fibrous tissue margin at day 7 or more (Anonymous 1986). Severe lesions of the coronary bands, as seen especially in pigs, may often lead to a separation of the horn during the acute inflammatory stage. If the horn is not shed, a ring will be formed in the horn that becomes visible below the coronary band approximately 1 week after the first appearance of clinical disease. This ring progresses down the hoof as the horn grows. Growth rate is approximately 1–2 mm per week, with faster horn growth in younger animals.

In young animals (pigs less than 8 weeks of age) that die from acute myocarditis, visual examination often reveals the heart to be soft and flaccid, with white or grayish stripes (the so-called tiger heart) or spots, seen mainly in the left ventricle and interventricular septum. In young animals dying from hyperacute disease, there may be no significant visible lesions in the heart and an absence of vesicular lesions, but the virus can usually be isolated from the myocardium or from blood, and lesions can be detected by histopathological examination (Donaldson et al. 1984). Occasionally, the skeletal muscles may also be affected. Furthermore, in ewes, FMDV can cross the placenta leading to infection and death in fetal lambs with no visible specific gross abnormalities in the infected fetuses (Ryan et al. 2007).

The significance of FMD acute myocarditis for the spread of the disease is not well understood. Possibly, there is little virus excretion, since death usually occurs early before the development of vesicular lesions. However, although in such cases FMDV mainly replicates in the heart, a significant viremia may occur (Donaldson et al. 1984), and virus may be present in respirations, saliva, nasal fluid, and so forth (Ryan et al. 2008b).

Microscopic lesions

The first histopathological changes in the cornified stratified squamous epithelium are ballooning degeneration and increased cytoplasmic eosinophilic staining of the cells in the stratum spinosum and the onset of intercellular edema within the dermis. These early lesions are detectable only by microscopic examination (Gailiunas 1968; Yilma 1980). This early stage may be followed by necrosis and subsequent mononuclear cell and granulocyte infiltration. The lesions, now macroscopically visible, develop further into vesicles by separation of the epithelium from the underlying tissue and filling of the cavity with vesicular fluid. In some cases, the vesicular fluid production may be high, and the

resulting vesicles large. In other cases, the amount of fluid may be limited, and the epithelium may undergo necrosis or be torn off by physical trauma without the formation of an obvious vesicle. The variability seen is most likely due to combinations of viral strain virulence, thickness of the affected skin, and husbandry conditions, especially as they affect physical stress on different regions of the skin. In young animals dying from acute disease, there is lymphohistiocytic myocarditis with hyaline degeneration, necrosis of myocytes, and infiltration with mononuclear cells.

In pigs, it is puzzling that no lesions develop on the soft palate or the dorsal part of the pharynx, despite the presence of significant amounts of virus. Possibly, the infection causes no acute cytopathology in the transitional epithelial cells found in these areas. Alternatively, the viral cytopathology is restricted to a few cells and is, therefore, not easily detected. Or perhaps, since the epithelia of this region are noncornified, the development of distinguishable lesions is somehow prevented. Regardless, the underlying mechanisms behind this apparent lack of cytopathology are unknown.

In infected sheep fetuses, no pathology was observed in the epithelium because FMDV RNA was located mainly in the subepithelial muscle of the tongue and in the myocardium. The replication of FMDV in the myocardium did not lead to classical "tiger heart" striations, as reported in piglets and calves (Ryan et al. 2007).

Diagnosis

Differential diagnosis

The clinical diagnosis of FMD is sometimes difficult, for example, in sheep and goats, in which clinical signs are often mild (Alexandersen et al. 2002b; Donaldson and Sellers 2000; Hughes et al. 2002). Moreover, certain strains of the virus may be of low virulence for some species (Donaldson 1998). In pigs, several other viral vesicular diseases, including swine vesicular disease (SVD), vesicular stomatitis, and SVV and vesivirus infection, cannot be distinguished from FMD on the basis of clinical findings. PEV infections have also been implicated in vesicular diseases in swine (so-called idiopathic vesicular disease). If the disease is not noted early, any vesicles present will have ruptured and will be indistinguishable from erosive lesions, such as those induced by trauma, caustic substances, and photosensitivity. Thus, a definitive diagnosis requires urgent laboratory investigation.

Pathological evaluation

The first step in the diagnostic evaluation is to determine whether vesicles are present. If no vesicles are present, it should be established whether there are lesions that could be consistent with older stages of vesicles, that is, after rupture and loss of the overlying lesion epithelia and thus resembling erosions. Although detailed gross examination of lesions may help in the evaluation, only laboratory testing can confirm or refute the presence of FMDV.

Virus detection

Definitive diagnosis of FMD must be carried out at specialized laboratories. Enzyme-linked immunosorbent assays (ELISAs) have supplanted the earlier use of the complement fixation test due to their sensitivity, specificity, and ability to test large numbers of samples (throughput), and in recent years initial virus detection is often done by real-time RT-PCR.

Laboratory diagnosis is traditionally made by ELISA detection of specific FMDV antigens in epithelial tissue suspensions, often with concurrent attempts at cell culture isolation and the application of ELISA to any samples showing cytopathic effect (Ferris and Dawson 1988; Hamblin et al. 1984; Roeder and Le Blanc Smith 1987). These tests are used to confirm the diagnosis and identify the FMDV serotype.

Given a sample with sufficient quantities of virus, a positive result for FMDV (including serotyping) can be obtained in 3–4 hours by antigen ELISA (Ferris and Dawson 1988; Hamblin et al. 1984; Have et al. 1984; Roeder and Le Blanc Smith 1987). However, samples with low quantities of virus may yield weak, inconclusive, or negative results. Thus, the antigen ELISA is highly specific and very suitable for confirming positive cases, but a negative result requires further examination in highly susceptible cell cultures or by real-time RT-PCR before FMD can be ruled out.

Most often, field samples are routinely inoculated onto primary BTY cells (Snowdon 1966) or bovine or lamb kidney cells, as well as on an established line of porcine kidney cells (IB-RS-2 cells) (De Castro 1964). Recently, two cell lines, ZZ-R 1272 and LFBK-αvβ6, have been reported to have high sensitivity to various FMDV strains (LaRocco et al. 2013, 2015). Cultures showing a cytopathic effect are confirmed by antigen ELISA or RT-PCR and sequencing using cell culture supernatant. For most FMDV strains, the BTY cell system is about 10 times more sensitive than other cultures (Burrows et al. 1981; Snowdon 1966). However, certain pig-adapted strains, for example, the O Taiwan 1997 strain (Dunn and Donaldson 1997), grow better in IB-RS-2 cells. Virus isolation in BTY and IB-RS-2 cell cultures essentially detects all positive samples with more than 1–5 infectious units per milliliter or per 0.1 g. Depending upon the amount of virus present, two 48 h passages may be required before a final result can be determined. However, if the specimens are of low quality or if the transport conditions were less than optimal, a small proportion of samples may give negative results for infectivity, but positive results by ELISA or RT-PCR.

RT-PCR assays have been developed for the detection of FMDV, but initially none seemed to be of sufficient sensitivity, specificity, and robustness for routine diagnostics (House and Meyer 1993; Moss and Haas 1999; Reid et al. 1998, 1999). RT-PCR assays for serotyping FMDV have been described, but the initial procedures were labor intensive (Callens and De Clercq 1997; Reid et al. 1998, 1999; Vangrysperre and De Clercq 1996). Alexandersen et al. (2000) developed an RT-PCR ELISA of increased sensitivity and included a simple and aqueous-phase (SNAP) hybridization step for optimal specificity, speed, and ease of use. Both conventional polymerase chain reaction (PCR) techniques and the SNAP method can provide serotype-specific results, but they are not of sufficiently high throughput for use in an epidemic.

Fluorogenic "real-time" RT-PCR methods combine the total RNA extraction and reverse transcription (RT) procedures of conventional RT-PCR with fluorogenic probe PCR amplification in real-time PCR equipment. This approach is able to achieve high sensitivity and specificity for the detection of FMDV genomes of all seven serotypes (Alexandersen et al. 2003b,c; Callahan et al. 2002; Hearps et al. 2002; Moniwa et al. 2007; Moonen et al. 2003; Rasmussen et al. 2003; Reid et al. 2002). The assay can be used for the detection of FMDV in tissue samples, serum samples, swab samples, and tissue culture supernatants and can include automated procedures for nucleic acid extraction, RT, and PCR amplification stages to increase sample throughput (Reid et al. 2003). In addition, some of these assays may be used on portable platforms (Ambagala et al. 2017; Callahan et al. 2002; Hearps et al. 2002; King et al. 2008).

Real-time RT-PCR methods are now as diagnostically effective as the combined ELISA/virus isolation system. These assays provide good agreement with virus isolation with same-day turnaround. With experimental samples, the sensitivity of RT-PCR is often higher than virus isolation. The timeliness of investigating suspect cases could be further improved by conducting the testing on or near the farm, but this type of testing, especially in a portable format, will require further evaluation. Serotyping RT-PCR methods and RT-LAMP assays have also been described and may have diagnostic potential (Bachanek-Bankowska et al. 2016; Dukes et al. 2006; Jamal and Belsham 2015; Reid et al. 2014; Waters et al. 2014).

In addition to the methods described above, "pen-side" antigen detection methods have been developed based essentially on the same principle as the antigen ELISA, but with a pan-serotype monoclonal antibody in a format adapted for field use (Ferris et al. 2009; Reid et al. 2001; Ryan et al. 2008a). Laboratory trials indicated that the sensitivity of this approach is equal to, or probably greater than, the traditional antigen ELISA. Field validation of such tests is a high priority. It has also been shown that intact viral RNA can be recovered from such pen-side devices and this can be used for further characterization (e.g. using RT-PCR and sequencing) of the virus (Fowler et al. 2014).

Antibody detection

The liquid-phase blocking ELISA was formerly in routine use at many laboratories for FMDV antibody detection (Hamblin et al. 1986). The diagnostic sensitivity of the assay is close to 100%, but the specificity is only approximately 95%. Since specimens with inconclusive ELISA results require testing by virus neutralization (VN) (Golding et al. 1976), the liquid-phase blocking ELISA is not the optimal test for large-scale screening because numerous VNs are likely to be required because of the relatively low specificity of the assay.

The OIE considers the VN the definitive "reference standard" for the final assessment of inconclusive ELISA results. At the World Reference Laboratory, the specificity of the VN for serotype O antibodies at a dilution of 1 : 45 was 100% (Paiba et al. 2004). However, it is likely that some of the newer tests may completely replace the VN when sufficient validation data become available.

Solid-phase competitive or blocking ELISAs (SP-C or SP-B ELISAs) possess both high sensitivity and specificity (Chénard et al. 2003; Have and Holm-Jensen 1983; Li et al. 2012; Mackay et al. 2001; Sorensen et al. 1992). Such assays may detect all experimentally infected animals at 5–8 days after infection and for many months thereafter (Paiba et al. 2004).

Research on vaccines from which FMDV nonstructural proteins (nsp) have been removed offers the future promise of a DIVA (differentiate infected from vaccinated animals) vaccine even in animals vaccinated more than once. Tests to detect antibodies against the conserved nsp of FMDV have already been reported (Berger et al. 1990; Bergmann et al. 1993; Lubroth and Brown 1995; Mackay 1998; Neitzert et al. 1991; Shen et al. 1999; Sorensen et al. 1998). In vaccinated populations, such tests could be used to differentiate vaccinates from infected animals on a herd basis. Unvaccinated populations can be screened by tests that detect antibodies to structural antigens, and a negative result will exclude FMDV in a statistically robust manner.

Immunity

The host immune response, including antibody production detected as early as 3–4 days after the first clinical signs, usually results in the clearance of FMDV, except in those infected ruminants that develop a persistent infection of the pharyngeal region. Clearance of virus from "peripheral" or "external" sites, for example, nasal

and oral surfaces, is less efficient. The virus may remain in the vesicular epithelium of foot lesions for 10–14 days, that is, longer than in oral lesions (Oliver et al. 1988).

Humoral response

ELISA can detect circulating antibodies 3–5 days after the appearance of clinical signs. Detection of antibodies by VN is usually 1–2 days later than ELISA. Coincident with the first detection of antibody, there is a progressive, rapid clearance of virus from the circulation and a significant reduction of virus in most organs, excretions, and secretions. The antibody response normally remains high for many months after infection and may still be detectable after several years. However, in fast-growing young pigs, antibodies may have a half-life as short as 1 week and may remain detectable for only a few months.

Immunity to FMDV is primarily mediated by circulating antibodies, and protection after infection or immunization (active or passive) is closely correlated with the antibody titer. Circulating antibodies capable of binding to the surface of virions will facilitate opsonization and uptake by phagocytes located in the liver, spleen, and elsewhere and thus rapidly reduce or prevent viremia (McCullough et al. 1992). As viremia is an important phase in the early infection process, its reduction or prevention will have a corresponding effect on acute generalized disease. However, since circulating antibodies will not prevent primary local infection (e.g. at the sites of intradermal entry or in the pharynx), it would seem that they prevent disease, but not infection (McVicar and Sutmoller 1976).

Infection with FMDV induces a strong mucosal immunoglobulin A (IgA) response that may protect against reinfection with the same virus, but traditional inactivated vaccines induce a very weak or no secretory IgA response. It is possible that vaccines with a high antigenic mass may induce some secretory IgA production in swine and that this response, if sufficiently high, may protect against infection (Eble et al. 2007; Pacheco et al. 2010).

Cell-mediated and innate responses

Although it is possible to protect pigs with passively acquired antibodies and although immunity to FMDV appears to be primarily mediated by circulating antibodies, immunity after vaccination or infection must necessarily involve more than just humoral immunity. However, little is known about the cell-mediated immune response to FMDV infection.

In swine, FMDV infection results in a significant lymphopenia. T-cell activity is significantly reduced during acute infection, possibly the result of increased interleukin (IL)-10 production by dendritic cells (Diaz-San Segundo et al. 2009; Golde et al. 2008; Grubman et al. 2008).

Both a CD4 and a CD8 T-cell response can be demonstrated in infected or vaccinated animals. However, the development of neutralizing antibodies and of class switching appears to be independent of a CD4 T-cell response, and the CD8 T-cell response may be of limited effect during acute infection, because acute FMDV infection rapidly results in reduced major histocompatibility complex (MHC) class I surface expression on infected cells. Moreover, the acute infection itself rapidly kills infected cells (Childerstone et al. 1999; Gerner et al. 2009; Guzman et al. 2008; Juleff et al. 2009; Sanz-Parra et al. 1998).

Likewise, the innate immune response is poorly described, and some studies suggest that the innate response plays a minor or no role in protection (Alves et al. 2009; Summerfield et al. 2009). Swine can be protected by interferons, as shown using replication-defective adenovirus expressing type I or type I and II interferons, a promising tool for potential new emergency vaccines or, rather, antivirals (Diaz-San Segundo et al. 2010). Furthermore, FMDV induces a rapid transient type I interferon response in pigs, which peaks 2–3 days post infection and may contribute to the rapid clearance of viremia (Nfon et al. 2010). Although FMDV is highly sensitive to interferons, the virus has efficient mechanisms to block interferon production in infected cells by blocking cellular protein synthesis via the FMDV leader protein (Belsham 2005). Other innate immune responses may work through natural killer cells (NK cells), but FMDV also appears able to counteract this mechanism by significantly reducing NK cell function during acute infection (Toka et al. 2009). Overall, it appears that FMDV has evolved efficient mechanisms for immune evasion during the acute stages of infection (Golde et al. 2008; Grubman et al. 2008). Nevertheless, emergency vaccines may provide early protection by induction of a combination of innate and adaptive immune responses.

Maternal immunity

Maternal immunity against FMDV is transferred to the offspring via colostral immunoglobulins from previously infected or vaccinated dams. Provided the levels are sufficiently high and directed toward the relevant challenge strain, this may protect piglets up to 8–12 weeks of age. Piglets respond poorly to FMD vaccination and are best protected by maternal immunity. They should not be vaccinated before 8–12 weeks of age, by which time maternal immunity is waning and they are able to respond to FMD vaccination (Francis and Black 1986; Kitching and Alexandersen 2002; Kitching and Salt 1995; Morgan and McKercher 1977).

Prevention and control

The most common methods of spreading FMDV are (1) movement of infected animals, (2) feeding of contaminated

animal products to susceptible livestock, and (3) movement of virus on fomites or mechanical vectors (humans and animals). Transmission by these routes can be prevented by application of strict disease control measures, that is, movement restrictions and biosecurity procedures. It should be borne in mind that virtually any surface or product could be contaminated with the virus, if the infection is present in the region or country. Airborne transmission of FMDV is essentially uncontrollable and not uncommon over short distances, but rarely occurs over long distances. However, when long-distance transmission occurs, the consequences can be dramatic (Anonymous 1969; Donaldson et al. 1982; Gloster et al. 1981, 1982).

FMDV has a wide host range, a low infectious dose, a rapid rate of replication, a high level of viral excretion, and multiple modes of transmission. Therefore, it is essential to identify suspect cases, conduct the diagnostic evaluation, and implement the appropriate response as quickly as possible. Extreme measures are required to eradicate FMDV, and, if they are not rapidly and effectively applied, there is a high probability that an outbreak will reach epidemic proportions.

There is currently no way of treating FMDV-infected pigs, and once the virus has been introduced into a swine farm, it is unlikely to disappear unless all the animals, both infected and uninfected, are euthanized, the carcasses removed (buried, rendered, or incinerated), and the premises disinfected (Alexandersen et al. 2003c; Kitching and Alexandersen 2002). Therefore, the response to the positive identification of FMDV is euthanasia of all susceptible animal species on infected farms, while vaccination may be used in noninfected farms. Depending on local regulations, animals suspected of having been exposed to FMDV could be transported to an abattoir for slaughter, if it is possible to avoid the risk of transmitting FMDV to other susceptible animals. Products from such animals must be heat treated and canned.

Control of FMDV is complicated by the existence of carrier ruminants (Alexandersen et al. 2002a; Sutmoller and Gaggero 1965; Van Bekkum et al. 1959). It is noteworthy that pigs do not become carriers (Alexandersen et al. 2003b; Stenfeldt et al. 2016). Recognition of the carrier state and the possible disease risk presented by carrier animals had a major impact on the design of control and eradication strategies for FMD. Experience has shown that total stamping out, whereby all susceptible species on infected premises are euthanized, both affected and apparently normal, is necessary to ensure the elimination of persistently infected carriers (Alexandersen et al. 2002a; Hedger and Stubbins 1971). These strategies have been shown to be effective under varying conditions in many countries. The risk posed by ruminant carriers has also had a marked influence on the safeguards taken to manage the risk of FMD associated with international livestock movements. These measures, mainly directed at animals originating from countries where the disease is either endemic or sporadic, range from complete embargo to quarantine and testing.

During the UK 2001 epidemic, mathematical models played a major part in influencing disease control strategy. However, they had not been used previously under operational conditions (Ferguson et al. 2001) and the assumptions behind them, as well as the justification for the use of such average-based, mathematically-derived, centrally controlled procedures and their actual impact on efficient disease management are still highly contentious.

Vaccines and vaccination

There are seven serotypes of FMDV, and infection or vaccination with one serotype does not protect against infection or disease with any of the other serotypes. Moreover, a wide range of strains may exist within a serotype, some of which may be sufficiently divergent to reduce the efficacy of a given vaccine (Kitching 1998; Kitching et al. 1989). As a general rule, protection against heterologous strains will be lower than against the homologous strain (Goris et al. 2008). Therefore, FMDV vaccine antigens need to be tailored to strains circulating in the region or strains with the potential to be introduced. Preparedness for emergency vaccination requires accurate intelligence with respect to the strains most likely to be introduced. An alternate strategy is to be broadly prepared against a large number of FMDV antigens.

The degree of cross-protection that one vaccine strain provides a vaccinated animal when challenged with another strain of the same serotype can only be assessed in animal trials, but most trials offer only limited insight because the low number of animals typically used offers low statistical power.

The antigenic relatedness of FMDV strains can be assessed in the laboratory using ELISA and VN methods. These results help select vaccine strain(s) most appropriate for use against a given field strain. Notably, these indirect methods are highly dependent on the individual laboratory doing the tests, the actual composition and potency of the vaccines, and other factors (Jamal et al. 2008; Maradei et al. 2008; Mattion et al. 2009; Paton et al. 2005).

Thus, although vaccines are available to control FMD, the existence of multiple serotypes of FMDV, each with multiple continuously evolving strains, should be considered when considering a control program that includes vaccination. Further, it should be recognized that vaccine-induced population protection usually only lasts 4–6 months and that susceptible animals are continuously introduced into swine populations through reproduction and animal movement. Consequently, vaccination programs often require two or more doses per year (Domenech et al. 2010). It should also be noted that

once established in the swine population, FMD is difficult to control by vaccination (Orsel and Bouma 2009; Orsel et al. 2007) and even more difficult to eradicate, as shown by the efforts in Taiwan, which, after nearly 15 years of vaccination following the 1997 introduction, has not eradicated FMD. Nevertheless, the successful eradication of FMD from the EU has clearly shown that eradication can be achieved using prophylactic vaccination with good quality vaccines, but it should be realized that this required a coordinated and efficient control effort, including stamping out of infected farms, over many years (Leforban and Gerbier 2002).

Swine vesicular disease virus

Relevance

In 1966 a new disease emerged in pigs in Italy. The disease was clinically recognized as FMD but was caused by an enterovirus (Nardelli et al. 1968). This disease was later called swine vesicular disease. The virus (SVDV) was subsequently isolated in Hong Kong in 1971 (Mowat et al. 1972). In 1972, SVD was diagnosed in the United Kingdom, Austria, Italy, Poland, and subsequently elsewhere (see Table 40.2). Recently, only Portugal and Italy have reported outbreaks of SVD (Table 40.2). The virus may still be present in some Asian countries although the last reported outbreak was in Taiwan in year 2000. North and South America are considered free of SVD. Most of the recent outbreaks in Italy were not discovered by clinical inspection, but by serological screening of subclinical infections. In the absence of serological screening, the disease would probably be underreported in Italy. Therefore, it is likely that the virus is present in more countries than those that have reported the disease.

Table 40.2 Year of first and last reports of swine vesicular disease outbreaks in the world. The data are based on the FAO Animal Health Yearbook (1971–1996) and information obtained from the European reference laboratory for vesicular diseases in Pirbright (United Kingdom).

Europe	Year of first report	Year of last report
Italy	1966	2015[a]
Portugal	1995	2007
Netherlands	1975	1994
Belgium	1979	1993
Spain	1993	1993
Romania	1973	1987
Germany	1973	1985
France	1973	1983
United Kingdom	1972	1982
Austria	1972	1979
Greece	1979	1979
Malta	1975	1975
Russia[b]	1975	1975
Switzerland	1974	1975
Ukraine	1972	1972
Poland	1972	1972
Bulgaria	1971	1971
Asia		
Taiwan	1997	2000
Hong Kong	1970	1991
Japan	1973	1975

[a] No outbreaks were recorded in 2016, and the latest reports in Italy were mostly triggered by detection of antibodies to the virus and not due to detection of clinical disease.
[b] These outbreaks were caused by SVDV-2, a variant of human coxsackievirus B4.

Significance to swine health and/or public health

Until 2015, SVD was listed by the OIE, because SVD lesions resemble FMD lesions. The disease was removed from the list as the production losses due to SVD are very limited and modern diagnostic techniques make it easy to differentiate between SVD and FMD infections; even "on-farm" diagnosis is possible (Ferris et al. 2009). In the FMD outbreak in 1997 in Taiwan, the presence of SVD in the country probably delayed the reporting of FMD cases, which may have led to more FMDV-infected farms. To avoid missing an FMD outbreak, SVD is still under official control in many OIE member states.

Shortly after SVDV was identified, it was shown that the structural proteins of SVDV are closely related to the structural proteins of coxsackievirus B5. Therefore, it was suggested that infection of humans by SVDV could be possible (Brown et al. 1976). There is one report where infection with SVDV might have been the cause of hospitalization of one person. Since the virus is closely related (see below) to the human coxsackievirus B5 (within the coding sequence for the structural proteins) and echovirus 9 (within the sequences encoding the nsp) (Zhang et al. 1999), infection of human cells is not unexpected. However, severe illness in humans has not been reported recently, not even from laboratories working with large amounts of live virus during large-scale screening. Thus, it remains uncertain whether the illness leading to the single hospitalization was caused by SVDV infection. Furthermore, recent studies suggest that the virus has further adapted to swine and that SVD viruses isolated after 1993 have lost the ability to infect human cells and have completely lost any original zoonotic potential. Consequently, currently circulating strains

and isolates obtained after 1993 should not be considered zoonotic (Jimenez-Clavero et al. 2005). SVDV is not the only human enterovirus that entered the swine population, but also a coxsackievirus B4-like strain (termed SVDV-2) causing vesicular lesions in pigs was detected in 1975 in Russia (Lomakina et al. 2016).

Etiology

SVDV is classified as an enterovirus within the family *Picornaviridae* and belongs to the species *Enterovirus B*. Like other picornaviruses, SVDV is non-enveloped. It has a single-stranded positive-sense RNA genome, and the virus particles are approximately 30–32 nm in diameter (Nardelli et al. 1968). Antigenic studies have revealed only small antigenic differences; therefore SVDV is considered a single serotype. Isolates, however, can be divided into four distinct phylogenetic groups by comparing monoclonal antibody reaction patterns or nucleotide sequences of the 1D (VP1) coding sequence (Borrego et al. 2002a,b; Brocchi et al. 1997).

The SVDV genome consists of approximately 7,400 nucleotides and encodes a single polyprotein of 2815 aa (Inoue et al. 1989). This polyprotein is posttranslationally cleaved into 11 mature proteins (plus various precursors). Four of these proteins – 1A, 1B, 1C, and 1D – form the virus capsid (Fry et al. 2003; Jimenez-Clavero et al. 2003), and one of these proteins, 3B (VPg), is linked directly to RNA and is, therefore, a component of the virion (but only 1 copy per particle; cf. 60 copies of each of the capsid proteins). The nsp are involved in virus replication and interruption of host cell functions (e.g. cellular protein synthesis).

The virus can be grown on primary or secondary porcine kidney cells and a wide range of pig kidney-derived cell lines and sometimes even on secondary lamb kidney cells (Aldo Dekker, unpublished observations). The virus is lethal to newborn mice (Nardelli et al. 1968). This links it to its ancestor virus coxsackievirus B5 because, in the enterovirus group, only coxsackieviruses can infect mice (Graves 1995). Sequence data show that SVDV has approximately 75–85% nucleotide identity with coxsackievirus B5 (Knowles and McCauley 1997) within the coding sequence for the structural proteins. There is also high sequence identity to echovirus 9 in the coding sequence for the nsp (Zhang et al. 1999). Phylogenetic analyses indicate that SVDV and recent coxsackie B5 isolates probably shared a common ancestor in the period 1945–1965, most likely close to 1961 (Bruhn et al. 2015; Zhang et al. 1999).

Epidemiology

Geographic distribution

SVDV has only been isolated in Asia and in Europe and outbreaks have been reported in a limited number of countries (Table 40.2). The only country that has reported SVDV detection recently is Italy, but it is possible that SVDV is present in other countries since only a few countries test for antibodies against SVDV.

Susceptible species

Not only Euro-Asian pigs but also American one-toed pigs are susceptible to the virus (Wilder et al. 1974). Relatively high titers of SVDV have been detected in the pharynx of sheep kept in close contact with SVDV-infected pigs (Burrows et al. 1974), and neutralizing antibodies were detected in some of these contact sheep, while similar experiments with cattle showed no signs of infection (Burrows et al. 1974). Thus, these studies might indicate that after close contact with infected swine, the virus may have replicated in the sheep; however, there is no indication that sheep or other ruminants play any role in the epidemiology of SVD.

Transmission

An epidemiological field study in the United Kingdom for the period 1972–1981 revealed that the main source of infection was movement of pigs (48%), either because infected pigs were transported (16%) or contaminated transport vehicles were used (21%) or through contacts at markets (11%). A second significant source of infection (15%) was feeding virus-contaminated waste food (Hedger and Mann 1989). The routes of transmission were very similar in the first phase of the Italian 2006 SVD outbreak: 12 of 36 outbreaks (33%) were caused by movement of infected pigs, 11 of 36 (31%) were caused by use of a contaminated transport vehicle, 11 of 36 (31%) were caused by infection via other indirect routes, while the route of introduction to 2 of 36 farms (5%) was unknown. In Italy, there was no indication that swill feeding played a role. Seventeen outbreaks occurred after more stringent control measures were implemented in the second phase: in 1 of 17, a person had previously visited an infected premises; in 5 of 17 cases the infection was transmitted between different locations owned by the same farmer (Bellini et al. 2010a); and the source of virus could not be determined for the remainder. The difference in transmission characteristics between the first and second phase of the Italian 2006 outbreak was further confirmed by spatial analysis of the outbreak farms. That is, in the first phase a higher probability for relative long-distance transmission was observed compared with the second phase (Nassuato et al. 2013).

The exceptional stability of the virus outside the host is the reason why indirect contact (e.g. through contaminated transport vehicles and swill feeding) plays an important role in the epidemiology of SVD. Transmission can occur not only by direct contact between infected and susceptible pigs but also via contact with a contaminated environment. The latter can lead to viremia within

1 day and clinical signs within 2 days – similar to infection by direct inoculation or direct contact (Dekker et al. 1995a). Studies on SVDV transmission within an outbreak farm showed that spread between pens most likely occurred when there was a shared open drainage system or frequent movement of pigs between pens. SVD, therefore, is considered a "pen disease" rather than a farm disease (Hedger and Mann 1989; Dekker et al. 2002).

Since clinically affected herds are culled immediately, it is not easy to study the transmission of SVD in field conditions. However, IgM and IgG ELISAs have been developed to study the time the virus was introduced (Brocchi et al. 1995; Dekker et al. 2002). These ELISAs allow for the estimation of the time of introduction; however, the isotype profiles of most infected animals are similar after approximately 50 days. Therefore, an estimation of the time of introduction beyond 50 days is not possible (Dekker et al. 2002).

The role of wildlife is most likely negligible, although there are not many studies in regions with SVD outbreaks. No SVDV antibodies have been detected in wild boar in countries not reporting the disease, for example, the Netherlands (Dekkers and Elbers 2000) and Croatia (Roic et al. 2012). Also, no SVDV antibodies were detected in wild boar sampled in 2005–2006 in Italy in the Campania region, although SVD was detected in domestic pigs in the same period (Montagnaro et al. 2010).

Duration and routes of shedding

The duration of SVDV shedding from vesicles is at least 7 days (Dekker et al. 1995a), but longer in feces. One report described recovery of virus up to 126 days after infection (Lin 1998), but it has been difficult to reproduce these findings (Lin et al. 2001). In a recent study, viral genome was detected 7–14 days after infection in oral fluids; interestingly these fluids could also be used to detect antibodies to the virus (mostly after day 7), making these fluids an interesting sample for early diagnosis (Senthilkumaran et al. 2017).

Persistence in the environment (inactivation by heat, UV, desiccation, etc.)

The virus remains infectious for months in carcasses and processed meat (e.g. salami or pepperoni sausages), which explains the role of swill feeding in the epidemiology of SVD (Hedger and Mann 1989; Mebus et al. 1997). SVDV remains infectious in slurry for a long time (Karpinski and Tereszczuk 1977), and inactivation of SVDV in slurry is more difficult than other significant pathogens (e.g. African swine fever virus) (Turner and Williams 1999).

Susceptibility to disinfectants

SVDV is stable over a wide range of pH, and consequently, acidic or alkaline disinfectants that work well for other pathogens (e.g. FMDV) are not effective for SVDV. As with many of the other picornaviruses, SVDV is also resistant to detergents and organic solvents, such as ether and chloroform. A 4-$\log_{10}$ reduction in infectivity in 5–6 minutes was not found with many commonly used disinfectants; only sodium hydroxide (1%) was able to inactivate SVDV adequately. If prolonged contact times between virus and disinfectant are an option, then inactivation is often possible to achieve, for example, formaldehyde (2%) was effective after 18 minutes (Terpstra 1992).

Pathogenesis

Virus entry, site of primary replication, spread within the body and sites of secondary virus replication

It has been suggested that SVDV enters the pig through the skin or the mucous membrane of the digestive tract (Chu et al. 1979; Lai et al. 1979; Mann and Hutchings 1980). Experimental SVDV infection can lead to clinical signs within 2 days, and SVDV has been isolated from a wide range of tissues (Burrows et al. 1974; Chu et al. 1979; Dekker et al. 1995a; Lai et al. 1979). After contact of pigs with a SVDV-contaminated environment, viremia can develop within 1 day, similar to the time frame observed when pigs are directly inoculated (Dekker et al. 1995a).

SVDV lesions are mainly observed in epithelial tissues and infection via the skin is very effective (Burrows et al. 1974). Virus titers in the myocardium and the brain can significantly exceed those in plasma. Epithelial tissues and sometimes the myocardium and brain are probably the sites of virus replication (Chu et al. 1979; Lai et al. 1979). Lymph nodes also may contain high titers of SVDV after experimental infection. It is not known, however, whether these tissues are positive because of the drainage of virus or because of virus replication (Dekker et al. 1995a). More research is necessary to identify the cells in lymph nodes (or other tissues) that support SVDV replication.

Factors affecting the severity of disease

SVD may run a subclinical, mild, or severe course. The latter was only seen when pigs were housed on a concrete floor in humid conditions (Hedger and Mann 1989; Kanno et al. 1996; Kodama et al. 1980). This suggests that the environment can be a factor in the observed virulence of the disease.

Experiments to show differences in virulence between strains showed that lesions were often difficult to observe without careful examination, so reports of differences in virulence in the field are probably biased by differences in the methods by which observations were collected. In the most recent SVDV detections in Italy, the cases were

never reported based on clinical disease, but always by the detection of SVDV-specific antibodies in the monitoring program. This suggests that the current strains mostly cause subclinical infections.

Host genetic factors associated with disease

Host genetic factors are not known to be associated with SVD.

Clinical signs

Incubation period

Experimental infection by intradermal injection in the heel bulb can lead to clinical signs in 1–2 days. In experiments in which pigs were exposed to a contaminated environment, lesions were first observed 2 days after exposure (Dekker et al. 1995a). This showed that the time to onset of clinical signs could also be very short in natural exposure. The 2006 outbreaks in Italy were detected in the beginning of October, but epidemiological studies indicated that the virus must have been present in August (Bellini et al. 2010a). This long period between introduction and detection was probably due to subclinical infection, as well as poor detection and reporting.

Morbidity and mortality rates

Mortality is not a reported feature of SVDV infection. Morbidity can be high, but depends on many factors, including virulence of the strain, type of farm, and, most importantly, time between infection and detection. The seroprevalence at the time of detection ranged from approximately 7% on a farm in which the disease was clinically detected to almost 90% in a collection center that was traced after receiving presumably infected pigs (Dekker et al. 2002).

Lesions

Gross lesions

In pigs infected with SVDV, vesicles appear around the coronary bands (Figures 40.5 and 40.6), on the skin of the metacarpus and metatarsus, and to a lesser extent on the snout, tongue, and lips. Lesions are indistinguishable from those induced by an FMDV infection. The clinical signs caused by SVDV are, however, much milder than those caused by FMDV. In experimental studies (Dekker et al. 1995a), fever was rare and lameness was almost never observed. Sudden death due to heart degeneration, often observed in young piglets infected with FMDV, is not seen with SVDV.

In typical cases of SVD, lesions are first noticed at the junction of the heel and the coronary band (Figures 40.5 and 40.6). The whole of the coronary band may eventually be involved, and the lesions may spread to the metatarsal and metacarpal regions. The hoof wall and sole may be damaged so extensively that the claw(s) sloughs off. In lactating sows, lesions on the udder and teats can be seen (Figure 40.7). Occasionally, the skin of the thorax and abdomen is involved. Lesions in the mouth and on the lips and snout occur in up to 10% of the cases. Those on the snout are mostly on the dorsal face of the rostrum and may be hemorrhagic in appearance. Tongue lesions are transient and heal rapidly (Hedger and Mann 1989).

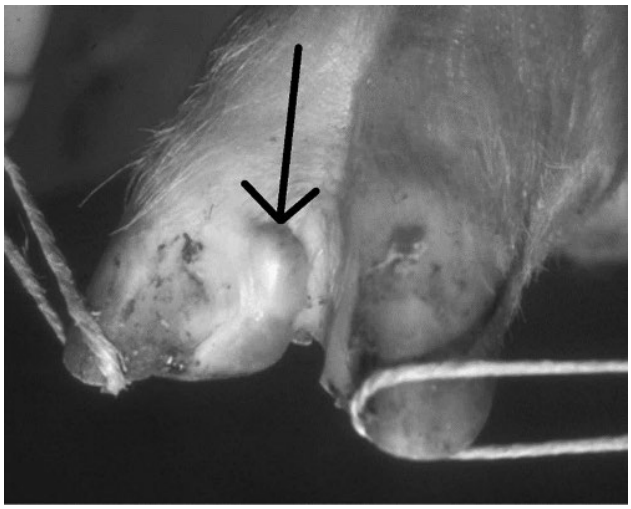


Figure 40.5 Vesicular lesion in the interdigital space of a pig infected with SVDV. A vesicle on the coronary band extending to the heel bulb.

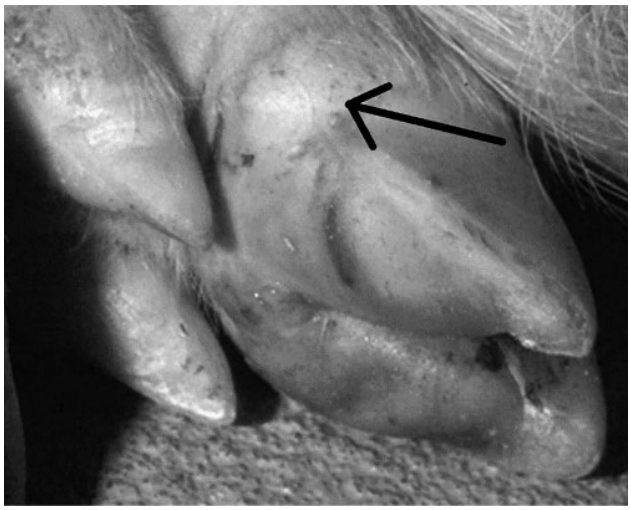


Figure 40.6 Vesicular lesion on the coronary band of a pig infected with SVDV.

Microscopic lesions

Early experimental infection by SVDV has been studied in skin sections showing replication in the epidermal layer of the skin with indication of rapid spread to the

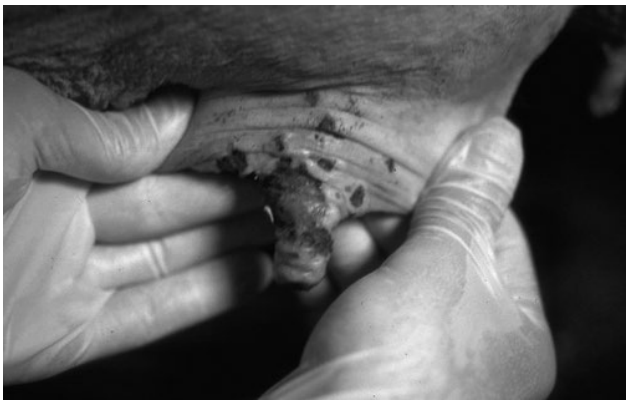


Figure 40.7 Teat lesions in a lactating sow infected with SVDV (outbreak NET/1/1992).

dermis (Mulder et al. 1997). Histological studies on the morphogenesis of vesicles in SVD have not been done, but it is considered to follow the same pattern as observed in FMD, starting with ballooning of epithelial cells and microvesicles in the stratum spinosum (Mohan et al. 2008). In experimentally infected animals, nonsuppurative meningoencephalitis may occur, but this does not result in signs of impaired central nervous system (CNS) function (Chu et al. 1979).

Diagnosis

Differential diagnosis

A farm with signs of a vesicular disease in pigs should be considered infected with FMDV until proven otherwise. Specific vesicular lesions are also seen in some other virus infections, for example, in vesicular stomatitis, SVV infection, and vesicular exanthema. FMDV can be found in many parts of the world and is the most relevant differential diagnosis. Vesicular stomatitis is endemic in and restricted to the Western Hemisphere; it has rarely been diagnosed elsewhere. Since 2008, SVV has been frequently associated with vesicular disease, not only in North and South America but also in China (Hause et al. 2016; Qian et al. 2016). Vesicular exanthema is caused by a calicivirus and was last observed in the United States in 1956.

Pathological evaluation

Lesions in pigs initially start with blanching of the coronary band, and since it is often the posterior part of the interdigital space that is affected, this is most easily observed in a recumbent pig. The areas develop into large fluid-filled vesicles that rupture in 1–2 days, after which erosion-like lesions are observed. True vesicular lesions are only caused by a limited number of diseases (see above), but erosions seen after vesicular lesions have ruptured are not specific for SVD and can be due to various causes. Foot lesions not caused by SVDV infection are often observed in sows, with more than 60% of sows with lesions at the sole–heel junction (Anil et al. 2007; Geudeke 1992). Therefore, it is unlikely that hoof lesions in sows will result in a clinical report; it is more likely that nose or udder lesions (Figure 40.7) will result in notification by the farmer or his veterinarian.

Laboratory confirmation

Methods of virus detection

Virus isolation on IB-RS-2 cells (De Castro 1964) is considered one of the most sensitive methods for laboratory diagnosis. SK6, PK-15, and primary or secondary porcine kidney cells are also susceptible to SVDV (Callens 1999; Nardelli et al. 1968). Several RT-PCR techniques have been developed to detect SVDV (Lin et al. 1997; McMenamy et al. 2011; Niedbalski 2009; Reid et al. 2004). Recent RT-PCR techniques are often multiplex real-time RT-PCRs designed to differentiate between various vesicular diseases (Fernandez et al. 2008; Peng et al. 2015; Wang et al. 2015). RT-PCR is the first choice for detection of SVDV in feces or organs. Virus isolation takes more time and has a similar sensitivity. In vesicular material, however, the amount of virus is very high, and an ELISA can therefore easily be used for antigen detection and identification (Roeder and Le Blanc Smith 1987).

Methods of antibody detection

SVD is considered one single serotype, and, after an outbreak, surveys based on the detection of SVDV-specific antibody are essential to prove that no infected farms have been missed. After an SVDV infection, high titers of neutralizing antibodies are found (Nardelli et al. 1968). Virus neutralization (VN) tests, however, are laborious, and ELISA tests have been developed (Armstrong and Barnett 1989; Brocchi et al. 1995; Chénard et al. 1998; Dekker et al. 1995b; Hamblin and Crowther 1982). An ELISA is easier to perform than a VN test but produces more false-positive results. The specificity of the ELISA can be increased by using monoclonal antibodies (Brocchi et al. 1995; Chénard et al. 1998) and is therefore adopted as the standard test by the OIE.

Immunity

Immune responses

The immune response to infection is rapid. IgM is detectable in 50% of the pigs 4 days post inoculation (DPI), followed by an IgG response detectable in 50% of the pigs after approximately 12 DPI (Brocchi et al. 1995; Dekker et al. 2002). Based on several experimental infection studies with various SVDV strains, approximately 50% of the pigs will be positive in the SVD ELISA by 7 DPI and by day 8 in the VN test (Chénard et al. 1998).

Protective immunity

Experimental SVDV vaccines have been developed (Delagneau et al. 1974; Gourreau et al. 1975; McKercher and Graves 1976; Mowat et al. 1974). In addition to monovalent SVD vaccines, combinations with FMD (McKercher and Graves 1976; Mitev et al. 1978) and more recently a SVD subunit vaccine have also been described. No SVD vaccine is commercially available, and, to date, vaccination of pigs has not been undertaken in the field.

Maternal immunity

Piglets born to SVDV-infected sows receive antibodies against SVDV in colostrum. The half-life of these antibodies in piglets is longer (30–50 days) (Bellini et al. 2010b) than the half-life of anti-FMDV antibodies (7–21 days) found in piglets following FMD vaccination (Francis and Black 1984). This means that maternally derived antibodies can be found in piglets up to almost 6 months of age. This should be taken into account when interpreting results of serological tests on outbreak farms.

Prevention and control

Methods to treat pigs or control outbreaks

SVD is no longer listed by the OIE, but SVD is still considered a notifiable disease in many countries, particularly countries that export pigs and/or pork. In the face of trade embargos resulting from SVD outbreaks, stamping out infected herd(s) is the quickest method to control the outbreak and regain an SVD-free status.

Methods to prevent introduction of agent or avoid outbreaks

Although SVDV is resistant to environmental factors and many commonly used disinfectants (Terpstra 1992), pigs transported within cleaned and properly disinfected vehicles do not often get infected. Even on farms where infection is present in one compartment, the infection does not spread easily to other compartments if strict hygienic measures are applied. In the past, swill feeding has contributed significantly to transmission of SVD. Even if swill feeding is not allowed, pigs should not be allowed to come into contact with foodstuffs left by visitors or farm personnel.

Methods to eliminate agent from herds

Stamping out whole farms followed by thorough disinfection and cleaning is practiced to rapidly eliminate SVDV. Indirect evidence suggests that elimination of SVDV by partial depopulation is probably possible. Specifically, in the Netherlands (1995), antibodies against SVD were detected in fattening pigs, indicating that they had been exposed to SVDV. A test and removal procedure was followed, with the removal of seropositive animals followed by disinfection and thorough cleaning of the areas in which the positive pigs had been housed. After 3–4 rounds of testing and removal, the serological prevalence was reduced to zero. The success of this case can be explained by studies that have shown that transmission of SVDV is limited in subclinically infected pigs (Burrows et al. 1974). In contrast, transmission occurs very quickly in cases of clinical infection. Therefore, it is unlikely that partial depopulation will be successful if pigs are clinically ill.

Encephalomyocarditis virus

Relevance

EMCV is a rodent-borne virus first described in 1940 (Jungeblut and Sanders 1940). Later, EMCV was isolated from a chimpanzee with myocarditis in Florida (Helwig and Schmidt 1945), and anti-EMCV antibodies or EMCV were subsequently detected in a variety of animal species (Tesh and Wallace 1978).

Infection of swine with EMCV is not uncommon, but clinical disease is infrequent. During an outbreak of acute disease in Panama in 1958, Murnane et al. (1960) isolated EMCV from the lung and spleen of a pig that suddenly collapsed and died within a few minutes. This was the first time that EMCV had been recognized as a swine pathogen. In pigs, disease due to EMCV may take one of two main forms: an acute myocarditis, usually causing sudden death in young pigs, and/or reproductive failure in sows.

EMCV outbreaks with high mortality in pigs have been reported in Australia, South Africa, New Zealand, Cuba, and Canada (Acland and Littlejohns 1975; Dea et al. 1991; Seaman et al. 1986). In Europe, clinical disease caused by EMCV was first observed among domestic pigs in 1986 and has since increased in frequency. Outbreaks of acute myocarditis have been reported in Italy, Greece, Belgium, and Cyprus (Koenen et al. 1999; Vansteenkiste et al. 2016). EMCV outbreaks are often clustered in so-called endemic areas (Maurice et al. 2007). In Belgium, EMCV was also frequently isolated in cases of reproductive failure (Koenen et al. 1999). In the endemic area of Italy, a seroprevalence of between 5 and 15%, exceptionally more than 60%, was found in farms with clinical EMC in a high density breeding area (Salogni et al. 2016). In 50% of the farms without clinical EMC, a similar seroprevalence level was detected (Maurice et al. 2005). In the United Kingdom, antibodies against EMCV were detected in 28% of clinically normal slaughterhouse pigs (Sangar et al. 1977); however no attempt was made to isolate virus. In Asia, outbreaks associated with reproductive failure have been reported in China (Feng et al. 2015), as well as the seroprevalence of 7.7% in wild boar in South Korea (Jeoung et al. 2015).

Etiology

EMCV (species *Cardiovirus A*) belongs to the genus *Cardiovirus* of the family *Picornaviridae*. Several antigenically similar viruses, including Columbia-SK and Mengo, were isolated during the 1940s and are considered to be in the same serotype as EMCV (i.e. EMCV-1). A second serotype, EMCV-2, has been isolated from a wood mouse in Germany (Philips et al. 2012). Many properties of EMCV are common to other picornaviruses. It is ether resistant and stable over a wide range of pH. It is inactivated after 30 minutes at 60 °C (140 °F), but some strains have shown a marked thermal stability (Joo 1999).

Although EMCV is antigenically stable, the 1D (VP1 capsid protein) coding region of EMCV displays considerable genetic variability. A single mutation in the nucleotide sequence can be involved in attenuation or confer diabetogenicity to a particular EMCV strain (Nelsen-Salz et al. 1996). In particular, the Greek isolates appear to differ genetically from those isolated in Belgium, Italy, and Cyprus (Knowles et al. 1998).

EMCV replicates well in cell cultures originating from several animal species, including rodents, swine, and primates. BHK-21 and Vero cells are used most commonly. The virus also replicates in mice and chicken embryos and is pathogenic in many laboratory animals. Acute fatal disease is produced in mice and hamsters after inoculation by various routes. Neurological disease due to encephalitis has been reported, but myocarditis is more frequently seen at necropsy. Pathogenicity in rats, guinea pigs, rabbits, and monkeys appears to vary depending on the age of the animals and the virus strains used.

The virus has hemagglutinating activity with guinea pig, rat, horse, and sheep erythrocytes, but differences in this activity among EMCV strains have been reported (Kim et al. 1991). Serial passage of EMCV in cell culture can alter *in vitro* growth characteristics, reduce virulence, and affect hemagglutinating activity (Zimmerman 1994).

Public health

The impact of EMCV on public health is believed to be minimal. Despite the frequency of infection in swine, Zimmerman (1994) found no association between infection and transmission of disease to humans even in persons at greatest risk (veterinarians, animal caretakers, and laboratory staff). A serological survey indicated a seroprevalence of >17% in cities located within the tropical rain forest in Peru (Czechowicz et al. 2011). In 2011, in Mexico, seroconversion in 47% of blood samples taken from 85 swine-specialist veterinarians sampled during a congress was detected (Rivera-Benitez et al. 2014). In 2009, EMCV was isolated from people with febrile illness in Peru (Oberste et al. 2009). Interestingly these viruses were most closely related to EMCVs isolated from pigs in Europe.

In the light of the ubiquitous presence of EMCV around the world in several animal species, including primates after a rodent plague (Canelli et al. 2010), secondary infections in immunocompromised persons can be expected to occur. In addition, the risk related to EMCV infection may become more important if pigs are used as donors for human xenografts. The experimental infection of a mouse through the transplantation of pig organs infected with EMCV validated this concern. A 2012 study shows that a myocardial (B279/95) and a rat strain (1086C) productively infect primary human cardiomyocytes and induce complete cytolysis (Brewer et al. 2003; Hammoumi et al. 2012).

Epidemiology

At one time considered a disease of subtropical or tropical areas, EMCVs are now noteworthy for their widespread geographical distribution and large number of susceptible hosts. EMCV infection has now been identified by virus isolation or the presence of antibodies throughout the world. In certain countries, a seasonal pattern of the outbreaks, with peaks in autumn, was noticed (Maurice et al. 2007).

EMCV is generally regarded as a rodent virus, although EMCVs have been isolated from over 30 species of mammals and birds. In mammals, the host range includes monkeys, chimpanzees, elephants, lions, squirrels, mongoose, raccoons, and swine (both domestic and wild boar). An episode of lion deaths at a zoo was found to be due to feeding carcasses of African elephants that had died of EMCV infection (Simpson et al. 1977). Among all outbreaks in wildlife, those in elephants are best documented (Grobler et al. 1995; Hunter et al. 1998; Reddacliff et al. 1997). In rodents, the virus usually persists without causing disease (Acland 1989; Zimmerman 1994). Infected rodents show high levels of the virus in the heart, spleen, lung, pancreas, Peyer's patches, and thymus and excrete the virus in their feces and urine (Spyrou et al. 2004).

Since infected pigs can excrete the virus, at least for a short period, direct pig-to-pig contact and contact with infected dead pigs are potential routes of virus spread at the farm level (Billinis et al. 1999; Maurice et al. 2002). Transplacental transmission may also occur (Christianson et al. 1992; Koenen and Vanderhallen 1997; Links et al. 1986). Although rigorous risk studies are scarce, a factor related to the introduction and/or spread of EMCV into pig farms includes the presence of rodents, which may play a role as a virus reservoir and contribute to spread either in their feces or as infected carcasses (Acland 1989; Spyrou et al. 2004). Feed and water contaminated with EMCV by rodents or infected rodent carcasses are considered important sources of swine infection. These findings are confirmed by a

matched case–control study (Maurice et al. 2007) in an area in Belgium where clinical EMCV outbreaks were regularly reported. The data indicated clusters of factors: (1) rodents, (2) general farm setup, and (3) general hygiene are associated with clinical EMCV. However, the conclusion was that the presence of mice was the most significant risk factor for clinical EMCV infection. A simulation suggested that in a compartmentalized pig housing unit, one single EMCV introduction is unlikely to cause a major outbreak by direct pig-to-pig transmission alone. Other mechanisms such as multiple introductions from a rodent reservoir may be required for large outbreaks to occur (Maurice et al. 2016).

Pathogenesis

Natural infection of swine is most likely to occur by the oral route. The course of the infection in swine appears to be influenced by virus strain, exposure dose, passage history, and susceptibility of the individual animal. For example, some strains cause either reproductive failure or myocardial death, while others can give rise to both (Koenen and Vanderhallen 1997). Australian strains were shown to be more virulent than New Zealand strains (Horner and Hunter 1979; Littlejohns and Acland 1975), and certain isolates in Florida were found to cause only myocarditis without death (Gainer et al. 1968). Other critical factors, such as the route of infection and age of the pigs, were found to be important factors in the spread of the virus under experimental conditions (Billinis et al. 2004; Littlejohns and Acland 1975).

After experimental oral infections in young pigs, virus was demonstrated as early as 6 hours post inoculation in the intestinal tract. In the heart and tonsils, focal positive reactions were found only in the cytoplasm of isolated macrophages and myocardial cells during the first 30 hours post inoculation. After 30 hours, some animals died with typical postmortem lesions, and clear positive immunohistochemical reactions were observed in the tonsils and heart. Three days post infection, the virus was also isolated from blood. The highest virus titers were recovered from heart muscle, in both experimental and natural infections. Myocardial lesions were predominant at necropsy. Gelmetti et al. (2006) concluded that virus replication in the heart, the target organ, is followed by myocarditis, with severe myocarditis resulting in sudden death in susceptible pigs. Proinflammatory cytokines such as IL-1β, tumor necrosis factor-α (TNF-α), and IL-6 have been implicated in the pathogenesis of myocarditis caused by EMCV infections (Robinson et al. 2009).

The consequences of transplacental EMCV infection are not well understood. Following intramuscular infection of pregnant sows with EMCV, a transplacental infection resulting in fetal death was observed 2 weeks post inoculation. Early farrowing, abortion, and mummification were observed in sows infected in mid- or late gestation, while evidence for fetal infection in sows during early pregnancy was not conclusive (Koenen and Vanderhallen 1997; Love and Grewal 1986). Antibodies and virus were recovered from fetuses, but the lesions varied from none to large diffuse patches in the heart, depending on the experiment (Kim et al. 1989a; Koenen and Vanderhallen 1997). Infection of swine fetuses *in utero* with laboratory-passaged strains produced little pathology.

In rats experimentally infected with a myocardial EMCV strain, no clinical or macroscopic lesions were observed in any organs. Virus was isolated from several tissues from 3 DPI until the end of the observation at day 62. EMCV was most frequently isolated from Peyer's patches and thymus, even in rats killed at 60 DPI. This finding indicated that this tissue represented a site of persistence after oral infection (Spyrou et al. 2004). The virus has seldom been pathogenic for rabbits and rhesus monkeys, causing inapparent infections despite high levels of viremia. In mice, certain strains cause predominantly fatal encephalitis, widespread myocardial damage, or even specific destruction of pancreatic beta cells (Cerutis et al. 1989).

Clinical signs

Many EMCV infections in a wide range of species are nonlethal and probably subclinical. Younger pigs are generally more susceptible to developing clinical disease, especially in the first weeks of life. In young pigs, the infection is most commonly characterized by acute disease with sudden death due to myocardial failure. Other clinical signs such as anorexia, listlessness, trembling, staggering, paralysis, or dyspnea have also been observed. Experimentally infected swine (Craighead et al. 1963; Littlejohns and Acland 1975) have shown temperatures up to 41 °C (106 °F) and death at 2–11 DPI (usually 3–5 days), or occasionally recovery with chronic myocarditis. Extremely high mortality, approaching 100%, can occur in pigs of preweaning age (Joo 1999). Infections in pigs from post weaning age to adulthood are usually subclinical, although mortality may occasionally be observed, even in adult pigs. In experimental infections, Billinis et al. (2004) found a high fatality rate in 20- and 40-day-old pigs, but none of the challenged 105-day-old pigs died.

In breeding females, clinical signs may vary from inapparent infection to various forms of reproductive failure, including abortion and increased numbers of mummified and stillborn fetuses (Dea et al. 1991; Koenen and Vanderhallen 1997).

Lesions

Pigs dying from the acute phase of cardiac failure may show only epicardial hemorrhage and no gross lesions.

Hydropericardium, hydrothorax, and pulmonary edema are frequently observed at necropsy. The heart is usually enlarged, soft, and pale. The most striking lesions are found in the myocardium where multiple foci of various sizes are found, especially in the right ventricle, which may extend to varying depths within the myocardium. They are often ill defined, circular, and linear and grayish/white in color (Figures 40.8 and 40.9). These lesions are observed more frequently in fattening pigs than in suckling piglets (Littlejohns and Acland 1975).

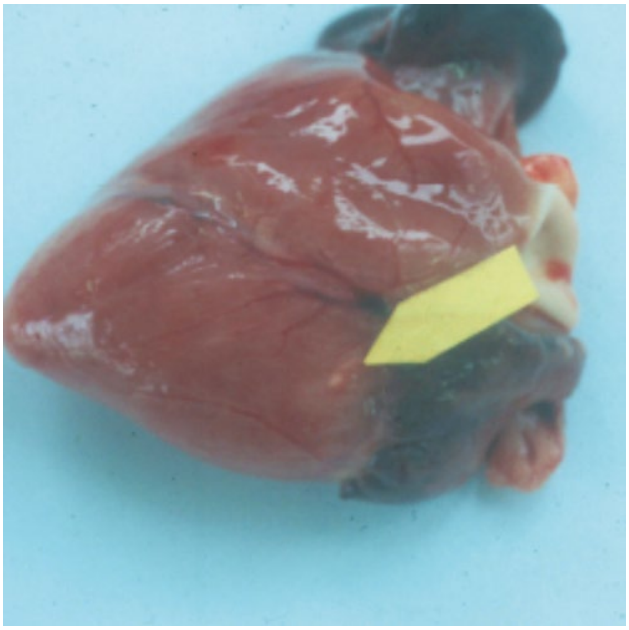


Figure 40.8 Heart of pig with EMCV infection showing typical white foci in myocardium. *Source:* Regional Animal Health Centre, Flanders, Torhout, Belgium. Reproduced with permission of John Wiley and Sons.

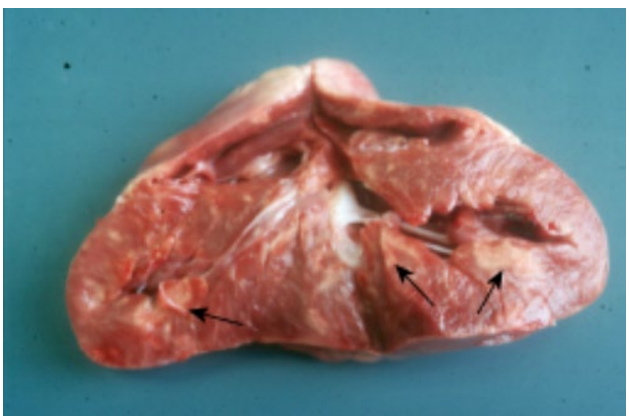


Figure 40.9 Sagittal section of the heart of pig with EMCV infection showing multiple foci of various sizes especially in the right ventricle, extending to varying depths. *Source:* Regional Animal Health Centre, Flanders, Torhout, Belgium. Reproduced with permission of John Wiley and Sons.

Infected fetuses are usually apparently normal, but can be hemorrhagic and edematous. With some virus strains, the fetuses can become mummified in various states of development, depending on the stage of infection. Macroscopic myocardial lesions are exceptional.

Histopathologically, the most significant findings in young pigs are seen in the heart. A positive immunohistochemical reaction is chiefly localized to the cytoplasm of myocardial cells. Its intensity and distribution is in accordance with the severity of the lesions. Sometimes, the positive reaction is detected in the Purkinje fibers and in the endothelial cells next to these. Mineralization of necrotic heart muscle is common (Figure 40.10) but not always present. In the tonsils, a positive immunohistochemical reaction is located in necrotic debris filling the crypts and in the cytoplasm of monocyte–macrophage lineage cells. This last finding was also noticed in lymph nodes (Gelmetti et al. 2006; Papaioannou et al. 2003; Psychas et al. 2001). Congestion with meningitis, perivascular infiltration with mononuclear cells, and some neural degeneration may be observed in the brain (Acland and Littlejohns 1975). Nonsuppurative encephalitis and myocarditis have also been described in swine fetuses with natural EMCV infection (Kim et al. 1989b).

Diagnosis

In newborn and suckling piglets, the disease is often characterized by sudden death between 3 days and 5 weeks of age. In most cases the piglets are found dead without any clinical signs. In finishing pigs, sudden death is also the most characteristic sign. All age categories can be affected, but mostly pigs of 60–70 kg (130–155 lbs.) are involved. The disease is often restricted to one barn, and deaths often occur in the late afternoon when the pigs are most active. In some pigs, squealing can be heard just before dying. In others dyspnea can be noticed.

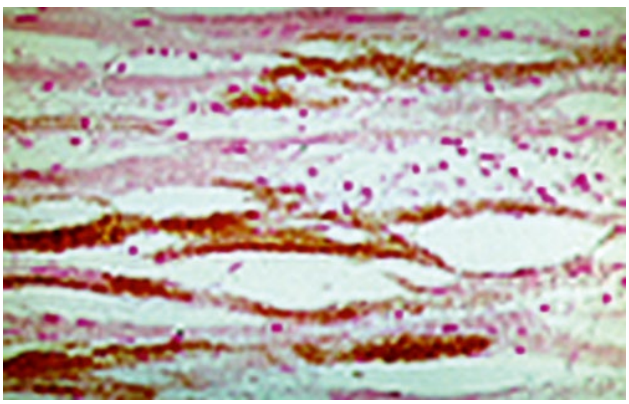


Figure 40.10 Interstitial myocarditis and calcification in the muscle fibers caused by EMCV infection. *Source:* Courtesy of Prof. Dr. R. Ducatelle, Faculty of Veterinary Medicine, Ghent University, Belgium.

A clinical history of reproductive failure and preweaning mortality is a useful indication of EMCV infection (Joo 1999). EMCV-induced reproductive problems should be differentiated from other pathogens causing reproductive problems in sows. EMCV causes reproductive failure in sows of all parities, while porcine parvovirus infection is manifested by an increase in mummification, mainly in gilt litters, without neonatal mortality. Other infections such as FMD, porcine reproductive and respiratory syndrome (PRRS), pseudorabies, porcine circovirus, and leptospirosis should also be considered.

Histopathological lesions may play an important role in making a diagnosis. A variable degree of nonsuppurative interstitial myocarditis or encephalitis (infiltration of lymphocytes, histiocytes, and plasma cells) is indicative of EMCV infection.

A conclusive diagnosis of EMCV should be demonstrated by virus isolation in mice or cell culture. BHK-21 cells are the most sensitive, but HeLa or Vero cell lines are also commonly used. Infected cell monolayers show a rapid and complete cytopathic effect (CPE). Virus identification can then be made by cross-serum neutralization with a reference antiserum or by staining with an anti-EMCV fluorescent antibody conjugate. Molecular methods, such as nucleic acid probes or RT-PCR for the detection of EMCV, have been reported (Kassimi et al. 2002; Vanderhallen and Koenen 1997; Yuan et al. 2014). Molecular methods are more sensitive and specific for diagnosis, especially when followed by sequencing.

Serological tests for the detection of serum antibodies against EMCV include hemagglutination inhibition (HI), ELISA, latex agglutination, immunofluorescent antibody (IFA) assay, agar-gel immunodiffusion (AGID), and VN. The VN and ELISA are the most commonly used methods and have been shown to be specific. For VN, antibody titers of ≥1 : 16 appear to be significant (Joo 1999).

Immunity

Neutralizing antibodies can be detected as early as 5–7 days after inoculation and may persist for an extended period (from 6 months to 1 year). Maternal antibodies remain for at least 2 months.

Proinflammatory cytokines such as IL-1β, TNF-α, and IL-6 have been implicated in the pathogenesis of myocarditis caused by EMCV infections (Robinson et al. 2009).

EMCV exists as two serotypes (although only a single isolation of EMCV-2 has been described; Philipps et al. 2012). Within EMCV-1 there is little antigenic variation; therefore cross-protection between all EMCV strains is likely to occur. No cross-neutralization was found between EMCV-1 and 62 human enterovirus serotypes or 11 porcine "enterovirus" serotypes (Zimmerman 1994). As discussed elsewhere in this chapter, most of these "enteroviruses" are now classified as teschoviruses.

Prevention and control

There is no treatment for the disease, but, in the acute phase, mortality may be minimized by avoiding stress or excitement of the pigs at risk. Rodents are often thought to play a role in the introduction and subsequent spread of EMCV on farms. Therefore, pig producers, especially in endemic areas, are advised to maintain rodent control to prevent clinical outbreaks of EMCV.

Pigs exposed to manure through slatted floors or to movement of manure between manure pits were found to be significantly protected against EMCV (Maurice et al. 2007), perhaps due to low-level exposure leading to subclinical infections.

The virus can be inactivated in water containing 0.5 ppm residual chlorine. For disinfectants, iodine-based preparations or mercuric chloride can be used.

An inactivated EMCV-1 vaccine is commercially available in the United States. The vaccine produced a strong humoral immune response in vaccinated pigs, and vaccinates were protected from clinical disease when challenged with virulent EMCV that killed 60% of unvaccinated controls. Also, protection against transplacental infection was demonstrated under experimental conditions. An EMCV vaccine candidate composed of noninfectious viruslike particles (VLPs) has been described (Jeoung et al. 2012).

Porcine teschovirus

Relevance

The first evidence of PTV infection was the occurrence of Teschen disease, a pig polioencephalomyelitis with high mortality, in Czechoslovakia over 75 years ago (Kouba 2009). PTVs are ubiquitous, and no conventional herd of pigs has been shown to be free of infection. While the majority of infections are subclinical, PTVs have been associated with a variety of clinical conditions, including polioencephalomyelitis, reproductive disease, enteric disease, and pneumonia. Strains that have not been shown to be pathogenic were previously referred to as enteric cytopathic swine orphan (ECSO) or enteric cytopathic porcine orphan (ECPO) viruses, but these terms are no longer in general use.

Etiology

PTVs were originally classified as PEVs. More recently, the genomic sequences of the prototype strains of all the PEV/PTV serotypes have been determined, as well as

partial genomic data on a number of additional isolates (Doherty et al. 1999; Kaku et al. 1999, 2001; Zell et al. 2001). Comparative analyses of these data showed that the PEVs fell into three genetic groups: (1) PEV types 1–7 and 11–13, (2) PEV type 8, and (3) PEV types 9 and 10. Prior to reclassification, PEVs were divided into three subgroups based on physicochemical properties, type of CPE produced in PK cells, and different cell culture host ranges (Knowles et al. 1979). The new classification scheme corresponds with the CPE groups defined earlier. The CPE group I viruses also possessed a leader polypeptide and a 2A/2B cleavage mechanism similar to the aphthoviruses, cardioviruses, and erboviruses.

Thus, the 10 former PEV serotypes 1–7 and 11–13 were renamed PTV-1 to PTV-10 and reclassified as a single species, *Teschovirus A* (former species name *Porcine teschovirus*), in a new genus, *Teschovirus*. Three additional types, PTV-11 to PTV-13, were also designated based on serological and/or molecular sequence data (Boros et al. 2012b; Cano-Gómez et al. 2011a; Zell et al. 2001). The remaining three PEV serotypes 8–10 are now classified in two species, *Sapelovirus A* (PEV-8, renamed PSV-1) and *Enterovirus G* (PEV-9 and PEV-10, renamed EV-G1 and -G2), in the genera *Sapelovirus* and *Enterovirus*, respectively.

As with all picornaviruses, the virions of PTVs are spherical and non-enveloped, with a diameter of 25–30 nm. A single-stranded RNA genome is surrounded by an icosahedral capsid consisting of 60 copies of four polypeptides. A small basic virus-encoded protein (VPg) is linked to the 5′ end of the genome. No three-dimensional structure data are yet available.

The serotypic classification of PTVs is based upon the VN tests (Dunne et al. 1971; Knowles et al. 1979). In the 1960s and 1970s, there were numerous attempts to achieve a uniform classification of PEVs, and these studies culminated in a classification of eight serotypes (Dunne et al. 1971). This was later extended to 13 serotypes (Auerbach et al. 1994; Knowles et al. 1979) (Table 40.1). A complement fixation test, suitable for rapid screening and typing of PTVs/PEVs, has also been described (Knowles and Buckley 1980). Subsequent findings (Knowles 1983) suggested that additional serotypes may exist. Honda et al. (1990b) compared the prototype strains found in Japan with 11 internationally recognized PTV serotypes by VN and suggested a further four candidate serotypes. Some limited cross-reactivity among the existing serotypes was evident, and Hazlett and Derbyshire (1978) showed that gastrointestinal antibodies were more broadly specific than serum antibodies.

PTVs are stable when treated with lipid solvents and relatively stable to heat. However, heating in the presence of halide ions tends to destabilize the virus. PTVs are stable at pH 2–9. Hemagglutination has not been demonstrated.

PTVs are readily cultivated in the laboratory in cell cultures of porcine origin. They are normally grown in primary or secondary PK cell cultures or in established cell lines such as IB-RS-2, but they may also be cultivated in other cells of porcine origin, such as the SST cell line, or in primary swine testes cells (Knowles et al. 1979).

Public health

PTVs are not known to be infectious for humans.

Epidemiology

Teschen disease has continued to occur sporadically, mainly in Central Europe but also in Africa. Milder forms of polioencephalomyelitis (Talfan disease, benign enzootic paresis), caused by serologically related but less virulent strains of PTV, have been reported in the last 50 years in Western Europe, North America, and Australia.

The only known natural host for PTVs is the pig. The virulent PTV-1 strains associated with classical Teschen disease appear to be restricted to those areas in which the disease occurs and they have not been isolated in North America. Less virulent PTV-1 strains and representatives of the other PTV serotypes appear to be ubiquitous.

Transmission of PTV infection is most frequently by the fecal–oral route, and indirect transmission by fomites is likely to occur since the viruses are relatively stable.

Endemic infection with several PTV serotypes can usually be demonstrated in conventional herds and is probably maintained in groups of weaned piglets. Singh and Bohl (1972) demonstrated waves of infection with six different serotypes over a period of 26 months in a long-term study of infection in a single herd. Piglets normally acquire infection shortly after weaning, when maternally derived antibodies are withdrawn and pigs from several litters are mixed, and it persists for at least several weeks. Adults rarely excrete virus but have high antibody levels. Pigs of any age are, however, fully susceptible to infection with a virus belonging to a serotype to which they have not previously been exposed.

PTVs are highly resistant to inactivation in the environment and may survive for long periods in liquid manure. Likewise, PTVs are relatively resistant to many disinfectants. Of 10 commonly used disinfectants tested by Derbyshire and Arkell (1971) against the Talfan virus, only sodium hypochlorite was effective.

Pathogenesis

Natural infection occurs by ingestion of the virus. It is well established (Long 1985) that initial replication occurs in the tonsil and intestinal tract. The large intestine and ileum are infected more frequently than the upper small intestine, and the former tissues contain

higher titers of virus. It has not been clearly established which cells in the intestine support viral replication, but by analogy with experiments on poliovirus (Kanamitsu et al. 1967), it is probable that the reticuloendothelial tissue of the lamina propria is involved. Epithelial cell destruction is not a feature of these infections. Viremia follows regularly in infections with the virulent PTV-1 strains, but less regularly with the less virulent strains, and leads to infection of the CNS (Holman et al. 1966).

It may be assumed that the pregnant uterus is also infected by viremic spread of the virus, since embryonic or fetal infections were demonstrated in gilts following nasal or oral inoculation of PTV (Huang et al. 1980). Intranasal inoculation of the virus may lead experimentally to lung infection (Meyer et al. 1966), but the significance of the natural inhalation of viral aerosols is not known. It has also been clearly demonstrated that when piglets are inoculated parenterally with PTVs, the virus rapidly infects the intestine. Extraintestinal infections are relatively transient, whereas the virus persists in the large intestine for several weeks.

Clinical signs

Although PTV infections are most frequently subclinical, various clinical syndromes have been associated with certain serotypes (Table 40.3) as outlined below.

Polioencephalomyelitis

The most severe form of polioencephalomyelitis is produced by the highly virulent PTV-1 strains that cause Teschen disease. This is a disease of high morbidity and high mortality, affecting all ages of swine and associated with major economic losses. The early signs of Teschen disease include fever, anorexia, and listlessness, rapidly followed by locomotor ataxia. In severe cases, there may be nystagmus, convulsions, opisthotonus, and coma. Paralysis ensues and the animal may assume a dog-sitting posture or remain in lateral recumbency. Stimulation by sound or touch may elicit uncoordinated limb movements or opisthotonus. Death is commonly within 3–4 days of the onset of clinical signs. Since the appetite returns after the acute phase, some animals may be kept alive by careful nursing, but these cases show muscle wasting and residual paralysis.

The less virulent PTV-1 strains (Talfan disease, benign enzootic paresis) and strains belonging to other serotypes associated with polioencephalomyelitis produce a milder disease with relatively low morbidity and mortality. Mainly young pigs are affected, and the disease rarely progresses to complete paralysis. Encephalomyelitis due to teschovirus infection has been reported in pigs in both the United States (Pogranichniy et al. 2003) and Japan (Yamada et al. 2004). Cesarean-derived colostrum-deprived pigs intravenously inoculated with PTV-2 and PTV-11, isolated from US swine, developed neurological signs consistent with teschovirus encephalomyelitis with more severe neurological signs observed in PTV-11-inoculated pigs (Matias Ferreyra et al. 2017).

Reproductive disorders

The term SMEDI was introduced initially (Dunne et al. 1965) to designate a group of viruses, subsequently shown to be PTVs, that had been isolated in association with stillbirth (S), mummified fetuses (M), embryonic death (ED), and infertility (I). Subsequent studies by the same group of workers and by others (Pensaert et al. 1973; Pensaert and De Meurichy 1973) indicated that the SMEDI could be reproduced experimentally. However, it is now well established that parvovirus infection may also lead to ED and fetal mummification and parvoviruses may be more frequently associated with these disorders of early and mid-gestation. Other findings (Cropper et al. 1976) substantiate a role for both PTV and parvoviruses in these disorders. Experimental (Bielaaski and Raeside 1977) and field (Kirkbride and McAdaragh 1978) data confirm an association between teschovirus infection and abortion in swine. These reproductive disorders are not usually accompanied by clinical signs in the sow or gilt. PTVs have also been isolated from the male genital tract (Phillips et al. 1972).

Table 40.3 Natural or experimental clinical syndromes associated with porcine enteric picornavirus infection.

Syndrome	PTV serotypes	Other picornaviruses
Polioencephalomyelitis	PTV-1, PTV-2, PTV-3, PTV-5, PTV-11	—
Reproductive disorders	PTV-1, PTV-3, PTV-6	PSV
Diarrhea	PTV-1, PTV-2, PTV-3, PTV-5	PSV
Pneumonia	PTV-1, PTV-2, PTV-3	PSV
Pericarditis and myocarditis	PTV-2, PTV-3	—
Cutaneous lesions	—	PEV-9, PEV-10

Diarrhea

The role of PTVs as enteric pathogens is uncertain. They have frequently been isolated from the feces of piglets with diarrhea, but since they can be readily isolated from normal piglets, particularly post weaning, and since diarrhea can be caused by a variety of other viral and bacterial agents, their presence may be coincidental. However, diarrhea has been produced experimentally by PTVs in piglets believed to be free of other pathogens. The diarrhea is mild and relatively transient, and it seems clear that PTVs are considerably less important enteric pathogens than rotaviruses or coronaviruses. When piglets were infected with PTVs together with rotaviruses, the disease was less severe than in piglets infected only with the rotavirus (Janke et al. 1988).

Pneumonia, pericarditis, and myocarditis

The role of PTVs as respiratory pathogens is also uncertain. It is probable that alone they rarely cause clinical signs of respiratory disease. Experimentally, two serotypes of PTV have been shown capable of producing pericarditis, and in one experiment myocardial involvement occurred (Long et al. 1969). These findings might lead to a suspicion of teschovirus infection in the case of sudden death in piglets, although EMCV would be higher on the list of differentials.

Possible association with cutaneous lesions

During the course of investigations of SVD outbreaks in the United Kingdom, many adventitious agents were isolated from samples of epithelium and feces. Most of these isolates were identified as teschoviruses or enteroviruses (Knowles 1983).

Lesions

No specific changes have been associated with intestinal PTV infections. They do not appear to cause the villous atrophy characteristic of primary intestinal pathogens, such as coronaviruses and rotaviruses. Other than muscle atrophy in chronic cases, no gross lesions are found in polioencephalomyelitis. The histological lesions associated with the latter are widely distributed in the CNS but are especially numerous in the ventral columns of the spinal cord, the cerebellar cortex, and the brain stem. The changes are more marked and extensive in Teschen disease than in milder encephalomyelitides, such as Talfan disease. The neurons show progressive diffuse chromatolysis (Koestner et al. 1966) and focal areas of gliosis and perivascular lymphocytes, particularly over the cerebellum, that may also occur.

The SMEDI syndrome is remarkable for the lack of specific lesions in stillborn or neonatal piglets, although mild focal gliosis and perivascular cuffing in the brain stem have been found occasionally. Placental changes are restricted to nonspecific degeneration.

Pneumonic lesions have been produced by several investigators. Smith et al. (1973) described areas of grayish-red consolidation in the ventral anterior lobes of lungs infected with a PTV-2 strain. Exudates were observed in the alveoli and bronchi, as well as slight perivascular and peribronchiolar cuffing and some hyperplasia of the bronchiolar epithelium.

A PTV-3 strain consistently produced serofibrinous pericarditis experimentally, and the more severely affected piglets showed focal myocardial necrosis (Long et al. 1969).

Diagnosis

Teschen disease (also known as teschovirus encephalomyelitis) is designated as a notifiable disease by the OIE and details of internationally accepted diagnostic methods are described (OIE 2008).

The occurrence of clinical signs associated with polioencephalomyelitis is suggestive of viral infection, but the differentiation of teschoviral infection from other neurotropic viruses requires isolation of the virus from the CNS, the demonstration of viral antigen by specific immunofluorescence, or the detection of viral RNA by RT-PCR. Similarly, with reproductive disorders, diarrhea, pneumonia, pericarditis, and myocarditis, there are no diagnostic clinical signs that would suggest PTV involvement, and laboratory diagnosis is required.

Virus detection

Virus isolation from the CNS requires the collection of tissues from a piglet showing early nervous signs. Animals that have been paralyzed for several days may no longer contain infectious virus in the CNS (Lynch et al. 1984). The virus may be isolated in PK cell cultures from suspensions of the spinal cord, brain stem, or cerebellum. The virus may subsequently be identified on the basis of its physicochemical characteristics or by immunostaining (Watanabe et al. 1971). Serological identification of the isolate is desirable. Isolation of a PTV from the gastrointestinal tract of a piglet with nervous signs does not establish the etiology of the disease, since the enteric infection may be coincidental.

In the SMEDI syndrome, mummified fetuses carried to term rarely contain live virus but may contain viral antigen detectable by immunofluorescence. Virus isolation in PK cell culture may be attempted from tissues of aborted or stillborn fetuses. Lung tissue appears to be the most reliable source for the isolation of PTVs from fetuses (Huang et al. 1980). VN test on the body fluids of such fetuses can be carried out against the SMEDI-associated PTV serotypes.

In the investigation of pneumonia or diarrhea, virus isolation from the respiratory or intestinal tract may be attempted, but the virological findings should be interpreted cautiously, especially in relation to diarrhea, since enteric infections with PTVs are common in healthy piglets. In one study PTVs and PEVs were isolated from 57% of porcine fecal samples submitted for SVD diagnosis over a 7-year period (Knowles 1983).

Isolated viruses may be identified by VN (Knowles et al. 1979), complement fixation (Knowles and Buckley 1980), or immunofluorescence (Auerbach et al. 1994; Dauber 1999) tests if suitable reference reagents are available. Monoclonal antibodies capable of detecting PTVs have been described (Dauber 1999).

Genomic sequence data are available for all the PTVs, and it is possible to use RT-PCR to detect viral RNA in clinical samples or to identify viruses isolated in cell culture. Palmquist et al. (2002) described an RT-PCR using a single set of primers for the simultaneous detection and differentiation (based on amplicon size) of PTVs and PSV. Nested RT-PCR assays to specifically detect PTV-1 and to differentiate PTV, PSV, and PEVs from each other using virus-specific primer sets have also been described (Zell et al. 2000). Improvements to these assays resulted in the development of a one-step real-time PCR (Cano-Gómez et al. 2011b; Chiu et al. 2014; Krumbholz et al. 2003; Zhang et al. 2013).

Antibody detection

Serology is of little value for diagnosis, unless paired sera are available and the serotype known, in which case VN would be suitable. An ELISA suitable for mass screening has been described for the detection of antibodies against Teschen disease virus (Hubschle et al. 1983). However, as these viruses are ubiquitous, serological surveys may not be very helpful.

Immunity

Infected pigs mount a classical humoral protective immune response (IgM and IgG). Mucosal immunity (IgA) may be produced and have a protective effect in the gastrointestinal tract. In an experimental study of PTV infection in piglets, it was shown that cell-mediated responses were weak, localized, and not associated with significant antiviral activity (Brundage et al. 1980).

Humoral antibody is thought to be important for protection. Immunosuppression of pigs infected with a PTV by cyclophosphamide treatment led to the lack of a serological response and a persistent infection of the intestinal tract (Derbyshire 1983). This resulted in severe diarrhea and, in one case, signs of encephalomyelitis. Presence of high levels of virus-neutralizing IgA antibody in the gastrointestinal tract may protect against oral challenge (Hazlett and Derbyshire 1977).

Since antibody is probably the most important factor in protection, at least for teschoviruses, the relatively large number of serotypes would suggest that cross-protection might not occur.

It has been reported for PTV-1 that maternal antibody has no effect on embryonic or fetal infections after the virus has reached the uterus (Huang et al. 1980). However, preinfection antibody in sows would probably limit infection and prevent the virus from reaching the uterus. Colostral antibody would be expected to protect piglets from infection.

Prevention and control

As with most viral infections, control measures for PTV depend on prevention rather than treatment. Potential antiviral chemotherapeutics for porcine enteric picornaviruses have received little attention. Piglets with mild polioencephalomyelitis may recover if nursing care is provided during the period of transient paresis.

Vaccination has been practiced in the field only for the control of Teschen disease. The earlier Teschen disease vaccines, containing inactivated virus of pig tissue origin, have been superseded by attenuated or inactivated cell culture vaccines. Mayr (1959) attenuated Teschen disease virus by cell culture passage and showed that live or formalin-inactivated vaccines prepared from this virus induced similar levels of protection in piglets. Success has been claimed for a Teschen disease eradication program involving ring vaccination and slaughter (Schaupp 1968). Restrictions on the import of swine and pork products from areas in which Teschen disease is endemic seem to be effective in limiting the spread of virulent PTV-1 strains.

Vaccination has not been practiced against the milder forms of polioencephalomyelitis or against the other clinical manifestations of PTV infection in swine. Only the SMEDI syndrome is of sufficient economic importance to justify specific control measures in the field, but the multiplicity of serotypes that may be involved complicates the development of an effective vaccine.

Currently, the best approach for the prevention of reproductive disorders associated with PTVs is the application of management practices that ensure that gilts are exposed to the infection with endemic viruses at least 1 month before breeding. This can be achieved naturally if the animals remain in a single building from birth to breeding, with thorough mixing of piglets from different litters at weaning. If breeding stock is segregated at an early age, they should be exposed to fecal material from recently weaned piglets. This can be readily accomplished by "feedback," that is, adding fresh feces to the feed of gilts or by dosing gilts with capsules of feces derived from a pooled sample collected from weaned piglets in several pens. The objective is to ensure exposure to the widest possible range of the viruses present in

the herd. The operation of a closed herd system reduces the risk of introducing extraneous viruses, but it is not possible to eliminate this risk, since the relatively resistant enteric picornaviruses can be transmitted by a variety of fomites. If the introduction of fresh stock is essential for breeding purposes, before the gilts or sows are bred, they should be exposed by feedback to any virus that may be present or introduced.

Exclusion of PTVs by repopulation of herds with specific-pathogen-free (SPF) stock seems to be difficult or impossible to achieve over a prolonged period. At least, these viruses have been isolated from commercial SPF herds, and the accidental introduction of Talfan virus into SPF gilts maintained under strict isolation has been described (Parker et al. 1981). Since transplacental infection of fetuses may occur, even gnotobiotic pigs may be infected.

Seneca Valley virus

Relevance

Between 1988 and 2001, 11 antigenically related picorna-like viruses were isolated from samples taken from pigs in various states in the United States. In 2002, a picorna-like virus (named Seneca Valley virus) was isolated at Genetic Therapy Inc. (Gaithersburg, MD, USA) while cultivating adenovirus 5-based vectors in PER.C6 cells (Hales et al. 2008). Comparison of the nucleotide sequences of these viruses led to the recognition that these were all the same virus, and the name Seneca Valley virus was adopted (Hales et al. 2008; Knowles et al. 2006).

Since 1997, an increasing association of SVV with idiopathic vesicular disease led to the recognition of the role of the virus in vesicular disease outbreaks in pigs (Knowles et al. 2006; Pasma et al. 2008; Singh et al. 2012). Coinfection with other porcine viruses seemed necessary for induction of vesicular lesions in pigs, and earlier efforts to reproduce the disease in pigs experimentally inoculated with SVV were futile (Yang et al. 2012). However, in recent outbreaks in Brazil and the United States in 2014 and 2015, SVV seemed to be the sole virus isolated from sick pigs, implicating SVV as a direct cause of vesicular disease in pigs (Leme et al. 2016a). Subsequently, vesicular lesions were induced in pigs following experimental inoculation with 2015 US isolates of SVV (Joshi et al. 2016b, Montiel et al. 2016).

Etiology

SVV is a picornavirus in the species *Senecavirus A* (formerly called *Seneca Valley virus*) in the genus *Senecavirus* (Knowles et al. 2012). All known SVV isolates belong to a single serotype, SVV-1. They also form a single genetic lineage, leading to the conclusion that they have a recent common origin (Knowles et al. 2016). Although referred to as Senecavirus A or Seneca A virus in recent publications, the virus common name is Seneca Valley virus.

The genome of SVV consists of approximately 7,310 nucleotides and encodes a single polyprotein of 2181 aa (Hales et al. 2008). This polyprotein is posttranslationally cleaved into 12 mature proteins (including a leader polypeptide with unknown function). Four of these proteins – 1A (VP4), 1B (VP2), 1C (VP3), and 1D (VP1) – form the virus capsid (Venkataraman et al. 2008), and one of these proteins, 3B (VPg), is linked directly to RNA. The nsp are involved in virus replication and interruption of host cell functions.

SVV can be grown in a number of different cells including PK-15, IB-RS-2, lamb kidney, Vero, LLC-MK2, and RK-13 (NJ Knowles, unpublished data), LFBKαVβ6, swine testis (ST), swine kidney (SK-RST), and human lung cancer cells (NCI-H1299) (Goolia et al. 2017; Yang et al. 2012). Identification may be achieved by standard serological assays (e.g. VN or ELISA) using suitable antisera such as monoclonal antibodies (Yang et al. 2012). Competitive ELISA, conventional RT-PCR, and real-time SYBR Green and Taqman® RT-PCR assays (rRT-PCR) have been developed (Bracht et al. 2016; Fowler et al. 2017; Leme et al. 2015; Yang et al. 2012).

Public health

SVV has no known public health significance. In fact one isolate, SVV-001, has been developed as a cancer therapy due to its ability to kill cancerous, but not normal, human cells (Burke 2016; Reddy et al. 2007).

Epidemiology

SVV was first found in 1988 in pigs in the United States. Since then SVV has been isolated from pigs in at least 10 states (California, Illinois, Iowa, Kansas, Louisiana, Minnesota, New Jersey, North Carolina, Ohio, and South Dakota). More recently there has been an increase of vesicular disease in the United States along with the isolation of SVV (Baker et al. 2017; Canning et al. 2016; Gimenez-Lirola et al. 2016; Guo et al. 2016; Hause et al. 2016; Wang et al. 2016). In 2007, SVV was isolated from imported pigs in Minnesota that were traced to pig farms in Manitoba, Canada, where the virus was also detected (Pasma et al. 2008). Later, SVV was also found in the brain of a pig in Canada in 2011 and 2016 (L.G. Koster and M.L. Killian, unpublished data). Since late 2014, SVV has also been found in pigs in the Goias, Minas Gerais, Paraná, and Santa Catarina states of Brazil (Laguardia-Nascimento et al. 2016; Leme et al.

2015, 2016b; Vannucci et al. 2015); in the Guangdong and Hubei provinces of the People's Republic of China (Qian et al. 2016; Wu et al. 2016, 2017); in Lamphun Province in the northern region of Thailand (Saeng-chuto et al. 2018); and in central western Colombia (Sun et al. 2017).

Only pigs are known to be infected by the virus, although there have not been comprehensible serological surveys of other domesticated or wild animals. Vesicular disease (indistinguishable from FMD, SVD, VS, and VES) has been reproduced experimentally in pigs (Joshi et al. 2016b; Montiel et al. 2016).

It is not understood how SVV is transmitted, but it is likely to be by the fecal–oral route or via cuts and abrasions. Vertical transmission has been suggested, but not proven (Leme et al. 2016b).

SVV RNA has been detected in the tonsils of experimentally infected pigs for up to 38 DPI by rRT-PCR and *in situ* hybridization (Joshi et al. 2016b). It has been postulated that virus shedding may occur via the feces or in urine (Leme et al. 2016b). Ruptured vesicles are also a source of virus.

Little is currently known about the persistence of the virus in the environment; however, SVV has been isolated from mice and houseflies on a farm where SVV-induced vesicular disease was present (Joshi et al. 2016a). Nucleotide sequence studies showed that the viruses present in pigs, mice, and flies were essentially identical (Joshi et al. 2016a).

Accelerated hydrogen peroxide® and Virkon® have been found to be effective disinfectants against SVV for use in the laboratory (Hole et al. 2017). Other commonly used disinfectants that are used for SVD (e.g. iodophors) may be suitable for use with SVV, but have not been tested.

Pathogenesis

Little is known about virus entry. Virus or virus RNA has been detected in epithelial tissues, tongue, gingiva, myocardium, lung, renal pelvis, liver, urinary bladder, brain, and small intestine (Leme et al. 2016b). There are no known virulence factors, but stress, especially during transportation, is postulated to be a predisposing factor for SVV-associated vesicular disease (Baker et al. 2017, Joshi et al. 2016b).

Clinical signs

The virus has a relatively short incubation period since in experimentally infected animals viremia was detected at 3 DPI and lesions observed at 4 DPI (Joshi et al. 2016b; Montiel et al. 2016). Clinical signs observed in the field included lameness in up to 80–90% of pigs, as well as vesicles and erosions on the coronary bands and snouts. Fever and loss of condition are negligible in adult pigs, and no adverse effects on farrowing were observed in pregnant sows (Baker et al. 2017; Pasma et al. 2008; Vannucci et al. 2015). Experimentally, clinical signs are characterized by lethargy and lameness at 4 DPI that persisted for 2–10 days (Joshi et al. 2016b). Vesicular lesions were first observed at 4 DPI on the snout and/or feet, affecting the coronary bands, dewclaws, interdigital space, and heel/sole of SVV-infected animals (Joshi et al. 2016b). A short viremia was observed between 3 and 10 DPI, whereas virus shedding was detected between 1 and 28 DPI in oral and nasal secretions and feces (Joshi et al. 2016b).

Although neonatal piglets do not typically show vesicular lesions like sows, other clinical signs of SVV infection have been observed in this age group. During outbreaks in Brazil in 2014–2015, one-week-old or younger piglets developed diarrhea accompanied by muscle weakness, reduced activity, increased salivation, and some neurologic signs. Redness of the skin and sudden deaths were also observed in this age group. Because SVV had been detected in sows from the same farms, it was suspected to be the cause of the clinical signs in neonates. Furthermore, SVV was detected in tissues from piglets that died suddenly. Piglets that survived usually recovered in 3–10 days (Leme et al. 2016a; Vannucci et al. 2015).

Morbidity and mortality

Some increase in mortality has been associated with SVV infection (Baker et al. 2017; Segalés et al. 2017). In Brazil, 20–30% mortality in neonatal piglets was recorded in farms where sows had developed SVV-associated vesicular lesions a week prior. Earlier reports in 2014 suggested even higher mortality (30–70%) in piglets ≤4 days of age. Pigs with sudden death were examined at necropsy, and SVV detected in multiple tissues, suggesting that SVV infection might have been responsible for the neonatal mortality (Leme et al. 2016a; Vannucci et al. 2015). Mortality is rare in adult pigs.

Lesions

In one study the principal gross findings included faint rib impressions on the pleural surface of the lungs, diphtheritic glossitis, and ulcerative lesions at the coronary band (Leme et al. 2016b). Histopathology revealed interstitial pneumonia, myocarditis, diphtheritic glossitis, encephalitis, and atrophy of intestinal villi with vacuolation of the superficial epithelial cells (Leme et al. 2016b). Immunohistochemistry with SVV-specific monoclonal antibodies demonstrated immunoreactivity of the choroid plexus of the cerebrum, degenerate epithelium of ulcerative lesions of the tongue, the urothelium of the kidney and urinary bladder, and the superficial cells of

the intestine (Leme et al. 2016b). Experimentally, within 4 DPI, 50% of pigs had intact or ruptured vesicular lesions on the coronary bands or the interdigital spaces (Montiel et al. 2016). Minimal to mild lameness occurred in some animals. After 5 DPI, almost 100% of pigs had vesicular lesions. Lesion severity ranged from blanched coronary bands to ulcerations and erosions from ruptured vesicles. Focal necrosis and crusting of either the interdigital space, the coronary band, or both developed in some animals. Severe lesions in a subset of the cohort progressed to multifocal deep ulcers. In general, vesicular lesions were 0.2–2 cm in diameter (Montiel et al. 2016).

Diagnosis

The vesicular lesions caused by SVV are indistinguishable from those produced by FMD, SVD, VS, and VES. Therefore, laboratory testing is mandatory. Inoculation of susceptible cell cultures, particular of porcine origin, will result in a rapid CPE. Virus identification can be achieved using VN, ELISA, or rRT-PCR. A conventional RT-PCR targeting the VP3/VP1 region (Leme et al. 2015), a SYBR Green-based rRT-PCR assay targeting the VP1 region (Bracht et al. 2016), and a Taqman® rRT-PCR targeting the 3D region (Dall-Agnol et al. 2017; Fowler et al. 2017) have all been described. The detection of SVV antibodies using competitive ELISA, VN, and immunofluorescent antibody test (IFAT) has been described (Goolia et al. 2017; Yang et al. 2012).

Immunity

Serological responses to SVV have been characterized by detection of neutralizing antibody 5 DPI, which coincided with decreased levels of viremia, virus shedding, and viral load in tissues (Joshi et al. 2016b).

Prevention and control

There have been few attempts to control outbreaks of SVV, and in the absence of clinical disease, the virus probably spreads easily in the pig population. No vaccines have been developed, but an experimental vaccine using inactivated cell culture-derived SVV has been successfully tested in pigs (Yang et al. 2018).

Porcine kobuvirus

The genus *Kobuvirus* initially consisted of the human Aichi virus (species *Aichivirus A*) and the bovine kobuvirus (species *Aichivirus B*). However, Reuter et al. (2009) reported the sequence of a PKV (species *Aichivirus C*) about 60% identical at the nucleotide sequence level to the Aichi virus and bovine kobuvirus. PKV was initially detected in an RT-PCR analysis for the presence of porcine sapovirus, a calicivirus, in fecal samples from healthy pigs in Hungary. A nonspecific PCR product was sequenced and found to be related to the known kobuviruses. Following the characterization of this virus, some 60% of the pigs at the farm were found to be carrying the virus, including about 90% of the animals under 3 weeks of age. Subsequently, an extremely high (99%) incidence of PKV was found in piglets with diarrhea in Thailand (Khamrin et al. 2009), and in a study from China (Yu et al. 2009), about 30% of fecal samples from healthy pigs were positive by RT-PCR using specific primers for this virus. However, no experimental studies demonstrating a causal role in disease have yet been published.

Porcine sapelovirus

Formerly called both PEV-8 and CPE type II group PEVs, PSV is a member (species *Sapelovirus A*) of genus *Sapelovirus*, along with some simian and avian picornaviruses. PSVs are antigenically diverse (Dunne et al. 1971) and have been associated with the SMEDI syndrome (Dunne et al. 1965) and diarrhea (Honda et al. 1990a). Experimentally, pregnant pigs infected with PSV developed fetal infections (Huang et al. 1980). In addition to cells of porcine origin, PSV can be cultivated in monkey kidney (e.g. Vero) and BHK-21 cells (Knowles et al. 1979). PSV was found in the spinal cords of pigs showing ataxia and paraparesis, and viral antigen co-localized in the spinal cord lesions of affected animals by an antibody known to react with PSV (Schock et al. 2014). PSV has also been detected in central nervous tissues of pigs with polioencephalomyelitis in the United States by PSV-specific RT-PCR and *in situ* hybridization (Arruda et al. 2017). A Korean PSV strain has been shown to induce diarrhea and intestinal pathology in experimentally infected piglets (Kim et al. 2016). The virus also displayed extraintestinal tropisms such as mild, nonsuppurative myelitis, encephalitis, and pneumonia in piglets. An RT-LAMP assay for the detection of PSV has been reported (Wang et al. 2014). A minor groove binder assay for real-time PCR detection of PSV has also been described (Chen et al. 2014).

Porcine enteroviruses

There is little evidence for the association of true PEVs with disease. During the course of investigations of SVD outbreaks in the United Kingdom, many adventitious agents were isolated from samples of epithelium and

feces. Most of these isolates were identified as enteric picornaviruses (Knowles 1983). PTVs and PSV were evenly distributed in feces samples (41 and 44%, respectively) and in epithelial samples (21% in each case); however, PEV-9 and PEV-10 were rarely found in feces (15%) and more commonly found in epithelial samples (58%) (Knowles 1988). Examination of another virus collection again revealed an approximately even distribution of PTV and PSV (57 and 43%, respectively), while PEV-9 and PEV-10 were not detected (Knowles 1988). It was assumed that most of the PTV and PSV isolates identified in epithelial samples were incidental contaminants, but the low frequency of PEV-9 and PEV-10 in feces could not explain the much higher isolation rate from epithelial tissue collected from atypical skin lesions. PEVs can be cultivated in pig, hamster (BHK-21), monkey (e.g. Vero), and some human (HeLa) cell cultures (Knowles et al. 1979).

Recently, the species *Porcine enterovirus B* (to which PEV-9 and PEV-10 belonged) has been renamed *Enterovirus G*, and the two types have been renamed EV-G1 (PEV-9) and EV-G2 (PEV-10). An additional 18 new types have been designated EV-G3 to EV-G20 (Boros et al. 2011, 2012a,c; Bunke et al. 2018; Moon et al. 2012; Shang et al. 2017; Van Dung et al. 2014, 2016; Wang et al. 2017). Among these 20 types, EV-G5 and EV-G7 have only been isolated from sheep, while EV-G20 has only been found in goats; the remaining 17 types have been detected in either domestic pigs or wild boar (Table 40.1). EV-G was reported for the first time in the United States in 2014 (Anbalagan et al. 2014).

Swine pasiviruses

Sauvage et al. (2012) recently described the molecular detection of a new picornavirus in healthy pigs in France. It was named swine pasivirus: "Pasivirus" for "Parecho sister-clade virus," with swine pasivirus 1 (PaV-1) proposed as the type species. A second swine pasivirus was identified in pig feces in China (Yu et al. 2013a,b) and a third in Hungary (Boros et al. 2015). The three viruses share 72–75% nucleotide identity and 74–77% aa identity in their VP1 regions, suggesting they belong to three distinct (geno)types: PaV-A1, PaV-A2, and PaV-A3. Swine pasiviruses have also been detected in pigs in the United States (Guo et al. 2018) and in Romania (L. Buburuzan, M. Eloit, M. Zaulet and V. Petrovan, unpublished data). They have not yet been cultivated in cell cultures. The role of pasiviruses in swine disease is not known; however, antibodies to PaV-1 have been detected in humans (Arnold et al. 2015; Yu et al. 2013b).

Porcine picornavirus Japan

PPVJ was found in the feces of healthy pigs in Japan in 2015 (Naoi et al. 2016). It is most closely related to members of the genera *Teschovirus* and *Hunnivirus* and the candidate genus *Lesavirus*. It has been suggested that it may represent a new species in a novel picornavirus genus (Naoi et al. 2016). The authors were not able to isolate the virus using a porcine kidney cell line (CPK) or Vero cells. However, using a conventional RT-PCR, targeted against the 3D coding region, the virus was detected in pigs on seven out of 12 farms examined. Nothing is known about the potential for PPVJ to cause disease in pigs.

Other picornaviruses detected in pigs

In a recent study of picornaviruses in humans and pigs in Bolivia, human parechovirus (type 4), human cosavirus (untyped), and a *Cardiovirus B* (untyped) were detected in pig feces (Nix et al. 2013). The significance of these findings is unclear as similar viruses were also detected in human fecal samples collected at the same time.

References

Acland HM. 1989. Encephalomyocarditis virus. In Horzinek MC, Pensaert MB, eds. *Virus Infections of Vertebrates*, Vol. 2. Amsterdam, The Netherlands: Elsevier Science Publishers BV, pp. 259–263.

Acland HM, Littlejohns IR. 1975. *Aust Vet J* 51: 409–415.

Alexandersen S, Donaldson AI. 2002. *Epidemiol Infect* 128:313–323.

Alexandersen S, Mowat N. 2005. *Curr Top Microbiol Immunol* 288:9–42.

Alexandersen S, Forsyth MA, Reid SM, et al. 2000. *J Clin Microbiol* 38:4604–4613.

Alexandersen S, Oleksiewicz MB, Donaldson AI. 2001. *J Gen Virol* 82:747–755.

Alexandersen S, Zhang Z, Donaldson AI. 2002a. *Microbes Infect* 4:1099–1110.

Alexandersen S, Zhang Z, Reid SM, et al. 2002b. *J Gen Virol* 83:1915–1923.

Alexandersen S, Kitching RP, Mansley LM, et al. 2003a. *Vet Rec* 152:489–496.

Alexandersen S, Quan M, Murphy C, et al. 2003b. *J Comp Pathol* 129:268–282.
Alexandersen S, Zhang Z, Donaldson AI, et al. 2003c. *J Comp Pathol* 129:1–36.
Alexandersen S, Wernery U, Nagy P, et al. 2008. *J Comp Pathol* 139:187–193.
Alves MP, Guzylack-Piriou L, Juillard V, et al. 2009. *Clin Vaccine Immunol* 16:1151–1157.
Ambagala A, Fisher M, Goolia M, et al. 2017. *Transbound Emerg Dis* 64:1610–1623.
Anbalagan S, Hesse RA, Hause BM. 2014. *PLoS One* 29:e97517.
Anil SS, Anil L, Deen J, et al. 2007. *J Swine Health Prod* 15:78–83.
Anonymous. 1969. Report of the Committee of Inquiry on Foot-and-Mouth Disease (1968), Ministry of Agriculture, Fisheries and Food. London: Her Majesty's Stationery Office, Part 1.
Anonymous. 1986. Foot-and-Mouth Disease. Ageing of lesions, Ministry of Agriculture, Fisheries and Food. London: Her Majesty's Stationery Office, Reference Book 400.
Armstrong RM, Barnett ITR. 1989. *J Virol Methods* 25:71–79.
Armstrong R, Davie J, Hedger RS. 1967. *Br Med J* 4:529–530.
Arnold F, Hober D, Chaussade H, et al. 2015. *J Clin Virol* 69:36–39.
Arruda PHE, Arruda BL, Schwartz KJ, et al. 2017. *Transbound Emerg Dis* 64:311–315.
Auerbach J, Prager D, Neuhaus S, et al. 1994. *Zentralbl Veterinarmed B* 41:277–282.
Bachanek-Bankowska K, Mero HR, Wadsworth J, et al. 2016. *J Virol Methods* 237:114–120.
Bachrach HL. 1968. *Annu Rev Microbiol* 22:201–244.
Bachrach HL, Breese SS, Callis JJ, et al. 1957. *Proc Soc Exp Biol Med* 95:147–152.
Baker KL, Mowrer C, Canon A, et al. 2017. *Transbound Emerg Dis* 64:11–18.
Bauer K. 1997. *Arch Virol Suppl* 13:95–97.
Beck E, Strohmaier K. 1987. *J Virol* 61:1621–1629.
Bellini S, Alborali L, Zanardi G, et al. 2010a. *Rev Off Int Epizoot* 29:639–648.
Bellini S, Grazioli S, Nassuato C, et al. 2010b. An experimental infection with swine vesicular disease virus in pregnant sows to determine the duration of passive immunity in piglets. Abstracts 4th Annual Meeting EPIZONE, Saint Malo, France, p. 178.
Belsham GJ. 1993. *Prog Biophys Mol Biol* 60:241–260.
Belsham GJ. 2005. *Curr Top Microbiol Immunol* 288:43–70.
Belsham GJ. 2009. *Virus Res* 139:183–192.
Bengis RG, Hedger RS, De Vos V, et al. 1984. The role of the African elephant *Loxodonta africana* in the epidemiology of foot-and-mouth disease in the Kruger national park. In Proceedings of the 13th World Buiatrics Congress, Vol. 13, pp. 39–44.
Berger HG, Straub OC, Ahl R, et al. 1990. *Vaccine* 8:213–216.
Bergmann IE, de Mello PA, Neitzert E, et al. 1993. *Am J Vet Res* 54:825–831.
Berryman S, Clark S, Monaghan P, et al. 2005. *J Virol* 79:8519–8534.
Bhattacharya S, Banerjee R, Ghosh R, et al. 2003. *Vet Rec* 153:504–505.
Bielaaski A, Raeside JI. 1977. *Res Vet Sci* 22:28–34.
Billinis C, Paschaleri-Papadopoulou E, Anastasiadis G, et al. 1999. *Vet Microbiol* 70:179–192.
Billinis C, Leontides L, Psychas V, et al. 2004. *Vet Microbiol* 99:187–195.
Borgen HC, Schwobel W. 1964. *Nature (Lond)* 202:932–933.
Boros Á, Pankovics P, Reuter G. 2011. *Infect Genet Evol* 11:1096–1102.
Boros Á, Nemes C, Pankovics P, et al. 2012a. *Arch Virol* 157:981–986.
Boros Á, Nemes C, Pankovics P, et al. 2012b. *Arch Virol* 157:1573–1578.
Boros Á, Pankovics P, Knowles NJ, et al. 2012c. *J Gen Virol* 93:1941–1951.
Boros Á, Fenyvesi H, Pankovics P, et al. 2015. *Arch Virol* 160:1363–1366.
Borrego B, Carra E, Garcia-Ranea JA, et al. 2002a. *J Gen Virol* 83:35–44.
Borrego B, Garcia Ranea JA, Douglas A, et al. 2002b. *J Gen Virol* 83:1387–1395.
Bøtner A, Belsham GJ. 2012. *Vet Microbiol* 157:41–9.
Bracht AJ, O'Hearn ES, Fabian AW, et al. 2016. *PLoS One* 11:e0146211.
Brehm KE, Ferris NP, Lenk M, et al. 2009. *J Clin Microbiol* 47:3156–3160.
Brewer L, Brown C, Murtaugh MP, et al. 2003. *Xenotransplantation* 10:569–576.
Brocchi E, Berlinzani A, Gamba D, et al. 1995. *J Virol Methods* 52:155–167.
Brocchi E, Zhang G, Knowles NJ, et al. 1997. *Epidemiol Infect* 118:51–61.
Brown F. 2003. *Virus Res* 91:3–7.
Brown F, Cartwright B, Stewart DL. 1963. *J Gen Microbiol* 31:179–186.
Brown F, Goodridge D, Burrows R. 1976. *J Comp Pathol* 86:409–414.
Bruhn CAW, Nielsen SCA, Samaniego JA, et al. 2015. *Evol Med Publ Health* 1:289–303.
Brundage LJ, Derbyshire JB, Wilkie BN. 1980. *Can J Comp Med* 44:61–69.
Bunke J, Receveur K, Oeser AC, et al. 2018. *Arch Virol* 163:489–493.
Burke MJ. 2016. *Oncolytic Virother* 5:81–9.
Burrows R. 1968. *Vet Rec* 83:387–388.

Burrows R, Mann JA, Goodridge D, et al. 1974. *J Hyg (Lond)* 73:101–107.
Burrows R, Mann JA, Garland AJ, et al. 1981. *J Comp Pathol* 91:599–609.
Callahan JD, Brown F, Osorio FA, et al. 2002. *J Am Vet Med Assoc* 220:1636–1642.
Callens M. 1999. *J Virol Methods* 77:87–99.
Callens M, De Clercq K. 1997. *J Virol Methods* 67:35–44.
Canelli E, Luppi A, Lavazza A, et al. 2010. *Virol J* 7:64.
Canning P, Canon A, Bates JL, et al. 2016. *Transbound Emerg Dis* 63:373–8.
Cano-Gómez C, Palero F, Buitrago MD, et al. 2011a. *Infect Genet Evol* 11:2144–2150.
Cano-Gómez C, Buitrago D, Fernández-Pinero J, et al. 2011b. *J Virol Methods* 176:131–134.
Cerutis DR, Bruner RH, Thomas DC, et al. 1989. *J Med Virol* 29:63–69.
Chard LS, Bordeleau ME, Pelletier J, et al. 2006. *J Gen Virol* 87:927–936.
Chen J, Chen F, Zhou Q, et al. 2014. *J Virol Methods* 198:69–74.
Chénard G, Bloemraad M, Kramps JA, et al. 1998. *J Virol Methods* 75:105–112.
Chénard G, Miedema K, Moonen P, et al. 2003. *J Virol Methods* 107:89–98.
Childerstone AJ, Cedillo-Baron L, Foster-Cuevas M, et al. 1999. *J Gen Virol* 80:663–669.
Chinsangaram J, Piccone ME, Grubman MJ. 1999. *J Virol* 73:9891–9898.
Chiu SC, Yang CL, Chen YM, et al. 2014. *Vet Microbiol* 168:69–77.
Christianson WT, Kim HS, Yoon IJ, et al. 1992. *Am J Vet Res* 53:44–47.
Chu RM, Moore DM, Conroy JD. 1979. *Can J Comp Med* 43:29–38.
Clavijo A, Sanchez-Vazquez MJ, Buzanovsky LP, et al. 2017. *Transbound Emerg Dis* 64:31–36.
Conceição-Neto N, Theuns S, Cui T, et al. 2017. *Virus Evol* 3:vex024.
Cottral GE. 1969. *Bull Off Int Epizoot* 70:549–568.
Cottral GE, Bachrach HL. 1968. Foot-and-mouth disease viremia. *Proc Annu Meet U S Anim Health Assoc* 72:383–399.
Cottral GE, Gailiunas P, Campion RL. 1963. Detection of foot-and-mouth disease virus in lymph nodes of cattle throughout course of infection. *Proc Annu Meet US Livest San Assoc* 67:463–472.
Cottral GE, Patty RE, Gailiunas P, et al. 1966. *Arch Gesamte Virusforsch* 18:276–293.
Craighead JE, Peralta PH, Murnane TG, et al. 1963. *J Infect Dis* 112:205–212.
Cropper M, Dunne HW, Leman AD, et al. 1976. *J Am Vet Med Assoc* 168:233–235.
Cunliffe HR, Blackwell JH, Walker JS. 1979. *Appl Environ Microbiol* 37:1044–1046.
Czechowicz J, Huaman JL, Forshey BM, et al. 2011 *Vector Borne Zoonotic Dis* 2011:367–74.
Dall-Agnol AM, Otonel RAA, Leme RA, et al. 2017. *Mol Cell Probes* 33:28–31.
Dauber M. 1999. *Vet Microbiol* 67:1–12.
De Castro MP. 1964. Behaviour of the foot and mouth disease virus in cell cultures: Susceptibility of the IB-RS-2 cell line. *Arq Inst Biol (Sao Paulo)* 31:63–78.
Dea SA, Bilodeau R, Martineau GP. 1991. *Arch Virol* 117:121–128.
Dekker A. 1998. *Vet Rec* 143:168–169.
Dekker A, Moonen P, Boer-Luijtze EA, et al. 1995a. *Vet Microbiol* 45:243–250.
Dekker A, Moonen PL, Terpstra C. 1995b. *J Virol Methods* 51:343–348.
Dekker A, Hemert-Kluitenberg F, Baars C, et al. 2002. *Epidemiol Infect* 128:277–284.
Dekkers LJ, Elbers AR. 2000. *Tijdschr Diergeneeskd* 125:2–4.
Delagneau JF, Guerche J, Adamowicz P, et al. 1974. *Annales de Microbiologie* 125B:559–574.
Derbyshire JB. 1983. *Can J Comp Med* 47:235–237.
Derbyshire JB, Arkell S. 1971. *Br Vet J* 127:137–142.
Diaz-San Segundo F, Rodriguez-Calvo T, de Avila A, et al. 2009. *PLoS One* 4:e5659.
Diaz-San Segundo F, Moraes MP, de Los ST, et al. 2010. *J Virol* 84:2063–2077.
Doherty M, Todd D, McFerran N, et al. 1999. *J Gen Virol* 80(Pt 8):1929–1941.
Domenech J, Lubroth J, Sumption K. 2010. *J Comp Pathol* 142(Suppl 1):S120–S124.
Donaldson AI. 1986. *Rev Sci Tech* 5:315–321.
Donaldson AI. 1987. *Ir Vet J* 41:325–327.
Donaldson AI. 1997. *Rev Sci Tech Off Int Epiz* 16:117–124.
Donaldson AI. 1998. Experimental and natural adaptation of strains of foot-and-mouth disease virus to different species. Session of the Research Group of the Standing Technical Committee, European Commission for the Control of Foot-and-Mouth Disease, pp. 18–22.
Donaldson AI, Alexandersen S. 2003. The virological determinants of the epidemiology of foot-and-mouth disease. In Dodet B, Vicari M, eds. *Foot-and-Mouth Disease: Control Strategies*. Paris, France: Elsevier, pp. 173–180.
Donaldson A, Knowles N. 2001. *Vet Rec* 148:319.
Donaldson AI, Sellers RF. 2000. Foot-and-mouth disease. In WB Martin, ID Aitken, eds. *Diseases of Sheep*, 3rd ed. Oxford, UK: Blackwell Science, pp. 254–258.
Donaldson AI, Herniman KA, Parker J, et al. 1970. *J Hyg (Lond)* 68:557–564.
Donaldson AI, Gloster J, Harvey LD, et al. 1982. *Vet Rec* 110:53–57.
Donaldson AI, Ferris NP, Wells GA. 1984. *Vet Rec* 115:509–512.
Donaldson AI, Gibson CF, Oliver R, et al. 1987. *Res Vet Sci* 43:339–346.

Donaldson AI, Alexandersen S, Sorensen JH, et al. 2001. *Vet Rec* 148:602–604.
Dukes JP, King DP, Alexandersen S. 2006. *Arch Virol* 151:1093–106.
Dunn CS, Donaldson AI. 1997. *Vet Rec* 141:174–175.
Dunne HW, Gobble JL, Hokanson JF, et al. 1965. *Am J Vet Res* 26:1284–1297.
Dunne HW, Wang JT, Ammerman EH. 1971. *Infect Immun* 4:619–631.
Duque H, Baxt B. 2003. *J Virol* 77:2500–2511.
Eble PL, Bouma A, Weerdmeester K, et al. 2007. *Vaccine* 25:1043–1054.
Ehrenfeld E, Domingo E, Ross RP, eds. 2010. *The Picornaviruses*. Washington, DC: ASM Press.
Fellowes ON. 1960. *Ann N Y Acad Sci* 83:595–608.
Feng R, Wei J, Zhang H, et al. 2015. *Arch Virol* 160:2957–2964.
Ferguson NM, Donnelly CA, Anderson RM. 2001. *Science* 292:1155–1160.
Fernandez J, Aguero M, Romero L, et al. 2008. *J Virol Methods* 147:301–311.
Ferris NP, Dawson M. 1988. *Vet Microbiol* 16:201–209.
Ferris NP, Nordengrahn A, Hutchings GH, et al. 2009. *J Virol Methods* 155:10–17.
Fowler VL, Bankowski BM, Armson B, et al. 2014. *PLoS One* 14 (9):e109322.
Fowler VL, Ransburgh RH, Poulsen EG, et al. 2017. *J Virol Methods* 239:34–37.
Francis MJ, Black L. 1984. *Res Vet Sci* 37:72–76.
Francis MJ, Black L. 1986. *Res Vet Sci* 41:33–39.
Fry EE, Knowles NJ, Newman JW, et al. 2003. *J Virol* 77:5475–5486.
Gailiunas P. 1968. *Arch Gesamte Virusforsch* 25:188–200.
Gainer JH, Sandefur JR, Bigler WJ. 1968. *Cornell Vet* 58:31–47.
Garland AJM, Donaldson AI. 1990. Foot-and-mouth disease. *Surveillance* 17:6–8.
Gelmetti D, Meroni A, Brocchi E, et al. 2006. *Vet Res* 37:15–23.
Gerner W, Hammer SE, Wiesmuller KH, et al. 2009. *J Virol* 83:4039–4050.
Geudeke MJ. 1992. De bruikbaarheid van slachthuisinformatie van zeugen voor de veterinair – zootechnische bedrijfsbegeleiding = The use of slaughterhouse information in monitoring systems for herd health control in sows. Veterinary Faculty. University of Utrecht, Utrecht, The Netherlands.
Gimenez-Lirola LG, Rademacher C, Linhares D, et al. 2016. *J Clin Microbiol* 54:2082–2089.
Gloster J, Alexandersen S. 2004. *Atmos Environ* 38:503–505.
Gloster J, Blackall J, Sellers RF, et al. 1981. *Vet Rec* 108:370–374.
Gloster J, Sellers RF, Donaldson AI. 1982. *Vet Rec* 110:47–52.
Gloster J, Champion HJ, Sorensen JH, et al. 2003. *Vet Rec* 152:525–533.
Gloster J, Freshwater A, Sellers RF, et al. 2005. *Epidemiol Infect* 133:767–783.
Golde WT, Nfon CK, Toka FN. 2008. *Immunol Rev* 225:85–95.
Golding SM, Hedger RS, Talbot P, et al. 1976. *Res Vet Sci* 20:142–147.
Goolia M, Vannucci F, Yang M, et al. 2017. *J Vet Diagn Invest* 29:250–253.
Goris N, Maradei E, D'Aloia R, et al. 2008. *Vaccine* 26:3432–3437.
Gourreau JM, Dhennin L, Labie J. 1975. Preparation of an inactivated virus vaccine against swine vesicular disease. *Rec Med Vet Ec Alfort* 151:85–89.
Graves JH. 1995. *Nature* 5424:314–315.
Grobler DG, Raath JP, Braack LE, et al. 1995. *Onderstepoort J Vet Res* 62:97–108.
Grubman MJ, Moraes MP, Diaz-San Segundo F, et al. 2008. *FEMS Immunol Med Microbiol* 53:8–17.
Guo B, Piñeyro PE, Rademacher CJ, et al. 2016. *Emerg Infect Dis* 22:1325–1327.
Guo B, Kim H, Zheng Y, et al. 2018. *Genome Announc* 6:e01569-17.
Guzman E, Taylor G, Charleston B, et al. 2008. *J Gen Virol* 89:667–675.
Hales LM, Knowles NJ, Reddy PS, et al. 2008. *J Gen Virol* 89:1265–1275.
Hamblin C, Crowther JR. 1982. *Br Vet J* 138:247–252.
Hamblin C, Armstrong RM, Hedger RS. 1984. *Vet Microbiol* 9:435–443.
Hamblin C, Barnett IT, Hedger RS. 1986. *J Immunol Methods* 93:115–121.
Hammoumi S, Guy M, Eloit M, et al. 2012. *Arch Virol* 157:43–52.
Hause BM, Myers O, Duff J, et al. 2016. *Emerg Infect Dis* 22:1323–1325.
Have P, Holm-Jensen M. 1983. Detection of antibodies to foot-and-mouth disease virus type 01 by enzyme linked immunosorbent assay (ELISA). Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Lelystad, The Netherlands, 20–22 September, 1983, Appendix VIII: 44–51, pp. 45–51.
Have P, Lei JC, Schjerning-Thiesen K. 1984. *Acta Vet Scand* 25:280–296.
Hazlett DT, Derbyshire JB. 1977. *Can J Comp Med* 41:264–273.
Hazlett DT, Derbyshire JB. 1978. *J Comp Pathol* 88:467–471.
Hearps A, Zhang Z, Alexandersen S. 2002. *Vet Rec* 150:625–628.
Hedger RS. 1972. *J Comp Pathol* 82:19–28.
Hedger RS, Brooksby JB. 1976. *Vet Rec* 99:93.

Hedger RS, Mann JA. 1989. Swine vesicular disease virus. In Pesaert MB, ed. *Virus Infections of Porcines*, 2nd ed. Amsterdam, The Netherlands: Elsevier Science Publishers BV, pp. 241–250.
Hedger RS, Stubbins AGJ. 1971. The carrier state in FMD, and the probang test. *State Vet J* 26:45–50.
Helwig FC, Schmidt CH. 1945. *Science* 102:31–33.
Henderson WM. 1948. Further consideration of some of the factors concerned in intracutaneous injection of cattle. *J Pathol Bacteriol* 60:137–139.
Henderson WM. 1949. *The Quantitative Study of Foot-and-Mouth Disease Virus*. London: Her Majesty's Stationery Office, p. 8.
Hofner MC, Fosbery MW, Eckersall PD, et al. 1994. *Res Vet Sci* 57:125–128.
Hole K, Ahmadpour F, Krishnan J, et al. 2017. *J Appl Microbiol* 122:634–639.
Holman JE, Koestner A, Kasza L. 1966. *Pathol Vet* 3:633–651.
Honda E, Hattori I, Oohara Y, et al. 1990a. *Nippon Juigaku Zasshi* 52:85–90.
Honda E, Kimata A, Hattori I, et al. 1990b. *Nippon Juigaku Zasshi* 52:49–54.
Horner GW, Hunter R. 1979. *N Z Vet J* 27:202–203.
House C, Meyer RF. 1993. *J Virol Methods* 43:1–6.
Howell PG, Young E, Hedger RS. 1973. *Onderstepoort J Vet Res* 40:41–52.
Huang J, Gentry RF, Zarkower A. 1980. *Am J Vet Res* 41:469–473.
Hubschle OJ, Rajanarison I, Koko M, et al. 1983. *Dtsch Tierarztl Wochenschr* 90:86–88.
Hughes GJ, Mioulet V, Kitching RP, et al. 2002. *Vet Rec* 150:724–727.
Hunter P, Swanepoel SP, Esterhuysen JJ, et al. 1998. *Vaccine* 16:55–61.
Hyslop NS. 1965. *J Comp Pathol* 75:111–117.
Hyslop NS. 1970. *Adv Vet Sci Comp Med* 14:261–307.
Inoue T, Suzuki T, Sekiguchi K. 1989. *J Gen Virol* 70:919–934.
Jackson T, Sheppard D, Denyer M, et al. 2000. *J Virol* 74:4949–4956.
Jamal SM, Belsham GJ. 2015. *PLoS One* 10:e0135559.
Jamal SM, Bouma A, van den Broek J, et al. 2008. *Vaccine* 26:6317–6321.
Janke BH, Morehouse LG, Solorzano RF. 1988. *Can J Vet Res* 52:364–369.
Jeoung HY, Shin BH, Jeong W, et al. 2012 *Res Vet Sci* 93:1508–1511.
Jeoung HY, Lim SI, Kim JJ, et al. 2015 *BMC Vet Res* 26 (11):78.
Jimenez-Clavero MA, Ley V, Fita I, et al. 2003. *Acta Crystallogr D Biol Crystallogr* 59:541–543.
Jimenez-Clavero MA, Escribano-Romero E, Ley V, et al. 2005. *J Gen Virol* 86:1369–1377.
Joo HS. 1999. Encephalomyocarditis virus. In Straw BE, D'Allaire S, Mengeling WL, et al., eds. *Diseases of Swine*, 8th ed. Ames, IA: Iowa State University Press, pp. 139–144.
Joshi LR, Mohr KA, Clement T, et al. 2016a. *J Clin Microbiol* 54:1536–1545.
Joshi LR, Fernandes MH, Clement T, et al. 2016b. *J Gen Virol* 97:3267–3279.
Juleff N, Windsor M, Lefevre EA, et al. 2009. *J Virol* 83:3626–3636.
Jungeblut CW, Sanders M. 1940. *J Exp Med* 72:407–436.
Kaku Y, Yamada S, Murakami Y. 1999. *Arch Virol* 144:1845–1852.
Kaku Y, Sarai A, Murakami Y. 2001. *J Gen Virol* 82:417–424.
Kanamitsu M, Kasamaki A, Ogawa M, et al. 1967. *Jpn J Med Sci Biol* 20:175–194.
Kanno T, Inoue T, Wang YF, et al. 1996. *J Gen Virol* 76:3099–3106.
Karpinski S, Tereszczuk S. 1977. Studies on the survival of swine vesicular disease (SVD) virus under various environmental conditions. *Med Weter* 33:26–29.
Kassimi LB, Gonzague M, Boutrouille A, et al. 2002. *J Virol Methods* 101:197–206.
Khamrin P, Maneekarn N, Kongkaew A, et al. 2009. *Emerg Infect Dis* 15:2075–2076.
Kim HS, Christianson WT, Joo HS. 1989a. *Arch Virol* 109:51–57.
Kim HS, Joo HS, Bergeland ME. 1989b. *J Vet Diagn Invest* 1:101–104.
Kim HS, Joo HS, Christianson WT, et al. 1991. *J Vet Diagn Invest* 3:283–286.
Kim DS, Kang MI, Son KY, et al. 2016. *J Gen Virol* 97:2566–2574.
Kindyakov VI. 1938. *Sovetsk Vet* 8–9:43. Cited in: Parker, J. 1971. Presence and inactivation of foot-and-mouth disease virus in animal feces. Vet Rec 88:659–662.
King DP, Dukes JP, Reid SM, et al. 2008. *Vet Rec* 162:315–316.
Kirkbride CA, McAdaragh JP. 1978. *J Am Vet Med Assoc* 172:480–483.
Kitching RP. 1998. *J Comp Pathol* 118:89–108.
Kitching RP, Alexandersen S. 2002. *Rev Sci Tech Off Int Epiz* 21:513–518.
Kitching RP, Salt JS. 1995. *Br Vet J* 151:379–389.
Kitching RP, Knowles NJ, Samuel AR, et al. 1989. *Trop Anim Health Prod* 21:153–166.
Knowles NJ. 1983. *Br Vet J* 139:19–22.
Knowles NJ. 1988. *Vet Rec* 122:441–442.
Knowles NJ, Buckley LS. 1980. *Res Vet Sci* 29:113–115.
Knowles NJ, McCauley JW. 1997. *Curr Top Microbiol Immunol* 223:153–167.
Knowles NJ, Buckley LS, Pereira HG. 1979. *Arch Virol* 62:201–208.
Knowles NJ, Dickinson ND, Wilsden G, et al. 1998. *Virus Res* 57:53–62.
Knowles NJ, Samuel AR, Davies PR, et al. 2001. *Vet Rec* 148:258–259.
Knowles NJ, Hales LM, Jones BH, et al. 2006. Northern Lights EUROPIC 2006: XIVth Meeting of the European

Study Group on the Molecular Biology of Picornaviruses, Saariselkä, Inari, Finland, 26th November-1st December 2006. Abstract G2.
Knowles NJ, Hovi T, Hyypiä T, et al. 2012. Family *Picornaviridae*. In King AMQ, Lefkowitz EJ, Adams MJ, et al., eds. *Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press, pp. 855–881.
Knowles NJ, Wadsworth J, Bachanek-Bankowska K. 2016. EUROPIC 2016: XIXth Meeting of the European Study Group on the Molecular Biology of Picornaviruses, Diablerets, Switzerland, 4-8th September 2016.
Knutson TP, Velayudhan BT, Marthaler DG. 2017. *J Gen Virol* 98:1305–1310.
Kodama M, Ogawa T, Saito T, et al. 1980. *Natl Inst Anim Health Q (Tokyo)* 20:1–10.
Koenen F, Vanderhallen H. 1997. *Zentralbl Veterinarmed B* 44:281–286.
Koenen F, Vanderhallen H, Castryck F, et al. 1999. *Zentralbl Veterinarmed B* 46:217–231.
Koestner A, Kasza L, Holman JE. 1966. *Am J Pathol* 49:325–337.
Kothmann VG, Kaaden OR, Eissner G. 1973. *Dtsch Tierartzl Wochenschr* 80:269–271.
Kouba V. 2009. Teschen disease (*Teschovirus encephalomyelitis*) eradication in Czechoslovakia: a historical report. *Vet Med (Praha)* 54:550–560.
Krumbholz A, Dauber M, Henke A, et al. 2002. *J Virol* 76:5813–5821.
Krumbholz A, Wurm R, Scheck O, et al. 2003. *J Virol Methods* 113:51–63.
Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874.
Laguardia-Nascimento M, Gasparini MR, Sales ÉB, et al. 2016. *Vet J* 216:207–9.
Lai SS, McKercher PD, Moore DM, et al. 1979. *Am J Vet Res* 40:463–468.
LaRocco M, Krug PK, Kramer E, et al. 2013. *J Clin Microbiol* 51:1714–1720.
LaRocco M, Krug PK, Kramer E, et al. 2015. *J Clin Microbiol* 53:755.
Larska M, Wernery U, Kinne J, et al. 2008. *Epidemiol Infect* 137:549–554.
Le Gall O, Christian P, Fauquet CM, et al. 2008. *Arch Virol* 153:715–727.
Leforban Y, Gerbier G. 2002. *Rev Sci Tech* 21:477–492.
Leme RA, Zotti E, Alcântara BK, et al. 2015. *Transbound Emerg Dis* 62:603–611.
Leme RA, Oliveira TE, Alcântara BK, et al. 2016a. *Emerg Infect Dis* 22:1238–1241.
Leme RA, Oliveira TE, Alfieri AF, et al. 2016b. *J Comp Pathol* 155:145–155.
Li Y, Swabey KG, Gibson D, et al. 2012. *J Virol Methods* 183:125–131.
Lin F. 1998. *Epidemiol Infect* 121:459–472.
Lin F, Mackay DK, Knowles NJ. 1997. *J Virol Methods* 65:111–121.
Lin F, Mackay DK, Knowles NJ, et al. 2001. *Epidemiol Infect* 127:135–145.
Links IJ, Whittington RJ, Kennedy DJ, et al. 1986. *Aust Vet J* 63:150–152.
Littlejohns IR, Acland HM. 1975. *Aust Vet J* 51:416–422.
Lomakina NF, Yu Shustova E, Strizhakova OM, et al. 2016. *J Gen Virol* 97:49–52.
Long JF. 1985. Pathogenesis of porcine polioencephalomyelitis. In Olsen RA, Krakowka S, Blakeslee JR, eds. *Comparative Pathology of Viral Diseases*. Vol. 1. Boca Raton, FL: CRC Press, pp. 179–197.
Long JF, Kasza L, Koestner A. 1969. *J Infect Dis* 120:245–249.
Love RJ, Grewal AS. 1986. *Aust Vet J* 63:128–129.
Lubroth J, Brown F. 1995. *Res Vet Sci* 59:70–78.
Lynch JA, Binnington BD, Hoover DM. 1984. *Can J Comp Med* 48:233–235.
Mackay DK. 1998. *Vet Q* 20(Suppl 2):2–5.
Mackay DK, Bulut AN, Rendle T, et al. 2001. *J Virol Methods* 97:33–48.
Mann JA, Hutchings GH. 1980. *J Hyg (Lond)* 84:355–363.
Maradei E, La Torre J, Robiolo B, et al. 2008. *Vaccine* 26:6577–6586.
Maragon S, Facchin E, Moutou F, et al. 1994. *Vet Rec* 135:53–57.
Matias Ferreyra F, Arruda B, Stevenson G, et al. 2017. *Viruses* 9:179.
Mattion N, Goris N, Willems T, et al. 2009. *Vaccine* 27:741–747.
Maurice H, Nielen M, Stegeman JA, et al. 2002. *Vet Microbiol* 88:301–314.
Maurice H, Nielen M, Brocchi E, et al. 2005. *Epidemiol Infect* 133:547–557.
Maurice H, Nielen M, Vyt P, et al. 2007. *Prev Vet Med* 78:24–34.
Maurice H, Thulke HH, Schmid JS, et al. 2016. *Prev Vet Med* 127:105–112.
Mayr A. 1959. *Zentralbl Bakteriol* 176:341–345.
McColl KA, Westbury HA, Kitching RP, et al. 1995. *Aust Vet J* 72:286–292.
McCullough KC, De Simone F, Brocchi E, et al. 1992. *J Virol* 66:1835–1840.
McKercher PD, Graves JH. 1976. A mixed vaccine for swine: an aid for control of foot and mouth and swine vesicular diseases. *Boletín del Centro Panamericano de Fiebre Aftosa* 23/24:37–49.
McLauchlan JD, Henderson WM. 1947. *J Hyg (Lond)* 45:474–479.
McMenamy MJ, McKillen J, Reid S, et al. 2011. *J Vir Meth* 171:219–224.

McVicar JW, Sutmoller P. 1976. *J Hyg (Lond)* 76:467–481.
McVicar JW, Richmond JY, Campbell CH, et al. 1973. *Can J Comp Med* 37:362–368.
McVicar JW, Eisner RJ, Johnson LA, et al. 1977. Foot-and-mouth disease and swine vesicular disease viruses in boar semen. *Proc Annu Meet US Anim Health Assoc* 81:221–230.
Mebus C, Arias M, Pineda JM, et al. 1997. Survival of several porcine viruses in different Spanish dry cured meat products. *Food Chem* 59:555–559.
Meyer RC, Woods GT, Simon J. 1966. *J Comp Pathol* 76:397–405.
Mitev G, Tekerlekov P, Dilovsky M, et al. 1978. *Arch Exp Veterinarmed* 32:29–33.
Moffat K, Knox C, Howell G, et al. 2007. *J. Virol* 81:1129–1139.
Mohan MS, Gajendragad MR, Gopalakrishna, S, et al. 2008. *Vet Res Commun* 32:481–489.
Monaghan P, Gold S, Simpson J, et al. 2005. *J Gen Virol* 86:2769–2780.
Moniwa M, Clavijo A, Li M, et al. 2007. *J Vet Diagn Invest* 19:9–20.
Montagnaro S, Sasso S, De Martino L, et al. 2010. *J Wildl Dis* 46:316–319.
Montiel N, Buckley A, Guo B, et al. 2016. *Emerg Infect Dis* 22:1246–1248.
Moon HJ, Song D, Seon BH, et al. 2012. *J Virol* 86:10250.
Moonen P, Boonstra J, van der Honing RH, et al. 2003. *J Virol Methods* 113:35–41.
Morgan DO, McKercher PD. 1977. Immune response of neonatal swine to inactivated foot-and-mouth disease virus vaccine with oil adjuvant. I. Influence of colostral antibody. *Proc Annu Meet U S Anim Health Assoc* 81:244–255.
Moss A, Haas B. 1999. *J Virol Methods* 80:59–67.
Mowat GN, Chapman WG. 1962. *Nature* 194:253–255.
Mowat GN, Darbyshire JH, Huntley JF. 1972. *Vet Rec* 90:618–621.
Mowat GN, Prince MJ, Spier RE, et al. 1974. *Arch Gesamte Virusforsch* 44:350–360.
Mulder WA, Van Poelwijk F, Moormann RJ, et al. 1997. *J Virol Methods* 68:169–175.
Murnane TG, Craighead JE, Mondragon H, et al. 1960. *Science* 131:498–499.
Murphy C, Bashiruddin JB, Quan M, et al. 2010. *Vet Rec* 166:10–14.
Naoi Y, Kishimoto M, Masuda T, et al. 2016. *Arch Virol* 161:1685–1690.
Nardelli L, Lodetti E, Gualandi GL, et al. 1968. *Nature* 219:1275–1276.
Nassuato C, Boender GJ, Eble PL, et al. 2013. *PLoS One* 8:e62878.
Neitzert E, Beck E, de Mello PA, et al. 1991. *Virology* 184:799–804.
Nelsen-Salz B, Zimmermann A, Wickert S, et al. 1996. *Virus Res* 41:109–122.
Nfon CK, Toka FN, Kenney M, et al. 2010. *Viral Immunol* 23:29–41.
Niedbalski W. 2009. *Pol J Vet Sci* 12:119–121.
Nix WA, Khetsuriani N, Peñaranda S, et al. 2013. *J Gen Virol* 94:2017–2028.
Oberste MS, Gotuzzo E, Blair P, et al. 2009. *Emerg Infect Dis* 15:640–646.
OIE. 2008. 2.8.10. Teschovirus encephalomyelitis (previously enterovirus encephalomyelitis or Teschen/Talfan). In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Vol. 2, 6th ed. OIE, World Organisation for Animal Health, Paris, France, pp. 1146–1152.
Olascoaga RC. 1984. *Prev Vet Med* 2:341–352.
Oleksiewicz MB, Donaldson AI, Alexandersen S. 2001. *J Virol Methods* 92:23–35.
Oliver RE, Donaldson AI, Gibson CF, et al. 1988. *Res Vet Sci* 44:315–319.
Orsel K, Bouma A. 2009. *Can Vet J* 50:1059–1063.
Orsel K, de Jong MC, Bouma A, et al. 2007. *Vaccine* 25:6381–6391.
Pacheco JM, Butler JE, Jew J, et al. 2010. *Clin Vaccine Immunol* 17:550–558.
Paiba GA, Anderson J, Paton DJ, et al. 2004. *J Virol Methods* 115:145–158.
Palmquist JM, Munir S, Taku A, et al. 2002. *J Vet Diagn Invest* 14:476–480.
Papaioannou N, Billinis C, Psychas V, et al. 2003. *J Comp Pathol* 129:161–168.
Parker J. 1971. *Vet Rec* 88:659–662.
Parker BN, Wrathall AE, Cartwright SF. 1981. *Br Vet J* 137:262–267.
Pasma T, Davidson S, Shaw SL. 2008. *Can Vet J* 49:84–85.
Paton DJ, Valarcher JF, Bergmann I, et al. 2005. *Rev Sci Tech* 24:981–993.
Peng S, Wang Y, Yang Z, et al. 2015. *Trop J Pharma Res* 14:853–858.
Pensaert M, de Meurichy W. 1973. *Zentralbl Veterinarmed B* 20:760–772.
Pensaert M, de Meurichy W, van Leeuwe G. 1973. *Zentralbl Veterinarmed B* 20:749–759.
Philipps A, Dauber M, Groth M, et al. 2012. *Vet Microbiol* 161:49–57.
Phillips RM, Foley CW, Lukert PD. 1972. *J Am Vet Med Assoc* 161:1306–1316.
Piragino S. 1970. FMD in a circus elephant. *Zooprofilassi* 25:17–22.
Platt H. 1956. A study of the pathological changes produced in young mice by the virus of foot-and-mouth disease. *J Pathol Bacteriol* 122:299–312.
Platt H. 1959. *Virology* 9:484–486.
Platt H. 1961. *Nature* 190:1075–1076.

Pogranichniy RM, Janke BH, Gillespie TG, et al. 2003. *J Vet Diagn Invest* 15:191–194.
Psychas V, Papaioannou N, Billinis C, et al. 2001. *Am J Vet Res* 62:1653–1657.
Qian S, Fan W, Qian P, et al. 2016. *Virol J* 13:173.
Quan M, Murphy C, Zhang Z, et al. 2004. *J Comp Pathol* 131:294–307.
Quan M, Murphy CM, Zhang Z, et al. 2009. *J Comp Pathol* 140:225–237.
Rasmussen TB, Uttenthal A, de Stricker K, et al. 2003. *Arch Virol* 148:2005–2021.
Reddacliff LA, Kirkland PD, Hartley WJ, et al. 1997. *J Zoo Wildl Med* 28:153–157.
Reddy PS, Burroughs KD, Hales LM, et al. 2007. *J Natl Cancer Inst* 99:1623–1633.
Reid SM, Forsyth MA, Hutchings GH, et al. 1998. *J Virol Methods* 70:213–217.
Reid SM, Hutchings GH, Ferris NP, et al. 1999. *J Virol Methods* 83:113–123.
Reid SM, Ferris NP, Bruning A, et al. 2001. *J Virol Methods* 96:189–202.
Reid S, Ferris N, Hutchings G, et al. 2002. *J Virol Methods* 105:67–80.
Reid SM, Grierson SS, Ferris NP, et al. 2003. *J Virol Methods* 107:129–139.
Reid SM, Paton DJ, Wilsden G, et al. 2004. *J Comp Pathol* 131:308–317.
Reid SM, Mioulet V, Knowles NJ, et al. 2014. *J Virol Methods* 207:146–153.
Reuter G, Boldizsar A, Pankovics P. 2009. *Arch Virol* 154:101–108.
Rhyan J, Deng M, Wang H, et al. 2008. *J Wildl Dis* 44:269–279.
Rivera-Benitez JF, Rosas-Estrada K, Pulido-Camarillo E, et al. 2014 *Zoonoses Public Health* 61:131–137.
Robinson P, Garza A, Moore J, et al. 2009. *Clin Exp Med* 2:76–86.
Roeder PL, Le Blanc Smith PM. 1987. *Res Vet Sci* 43:225–232.
Roic B, Jemersic L, Terzic S, et al. 2012. *J Wildl Dis* 48:131–137.
Ryan E, Zhang Z, Brooks HW, et al. 2007. *J Comp Pathol* 136:256–265.
Ryan E, Gloster J, Reid SM, et al. 2008a. *Vet Rec* 163:139–147.
Ryan E, Horsington J, Durand S, et al. 2008b. *Vet Microbiol* 127:258–274.
Saeng-chuto, RP, Temeeyasen G, et al. 2018. *Transbound Emerg Dis* 65:285–288.
Salogni C, Lazzaro M, Giacomini E, et al. 2016. *J Vet Diagn Invest* 28:550–554.
Sangar DV, Rowlands DJ, Brown F. 1977. *Vet Rec* 100:240–241.
Sangula AK, Siegismund HR, Belsham GJ, et al. 2011. *Epidemiol Infect* 139:189–196.
Sanson RL. 1994. *N Z Vet J* 42:41–53.
Sanz-Parra A, Sobrino F, Ley V. 1998. *J Gen Virol* 79:433–436.
Sauvage V, Ar Gouilh M, Cheval J, et al. 2012. *J Virol* 86:10036–10046.
Schaupp W. 1968. *Wien Tierarztl Monatsschr* 55:346–356.
Schock A, Gurrala R, Fuller H, et al. 2014. *Vet Microbiol* 172:381–389.
Scott FW, Cottral GE, Gailiunas P. 1966. *Am J Vet Res* 27:1531–1536.
Seaman JT, Boulton JG, Carrigan MJ. 1986. *Aust Vet J* 63:292–294.
Segalés J, Barcellos D, Alfieri A, et al. 2017. *Vet Pathol* 54:11–21.
Seibold HR, Cottral GE, Patty RE, et al. 1964. *Am J Vet Res* 25:806–814.
Sellers RF. 1963. *Nature* 198:1228–1229.
Sellers RF. 1968. *Vet Rec* 83:504–506.
Sellers RF. 1971. *Vet Bull* 41:431–439.
Sellers RF, Forman AJ. 1973. *J Hyg (Lond)* 71:15–34.
Sellers RF, Parker J. 1969. *J Hyg (Lond)* 67:671–677.
Sellers RF, Burt LM, Cumming A, et al. 1959. *Arch Gesamte Virusforsch* 9:637–646.
Sellers RF, Donaldson AI, Herniman KA. 1970. *J Hyg (Lond)* 68:565–573.
Sellers RF, Herniman KA, Mann JA. 1971. *Vet Rec* 89:447–449.
Senthilkumaran C, Bittner H, Ambagala A, et al. 2017. *Transbound Emerg Dis* 64:1762–1770.
Shang P, Misra S, Hause B, Fang Y. 2017. *J Virol* 91:e00450-17.
Shen F, Chen PD, Walfield AM, et al. 1999. *Vaccine* 17:3039–3049.
Simpson CF, Lewis AL, Gaskin JM. 1977. *J Am Vet Med Assoc* 171:902–905.
Singh KV, Bohl EH. 1972. *Can J Comp Med* 36:243–248.
Singh K, Corner S, Clark SG, et al. 2012. *J Vet Sci Technol* 3:6.
Skinner HH. 1951. *Proc R Soc Med* 44:1041–1044.
Skinner HH. 1953. One-week-old white mice as test animals in foot-and-mouth disease research. In Proceedings from XVth International Veterinary Congress IB45, pp. 3–8.
Skinner HH. 1954. *Nature* 174:1052–1054.
Smith IM, Betts AO, Watt RG, et al. 1973. *J Comp Pathol* 83:1–12.
Snowdon WA. 1966. *Nature* 210:1079–1080.
Snowdon WA. 1968. *Aust J Exp Biol Med Sci* 46:667–687.
Sorensen KJ, Madekurozwa RL, Dawe P. 1992. *Vet Microbiol* 32:253–265.
Sorensen KJ, Madsen KG, Madsen ES, et al. 1998. *Arch Virol* 143:1461–1476.
Spyrou V, Maurice H, Billinis C, et al. 2004. *Vet Res* 35:113–122.

Stenfeldt C, Heegaard PM, Stockmarr A, et al. 2011. *Vet Res* 42:66.
Stenfeldt, C, Pacheco JM, Smoliga, GR, et al. 2016. *Transbound Emerg Dis* 63:152–64.
Summerfield A, Guzylack-Piriou L, Harwood L, et al. 2009. *Vet Immunol Immunopathol* 128:205–210.
Sun D, Vannucci F, Knutson TP, et al. 2017. *Transbound Emerg Dis* 64:1346–1349.
Sutmoller P, Gaggero A. 1965. *Vet Rec* 77:968–969.
Sutmoller P, Barteling SS, Olascoaga RC, et al. 2003. *Virus Res* 91:101–144.
Sweeney TR, Dhote V, Yu Y, et al. 2012. *J Virol* 86:1468–86.
Terpstra C. 1992. *Tijdschr Diergeneeskd* 117:623–626.
Tesh RB, Wallace GD. 1978. *Am J Trop Med Hyg* 27:133–143.
Thomson GR. 1994. Foot-and-mouth disease. In Coetzer JAW, Thomson GR, Tustin RC, et al., eds. *Infectious Diseases of Livestock with Special Reference to Southern Africa*. Cape Town, South Africa: Oxford University Press, pp. 825–852.
Thomson GR, Vosloo W, Bastos AD. 2003. *Virus Res* 91:145–161.
Toka FN, Nfon C, Dawson H, et al. 2009. *Clin Vaccine Immunol* 16:1738–1749.
Tsuchiaka S, Naoi Y, Imai R, et al. 2018. *PLoS One* 13:e0190819.
Turbitt D. 2001. No human cases so far in foot and mouth epidemic in the United Kingdom. *Euro Surveill* 5:1.
Turner C, Williams SM. 1999. *J Appl Micro* 87:148–157.
Van Bekkum JG, Frenkel HS, Frederiks HHJ, et al. 1959. Observations on the carrier state of cattle exposed to foot-and-mouth disease virus. *Bull Off Int Epizoot* 51:917–922.
Van Dung N, Ang PH, Van Cuong N, et al. 2014. *J Gen Virol* 95:549–556.
Van Dung N, Anh PH, Van Cuong N, et al. 2016. *J Gen Virol* 97:378–388.
Vanderhallen H, Koenen F. 1997. *J Virol Methods* 66:83–89.
Vangrysperre W, De Clercq K. 1996. *Arch Virol* 141:331–344.
Vannucci FA, Linhares DCL, Barcellos D, et al. 2015. *Transbound Emerg Dis* 62:589–593.
Vansteenkiste K, Van Limbergen T, Decaluwé R, et al. 2016. Clinical problems due to encephalomyocarditis virus infections in two pig herds. *Porcine Health Manage* 2:19.
Venkataraman S, Reddy SP, Loo J, et al. 2008. *Structure* 16:1555–1561.
Wang C, Jiang P, Sand C, et al. 2012. *J Virol* 86:9964–9975.
Wang C, Yu D, Cui L, et al. 2014. *J Virol Methods* 203:5–8.
Wang N, Huang C, Wu D, et al. 2015. *J Food Saf Qual* 6:466–471.
Wang L, Prarat M, Hayes J, et al. 2016. *Emerg Inf Dis* 22:1321–1323.
Wang M, He J, Lu H, et al. 2017. *PLoS One* 12:e0174600.
Watanabe H, Pospisil Z, Mensik J. 1971. *Jpn J Vet Res* 19:107–112.
Waters RA, Fowler VL, Armson B, et al. 2014. *PLoS One* 9:e105630.
Wilder FW, Dardiri AH, Gay JG, et al. 1974. Susceptibility of one-toed pigs to certain diseases exotic to the United States. *Proc Annu Meet U S Anim Health Assoc* 78:195–199.
Wright WC. 1930. *Hieronymi Fracastorii—De contagione et contagiosis morbis et eorum curatione, libre III*, 1st ed. New York and London: G. P. Putnam's Sons.
Wright CF, Gloster J, Mazelet L, et al. 2010. *Vet Rec* 167:928–931.
Wu Q, Zhao X, Chen Y, et al. 2016. *Genome Announc* 4:e01509-15.
Wu Q, Zhao X, Bai Y, et al. 2017. *Transbound Emerg Dis* 64:1633–1640.
Yamada M, Kozakura R, Ikegami R, et al. 2004. *Vet Rec* 155:304–306.
Yang M, van Bruggen R, Xu W. 2012. *J Vet Diagn Invest* 24:42–50.
Yang F, Zhu Z, Cao W, et al. 2018. *Vaccine* 36:841–846
Yilma T. 1980. *Am J Vet Res* 41:1537–1542.
Yu JM, Jin M, Zhang Q, et al. 2009. *Emerg Infect Dis* 15:823–825.
Yu JM, Li JS, Ao YY, et al. 2013a. *Virol J* 10:39.
Yu JM, Li XY, Ao YY, et al. 2013b. *PLoS One* 8:e70137.
Yuan W, Zheng Y, Sun M, et al. 2014. *J Virol Methods* 207:60–65.
Zell R, Krumbholz A, Henke A, et al. 2000. *J Virol Methods* 88:205–218.
Zell R, Dauber M, Krumbholz A, et al. 2001. *J Virol* 75:1620–1631.
Zhang Z, Bashiruddin JB. 2009. *Vet J* 180:130–132.
Zhang G, Haydon DT, Knowles NJ, et al. 1999. *J Gen Virol* 80:639–651.
Zhang C, Wang Z, Hu F, et al. 2013. *Trop Anim Health Prod* 45:1057–1061.
Zimmerman JJ. 1994. Encephalomyocarditis. In Beran GW, Steele JH, eds. *Handbook of Zoonoses*, 2nd ed. Boca Raton, FL: CRC Press, Inc., pp. 423–436.

41

Porcine Reproductive and Respiratory Syndrome Viruses (Porcine Arteriviruses)

Jeffrey J. Zimmerman, Scott A. Dee, Derald J. Holtkamp, Michael P. Murtaugh, Tomasz Stadejek, Gregory W. Stevenson, Montserrat Torremorell, Hanchun Yang, and Jianqiang Zhang

Relevance

In the late 1980s, severe outbreaks characterized by severe reproductive losses, respiratory disease, reduction in growth rate, and increased mortality were reported in the United States (Hill 1990; Keffaber 1989). Initial efforts to identify the etiology were unsuccessful. Clinically similar outbreaks were reported in Germany in November 1990, but no common link was found between the outbreaks in Germany and the United States (Anon 1991). The etiology of the disease was established in 1991 when Koch's postulates were fulfilled with a previously unrecognized RNA virus (Terpstra et al. 1991a; Wensvoort et al. 1991). European researchers introduced the term "porcine reproductive and respiratory syndrome" (PRRS) in 1991 (Terpstra et al. 1991b). The origin of porcine reproductive and respiratory syndrome virus (PRRSV) remains unknown. In the years following the initial reports, outbreaks were widely documented, and the virus is now endemic in most swine-producing regions of the world. Control of PRRSV remains problematic, and economic studies have uniformly shown that PRRSV inflicts major losses on swine health and productivity (Holtkamp et al. 2013; Nathues et al. 2017; Neumann et al. 2005; Nieuwenhuis et al. 2012; Zhang and Kono, 2012). In Europe and North America, the cost of PRRSV to the industry has been estimated at $6.25–$15.25 USD per pig marketed (Holtkamp et al. 2013; Nathues et al. 2017). For this reason, elimination of PRRSV from herds, regions, and countries is considered the optimal solution.

Etiology

Taxonomy and classification

PRRSV type 1 (PRRSV-1) and PRRSV type 2 (PRRSV-2) are currently regarded as two species classified with 15 other species of primate, rodent, and equine viruses in the family *Arteriviridae*. The two prototype genomes (PRRSV-1 strain Lelystad and PRRSV-2 strain VR-2332) were both discovered around 1991 in Europe (PRRSV-1) and North America (PRRSV-2), but vary by approximately 44% in nucleotide sequence. Today, both species share worldwide distribution, with PRRSV-1 being predominant in Europe and PRRSV-2 predominant in the Americas and Asia.

Phylogenetic analyses, based largely on open reading frame (ORF) 5, which encodes the major envelope glycoprotein (GP5), show that both PRRSV species are remarkably diverse. Intra-type pairwise nucleotide sequence variation up to 30% is present in PRRSV-1 viruses, and it exceeds 21% in PRRSV-2 viruses. Although the origin of PRRSV is not known, the extensive differences between PRRSV-1 and PRRSV-2 suggest that their ancestor(s) evolved independently in ecologically or geographically distinct environments for an extended period of time in an as yet unidentified host. (Note: The possibility of recombination between PRRSV-1 and PRRSV-2 isolates cannot be completely excluded, but is considered highly unlikely [van Vugt et al. 2001].)

Four genetic subtypes are described in PRRSV-1. The subtypes 2, 3, and 4 present in Russia, Belarus, Ukraine, Lithuania, and Latvia have significantly larger diversity and likely earlier divergence times than globally spread subtype 1. These data suggest that PRRSV-1 was established in countries in the former Soviet Union long before the first reported epidemic outbreak in Western Europe, suggesting that this region was the primordial reservoir in which PRRSV-1 evolved (Stadejek et al. 2006). The apparent segregation of independent genetic viral subtypes between Western and Eastern Europe is consistent with biologically independent swine populations until political changes in Europe in the 1990s facilitated increased movement of animals across borders.

In the phylogeny of ORF5 of subtype 1 strains, 12 diverse clades have been defined (Shi et al. 2010b). Nine

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

diverse lineages have been defined in the phylogeny of PRRSV-2 ORF5, corresponding to groups in subtype 1 of PRRSV-1 (Shi et al. 2010a,b). Seven lineages are primarily in North America and two are exclusively found in East Asia. Phylogenetic analysis indicated that PRRSV-2 lineages, like PRRSV-1 subtypes, may have diverged prior to the discovery of PRRSV-2 in North America. Thus, transmission and evolution of PRRSV-2 was likely extensive prior to the recognition of PRRS as a new swine disease. The presence of PRRSV-2 in Asia appears to be due primarily to introductions of North American lineages, followed by local diversification, leading to new disease outbreaks and increased virulence (An et al. 2007, 2010; Hu et al. 2009; Shi et al. 2010a; Zhao et al. 2015).

The taxonomic emphasis on ORF5 may obscure important genetic variation in other regions of the PRRSV genome, since strains within either PRRSV species can recombine. Thus, a fuller picture of PRRSV genetic relationships and evolutionary origins may be facilitated by whole genome analyses and comparisons of multiple protein coding regions, including the polymerase gene, which is widely used in RNA viral evolutionary analyses.

Other methods of describing PRRSV isolates

Restriction fragment length polymorphism (RFLP) typing is based on identifying restriction endonuclease cleavage patterns of three restriction enzymes (MluI, HincII, and SacII) in PRRSV ORF5 (Wesley et al. 1998). Restriction endonucleases cleave a nucleic acid chain at a predetermined sequence of nucleotides; thus viruses with the same RFLP pattern contain the same restriction cleavage sites. However, RFLP typing has several major weaknesses: (1) RFLP types do not correlate with genetic relationships of field isolates; (2) the RFLP pattern is not a stable characteristic, even changing as a virus is passed in pigs; (3) it is not broadly adopted outside of North America; and (4) the RFLP identification terminology is too complex. In 2018, five MluI digestion patterns, 163 HincII digestion patterns, and 18 SacII digestion patterns were recognized in PRRSV-2 (Stephanie Rossow, personal communication).

Serotyping has been explored as a method of grouping PRRSV isolates, but it has been difficult to identify distinct serologic relationships. This problem is exacerbated by differences in the strength of the antibody response among individual pigs to PRRSV infection (Johnson et al. 2007). Serotyping based on virus neutralization (VN) is beset by variable and inconsistent appearance of neutralizing antibodies in immune pigs (Nelson et al. 1994; Robinson et al. 2015). Also, VN has not been shown to reproducibly identify related clusters of viruses, and cross-neutralization has not been related to efficacious cross-protection against challenge with virulent PRRSV. On the contrary, immune cross-protection appears to be broad between viruses in which there is no evidence of serological cross-neutralization (Opriessnig et al. 2005).

Other methods include glycotyping (i.e. clustering of PRRSV isolates by predicted N-linked glycosylation patterns in GP5) and typing by a specific amino acid sequence in GP5 (Kim 2008). For both methods, there is an absence of published data supporting their value.

The issue of quasispecies confounds attempts at grouping PRRSVs within a species. RNA viruses with high mutation rates are proposed to exist in an infected host as a cloud of mutationally distinct genomes. Theoretically, genetic diversity increases reproductive fitness and may be necessary for evolutionary success. Quasispecies might help explain the exceptional genetic diversity of PRRSV and facilitate emergence of mutants resistant to existing immunity. However, sequential pig-to-pig passages of PRRSV did not increase the genetic variation within individual pigs, and no evidence for immunological selection of escape mutants has been described in experimental or field settings (Chang et al. 2002; Goldberg et al. 2003). Thus, the role or significance of quasispecies in PRRSV etiology is unclear.

Physicochemical and biological properties

PRRSV is a small, enveloped, positive-sense single-stranded RNA virus. The virion includes an infectious RNA genome of approximately 15 kb in a proteinaceous nucleocapsid, surrounded by a lipid-containing envelope with five or six structural proteins. Virions are small, pleomorphic spheroids approximately 50–70 nm in diameter, with inapparent surface projections that cover the virion surface (Benfield et al. 1992; Spilman et al. 2009). The buoyant density of infectious viral particles is approximately 1.18–1.22 g/cm^3 in cesium chloride (Benfield et al. 1992). PRRSV is stable in media, serum, and tissue homogenates at −70 °C (−94 °F), but its half-life decreases with increasing temperature. Jacobs et al. (2010) observed no difference in inactivation rate among 4 PRRSV-2 isolates and estimated the virus half-life at 4 °C (39 °F), 155 hours; 10 °C (50 °F), 84.5 hours; 20 °C (68 °F), 27.4 hours; and 30 °C (86 °F), 1.6 hours. The virus is stable at neutral pH but is inactivated below pH 6 or above pH 7.5 (Benfield et al. 1992; Bloemraad et al. 1994). It is inactivated by lipid solvents and is highly unstable in solutions containing low concentrations of ionic or non-ionic detergents due to loss of infectivity through disruption of the viral envelope.

Genomic organization and gene expression

The genomic organization of PRRSV is similar to that of other arteriviruses, consisting of approximately 15,000 nucleotides organized into about 11 ORFs (1a, 1b, 2a, 2b,

3, 4, 5a, 5, 6, 7 and a short transframe ORF) that are expressed from genomic and subgenomic (sg) mRNAs (sgmRNAs). ORFs 1a and 1b comprise 80% of the genome and encode the protein cleavage, homologous recombination, and RNA replicase machinery for viral transcription, replication, and immunomodulation. ORF1a and ORF1b are translated as large polypeptides, which are then proteolytically processed into approximately 12 nonstructural proteins (nsp).

Eight ORFs (2a, 2b, 3–7, and 5a) located downstream of ORF1b at the 3′ end of the genome encode the viral structural proteins. Expression is accomplished by formation of sgmRNAs containing the viral 5′ leader sequence ligated at a conserved leader–body junction site upstream of each ORF. Protein expression occurs by translation of the first ORF downstream of the leader sequence in each sgmRNA, except in sgmRNA2 and sgmRNA5, each of which encodes two proteins in different reading frames (Wu et al. 2005; Johnson et al. 2011). Subgenomic mRNA7 expression is most abundant, followed by sgmRNA6 and sgmRNA5 and then by sgmRNA2, sgmRNA3, and sgmRNA4. The noncoding untranslated 5′ leader sequence and 3′ untranslated sequence downstream of ORF7 are conserved within PRRSV-1 and PRRSV-2 strains. These sequences are presumed to be essential for viral replication.

A third RNA species, heteroclite RNA, consists of genomic RNA ligated at noncanonical junction sites downstream of the 5′ leader and within structural protein coding ORFs in the 3′ end (Yuan et al. 2000). Heteroclite RNAs are produced under all conditions of viral growth and vary in size, but all lack nsp9 encoding the polymerase. They appear to be incorporated into virions and can be translated, but their significance is not known (Yuan et al. 2004).

Nonstructural proteins

PRRSV nsps are encoded in one of two large polyproteins translated from the full-length genomic RNA molecule. ORF1a is translated into a large polyprotein (pp) 1a of approximately 260 to 277 kDa that is proteolytically cleaved into smaller active proteins including four proteases (nsp1-alpha, nsp1-beta, nsp2, and nsp4) that carry out the cleavage events in ORF1a- and ORF1b-encoded proteins. PRRSV has a 'slippery' sequence and an unusual RNA loop structure at the ORF1a/1b overlapping region that direct a -1 programmed ribosomal frameshifting during translation of ORF1b, resulting in an pp1ab polyprotein containing an additional approximately 160–170 kDa of protein. The proteases in ORF1a cleave the additional polyprotein (the ORF1b-encoded portion of pp1ab) into approximately six proteins that contain the replicase activity, including an RNA-dependent RNA polymerase, a helicase, and an endoribonuclease. The replication complex is in cytoplasmic compartments that are continuous with endoplasmic reticulum (van der Hoeven et al. 2016).

In addition to endoproteolytic activity, nsp1-alpha and nsp1-beta may contribute to viral pathogenicity by blocking type 1 interferon synthesis directly or via inhibition of signaling pathways (Beura et al. 2010; Chen et al. 2010). Nsp2 shows extensive size polymorphisms due to variable in-frame deletions that are frequently observed in highly virulent strains. However, the nsp2 region does not appear to determine virulence (Zhou et al. 2009b).

Structural proteins

The most abundant structural protein is N, a small (15 kDa), highly basic, genetically diverse polypeptide that interacts with the viral RNA in the assembly of infectious particles (Stadejek et al. 2013). It is expressed at high levels in infected cells and represents 20–40% of the total protein content of the virion. It is active in nuclear shuttling and localization to the nucleolus and may influence nuclear processes during replication, possibly through ribosomal RNA precursor processing and ribosome biogenesis (Yoo et al. 2003). Its abundant expression and antigenicity make it a good target for immunodiagnostic assays, but a role in immune protection has not been demonstrated.

The two major envelope proteins are a non-glycosylated matrix (M) envelope protein, which lacks a signal sequence and accumulates in the endoplasmic reticulum, and GP5, with which M forms disulfide-linked heterodimers that are incorporated into the virion envelope. GP5, after cleavage of the signal peptide, contains an ectodomain of about 30 amino acids in length and is predicted to contain 2–5 N-linked glycans. The GP5/M heterodimer is essential for virion formation, but GP5/M alone is not sufficient for viral infectivity (Das et al. 2010; Wissink et al. 2005).

The 29–30 kDa GP2, 45–50 kDa GP3, and 31–35 kDa GP4 glycoproteins are present in low amounts and form a trimeric envelope protein complex. The presence of all three proteins is required for their assembly and incorporation into the virion and for viral infectivity (Wissink et al. 2005). The trimeric structure mediates infection alone or through an interaction with GP5 (Das et al. 2010; Wissink et al. 2005). These observations explain why replacement of EAV GP5 with PRRSV GP5 in EAV did not change the tropism of EAV to porcine macrophages (Dobbe et al. 2001). The minor envelope glycoprotein complex mediates infection of permissive porcine cells through an interaction with CD163 (Calvert et al. 2007; Das et al. 2010; Tian et al. 2012).

Laboratory cultivation

PRRSV-1 and PRRSV-2 grow primarily in porcine macrophages of the lung and lymphoid tissues and in dendritic cells. In the laboratory, PRRSV is cultivated on

alveolar macrophages isolated from young pigs and on simian cells, especially the African green monkey MA-104 cell line and its derivative, MARC-145. PRRSV-1 isolates grow readily on porcine macrophages but are difficult to adapt to simian cell culture. PRRSV-2 field isolates grow preferentially on macrophages but are frequently adaptable to simian cell culture with several blind passages, whereas cell culture-adapted vaccine strains are preferentially isolated on MARC-145 cells (de Abin et al. 2009). Variation in the ability of both PRRSV species to grow *in vitro* suggests that both porcine macrophages and simian cells should be used for virus isolation (VI) whenever possible.

Characterization of host cell surface molecules involved in PRRSV infection, especially CD163, has resulted in the development of transgenic PK-15 and CHO cell lines that permit infection by vaccine strains and field isolates (Delrue et al. 2010; Van Gorp et al. 2008). Established cell lines that are readily permissive to PRRSV-1 and PRRSV-2 field isolates will facilitate VI, especially for PRRSV-1 isolates.

Public health

PRRSV is not infectious for humans or cells of human origin and has no public health significance.

Epidemiology

Geographic distribution

As diagnostic assays became available during the 1990s, PRRSV was found to have spread nearly everywhere domestic pigs are raised. Retrospective serologic studies found that PRRSV was present in Canada by 1979 (Carman et al. 1995), the United States by 1985 (Zimmerman et al. 1997a), and the former German Democratic Republic by 1987 (Ohlinger et al. 2000). In Asia, anti-PRRSV antibodies were retrospectively documented in serum from pigs imported into the Republic of Korea (South Korea) in 1985 (Shin et al. 1993), in serum samples collected in 1987 in Taiwan (Chiou 2003), and in samples collected in 1988 in Japan (Hirose et al. 1995). In all cases, the serologic evidence of PRRSV preceded the recognition of clinical PRRS.

Some countries are believed to be free of PRRSV, including Argentina (Perfumo and Sanguinetti 2003), Australia (Garner et al. 1997), Brazil (Ciacci-Zanella et al. 2004), Cuba (Alfonso and Frías-Lepoureau 2003), Finland (Bøtner 2003), New Zealand (Motha et al. 1997), Norway (OIE 1997), Sweden (Carlsson et al. 2009), and Switzerland (Nathues et al. 2016). Experience has shown that remaining free of PRRSV requires vigilance (Carlsson et al. 2009; Nathues et al. 2016; Ramos et al. 2018).

Susceptible species

Pigs (*Sus scrofa*) and the collared peccary (*Pecari tajacu*) are susceptible to PRRSV (Molina-Barrios et al. 2018). The prevalence of PRRSV in free-ranging feral swine is low, and their contribution to PRRSV ecology is undefined (Albina et al. 2000; Pedersen et al. 2018; Stankevicius et al. 2014; Wyckoff et al. 2009). The susceptibility of other species within superfamily *Suoidea* (i.e. families *Suidae* and *Tayassuidae*) has not been established. Zimmerman et al. (1997b) reported PRRSV infection in mallard ducks (*Anas platyrhynchos*), but subsequent workers have not replicated these results. Species considered insusceptible to PRRSV include cats, dogs, guinea pigs, house sparrows and starlings, mice, opossums, raccoons, rats, and skunks (Hooper et al. 1994; Rosenfeld et al. 2009; Wills et al. 2000a).

Routes of shedding

Infected animals shed virus in oral and nasal secretions (Christianson et al. 1993; Rossow et al. 1994; Wills et al. 1997a), urine (Wills et al. 1997a), semen (Swenson et al. 1994a), and occasionally feces (Christianson et al. 1993). PRRSV-naïve females inoculated in late gestation shed virus in mammary secretions (Wagstrom et al. 2001). Virus isolates can vary significantly in the level and duration of shedding by pigs (Cho et al. 2006a,b).

Shedding of PRRSV in semen is of particular concern because of the potential for long-distance virus transmission through artificial insemination (Nathues et al. 2014, 2016). The duration of semen shedding varies widely among boars (Christopher-Hennings et al. 1996). Swenson et al. (1994a) found infectious virus in the semen of experimentally infected boars for up to 43 days following exposure. By PCR, Christopher-Hennings et al. (1995a) detected viral RNA in the semen of experimentally infected boars for up to 92 days post inoculation (DPI) and isolated PRRSV from the bulbourethral gland of a boar euthanized 101 DPI. Semen shedding of modified live virus (MLV) vaccine virus occurred for up to 39 days in one study, but prior vaccination eliminated or reduced shedding upon challenge (Christopher-Hennings et al. 1997).

Persistent infection

Persistence is the most significant epidemiological feature of PRRSV infection. Persistence reflects the inability of the immune system to clear the primary infection, with virus continuing to replicate in specific cells over time. PRRSV produces a "chronic persistent" infection – a type of persistent infection in which the virus is eventually cleared. None of the diagnostic tests currently available can differentiate carriers from animals that have successfully cleared the virus.

Persistent PRRSV infection has been documented through transmission experiments and by detection of virus in animals. A number of studies have reported detection of infectious virus for 100–165 DPI, particularly from tonsil or lymphoid tissues (Allende et al. 2000; Benfield et al. 2000b; Fangman et al. 2007; Horter et al. 2002; Wills et al. 1997b). Persistent infection is not a function of pig age at the time of infection. Persistence occurs regardless of whether the pig is exposed *in utero* (Benfield et al. 1997, 2000b; Rowland et al. 1999), as a young animal, or as an adult (Bierk et al. 2001; Christopher-Hennings et al. 1995a; Fairbanks et al. 2002; Zimmerman et al. 1992). The mechanism(s) by which the virus is able to persevere in the face of an active immune response has not been identified, but apparently does not involve evasion of immunity through continual *in vivo* viral mutation. Chang et al. (2002, 2009) found relatively low rates of mutation in persistently infected animals.

Transmission

Swine are susceptible to PRRSV by intranasal, intramuscular, oral, intrauterine, and vaginal routes of exposure, but the probability that a given dose will result in infection differs by route. Hermann et al. (2005) estimated the "infectious dose 50" (ID_{50}), i.e. the dose required to infect one-half of the exposed animals, for oral and intranasal routes of exposure to be $1 \times 10^{5.3}$ $TCID_{50}$ and $1 \times 10^{4.0}$ $TCID_{50}$, respectively. Based on data from Benfield et al. (2000a), the ID_{50} for exposure via artificial insemination is approximately $1 \times 10^{4.5}$ $TCID_{50}$. Pigs are particularly susceptible to infection via parenteral exposure (breaks in the skin barrier). Thus, Yoon et al. (1999) found that intramuscular inoculation with ≤20 PRRSV particles resulted in infection. In the field, potential parenteral exposures include standard husbandry practices such as ear notching, tail docking, teeth clipping, tattooing, and inoculations with medications and biologics. Likewise, because PRRSV is present in oropharyngeal fluids for weeks following infection, parenteral exposure may occur via bites, cuts, scrapes, and/or abrasions that occur during aggressive interactions among infected and susceptible pigs. Bierk et al. (2001) associated transmission with aggressive behavior between carrier sows and susceptible contacts.

Indirect transmission involves transmission by inanimate objects (e.g. equipment, instruments, clothing) or substances (e.g. water, food), aerosols, and possibly arthropod vectors. Aerosol transmission is dependent on the viral variant and environmental factors. Dee et al. (2010) found that the meteorological conditions supportive of airborne transport and transmission included low temperatures, moderate levels of relative humidity, a rising barometric pressure, and slow directional winds in combination with low sunlight levels representative of cloudy days or early morning periods prior to sunrise.

Vertical transmission

PRRSV is transmitted from viremic dams transplacentally to fetuses, resulting in fetal death or the birth of infected pigs that are weak or may appear normal (Bøtner et al. 1994; Christianson et al. 1992; Harding et al. 2017; Terpstra et al. 1991a). Some pigs in affected litters may escape infection. PRRSV can replicate in fetuses 14 days of gestational age or older, but infection of fetuses during the first two-thirds of gestation is uncommon because most PRRSVs cross the placenta efficiently only in the last trimester of pregnancy (Christianson et al. 1993; Lager and Mengeling 1995; Mengeling et al. 1994; Prieto et al. 1996a,b). Transit is independent of the reproductive virulence of the virus isolate. Park et al. (1996) showed that PRRSV isolates of low and high virulence for fetuses crossed the placenta with equal efficiency when sows were inoculated at 90 days of gestation.

Transmission within herds

Once infected, PRRSV tends to circulate within a herd indefinitely. Endemicity is driven by persistent PRRSV infections (carrier animals) and the continual availability of susceptible animals introduced through birth or purchase. The virus is perpetuated by a cycle of transmission from dams to pigs either *in utero* or postpartum or by commingling susceptible animals with infected animals. Under conditions in which susceptible and infectious pigs are mixed, e.g. at weaning, a large proportion of the population may quickly become infected. Dee and Joo (1994a) reported 80–100% of pigs in three swine herds were infected by 8–9 weeks of age, and Maes (1997) found 96% of market hogs sampled from 50 herds to be positive. However, marked differences in infection rates between groups, pens, or rooms of animals may be observed in endemically infected herds. Houben et al. (1995) even found transmission to vary within litters. Some littermates seroconverted as early as 6–8 weeks of age, but other individuals reached 12 weeks of age, the end of the monitoring period, still free of PRRSV infection.

Transmission between herds

The role of infected pigs, virus-contaminated semen, and aerosols in herd-to-herd transmission is firmly established (Dee et al. 2010; Mousing et al. 1997; Otake et al. 2010; Weigel et al. 2000). Goldberg et al. (2000) evaluated the ORF5 gene sequences from 55 field isolates and concluded that PRRSV was most commonly

introduced into herds through animals or semen. Mortensen et al. (2002) found that PRRSV entered negative herds through the introduction of animals and semen and through area spread from neighboring farms, which they attributed to aerosol transmission. Torremorell et al. (2004) attributed over 80% of new infections in commercial systems to area spread from neighboring units, the movement of pigs in PRRSV-infected transports, the lack of compliance of the biosecurity protocols, or possibly introduction via insects. Le Potier et al. (1997) found that 45% of herds suspected to have become infected through area spread were located within 500 m (0.3 miles) of the postulated source herd and only 2% were 1 km from the initial outbreak. Herd density and proximity to infected herds are definitive risk factors, but recent studies have also underlined the extensive connectivity of contemporary production systems and the potential to move PRRSV through the movement of animals, personnel, equipment, transport, and other links (Amirpour Haredasht et al. 2017; Arruda et al. 2016, 2017, 2018; Lee et al. 2017; Lowe et al. 2017).

Stability in the environment

Shedding of virus in oropharyngeal fluids, in urine, and to a much lesser extent in feces results in environmental contamination. PRRSV is stable at pH 6.5–7.5, with infectivity rapidly lost at pH outside this range (Benfield et al. 1992; Bloemraad et al. 1994). The virus is stable for months to years at temperatures of –70 °C and – 20 °C, but quickly inactivated by heat and drying. At 25–27 °C, infectious virus was not detected on dry materials (e.g. plastic, stainless steel, rubber, alfalfa, wood shavings, straw, corn, swine starter feed, or denim cloth) beyond day zero (Pirtle and Beran 1996).

In the presence of moisture, the stability of infectious PRRSV is dependent on specific conditions of temperature, the matrix, and pH. Jacobs et al. (2010) observed no difference in inactivation rate among 4 type 2 isolates in solution and estimated the virus half-life at 4 °C (39 °F), 155 hours; 10 °C (50 °F), 84.5 hours; 20 °C (68 °F), 27.4 hours; and 30 °C (86 °F), 1.6 hours. As reviewed by Linhares et al. (2012a), half-life estimates are relatively consistent among reports. As noted by Linhares et al. (2012a), PRRSV half-life in manure was slightly shorter than virus in solution for any given temperature.

The thermal stability of PRRSV in serum and tissues is similar to that described for virus stored in media. PRRSV was isolated from 47, 14, and 7% of porcine serum samples stored at 25 °C for 24, 48, and 72 hours, respectively. When serum was stored at 4 °C or – 20 °C, PRRSV was isolated from 85% of the samples after 72 hours (Van Alstine et al. 1993).

Disinfection

PRRSV is inactivated by lipid solvents, e.g. chloroform and ether (Benfield et al. 1992). PRRSV is highly unstable in solutions containing low concentrations of detergents, which disrupt the envelope with concomitant release of the noninfectious core particles and loss of infectivity (Snijder and Meulenberg 2001). At "room temperature," Shirai et al. (2000) reported complete inactivation of PRRSV with chlorine (0.03%) in 10 minutes, iodine (0.0075%) in 1 minute, and a quaternary ammonium compound (0.0063%) in 1 minute. Decontamination protocols involving drying, thermo-assisted drying, and foaming disinfectants containing glutaraldehyde and quaternary ammonium chloride compounds are effective at inactivating PRRSV in farrowing rooms and transport vehicles in cold and warm climates (Dee et al. 2004, 2005a; Schneider et al. 2015).

Pathogenesis

PRRSV replicates in a subset of monocyte-derived cells that display CD163, the only required cell receptor for PRRSV binding, internalization, and replication (Calvert et al. 2007; Prather et al. 2013; Van Gorp et al. 2008). Although not required, co-expression of cell surface sialoadhesin (Sn, CD169) may augment viral internalization (Van Breedam et al. 2013). In an *in vitro* study, non-permissive PK-15 cells co-expressing CD163 and Sn produced 10–100× more virus compared with cells expressing only CD163 (Van Gorp et al. 2008). The predominant cells known to support PRRSV replication *in vivo* include pulmonary alveolar macrophages (PAM) and pulmonary intravascular macrophages (PIM) in the lung (Thanawongnuwech et al. 1997b, 2000b; Wensvoort et al. 1991) and monocyte-derived macrophages (MDM) in lymphoid tissues (Duan et al. 1997). PRRSV replicates to a lesser extent in dendritic cells (Loving et al. 2007) and in MDM that reside in most organs in perivascular locations.

The frequent recombination and mutation of the PRRSV genome has led to periodic emergence of strains with atypically high virulence, including PRRSV-2 strains in North America (Mengeling et al. 1998; Wang et al. 2015c), PRRSV-1 strains in Europe (Karniychuk et al. 2010), and PRRSV-2 strains in China (Li et al. 2007, Tian et al. 2007) and elsewhere in Southeast Asia, where they are known as high pathogenicity (HP-PRRS).

Postnatal PRRS

PRRSV viral infection can be divided into three distinct stages: acute infection, persistence, and extinction (Lunney et al. 2016). Acute infection follows exposure

and is characterized by rapid spread to primary sites of replication in lung and lymphoid tissues. Viremia can usually be detected 6–48 hours post exposure. Viral loads peak by 4–14 DPI, with typical levels of 1×10^2–10^5 $TCID_{50}$ per ml of serum or gram of lung tissue, but may be $\geq 10^8$ $TCID_{50}$ in HP-PRRS (Guo et al. 2013a; Hu et al. 2013). Increased replication in high virulence strains may be due to a broader cell tropism. Studies comparing an atypical PRRSV-1 strain with lower virulence PRRSV-1 strains revealed that the atypically virulent strain replicated in macrophages with a CD163+Sn- phenotype, in addition to the CD163+Sn+ phenotype in which the lower virulence strains replicated (Frydas et al. 2013, 2015; Frydas and Nauwynck 2016). Lesser amounts of virus can be detected in a variety of other tissues. Certain strains of PRRSV may exhibit atypical tissue tropisms, such as so-called neurotropic strains of PRRSV that replicate in MDM in the brain (Rossow et al. 1999; Thanawongnuwech et al. 1997a). Some HP-PRRSV strains also exhibit increased tropism for the brain (Brockmeier et al. 2017; Lunney et al. 2016). Clinical disease is observed at the times of highest viral titers, i.e. in the early acute infection. After peaking, virus titers in serum decrease rapidly. Most pigs are no longer viremic by 21–28 DPI. Pig age at the time of PRRSV exposure affects disease; younger pigs replicate virus to higher titers and have longer duration of viremia and shedding when compared with older pigs (Cho et al. 2006b; Klinge et al. 2009; van der Linden et al. 2003).

The persistence stage begins when viremia ends and is characterized by absence of clinical disease, progressively diminishing PRRSV replication in lymph nodes and tonsil, and progressively diminishing viral shedding (Allende et al. 2000; Rowland and Yoo 2003; Wills et al. 1997b).

The extinction phase begins when viral shedding ends and is complete when virus is cleared, the duration varies between pigs, but can be as long as 250 days post exposure (Wills et al. 2003). No diagnostic test is able to reveal the initiation of the extinction phase.

Disease is produced by a variety of mechanisms. Cell death in PRRSV-infected macrophages is by necrosis, as well as apoptosis, involving activation of caspases and a mitochondria-mediated pathway (Costers et al. 2008; Lee and Kleiboeker 2007). However a majority of apoptotic cells in PRRSV-infected tissues are not infected with virus (Duan et al. 1997; Mengeling et al. 1995), but are killed indirectly by bystander apoptosis. In the lung, cells killed by bystander apoptosis are mostly macrophages with fewer lymphocytes and alveolar pneumocytes; in lymph nodes and thymus, cells are mostly lymphocytes, with fewer macrophages (Feng et al. 2002; Labarque et al. 2003; Sirinarumitr et al. 1998; Sur et al. 1998; Wang et al. 2014). A feature of HP-PRRS strains is induction of higher levels of bystander apoptosis in a variety of tissues. For example, bystander apoptosis in thymic CD3+ thymocytes is 5–40× that of lower pathogenicity PRRSV-2 strains (He et al. 2012; Li et al. 2014; Wang et al. 2015a).

PRRSV markedly alters innate immunity and inflammatory and immunoregulatory cytokines in a strain-specific manner (see reviews by Butler et al. 2014; Lunney et al. 2016). Secretion of proinflammatory cytokines from PRRSV-infected macrophages, including TNF-alpha, IL-1, and IL-6 (Choi et al. 2001; Suradhat and Thanawongnuwech 2003; Thanawongnuwech et al. 2004; van Gucht et al. 2003), is known to promote influx and activation of leukocytes, increased microvascular permeability (pulmonary edema), and induction of systemic effects such as pyrexia, anorexia, and lethargy. Alteration of levels of these and others in HP-PRRS is exacerbated and suggested as a potential cause of death similar to "macrophage activation syndrome" or "cytokine storms" in humans (Behrens et al. 2011; Guo et al. 2013a). Among other mechanisms, PRRSV-induced alterations of immunoregulatory cytokines, as well as alteration in numbers of various lymphocyte subsets, result in a delay of effective adaptive immunity, including delayed production of neutralizing antibodies and delay of a sterilizing cell-mediated immune (CMI) response. Delay in neutralizing antibody response is credited for the extended duration of viremia and delay in CMI for the extended persistence in lymphoid tissues.

PRRSV infection predisposes to *S. suis* and possibly other septicemias and opportunistic bacterial pneumonia in a strain-dependent manner (Brockmeier et al. 2000; Feng et al. 2001; Galina et al. 1994; Thanawongnuwech et al. 2000a; Wills et al. 2000b). This is believed to be due to PRRSV-induced reduction in bacterial phagocytic and killing capacity by PIM and PAM (Thanawongnuwech et al. 1998a,b, 2000b) and likely other MDM. Outbreaks of HP-PRRS in Southeast Asia, known as "porcine high fever disease" (PHFD), were associated with concurrent septicemia and/or bacterial bronchopneumonia (Li et al. 2007; Metwally et al. 2010; Xu et al. 2010). In a study by Brockmeier et al. (2017), both atypical US and HP-PRRS strains of PRRSV induced more severe secondary bacterial bronchopneumonia when compared with lower virulence US and Chinese PRRSV strains in a dual bacteria + PRRSV inoculation model.

Gram-negative bacterial coinfections may enhance PRRS through the action of bacterial lipopolysaccharide (LPS). Bacterial LPS (endotoxin) is a major component of gram-negative bacterial cell walls that acts systemically in sepsis and locally in the respiratory tract when inhaled in high levels in dust in poorly ventilated swine buildings (Zejda et al. 1994). Intratracheal administration of LPS in PRRSV-inoculated pigs resulted in more severe respiratory disease associated with 10–100× elevations in inflammatory cytokines IL-1, IL-6, and

TNF-alpha compared with pigs given only PRRSV or LPS (Labarque et al. 2002; van Gucht et al. 2003). *In vitro* studies in PAM cultures suggest that PRRSV infection causes elevated secretion of inflammatory cytokines through upregulation of CD14, the LPS receptor, on PAM cell membranes (Qiao et al. 2011).

In addition to bacterial diseases, PRRSV also significantly enhances replication of porcine circovirus type 2 (PCV2), resulting in more severe PRRS viral pneumonia, as well as lesions of PCV2-associated porcine multisystemic wasting syndrome (Allan et al. 2000; Harms et al. 2001).

The severity of PRRS can also be influenced by host susceptibility. The first indication of natural genetic resistance to PRRSV was provided by Halbur et al. (1998) who demonstrated that Meishan pigs had lower viral titers and less severe lesions than Duroc pigs when inoculated with PRRSV. Since then, a region on *Sus scrofa* chromosome 4 (SSC4) surrounding the single nucleotide polymorphism (SNP) marker WUR10000125 (WUR) has been identified that is strongly associated with improved weight gain and lower viral loads in PRRSV-inoculated pigs that are homozygous or heterozygous for the dominant allele (Boddicker et al. 2012, 2014a,b; Rowland et al. 2012; Schroyen et al. 2016). However, this trait varies in efficacy based on PRRSV strain (Hess et al. 2016).

Reproductive PRRS

Acute PRRS in boars and sows can lead to reproductive failure in a strain-dependent manner (Mengeling et al. 1996, 1998; Park et al. 1996). During acute PRRS, viral shedding in semen can result in venereal transmission to sows (Swenson et al. 1994b; Yaeger et al. 1993). Venereal transmission infects sows by direct transit through the endometrium, since preimplantation embryos are refractory to PRRSV infection (Lager et al. 1999; Mateusen et al. 2007; Prieto et al. 1996b). This is likely due to their lack of Sn+ macrophages (Mateusen et al. 2007). Postimplantation fetuses of all ages are susceptible to PRRSV infection (Christianson et al. 1993; Lager and Mengeling 1995; Lager et al. 1996; Mateusen et al. 2007; Prieto et al. 1996b). However, PRRSV crosses the maternal–fetal interface (MFI) in pregnant females to infect fetuses with high efficiency only in the third trimester (Christianson et al. 1992; Kranker et al. 1998. Lager et al. 1997a,b; Mengeling et al. 1994, 1998; Terpstra et al. 1991a; Wang et al. 2015a). But a minority of strains, including some HP-PRRS strains, are able to cross the placenta mid-gestation with moderate efficiency and kill fetuses (Han et al. 2017; Wang et al. 2015b).

Resistance to PRRSV transit at the MFI seems to be two way. Intra-fetal or intra-amniotic inoculation of fetuses with PRRSV 45–50 days of gestation did not result in passage of PRRSV from fetus to dam (Christianson et al. 1993). The reason for resistance to PRRSV transit at the MFI during early and mid-gestation and for efficient transit during late gestation may be due, in part, to numbers of PRRSV-permissive cells in the fetal placenta and other fetal tissues. Karniychuk et al. (2009) demonstrated that during early and mid-gestation, the predominant macrophage phenotype is the less permissive CD163+Sn–, whereas in late gestation it is the highly permissive CD163+Sn+.

Fetuses are infected by transit of PRRSV from the dam via the MFI to fetal placenta or by transmission from adjacent intrauterine PRRSV-infected fetuses (Ladinig et al. 2014, 2015a). PRRSV replicates to its highest titers in fetal thymus, tonsils, and lymph nodes (Karniychuk et al. 2011; Kranker et al. 1998; Mengeling et al. 1994; Rowland 2010), contributing to fetal death or, if pregnancy continues to term, birth of PRRSV-infected piglets (Kranker et al. 1998; Mengeling et al. 1994). Lesions are observed in a minority of PRRSV-infected fetuses (Christianson et al. 1992; Lager et al. 1996; Novakovic et al. 2016; Rossow et al. 1996), leading some to question whether viral replication in fetuses is the sole or significant cause of fetal death (Karniychuk et al. 2011; Karniychuk and Nauwynck 2013; Novakovic et al. 2017).

Several studies have provided evidence that events at the MFI may determine transit of PRRSV from dam to fetus and/or contribute to fetal outcome. A study of the MFI in gilts inoculated with PRRSV at 90 days of gestation confirmed direct apoptosis in CD163+Sn+ cells with spatially and numerically correlated bystander apoptosis in both the maternal endometrium and interfacing fetal placenta (Karniychuk et al. 2011). A much larger study confirmed in PRRSV-infected dams that, at the MFI for a particular fetus, the amount of direct and bystander apoptosis is directly proportional to the number of PRRSV-infected macrophages in the maternal endometrium, the PRRSV load in the respective fetal thymus, and the risk for fetal death (Ladinig et al. 2015b; Novakovic et al. 2017). Furthermore, bystander apoptosis at the MFI included endometrial epithelial cells and placental trophoblasts that were associated with micro or rarely larger separations of the placenta from the endometrium. These findings suggested that PRRSV replication in macrophages in the maternal endometrium, by causing indirect killing of maternal and fetal cells at the MFI, could provide the means of PRRSV transit to the fetus and/or contribute, along with PRRSV replication in fetal tissues, to fetal death.

Clinical signs

Descriptions of the clinical signs of PRRS in swine herds are generally similar in North America (Bilodeau et al. 1991; Keffaber 1989; Loula 1991; Moore 1990; Sanford 1992), South America (Dewey 2000), Europe

(Anon 1992; Busse et al. 1992; de Jong et al. 1991; Gordon 1992; Hopper et al. 1992; Leyk 1991; Wensvoort et al. 1991; White 1992a,b), and Asia (Chiou 2003; Thanawongnuwech and Thacker 2003; Tong and Qiu 2003; Yang et al. 2003).

Clinical presentation of PRRS varies among herds, ranging from subclinical to devastating. Clinical signs of PRRSV are influenced by virulence of virus strain, host immune status, host susceptibility, exposure to LPS, concurrent infections, and other management factors (White 1992a).

Clinical epidemics occur in immunologically naive populations, and all ages are affected, whereas endemic PRRS occurs in herds that have some degree of immunity to the infecting PRRSV. In endemic PRRS, clinical disease is observed in susceptible subpopulations, usually in nursery–grower pigs when maternal immunity decays, and/or in replacement gilts or sows that have previously escaped infection, as well as their congenitally infected progeny.

Antigenic variation is great enough among variants of PRRSV that entry or emergence of a new, relatively unrelated virus can cause epidemics in endemically PRRSV-infected herds or regions (Li et al. 2007; Tian et al. 2007; Wang et al. 2015c; Zhou et al. 2008).

"Atypical" highly virulent strains periodically emerge that extend the severity or range of clinical signs (Karniychuk et al. 2010; Mengeling et al. 1998; Wang et al. 2015c). Notable among these are HP-PRRSV strains that emerged in China in 2006 (Li et al. 2007; Tian et al. 2007) and elsewhere in Southeast Asia (Feng et al. 2008; Metwally et al. 2010; Ni et al. 2012). These viruses are genetically homologous, contain a discontinuous 30-amino-acid deletion in the nsp2 gene (Zhou et al. 2009a), and are typically fatal for pigs inoculated under experimental conditions (Li et al. 2007; Tian et al. 2007; Zhou et al. 2008; Zhou et al. 2009b). In "high fever disease" outbreaks, HP-PRRSV is often found concurrently with other pathogens, e.g. classical swine fever virus, Aujeszky's disease virus, streptococci, *H. parasuis*, and others.

Epidemic infection

The first phase of acute illness in a PRRS epidemic lasts two or more weeks and affects animals of all ages. It begins in one or more stages of production and quickly spreads in 3–7 or more days, depending on size and composition of the site, to all stages of production. The spread of the disease through a segregated group of pigs usually requires 7–10 or more days, although individuals may escape infection for weeks or months, depending on virus strain, herd size, and type of housing.

The second phase of reproductive failure may begin before the first phase of acute illness is completed and continues for 1–4 months. This phase is characterized by reproductive failure, primarily in sows that were viremic in their third trimester, and by high preweaning mortality in their live-born progeny. When reproductive performance and preweaning mortality return to near pre-outbreak levels, endemic infection of most herds continues.

Sows and boars

During the phase of acute illness, 1–3% of litters may be lost in sows that are at 21–109 days of gestation. This presents as overt abortions or, later, as irregular returns to estrus or nonpregnant sows (Hopper et al. 1992; Keffaber 1989; Loula 1991; White 1992a). Also observed inconsistently in acutely ill sows are agalactia (Hopper et al. 1992), incoordination (de Jong et al. 1991), and/or a dramatic exacerbation of endemic diseases, such as sarcoptic mange, atrophic rhinitis, or cystitis/pyelonephritis (White 1992a).

Mortality in sows is typically 1–4% during acute illness and is sometimes associated with pulmonary edema and/or cystitis/nephritis (Hopper et al. 1992; Loula 1991). A few cases of severe acute PRRS in sows have been described with 10–50% abortions, up to 10% mortality, and nervous signs such as ataxia, circling, and paresis (Epperson and Holler 1997; Halbur and Bush 1997). Abortion rates of 40–100% and sow mortality of ≥10% are reported in Southeast Asia for acute HP-PRRS (Han et al. 2017).

Not all affected sows are clinically ill during acute PRRS. Typically, 5–80% of sows farrow on day 100–118 of gestation and have litters composed of any combination of normal pigs, weak variably sized pigs, and dead pigs that are fresh stillborn (intrapartum death), autolytic (brown), partially mummified, or completely mummified fetuses. Typically, pigs born dead comprise 0–100% of each affected litter and 7–35% of the total pigs born in a farrowing group. In time, there is a shift from predominantly stillborn pigs and large partially mummified pigs to smaller more completely mummified pigs, to small weak-born pigs, and to pigs of normal size and vigor (Keffaber 1989; Loula 1991; White 1992a). In some herds, the majority of abnormal pigs are born alive, premature, weak, and small, but few are born dead (Gordon 1992). Periparturient mortality in sows may be 1–2% (de Jong et al. 1991; Keffaber 1989). Surviving sows on the subsequent breeding often have delayed return to estrus and low conception rates.

Boars acutely infected by PRRSV, in addition to anorexia, lethargy, and respiratory clinical signs, may lack libido and have variable reduction in semen quality (de Jong et al. 1991; Feitsma et al. 1992; Prieto et al. 1994). Changes in sperm occur 2–10 weeks after infection with virus and include reduced motility and acrosomal defects, but it is unclear whether conception rates are affected (Lager et al. 1996; Prieto et al. 1996a,b; Swenson et al. 1994b; Yaeger et al. 1993).

Suckling pigs

During the 1- to 4-month phase of late-term reproductive failure, there is high preweaning mortality (up to 60%) in pigs born prematurely and at term associated most consistently with listlessness, emaciation/starvation, splayleg posture, hyperpnea, dyspnea ("thumping"), and chemosis. Seen less commonly are somnolence, tremors or paddling (Keffaber 1989; Loula 1991; Rossow et al. 1999), anemia and thrombocytopenia with consequent hemorrhage from navels and elsewhere, and an increase in bacterial polyarthritis and meningitis (Hopper et al. 1992; White 1992a). Watery diarrhea was commonly reported in the United Kingdom (Gordon 1992; Hopper et al. 1992; White 1992a) and less commonly elsewhere (Keffaber 1989; Leyk 1991). Mortality reported for HP-PRRS in suckling pigs is often as high as 100% (Han et al. 2017).

Weanling and grower pigs

Acute PRRSV infection in nursery or grower–finisher pigs is characterized most consistently by anorexia, lethargy, cutaneous hyperemia, hyperpnea and/or dyspnea, variable coughing, rough hair coats, variable reduction in average daily gain, and elevated mortality of 12–20% (Moore 1990; White 1992b). Uncommonly, nervous signs including tremors, ataxia, and convulsions are reported (Thanawongnuwech et al. 1997a). Additionally with HP-PRRS strains, a prolonged high fever of 40–42 °C (104–108 °F), rapid weight loss, and high mortality are consistently observed, and inconsistently observed are cyanosis, vomiting, diarrhea, constipation, tremors, and conjunctivitis (Brockmeier et al. 2017; Guo et al. 2013a,b; Hu et al. 2013; Li et al. 2007; Tian et al. 2007; Zhou et al. 2008). Concurrent with acute PRRS, a higher than usual incidence of endemic diseases is often reported (Brockmeier et al. 2017; Han et al. 2017; Keffaber et al. 1992; Loula 1991; Moore 1990; Stevenson et al. 1993; White 1992a). Diseases most commonly reported include streptococcal meningitis, Glässer's disease, exudative dermatitis, sarcoptic mange, and bacterial bronchopneumonia.

Endemic infection of herds

Once introduced into a herd, PRRSV becomes endemic in nearly all cases. In endemically infected herds, PRRS is most often seen as regular or occasional outbreaks of typical acute PRRS in susceptible nursery or grower–finisher pigs (Keffaber et al. 1992; Stevenson et al. 1993). Clinical signs are also observed in groups of susceptible gilts or replacement boars exposed to PRRSV after introduction into the herd (Dee et al. 1996; Dee and Joo 1994b; Grosse-Beilage and Grosse-Beilage 1992) but may also be seen in susceptible sows. Acute clinical disease in gilts or boars is as described for epidemics. The reproductive consequences depend on the number of gilts/sows infected and the stage of their reproductive cycle when infected, both of which may vary widely (Torrison et al. 1994). If few gilts are infected on an ongoing basis, then there may be scattered abortions, irregular returns to estrus, nonpregnant gilts, and late-term reproductive failure with abnormal litters typical of PRRS. These may only be recognized if records are evaluated on a parity-specific basis (White 1992b). Alternatively, gilts may escape exposure to PRRSV until there is a significant subpopulation of susceptible gilts in various stages of gestation. In this situation, endemic PRRS in the breeding herd manifests as periodic mini-outbreaks of PRRS in gilts and, less commonly, sows that are identical to those in an epidemic (Dee and Joo 1994b).

Lesions

Postnatal lesions

Similar lesions are described in all ages of pigs with PRRSV infection. The severity and distribution of lesions varies with the virulence of the infecting virus (Brockmeier et al. 2017; Done and Paton 1995; Guo et al. 2013b; Halbur et al. 1996b; Karniychuk et al. 2010; Morgan et al. 2014; Pol et al. 1991). Most inoculation studies in which lesions have been described were in suckling or weaned pigs 1–105 days of age (Collins et al. 1992; Dea et al. 1992; Guo et al. 2013b; Halbur et al. 1995b, 1996a,b; Li et al. 2007; Karniychuk et al. 2010; Morgan et al. 2014; Pol et al. 1991; Rossow et al. 1994, 1995; Zhou et al. 2008).

Consistent gross and microscopic lesions are observed in the lungs and lymph nodes 4 to ≥28 DPI. There is interstitial pneumonia that varies in severity. Distribution may be cranialventral to diffuse, consistency varies from slightly firm and resilient to moderately firm and rubbery, color varies from tan to dark redpurple, and pulmonary edema varies from mild to severe with separation of lobules. In HP-PRRS, the lungs may be hemorrhagic. Many lymph nodes in most pigs are enlarged 2–10× normal. Early post exposure, enlarged nodes are edematous, tan, and moderately firm and in HP-PRRS may be hemorrhagic. Later, nodes are firm and white or light tan in a nodular or diffuse pattern. Uncommonly, there are multiple fluid-filled 2–5 mm diameter cortical cysts. Gross lesions unique to pigs infected at ≤13 days of age include periocular edema 6–23 DPI, scrotal edema 11–14 DPI, and subcutaneous edema 2–7 DPI (Rossow et al. 1994, 1995). Additional gross lesions frequently observed with HP-PRRS include multifocal cutaneous, epicardial, and renal cortical hemorrhages, chemosis, conjunctivitis, and thymic atrophy (Guo et al. 2013b; Han et al. 2017; Li et al. 2007; Zhou et al. 2008).

The most consistent microscopic lesions are observed in the lungs and lymphoid tissues. Alveolar septa are

expanded by macrophages, lymphocytes, and plasma cells and may be lined by hyperplastic type II pneumocytes. Alveoli may contain necrotic macrophages, cell debris, and serous fluid. Lymphocytes and plasma cells form cuffs around airways and blood vessels. Edema may expand interlobular connective tissues and with HP-PRRS there may be multifocal hemorrhage. Germinal centers in lymph nodes early in the course of infection are necrotic and depleted. Later germinal centers are large and composed of blast-type lymphocytes. There may be lymphoid necrosis and depletion in the thymus, from mild to moderate and multifocal to diffuse and severe, resulting in nearly complete cortical atrophy as described for some HP-PRRS strains (Brockmeier et al. 2017; He et al. 2012; Wang et al. 2011). Lymphoid depletion followed later by hyperplasia may also be observed in periarteriolar lymphoid sheaths of the spleen and in lymphoid follicles in tonsil and Peyer's patches (Halbur et al. 1995b; Pol et al. 1991).

Other microscopic lesions include segmental lymphohistiocytic perivascular cuffing in the epicardium and myocardium with occasional cuffing of Purkinje fibers (Halbur et al. 1995a, 1996b; Rossow et al. 1994, 1995). Kidneys may have mild periglomerular and peritubular lymphohistiocytic aggregates and mild to severe segmental lymphohistiocytic necrotizing vasculitis that is most severe in the renal pelvis and medulla (Cooper et al. 1997; Rossow et al. 1995). Mild lymphohistiocytic encephalitis characterized by multifocal gliosis and segmental lymphohistiocytic perivascular cuffs may also be observed (Brockmeier et al. 2017; Collins et al. 1992; Halbur et al. 1996b; Han et al. 2017; Rossow et al. 1995, 1999; Thanawongnuwech et al. 1997a). More severe encephalitis may uncommonly be observed that also includes lymphohistiocytic necrotizing vasculitis (Thanawongnuwech et al. 1997a).

Pregnant sows frequently have microscopic lesions in the uterus (Christianson et al. 1992; Lager and Halbur 1996; Novakovic et al. 2016, 2017; Stockhofe-Zurwieden et al. 1993). The myometrium and/or endometrium are edematous with lymphohistiocytic perivascular cuffs. Less commonly, there are segmental lymphohistiocytic vasculitis in small vessels and microseparations at the MFI between endometrial epithelium and placental trophoblasts that contain eosinophilic proteinaceous fluid and cell debris.

Atrophy of seminiferous tubules may be observed in 5- to 6-month-old boars 7–25 DPI (Sur et al. 1997). Atrophic tubules have PRRSV antigen and nucleic acid in germinal epithelial cells, giant cells with 2–15 nuclei, and apoptosis and depletion of germ cells.

Fetal lesions

Gestational age at expulsion and composition of litters is typically more suggestive of PRRS than gross or microscopic lesions in fetuses. In most cases of PRRS reproductive failure, litters are delivered in late gestation, but before term, and are composed of variable proportions of clinically normal pigs, small- or normal-sized weak pigs, dead variably autolyzed pigs, and mummies. Dead pigs may be coated with a thick brown mixture of meconium and amniotic fluid, a nonspecific finding that suggests fetal stress and/or hypoxia (Lager and Halbur 1996; Stockhofe-Zurwieden et al. 1993). Lesions in fetuses and stillborn pigs are uncommon and rarely contribute to a definitive diagnosis of PRRS.

PRRSV-specific gross lesions are best observed in fetuses with little or no *in utero* autolysis (Bøtner et al. 1994; Collins et al. 1992; Done and Paton 1995), but are more commonly seen in live-born PRRSV-infected littermates that die or are sacrificed within a few days after birth. Gross lesions may include perirenal edema, edema of the splenic ligament, mesenteric edema, ascites, hydrothorax, hydroperitoneum, and segmental enlargement of the umbilical cord by hemorrhage (Dea et al. 1992; Lager and Halbur 1996; Plana Duran et al. 1992).

Microscopic lesions are mild and may include lymphoid depletion and follicular atrophy in mesenteric lymph nodes; lymphohistiocytic segmental arteritis and periarteritis in umbilical cord, lung, heart, and kidney; multifocal interstitial pneumonia with occasional hyperplasia of type II pneumocytes; mild periportal hepatitis; myocarditis with loss of myocardial fibers; multifocal leukoencephalitis; and thymic atrophy (Lager and Halbur 1996; Novakovic et al. 2016; Plana Duran et al. 1992; Rossow et al. 1996; Sur et al. 1996).

Diagnosis

The possible involvement of PRRSV is suggested in any herd with reproductive disease in breeding swine and/or respiratory disease in pigs of any age. Infection with some PRRSV strains has been associated with neurological signs (e.g. shivering, lameness, and opisthotonos) (Cao et al. 2012; Tian et al. 2007). Analysis of production records in herds with clinically active PRRSV may show increased abortions, early farrowings, stillbirths, preweaning mortality, and nonproductive sow days. However, the absence of clinical signs does not mean that a population is free of PRRSV: this is established by testing. Notably absent is a test capable of differentiating inapparently infected (non-viremic) carrier animals from animals that have cleared the virus (Molina et al. 2008).

Pathological evaluation

PRRSV infection does not produce pathognomonic gross or microscopic lesions, and aborted fetuses and stillborn pigs rarely have lesions of diagnostic value. Gross lesions of interstitial pneumonia and enlarged

lymph nodes may be observed in infected pigs of all ages (Lager and Halbur 1996; Stevenson et al. 1993). Microscopically, interstitial pneumonia is the primary lesion. Typically, concurrent viral and/or bacterial infections complicate the clinical picture and diagnosis.

Depending on the region, the differential diagnosis of PRRSV infection may include classical swine fever virus, cytomegalovirus, hemagglutinating encephalomyelitis virus, leptospirosis, parvovirus, PCV2, pseudorabies (Aujeszky's disease) virus, swine influenza virus, and teschovirus (Halbur 2003). Therefore, a definitive diagnosis requires laboratory confirmation of the presence of virus, viral products, and/or antibodies.

Diagnostic specimens

Specimens typically collected for laboratory testing include serum or blood, tissues (e.g. lung, tonsil, and lymph nodes), bronchoalveolar lavage (BAL), semen, and oral fluid specimens from individual or groups of pigs (Decorte et al. 2015; Fablet et al. 2017; Kittawornrat et al. 2010; Olsen et al. 2013a,b; Prickett and Zimmerman 2010). "Processing fluid," a composite sample composed of the exudate from tissues collected at the time of piglet castration and tail docking, has also been shown to be useful for the detection of PRRSV RNA and antibody (Lopez et al. 2018). In reproductive failure, PRRSV detection is recommended on three sample sources: fetal/stillborn thymus and lung pools, serum pools from dams, and processing fluids from neonatal live-born littermates or cohorts.

Virus isolation

The success rate for VI varies among specimens, with serum the preferred antemortem sample because of the prolonged period of PRRSV viremia, but lung and lymphoid tissue samples collected at necropsy may also be used. Specimens for VI should be refrigerated (4°C) immediately after collection and shipped for testing within 24–48 hours. The virus is heat labile and has a narrow range of pH stability (Benfield et al. 1992; Bloemraad et al. 1994; Jacobs et al. 2010; Van Alstine et al. 1993).

PRRSV can be isolated on porcine alveolar macrophages or sublines (CL-2621, MARC-145) of the African monkey kidney cell line MA-104 (Benfield et al. 1992; Kim et al. 1993). PRRSVs vary in their ability to replicate in PAMs and MA-104 cells (Bautista et al. 1993), and, ideally, VI should be attempted on both cell types for optimum recovery of virus. PAMs are reportedly more sensitive than MARC-145 cells for VI (de Abin et al. 2009; Yoon et al. 2003). However, PAMs are primary cells and cannot be continuously passed, but need to be prepared periodically, and batches of PAMs vary in their susceptibility to the virus. Recently, an immortalized cell line (ZMAC-1, ATCC® PTA-8764) derived from PAMs became available for VI (Calzada-Nova et al. 2012). Preliminary data showed that the rate of PRRSV-2 and PRRSV-1 VI from PRRSV RNA-positive serum and lung samples was significantly higher in ZMAC-1 cells than in MARC-145 cells (Yim-Im et al. 2017). Isolation of PRRSV on cell culture may be confirmed by reverse transcription PCR (RT-PCR) or by visualizing viral antigens in the cytoplasm of infected cells by fluorescent antibody (FA) or immunohistochemistry (IHC) using PRRSV-specific monoclonal antibodies. Negative stain electron microscopy (EM) can also be used to visualize virus particles in cell culture fluids.

Detection of viral antigens

Methods for the routine detection of PRRSV antigens include IHC and FA staining. Tissues (lung, tonsil, lymph nodes, heart, brain, thymus, spleen, and kidney) can be fixed in 10% neutral buffered formalin for microscopic evaluation and IHC staining using PRRSV-specific monoclonal antibody against the highly conserved nucleocapsid (N) protein (Halbur et al. 1994; Van Alstine et al. 2002; Yaeger 2002). The combination of histopathology and IHC allows visualization of viral antigens in the cytoplasm of cells within, or contiguous to, microscopic lesions (Halbur et al. 1994). Tissues should be processed within 48 hours of fixation to avoid degradation of PRRSV antigens and loss of IHC-positive cells (Van Alstine et al. 2002). Lesions and viral antigens are best observed during acute infection (4–14 DPI). Viral antigen can also be detected in frozen tissue sections by FA (Benfield et al. 1992; Halbur et al. 1996a; Rossow et al. 1995). The FA test is faster and less expensive than IHC but requires unfixed tissues. Detection of viral antigen by IHC or FA is significantly less analytically and diagnostically sensitive than methods for detection of viral nucleic acids.

Detection and characterization of viral nucleic acids

Nucleic acid-based PRRSV detection methods include RT-PCR, sequencing, *in situ* hybridization (ISH), and loop-mediated isothermal amplification (LAMP). The specimen types used for PRRSV VI can also be used for PRRSV RNA detection.

RT-PCR

The PRRSV RT-PCR was initially developed to detect PRRSV RNA in semen and serum of boars (Christopher-Hennings et al. 1995b, 1996, 2001). Various PRRSV PCR formats have since been described. Real-time RT-PCR (rRT-PCR) is widely used because it is analytically sensitive, analytically specific, and compatible with high-throughput testing. PRRSV rRT-PCR can also

quantify PRRSV genomic copies. Notably, current assays cannot differentiate RNA from infectious virus vs. noninfectious (inactivated) PRRSV. Commercial rRT-PCR assays are available for the detection of PRRSV-1 and/or PRRSV-2, but variation in diagnostic performance has been reported (Gerber et al. 2013; Harmon et al. 2012).

PRRSV RT-PCR can be conducted on all specimens typically collected for laboratory testing. During acute infection, serum and tissues are the preferred specimens. The duration of RT-PCR-detectable viremia differs among PRRSV strains, but conservatively is from 2 to 21 DPI. Under experimental conditions, approximately 50% and 10% of pigs were PCR positive at approximately 50 DPI and 100 DPI in serum, respectively (Horter et al. 2002; Molina et al. 2008). Virus persists longer in the tonsil and lymph nodes than in serum, lung, and other specimens. For example, virus was detected in tonsil and oropharyngeal scrapings at 130 and 157 DPI, respectively, under experimental conditions (Rowland and Yoo 2003; Wills et al. 2003).

Pooling (i.e. combining ≥2 individual samples into one sample) can not only improve testing efficiency and reduce costs but can also lead to false-negative test results. Gerber et al. (2013) reported that pooling five serum or blood swab samples did not reduce PRRSV rRT-PCR detection rates if the pool contained at least one positive sample with a relatively high concentration of viral RNA. Pooling of population-based samples (e.g. oral fluid and processing fluid samples) for PRRSV RT-PCR testing is not recommended.

Sequencing

Prior to the ready availability of sequencing, PRRSV isolates were identified and differentiated by ORF5 RFLP (Umthun and Mengeling 1999; Wesley et al. 1998). RFLP patterns do not characterize genetic relatedness among viruses or predict viral virulence (van Geelen et al. 2018). Although sequencing renders the use of RFLP highly questionable, RFLP will continue to be used until an equally convenient system for describing PRRSV isolates is established.

Sequencing and phylogenetic analyses provide precise and accurate genetic characterizations of PRRSV isolates and the means for comparing them. Sequence identity matrices and dendrograms (phylogenetic trees) are used to compare PRRSV isolates at the genetic level. ORF5 (major envelope protein) is the most common target for PRRSV sequencing. ORF5 sequences are highly variable, and there is an extensive databank of sequences available for comparison. However, ORF5 is only approximately 4% of the entire PRRSV genome, or 12% of the structural genes, and thus may be insufficient to provide the breadth of evidence needed for differentiating PRRSV strains.

Whole genome sequencing, an approach that provides more comprehensive information for genetic comparisons, has become more commonplace with the development of next-generation sequencing (NGS) technology. NGS can determine whole genome sequences faster and at lower cost than previous technologies. High-throughput whole genome sequencing of PRRSV from cell culture materials and clinical specimens using NGS has been described (Zhang et al. 2017).

In situ hybridization (ISH)

An ISH test using a PRRSV-specific nucleic acid probe has been described for the detection of PRRSV in cell culture and formalin-fixed tissues (Larochelle et al. 1996), but is not widely used in diagnostic laboratories.

Reverse transcription loop-mediated isothermal amplification

LAMP assay is similar to RT-PCR in that a specific fragment of the DNA is amplified, but LAMP can be done at a constant temperature in a water bath or heat block with no need of a thermal cycler. A number of RT-LAMP assays have been described for the detection of PRRSV (Li et al. 2009; Park et al. 2016; Rovira et al. 2009). While RT-LAMP tests have lower diagnostic sensitivity than RT-PCR, there is potential for this technique to be adapted in laboratories where RT-PCR instrumentation is too expensive or technical to be implemented (Rovira et al. 2009).

Detection of antibody

PRRSV antibodies can be routinely detected in serum and oral fluid specimens (Benfield et al. 1992; Gerber et al. 2014; Kittawornrat et al. 2012a,b; Langenhorst et al. 2012; Ouyang et al. 2013; Rotolo et al. 2018; Sattler et al. 2015). Single serum samples are of limited value due to the high prevalence of PRRSV in herds. For that reason, a single positive antibody result does not prove a causal role for PRRSV in a clinical diagnosis. In addition, detection of antibodies in weaned pigs may represent circulating maternal (IgG) PRRSV antibodies (Rotolo et al. 2018). Therefore, seroconversion (negative to positive) and/or rising levels of PRRSV-specific antibody in serum or oral fluid samples collected over time are used to diagnose or track PRRSV infections. Notably, DIVA vaccines have not been developed, and current antibody assays cannot differentiate antibodies produced by infection from those produced by MLV vaccines.

The tests most commonly used to detect antibodies to PRRSV are immunoperoxidase monolayer assay (IPMA), indirect fluorescent antibody (IFA), ELISA, and VN. The fluorescent microsphere immunoassay (FMIA) has been used to detect PRRSV antibodies in serum and oral fluid samples (Gerber et al. 2014; Langenhorst et al. 2012), but

has not been widely implemented in diagnostic laboratories.

IPMA and IFA may be performed using either PAM or MA-104 cells (Pejsak and Podgórska 2015). In particular, IFA performance (diagnostic sensitivity, diagnostic specificity, and reproducibility) can be affected by the technical skill of laboratory personnel and antigenic differences between the PRRSV isolate used in the IFA and the field virus that induced antibodies in the pig. To a large extent, these tests have been replaced by antibody ELISAs.

Commercial antibody ELISAs are widely available for the detection of IgG antibodies against PRRSV nucleocapsid antigens in serum and oral fluid samples, albeit commercial PRRSV ELISAs vary in diagnostic performance (Sattler et al. 2014). ELISAs based on nsp 1, 2, 7, and 9 (Brown et al. 2009; Cong et al. 2013; Janková and Celer 2012; Molina et al. 2008) have been used in research, but have not been commercialized.

The ELISA format is considered diagnostically sensitive, diagnostically specific, and compatible with high-throughput testing. Most commercial ELISAs are designed to detect antibodies to both PRRSV-1 and PRRSV-2, but some are genotype specific. Antibody can be detected by ELISA in serum or oral fluid samples as early as 9 DPI, but the response varies among individual pigs and among virus isolates (Horter et al. 2002; Johnson et al. 2004). Johnson et al. (2004) found that virulent PRRSV isolates induced a faster and stronger humoral immune response. There is no detectable difference in antibody response between persistently infected animals and those that have cleared the virus, i.e. ELISA results cannot be used to predict carrier status (Fangman et al. 2007; Horter et al. 2002; Molina et al. 2008).

Commercial ELISAs typically target IgG antibodies because of the higher concentration of IgG compared with IgM and IgA in the samples. The presence of maternal IgG antibody in young pigs can make it difficult to determine whether a positive ELISA result is due to infection, vaccination, or maternal antibody. However, Rotolo et al. (2018) reported that a combined IgM–IgA oral fluid ELISA was able to detect PRRSV-specific IgM and IgA antibody produced by pigs in response to PRRSV infection, despite the presence of maternal IgG.

The VN test detects antibodies capable of neutralizing a constant amount of PRRSV in cell culture. Neutralizing antibody appears approximately 4 weeks after infection and persists for at least 210 days (Meier et al. 2003; Molina et al. 2008; Wills et al. 1997b). Neutralizing antibodies can completely inactivate homologous virus but may only partially neutralize heterologous virus isolates (Lopez and Osorio, 2004; Robinson et al. 2015). Therefore, like IFA, the magnitude of the VN response will depend, in part, on the homology between the PRRSV isolate used in the test and the field virus that induced antibodies in the pig. The VN test has not been standardized between laboratories and is not generally used as a routine diagnostic.

Immunity

Infection with PRRSV induces immunity that eventually controls the initial infection, eliminates the virus, and establishes memory that is variably protective against future infection. Live virus is necessary for induction of immune protection. Killed virus, subunit proteins, and other experimental vaccines that do not contain live PRRSV do not induce effective protection against PRRSV. The properties that confer protection have not been identified despite extensive examination of major and minor envelope proteins and testing of recombinant viruses containing nonstructural and structural coding regions implicated in virulence or protection.

Resistance to infection increases with age, i.e. sows are more resistant to viral replication and disease than weaned pigs (Klinge et al. 2009). Immune induction may be independent of age, but findings related to infection and immunity in young pigs should be interpreted cautiously when applied to older swine. Immunity to PRRSV appears to be the same for PRRSV-1 and PRRSV-2, but the majority of the research has been conducted on PRRSV-2 isolates and strains.

Humoral immune response

Humoral IgM antibodies appear within 1 week of infection against many PRRSV structural and nonstructural proteins (Brown et al. 2009; de Lima et al. 2006; Johnson et al. 2007; Molina et al. 2008; Mulupuri et al. 2008). The strength and kinetics of antibody responses is variable among individual proteins in any given pig and among individual pigs in a group.

It appears that humoral immunity to PRRSV, once acquired, is highly durable. However, anti-N antibodies may decay more rapidly even though virus is present in lymphoid tissues. Since many serodiagnostics are based on nucleocapsid antigen, it is possible to misdiagnose immune animals as nonimmune several months after exposure (Batista et al. 2004).

Anatomically, actively secreting and memory PRRSV-specific B cells reside in diverse lymphoid tissues, especially lymph nodes draining the lungs and genital areas, spleen, and tonsil (Mulupuri et al. 2008).

Neutralizing antibodies are key to the control of many viral infections, but their role in controlling PRRSV infection is controversial. The appearance of neutralizing antibodies in primary infection mainly occurs after viremia is already resolved, but neutralizing antibody titer is the best predictor of level and duration of viremia (Molina et al. 2008). Passive transfer of homologous

neutralizing antibodies was shown to prevent reproductive disease and viral transmission to offspring (Osorio et al. 2002). High titers of broadly neutralizing activity in naturally infected pigs can provide cross-protection against heterologous PRRSV (Robinson et al. 2015, 2018). The viral neutralization target appears to be one or more epitopes in the minor envelope glycoproteins (GP2, GP3, GP4) that form a trimeric complex that mediates cellular infection (Das et al. 2010). GP5 is not required for macrophage infection and anti-GP5 antibodies do not neutralize infection (Dobbe et al. 2001; Li and Murtaugh 2012). It seems likely that additional factors, including genetic variation in neutralizing epitopes, variation in immunodominance, epitope masking by glycosylation, and variation in host response, may contribute to individual variation in viral neutralizing capability. Since broadly neutralizing immune serum can control viremia, there may be a role for antibody-based strategies as an immunotherapeutic.

Cell-mediated immune response

T-cell immunity is poorly understood in swine due to an inability to expand antigen-specific T-cell populations *in vitro* and a lack of tools and reagents to examine antigen-specific responses *in vitro* or *in vivo*. In the case of PRRSV, except for one study, it has been difficult to demonstrate protein-specific T-cell proliferation or cytotoxicity in classical cell culture systems (Bautista et al. 1999). New approaches may address this problem (Jeong et al. 2010). Interferon-γ ELISPOT using live PRRSV in leukocyte cultures has shown a consistent T-cell response to PRRSV infection, but its significance is uncertain (Xiao et al. 2004). The source of interferon-γ is difficult to determine since it is produced by Th1 helper T cells, activated cytotoxic T cells, and natural killer cells. IFN-γ-secreting cells often increase with pig age, but there is no correlation with resolution of PRRSV infection (Klinge et al. 2009).

Cytokine expression that might indicate T-cell responses indicative of Th1 or Th2 regulation may not be informative in pigs (Klinge et al. 2009; Murtaugh et al. 2009). Regulatory T cells (Tregs) and the Th3 cytokine IL-10 are induced by PRRSV or PRRSV-infected dendritic cells (Gomez-Laguna et al. 2009; Silva-Campa et al. 2009; Wongyanin et al. 2010). Tregs may be important in PRRSV since the delayed induction of immunity and prolonged infection indicate the potential for a T-cell response that is suppressive rather than ameliorative.

Protective immunity

At the individual pig level, exposure to PRRSV elicits protection against subsequent challenge. Immunological protection is premised on the induction of memory B and T cells that persist in the body after all evidence of primary infection is gone. Memory B cells against structural and nonstructural viral proteins are present before viremia is extinguished (Mulupuri et al. 2008). Even though memory B cells appear to be quite abundant, there is no anamnestic response to viral challenge (Foss et al. 2002). Pigs consistently show substantial resistance to infection, but show no significant change in antibody levels, which is a hallmark of memory. These observations are relevant to vaccine development since they raise the possibility that the mechanism of immunological protection may not be entirely dependent on memory lymphocytes. In the related arterivirus of mice (lactate dehydrogenase-elevating virus (LDV)), resistance to infection is attributed to the depletion of permissive macrophages (Cafruny et al. 2003). No data shows that a similar mechanism exists against PRRSV in swine.

In the time before sterilizing immunity is established, it is possible that resistance to challenge could be due to ongoing immunity to the first exposure. One flaw in the understanding of protection is that almost all challenge experiments are performed before the first infection has been resolved. In these cases, it is not certain that memory is required. In commercial pig production, the duration of PRRSV infection (persistent infection) often exceeds the life span of market pigs (Molina et al. 2008).

Cross-protection

Vaccines based on specific PRRSV strains are dependent on induction of cross-protective immunity for efficacy. Cross-protection studies in the growing pig consistently show significant improvement in clinical indicators of health, lung pathology and histopathology, and growth performance (Johnson et al. 2004; Mengeling et al. 2003; Opriessnig et al. 2005; Wei et al. 2013). Studies in pregnant sows are less common but also show significant improvement in reproductive performance (Lager et al. 1999; Mengeling et al. 1999).

In the field, the degree of cross-protection is a major concern, particularly for reproductive PRRS, since outbreaks are known to occur in herds with solid immunity induced by regular vaccination or inoculation programs. Incomplete protection can give rise to viremic piglets that cause outbreaks in downstream nurseries. In contrast, vaccine intervention in a finisher outbreak that results in partial reduction in disease severity and reduces economic losses at market may be judged a success.

High levels of cross-protective antibodies are present in immune pigs (Robinson et al. 2015, 2018). However, the uneven translation to cross-protection in the field indicates that biological factors, in addition to viral genetic variation, affect its efficacy. These factors may include physiological variation in pig susceptibility to

infection (Klinge et al. 2009) and genetic variation in host tolerance to PRRSV infection (Halbur et al. 1998; Lewis et al. 2009; Petry et al. 2007).

Maternal immunity

The appearance of PRRSV in weaned pigs has been associated with the loss of maternal antibody, and the duration of maternal protection was correlated with neutralizing antibody titer (Chung et al. 1997). However, piglets nursing on nonimmune sows showed less severe disease and shorter duration of viremia after challenge than piglets nursing on immune sows (Shibata et al. 1998). The finding may indicate that infection occurred *in utero* or from virus shed in milk (Wagstrom et al. 2001). In PRRSV-1-vaccinated sows, maternally derived antibodies impaired humoral and cellular immune responses, though the impact on protection and viral transmission was not examined (Fablet et al. 2016).

Genetic resistance

The challenge of achieving effective immunological control of PRRSV has so far proven insurmountable. The discovery that CD163, a macrophage scavenger receptor, is the PRRSV receptor on permissive cells, combined with new technologies for germ-line modification of animals, has provided proof that pigs lacking CD163 alone are completely resistant to both PRRSV-1 and PRRSV-2 (Calvert et al. 2007; Prather et al. 2013; Wells et al. 2017; Whitworth et al. 2016). Deletion of CD163 compromises pig health so that gene-knockout pigs are not suitable for commercial production. A more subtle partial deletion of CD163 confers resistance to PRRSV-1 but not PRRSV-2 (Wells et al. 2017), although its potential side effects are not yet known. Given the power of these new technologies, developments in the search for pigs that are commercially viable yet genetically resistant to PRRSV and other pathogens will continue apace.

Prevention and control

Prevention

Prevention is the implementation of actions strategically designed to reduce the probability of introducing PRRSV into negative herds or new PRRSV variants into positive herds. Every swine premises is different, so the first step is to identify site-specific hazards. Examples of routine events with the potential to carry virus into a herd are listed in Table 41.1. The second step in biosecurity is to recognize that a series of failures must occur for PRRSV to be introduced into a population (Figure 41.1). These are the critical control points at which specific actions can be taken to prevent the introduction of the virus. Examples of such actions include the use of quarantine facilities and testing protocols for incoming breeding stock, sanitation and drying protocols for transport vehicles and incoming supplies, personnel entry protocols such as shower-in facilities or Danish entry systems, and insect control programs, i.e. screens, habitat management, and insecticides. For herds in swine-dense regions, PRRSV prevention may include the use of air filtration or air treatment systems. Filtration has been shown to effectively reduce the risk of the introduction of PRRSV and other airborne agents, such as *Mycoplasma hyopneumoniae*, under both laboratory and field conditions (Dee et al. 2010; Pitkin et al. 2009; Spronk et al. 2010).

Table 41.1 Examples of routine events with the potential to carry PRRSV into a swine herd.

Swine movement	People movement
Semen delivery	Entry of on-farm employees
Gilt delivery	Repair inside/outside barns
Cull removal	Other visitors (vets, vendors, etc.)
Weaned pig removal	
Vehicles/deliveries	Other hazards
Mortality removal	Pork/food product entry
Feed delivery	Manure removal
Propane/fuel delivery	Entry of other animals
Garbage removal	Entry of air/water
Tools/supplies delivery	

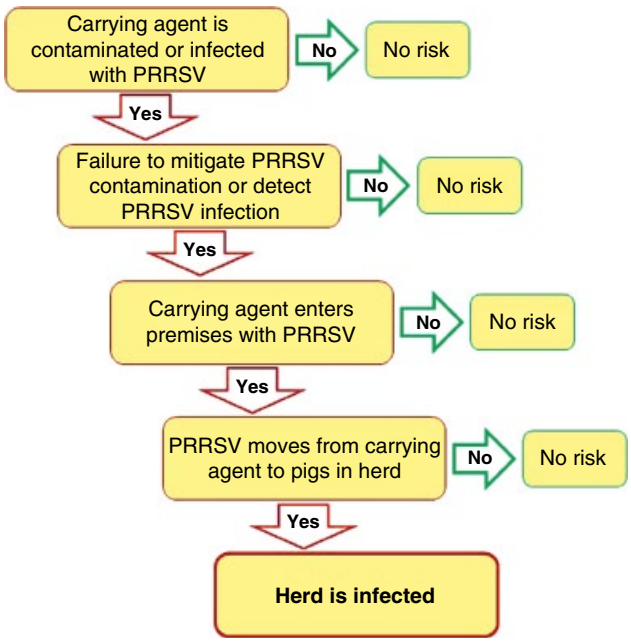


Figure 41.1 Chronological series of events required for PRRSV to be introduced into a swine herd.

Control

Specific treatments or methods to reduce the clinical effects of PRRSV are limited. Therefore, the objective of control is to ameliorate the adverse effects of the virus in the various stages of production. During an acute outbreak, it is common to administer anti-inflammatories to control fever along with antibiotics to manage secondary bacterial infections. As hematogenous spread of PRRSV via contaminated needles is well documented (Otake et al. 2002), changing needles between animals and/or the use of needle-free technologies may assist in controlling spread.

Chronically infected herds are best managed through pig flow strategies, such as McREBEL management in the farrowing house and all-in/all-out animal flow or partial depopulation, to prevent endemic viral circulation post weaning (Dee et al. 1994; McCaw 2000). The use of modified live vaccines has been demonstrated to reduce production and economic losses and the shedding of wild-type virus (Linhares et al. 2012b). Proper sanitation and disinfection of facilities is critical for reduction of viral load and subsequent challenge to incoming groups of healthy pigs.

Gilt acclimatization

A key aspect to any control program is management of the gilt pool (Dee et al. 1994). The introduction of naïve replacement gilts into an infected breeding population results in recurrent episodes of reproductive failure and both horizontal and vertical transmission of virus from dam to offspring. To properly acclimatize incoming replacement gilts, many production systems use designated facilities for gilt development units (GDUs) (Dee et al. 1997). Replacement gilts enter these facilities at a young age, i.e. at weaning or 25 kg. This allows adequate time (4–6 months) for the development of protective immunity through the use of modified live vaccines and/or exposure to farm-specific wild-type virus via live virus inoculation (LVI).

LVI typically involves the administration of a standardized dose of a farm-specific PRRSV strain using serum from an infected animal. This method has inherent risks and requires a thoughtful approach and high-quality control standards. That is, while the safety and efficacy of commercial vaccines is well established, the same cannot be said for LVI. Therefore, to improve the safety of this approach, it is important to properly characterize the virus in the inoculum through nucleic acid sequencing, quantify the dose, and screen the material for the presence of adventitious agents.

Along with the use of a GDU to manage the immunity of replacement gilts, it is also important to develop a controlled approach of managing immunity in the breeding herd. Similar protocols (whole herd vaccination and/or LVI) have been applied to eliminate subpopulations of naïve animals within the chronically infected herd (Dee et al. 1996). Once again, proper preparation of the live virus inoculum as well as discussion of the potential side effects of whole herd administration of PRRSV-positive serum with farm personnel and ownership groups is important to properly manage expectations. Application of a comprehensive herd immunization approach has been shown to be successful in decreasing the time to PRRSV-negative weaned pig production and the improved biological and financial performance of the infected farm. More specifically the use of whole herd vaccination using a modified live vaccine in the face of outbreaks resulted in the recovery of production losses more rapidly and the reduction of total piglet loss than herds where immunization was performed by LVI. However, the production of negative groups of pigs (i.e. farm PRRSV stability) was achieved sooner when an LVI protocol was implemented (Linhares et al. 2014).

Eradication

Elimination of PRRSV from a population is justified by clear improvements in pig health and productivity. Spontaneous elimination of PRRSV from a herd has been described (Freese and Joo 1994), but is a near impossibility in contemporary production systems.

Several methods have been used to eliminate PRRSV (Corzo et al. 2010). These include total depopulation/repopulation, partial depopulation, test and removal, and herd closure. Among these, herd closure is the most common method to eliminate PRRSV from breeding herds.

Herd closure consists of the temporal interruption of introduction of replacement animals into an infected herd, while herd immunity builds after an outbreak or the intentional exposure of resident animals to PRRSV or after vaccination with a modified live vaccine (Corzo et al. 2010; Linhares et al. 2014; Molina et al. 2008; Torremorell et al. 2003). Herd closure is based on the premise that, although PRRSV produces a chronic persistent infection, the immune system is eventually able to clear the virus from all tissues.

Herd closure periods vary between herds based on different factors such as exposure method, the presence of prior immunity, type of virus, or management factors. The period for herd closure may range between 12 and 42 weeks, and a median of 26.6 weeks has been documented for a group of herds (Linhares et al. 2014). After that period, introduction of negative replacement animals follows the attrition or scheduled culling of the previously infected animals. The herd closure method is also referred as "load–close–expose" when groups of gilts are introduced prior to closure and exposure.

Total herd depopulation and repopulation is also a successful technique, but it is costly and may only be

justifiable if the elimination of other concurrent diseases is desired.

Partial depopulation is indicated for the elimination of the virus from growing pigs when shedding from the breeding population has completely stopped. This strategy is commonly used in combination with herd closure or mass vaccination and unidirectional pig flow protocols (Dee et al. 1993, 1997; Dee and Molitor 1998).

Elimination by test and removal has also been documented and consists of testing all individuals in the entire breeding herd to identify PRRSV-infected animals using antibody and nucleic acid tests and then removing any positive animals from the farm (Dee and Phillips 1998; Dee et al. 2004, 2005b). This method is more costly and labor intensive than herd closure, but it is an option for herds where the presence of persistently infected animals is considered a potential risk for failure of the program or where there is a desire to expedite the removal of serologically positive animals.

Surveillance

Prevention, control, and/or eradication efforts need ongoing population data to set baselines, track changes, and measure success (or failure). PRRSV infection is often silent, i.e. does not produce clinical signs. Therefore, assessments of population status vis-à-vis PRRSV must be based on testing. Serum is the traditional surveillance specimen, although other individual animal specimens may be used, e.g. semen and blood swabs. "Pooling" samples prior to testing, i.e. combining two or more discrete samples into one prior to testing, is one strategy for reducing test costs. However, more recent work has shown that oral fluid specimens and/or neonatal processing fluids provide more sensitive surveillance and with less effort expended on sample collection and at a lower testing cost (Kittawornrat et al. 2014; Lopez et al. 2018; Olsen et al. 2013a,b; Rotolo et al. 2017, 2018). Regardless of the approach selected, surveillance should be performed on a routine basis.

References

de Abin MF, Spronk G, Wagner M, et al. 2009. *Can J Vet Res* 73:200–204.

Albina E, Mesplede A, Chenut G, et al. 2000. *Vet Microbiol* 77:43–57.

Alfonso P, Frías-Lepoureau MT. 2003. PRRS in Central America and the Caribbean region. In Zimmerman J, Yoon K-J, ed. *The PRRS Compendium*, 2nd ed. Des Moines, IA: National Pork Board, pp. 217–220.

Allan G, McNeilly F, Ellis J, et al. 2000. *Arch Virol* 145:2421–2429.

Allende R, Laegreid WW, Kutish GF, et al. 2000. *J Virol* 74:10834–10837.

Amirpour Haredasht S, Polson D, Main R, et al. 2017. *BMC Vet Res* 13:163.

An TQ, Zhou YJ, Liu GQ, et al. 2007. *Vet Microbiol* 123:43–52.

An TQ, Tian ZJ, Xiao Y, et al. 2010. *Emerg Infect Dis* 16:365–367.

Anon. 1991. The new pig disease: conclusions reached at the seminar held. In The new pig disease. Porcine reproductive and respiratory syndrome. A report on the seminar/workshop held in Brussels on 29–30 April and organized by the European Commission (Directorate General for Agriculture), pp. 82–86.

Anon. 1992. Porcine reproductive and respiratory syndrome (PRRS or blue-eared pig disease). *Vet Rec* 130:87–89.

Arruda AG, Friendship R, Carpenter J, et al. 2016. *Prev Vet Med* 128:41–50.

Arruda AG, Friendship R, Carpenter J, et al. 2017. *Transbound Emerg Dis* 64:89–100.

Arruda AG, Sanhueza J, Corzo C, et al. 2018. *Transbound Emerg Dis* https://doi.org/10.1111/tbed.12875.

Batista L, Dee SA, Rossow KD, et al. 2004. *Vet Rec* 154:25–26.

Bautista EM, Goyal SM, Yoon I-J, et al. 1993. *J Vet Diagn Invest* 5:163–165.

Bautista EM, Suarez P, Molitor TW. 1999. *Arch Virol* 144:117–134.

Behrens EM, Canna SW, Slade K, et al. 2011. *J Clin Invest* 121:2264–2277.

Benfield DA, Nelson E, Collins JE, et al. 1992. *J Vet Diagn Invest* 4:127–133.

Benfield DA, Christopher-Hennings J, Nelson EA, et al. 1997. Persistent fetal infection of porcine reproductive and respiratory syndrome (PRRS) virus. In Proceedings of American Association of Swine Practitioner Annual Meeting, pp. 455–458.

Benfield DA, Nelson C, Steffen M, et al. 2000a. Transmission of PRRSV by artificial insemination using extended semen seeded with different concentrations of PRRSV. In Proceedings of American Association of Swine Practitioner Annual Meeting, pp. 405–408.

Benfield D, Nelson J, Rossow K, et al. 2000b. *Vet Res* 31:71.

Beura LK, Sarkar SN, Kwon B, et al. 2010. *J Virol* 84:1574–1584.

Bierk MD, Dee SA, Rossow KD, et al. 2001. *Can J Vet Res* 65:261–266.

Bilodeau R, Dea S, Sauvageau RA, et al. 1991. Porcine reproductive and respiratory syndrome in Quebec [letter]. *Vet Rec* 129:102–103.

Bloemraad M, de Kluijver EP, Petersen A, et al. 1994. *Vet Microbiol* 42:361–371.

Boddicker NJ, Waide EH, Rowland RRR, et al. 2012. *J Anim Sci* 90:1733–1746.

Boddicker NJ, Bjorkquist A, Rowland RRR, et al. 2014a. *Genet Sel Evol* 46:18.

Boddicker NJ, Garick DJ, Rowland RRR, et al. 2014b. *Anim Genet* 45:48–58.

Bøtner A. 2003. The PRRS situation in Denmark, Norway, Finland, and Sweden. In Zimmerman J, Yoon K-J, eds. *The PRRS Compendium*, 2nd ed. Des Moines, IA: National Pork Board, pp. 233–238.

Bøtner A, Nielsen J, Bille-Hansen V. 1994. *Vet Microbiol* 40:351–360.

Brockmeier S, Palmer M, Bolin S. 2000. *Am J Vet Res* 61:892–899.

Brockmeier SL, Loving CL, Palmer MV, et al. 2017. *Vet Microbiol* 203:6–17.

Brown E, Lawson S, Welbon C, et al. 2009. *Clin Vaccine Immunol* 16:628–635.

Busse FW, Alt M, Janthur I, et al. 1992. Epidemiologic studies on porcine epidemic abortion and respiratory syndrome (PEARS) in Lower Saxony of Germany. *Proc Congr Int Pig Vet Soc* 12:115.

Butler JE, Lager KM, Golde W, et al. 2014. *Immun Res* 59:81–108.

Cafruny WA, Jones QA, Haven TR, et al. 2003. *Virus Res* 92:83–87.

Calvert JG, Slade DE, Shields SL, et al. 2007. *J Virol* 81:7371–7379.

Calzada-Nova G, Husmann RJ, Schnitzlein WM, et al. 2012. *Vet Immunol Immunopathol* 148:116–125.

Cao J, Li B, Fang L, et al. 2012. *J Vet Diagn Invest* 24:767–771.

Carlsson U, Wallgren P, Renström LHM, et al. 2009. *Transbound Emerg Dis* 56:121–131.

Carman S, Sanford SE, Dea S. 1995. *Can Vet J* 36:776–777.

Chang CC, Yoon KJ, Zimmerman JJ, et al. 2002. *J Virol* 76:4750–4763.

Chang C-C, Yoon K-J, Zimmerman JJ. 2009. *J Swine Health Prod* 17:318–324.

Chen Z, Lawson S, Sun Z, et al. 2010. *Virology* 398:87–97.

Chiou M-T. 2003. An overview of PRRS in Taiwan. In Zimmerman J, Yoon K-J, eds. *The PRRS Compendium*, 2nd ed. Des Moines, IA: National Pork Board, pp. 281–283.

Cho JG, Dee SA, Deen J, et al. 2006a. *Am J Vet Res* 67:489–493.

Cho JG, Dee SA, Deen J, et al. 2006b. *Can J Vet Res* 70:297–301.

Choi C, Cho W, Kim B, et al. 2001. *J Comp Path* 127:106–113.

Christianson WT, Collins JE, Benfield DA, et al. 1992. *Am J Vet Res* 53:485–488.

Christianson WT, Choi CS, Collins JE, et al. 1993. *Can J Vet Res* 57:262–268.

Christopher-Hennings J, Nelson EA, Hines RJ, et al. 1995a. *J Vet Diagn Invest* 7:456–464.

Christopher-Hennings J, Nelson EA, Nelson JA, et al. 1995b. *J Clin Microbiol* 33:1730–1734.

Christopher-Hennings J, Nelson EA, Benfield DA. 1996. Detecting PRRSV in boar semen. *Swine Health Prod* 4(1):37–39.

Christopher-Hennings J, Nelson EA, Nelson JK, et al. 1997. *Am J Vet Res* 58:40–45.

Christopher-Hennings J, Holler L, Benfield D, et al. 2001. *J Vet Diagn Invest* 13:133–142.

Chung WB, Lin MW, Chang WF, et al. 1997. *Can J Vet Res* 61:292–298.

Ciacci-Zanella JR, Trombetta C, Vargas I, et al. 2004. Lack of evidence of porcine reproductive and respiratory syndrome virus (PRRSV) infection in domestic swine in Brazil. *Ciencia Rural* 34:449–455.

Collins JE, Benfield DA, Christianson WT, et al. 1992. *J Vet Diagn Invest* 4:117–126.

Cong Y, Huang Z, Sun Y, et al. 2013. *Virology* 444:310–316.

Cooper VL, Hesse RA, Doster AR. 1997. *J Vet Diagn Invest* 9:198–201.

Corzo C, Mondaca E, Wayne S, et al. 2010. *Virus Res*, 154:185–192.

Costers S, Lefebvre DJ, Delputte PL, Nauwynck HJ. 2008. *Arch Virol* 153:1453–1465.

Das PB, Dinh PX, Ansari IH, et al. 2010. *J Virol* 84:1731–1740.

Dea S, Bilodeau R, Athanassious R, et al. 1992. *Can Vet J* 33:552–553.

Decorte I, Van Campe W, Mostin L, et al. 2015. *J Vet Diagn Invest* 27:47–54.

Dee SA, Joo HS. 1994a. *Vet Rec* 135:6–9.

Dee SA, Joo HS. 1994b. *J Am Vet Med Assoc* 205:1017–1018.

Dee SA, Molitor TW. 1998. *Vet Rec* 143:474–476.

Dee SA, Phillips R. 1998. Using vaccination and unidirectional pig flow to control PRRSV transmission. *Swine Health Prod* 1(5):21–25.

Dee SA, Morrison RB, Joo HS. 1993. Eradication of PRRS virus using multi-site production and nursery depopulation. *Swine Health Prod* 1(5):20–23.

Dee SA, Joo HS, Pijoan C. 1994. Controlling the spread of PRRS virus in the breeding herd through management of the gilt pool. *Swine Health Prod* 3(2):64–69.

Dee SA, Joo HS, Henry S, et al. 1996. Detecting subpopulations after PRRS virus infection in large breeding herds using multiple serologic tests. *Swine Health Prod* 4(4):181–184.

Dee SA, Joo HS, Polson DD, et al. 1997. *Vet Rec* 140:247–248.

Dee S, Deen J, Burns D, et al. 2004. *Can J Vet Res* 68:208–214.

Dee SA, Torremorell M, Thompson R, et al. 2005a. *Can J Vet Res* 69:58.

Dee SA, Bierk MD, Deen J, et al. 2005b. *Can H Vet Res* 65:22–27.

Dee SA, Otake S, Deen J. 2010. *Virus Res* 154:177–184.

Delrue I, Van Gorp H, Van Doorsselaere J, et al. 2010. *BMC Biotechnol* 10:48.

van der Hoeven B, Oudshoorn D, Koster AJ, et al. 2016. *Virus Res* 220:70–90.

van der Linden IFA, Voermans JJM, van der Linde-Bril EM, et al. 2003. *Vaccine* 21:1952–1957.

Dewey C. 2000. *Vet Res* 31:84–85.

Dobbe JC, van der Meer Y, Spaan WJ, et al. 2001. *Virology* 288:283–294.

Done SH, Paton DJ. 1995. *Vet Rec* 136:32–35.

Duan X, Nauwynck HJ, Pensaert MB. 1997. *Vet Microbiol* 56:9–19.
Epperson B, Holler L. 1997. An abortion storm and sow mortality syndrome. In Proceedings of American Association of Swine Practitioner Annual Meeting, pp. 479–484.
Fablet C, Renson P, Eono F, et al. 2016. *Vet Microbiol* 192:175–180.
Fablet C, Renson P, Pol F, et al. 2017. *Vet Microbiol* 204:25–34.
Fairbanks K, Chase C, Benfield DA. 2002. *J Swine Health Prod* 10(2):87–88.
Fangman TJ, Kleiboeker SB, Coleman M. 2007. *J Swine Health Prod* 15:219–223.
Feitsma H, Grooten HJ, Schie FW. 1992. The effect of porcine epidemic abortion and respiratory syndrome (PEARS) on sperm production. In Proceedings of 12th International Congress on Animal Reproduction, pp. 1710–1712.
Feng WH, Laster SM, Tompkins M, et al. 2001. *J Virol* 75:4889–4895.
Feng WH, Tompkings MB, Xu JS, et al. 2002. *Virology* 302:363–372.
Feng Y, Zhao T, Nguyen T, et al. 2008. *Emerg Infect Dis* 14:1774–1776.
Foss DL, Zilliox MJ, Meier W, et al. 2002. *Viral Immunol* 15:557–566.
Freese WR, Joo HS. 1994. Cessation of porcine reproductive and respiratory syndrome (PRRS) virus spread in a commercial swine herd. *Swine Health Prod* 2(1):13–15.
Frydas IS, Nauwynck HJ. 2016. *Vet Microbiol* 182:156–162.
Frydas IS, Verbeeck M, Cao J, et al. 2013. *Vet Res* 44:73
Frydas IS, Trus I, Kvisgaard LK et al. 2015. *Vet Res* 46:37
Galina L, Pijoan C, Sitjar M, et al. 1994. *Vet Rec* 134:60–64.
Garner MG, Gleeson LJ, Holyoake PK, et al. 1997. *Aust Vet J* 75:596–600.
van Geelen AGM, Anderson TK, Lager KM, et al. 2018. *Virology* 513:168–174.
Gerber PF, O'Neil K, Owolodun O, et al. 2013. *J Clin Microbiol* 51:547.
Gerber PF, Giménez-Lirola LG, Halbur PG, et al. 2014. *J Virol Methods* 197:63–66.
Goldberg TL, Hahn EC, Weigel RM, et al. 2000. *J Gen Virol* 81:171–179.
Goldberg TL, Lowe JF, Milburn SM, et al. 2003. *Virology* 317:197–207.
Gomez-Laguna J, Salguero FJ, De Marco MF, et al. 2009. *Viral Immunol* 22:261–271.
Gordon SC. 1992. *Vet Rec* 130:513–514.
Grosse-Beilage E, Grosse-Beilage T. 1992. Epidemiological investigations into PEARS in Germany: Influence on reproduction. In Proceedings of 12th International Pig Veterinary Society Congress, p. 125.
van Gucht S, Van Reeth K, Pensaert M. 2003. *J Clin Microbiol* 41:960–966.
Guo B, Lager KM, Henningson JN, et al. 2013a. *Virol* 435:372–384.
Guo B, Lager KM, Schlink SN, et al. 2013b. *Virol* 446:238–250.
Halbur PG. 2003. Factors that influence the severity of clinical disease. In Zimmerman J, Yoon K-J, eds. *The PRRS Compendium*, 2nd ed. Des Moines, IA: National Pork Board, pp. 17–25.
Halbur PG, Bush E. 1997. Update on abortion storms and sow mortality. *Swine Health Prod* 5(2):73.
Halbur PG, Andrews JJ, Huffman EL, et al. 1994. *J Vet Diagn Invest* 6:254–257.
Halbur PG, Miller LD, Paul PS, et al. 1995a. *Vet Pathol* 32:200–204.
Halbur PG, Paul PS, Frey ML, et al. 1995b. *Vet Pathol* 32:648–660.
Halbur PG, Paul PS, Frey ML, et al. 1996a. *Vet Pathol* 33:159–170.
Halbur PG, Paul PS, Meng XJ, et al. 1996b. *J Vet Diagn Invest* 8:11–20.
Halbur PG, Rotschild MF, Thacker BJ, et al. 1998. *J Anim Breed Genet* 115:181–189.
Han J, Zhou L, Ge X, et al. 2017. *Vet Microbiol* 209:30–47.
Harding JCS, Ladining A, Novakovic P, et al. 2017. *Vet Microbiol* 209:114–123.
Harmon KM, Abate SA, Chriswell AJ, et al. 2012. *J Swine Health Prod* 20:184–188.
Harms P, Sorden S, Halbur P, et al. 2001. *Vet Pathol* 38:528–539.
He Y, Wang G, Liu Y, et al. 2012. *Vet Microbiol* 160:455–462.
Hermann JR, Muñoz-Zanzi CA, Roof MB, et al. 2005. *Vet Microbiol* 110:7–16.
Hess AS, Islam Z, Hess MK, et al. 2016. *Genet Sel Evol* 48:43.
Hill H. 1990. Overview and history of Mystery Swine Disease (Swine infertility/respiratory syndrome). In Proceedings of the Mystery Swine Disease Committee Meeting, Livestock Conservation Institute, Denver, Colorado, pp. 29–31.
Hirose O, Kudo H, Yoshizawa S, et al. 1995. *J Jpn Vet Med Assoc* 48:650–653.
Holtkamp DJ, Kliebenstein JB, Neumann EJ, et al. 2013. *J Swine Health Prod* 21:72–84.
Hooper CC, Van Alstine WG, Stevenson GW, et al. 1994. *J Vet Diagn Invest* 6:13–15.
Hopper SA, White ME, Twiddy N. 1992. *Vet Rec* 131:140–144.
Horter DC, Pogranichniy RC, Chang CC, et al. 2002. *Vet Microbiol* 86:213–218.
Houben S, van Reeth K, Pensaert MB. 1995. *Zentralbl Veterinarmed B* 42:209–215.
Hu H, Li X, Zhang Z, et al. 2009. *Arch Virol* 154:391–398.
Hu SP, Zhang Z, Liu YG, et al. 2013. *Transbound Emerg Dis* 60:351–359.
Jacobs AC, Hermann JR, Muñoz-Zanzi C, et al. 2010. *J Vet Diagn Invest* 22:257–260.
Janková J, Celer V. 2012. *Res Vet Sci* 93:1537–1542.
Jeong HJ, Song YJ, Lee SW, et al. 2010. *Clin Vaccine Immunol* 17:503–512.
Johnson W, Roof M, Vaughn E, et al. 2004. *Vet Immunol Immunopathol* 102:233–247.
Johnson CR, Yu W, Murtaugh MP. 2007. *J Gen Virol* 88:1184–1195.

Johnson CR, Griggs TF, Gnanandarajah JS, et al. 2011. *J Gen Virol* 92:1107–1116.
de Jong MF, Cromwijk W, Van't Veld P. 1991. The new pig disease: Epidemiology and production losses in the Netherlands. In The new pig disease. Porcine reproductive and respiratory syndrome. A report on the seminar/workshop held in Brussels on 29–30 April and organized by the European Commission (Directorate General for Agriculture), pp. 9–19.
Karniychuk UU, Nauwynck HJ. 2013. *Vet Res* 44:95.
Karniychuk UU, Geldhof M, Vanhee M, et al. 2010. *BMC Vet Res* 6:30
Karniychuk UU, Saha D, Galdhof M, et al. 2011. *Microbial Path* 51:194–202.
Keffaber KK. 1989. Reproductive failure of unknown etiology. *Am Assoc Swine Pract News Lett* 1:1–10.
Keffaber K, Stevenson G, Van Alstine W, et al. 1992. SIRS virus infection in nursery/grower pigs. *Am Assoc Swine Pract News Lett* 4:38–39.
Kim B. 2008. Making sense of PRRS virus sequences and a new view for PRRS inactivated vaccine – MJPRRS™: Old problem – new approach. *Proc Allen D. Leman Swine Conference* 35:28.
Kim HS, Kwang J, Yoon IJ, et al. 1993. *Arch Virol* 133:477–483.
Kittawornrat A, Engle M, Johnson J, et al. 2010. *Virus Res* 154:170–176.
Kittawornrat A, Prickett J, Wang C, et al. 2012a. *J Vet Diagn Invest* 24:262–269.
Kittawornrat A, Wang C, Anderson G, et al. 2012b. *J Vet Diagn Invest* 24:1057–1063.
Kittawornrat A, Panyasing Y, Goodell C, et al. 2014. *Vet Microbiol* 168:331–339.
Klinge KL, Vaughn EM, Roof MB, et al. 2009. *Virol J* 6:177.
Kranker S. Nielsen J, Bille-Hansen V, et al. 1998. *Vet Microbiol* 61:21–31.
Labarque G, Van Reeth K, Van Gucht S, et al. 2002. *Vet Microbiol* 88:1–12.
Labarque G, Van Gucht S, Nauwynck H, et al. 2003. *Vet Res* 34:249–260.
Ladinig A, Wilkinson J, Ashley C, et al. 2014. *PLoS One* 9:e96104.
Ladinig A, Ashley C, Detmer SE, et al. 2015a. *Vet Res* 46:107.
Ladinig A, Detmer SE, Clarke K, et al. 2015b. *Virus Res* 203:24–35.
Lager KM, Halbur PG. 1996. *J Vet Diagn Invest* 8:275–282.
Lager KM, Mengeling WL. 1995. *Can J Vet Res* 59:187–192.
Lager KM, Mengeling WL, Brockmeier SL. 1996. *Vet Rec* 138:227–228.
Lager KM, Mengeling WL, Brockmeier SL. 1997a. *Vet Microbiol* 58:113–125.
Lager KM, Mengeling WL, Brockmeier SL. 1997b. *Vet Microbiol* 58:127–133.
Lager KM, Mengeling WL, Brockmeier SL. 1999. *Am J Vet Res* 60:1022–1027.
Langenhorst RJ, Lawson S, Kittawornrat A, et al. 2012. *Clin Vaccine Immunol* 19:180–189.
Larochelle R, Mardassi H, Dea S, et al. 1996. *J Vet Diagn Invest* 8:3–10.
Le Potier M-F, Blanquefort P, Morvan E, et al. 1997. *Vet Microbiol* 55:355–360.
Lee SM, Kleiboeker SB. 2007. *Virology* 365:419–434.
Lee K, Polson D, Lowe E, et al. 2017. *Prev Vet Med* 138:113–123.
Lewis CR, Torremorell M, Galina-Pantoja L, et al. 2009. *J Anim Sci* 87: 876–884.
Leyk W. 1991. Observations in three affected herds in North Rhine Westphalia. In The new pig disease. Porcine reproductive and respiratory syndrome. A report on the seminar/workshop held in Brussels on 29–30 April and organized by the European Commission (Directorate General for Agriculture), pp. 3–4.
Li J, Murtaugh MP. 2012. *Virology* 433:367–376.
Li Y, Wang X, Bo K, et al. 2007. *Vet J* 174:577–84.
Li Q, Zhou Q-F, Xue C-Y, et al. 2009. *J Virol Methods* 155:55–60.
Li Y, Wang G, Liu Y, et al. 2014. *Virus Res* 189:29–33.
de Lima M, Pattnaik AK, Flores EF, et al. 2006. *Virology* 353:410–421.
Linhares DCL, Torremorell M, Joo HS, et al. 2012a. *Vet Microbiol* 160:23–28.
Linhares DCL, Cano JP, Wetzell T, et al. 2012b. *Vaccine* 30:407–413.
Linhares DCL, Cano JP, Torremorell M, et al. 2014. *Prev Vet Med* 116:111–119.
Lopez OJ, Osorio FA. 2004. *Vet Immunol Immunopathol* 102:155–163.
Lopez WA, Angulo J, Zimmerman JJ, et al. 2018. *J Swine Health Prod* 26:146–150.
Loula T. 1991. Mystery pig disease. *Agri-practice* 12:23–34.
Loving CL, Boockmeier SL, Sacco RE. 2007. *Immunology* 120:217–229.
Lowe J, McCann R, Greiner L. 2017. *J Swine Health Prod* 25:19–23.
Lunney JK, Fang Y, Ladinig A, et al. 2016. *Annu Rev Anim Biosci* 4:129–154.
Maes D. 1997. *Epidémiol Santé Anim* 31–32.
Mateusen B, Van Soom A, Maes DGD, et al. 2007. *Biol Reprod* 76:415–423.
McCaw MB. 2000. *J Swine Health Prod* 8(1):15–21
Meier WA, Galeota J, Osoriao FA, et al. 2003. *Virology* 309:18–31.
Mengeling WL, Lager KM, Vorwald AC. 1994. *Am J Vet Res* 55:1391–1398.
Mengeling WL, Lager KM, Vorwald AC. 1995. *J Vet Diagn Invest* 7:3–16.
Mengeling WL, Vorwald AC, Lager KM, et al. 1996. *Am J Vet Res* 57:834–839.
Mengeling WL, Lager KM, Vorwald AC. 1998. *Am J Vet Res* 59:1540–1544.
Mengeling WL, Lager KM, Vorwald AC. 1999. *Am J Vet Res* 60:796–801.
Mengeling WL, Lager KM, Vorwald AC, et al. 2003. *Vet Microbiol* 93:25–38.

Metwally S, Mohamed F, Faaberg K, et al. 2010. *Transbound Emerg Dis* 57:315–329.
Molina RM, Cha SH, Chittick W, et al. 2008. *Vet Immunol Immunopathol* 126:283–292.
Molina-Barrios RM, Luevano-Adame J, Henao-Díaz A, et al. 2018. Collared peccary (Pecari tajacu) are susceptible to porcine reproductive and respiratory syndrome virus (PRRSV). *Trans Emerg Dis*. doi: 10.1111/tbed.12944.
Moore C. 1990. Clinical presentation of mystery swine disease in the growing pig. In Proceedings of the Mystery Swine Disease Committee Meeting, Livestock Conservation Institute, Denver, Colorado, pp. 41–49.
Morgan SB, Frossard JP, Pallares FJ, et al. 2014. *Transbound Emerg Dis* 63:285–295.
Mortensen S, Stryhn H, Sogaard R, et al. 2002. *Prev Vet Med* 53:83–101.
Motha J, Stark K, Thompson J. 1997. New Zealand is free from PRRS, TGE, and PRCV. *Surveillance* 24:10–11.
Mousing J, Permin A, Mortensen S, et al. 1997. *Vet Microbiol* 55:323–328.
Mulupuri P, Zimmerman JJ, Hermann J, et al. 2008. *J Virol* 82:358–370.
Murtaugh MP, Johnson CR, Xiao Z, et al. 2009. *Dev Comp Immunol* 33:344–352.
Nathues C, Zimmerli U, Hauser R, et al. 2014. *Transbound Emerg Dis* 61:546–554.
Nathues C, Perler L, Bruhn S, et al. 2016. *Transbound Emerg Dis* 63:e251–e261.
Nathues H, Alarcon P, Rushton J, et al. 2017. *Prev Vet Med* 142:16–29.
Nelson EA, Christopher-Hennings J, Benfield DA. 1994. *J Vet Diagn Invest* 6:410–415.
Neumann EJ, Kliebenstein JB, Johnson CD, et al. 2005. *J Am Vet Med Assoc* 227:385–392.
Ni J, Yang S, Bounlom D, et al. 2012. *J Vet Diagn Invest* 24:349–354.
Nieuwenhuis N, Duinhof TF, van Nes A. 2012. *Vet Rec* 170:225.
Novakovic P, Harding JCS, Al-Dissi AN, et al. 2016. *PLoS One* 11(3):e0151198.
Novakovic P, Harding JCS, Al-Dissi AN, et al. 2017. *PLoS One* 12(3):e0173360.
Ohlinger VF, Pesch S, Bischoff C. 2000. *Vet Res* 31:86–87.
OIE (Office International des Épizooties). 1997. World Animal Health in 1996. Part 1. Reports on the Animal Health Status and Disease Control Methods and List A Disease Outbreaks – Statistics, p. 249.
Olsen C, Karriker L, Wang C, et al. 2013a. *Vet J* 19:158–163.
Olsen C, Wang C, Christopher-Hennings J, et al. 2013b. *J Vet Diagn Invest* 25:328–335.
Opriessnig T, Pallares FJ, Nilubol D, et al. 2005. *J Swine Health Prod* 13:246–253.
Osorio FA, Galeota JA, Nelson E, et al. 2002. *Virology* 302:9–20.
Otake S, Dee SA, Rossow KD, et al. 2002. *Vet Rec* 150:114–115.
Otake S, Dee S, Corzo C, et al. 2010. *Vet Microbiol* 145:198–208.
Ouyang K, Binjawadagi B, Kittawornrat A, et al. 2013. *Clin Vaccine Immunol* 20:1305–1313.
Park BK, Yoon IJ, Joo HS. 1996. *Am J Vet Res* 57:320–323.
Park JY, Park S, Park YR, et al. 2016. *J Virol Methods* 237:10–13.
Pedersen K, Miller RS, Musante AR, et al. 2018. *J Swine Health Prod* 26:41–44.
Pejsak Z, Podgórska K. (updated by Ruggli N, Stadejek T). 2015. Chapter 2.8.6: Porcine reproductive and respiratory syndrome virus. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2017. World Organisation for Animal Health (on-line).
Perfumo CJ, Sanguinetti HR. 2003. Argentina: serological studies on PRRS virus. In Zimmerman J, Yoon K-J, eds. *The PRRS Compendium*, 2nd ed. Des Moines, IA: National Pork Board, pp. 209–211.
Petry DB, Lunney J, Boyd P, et al. 2007. *J Anim Sci* 85:2075–2092.
Pirtle EC, Beran GW. 1996. *J Am Vet Med Assoc* 208:390–392.
Pitkin AN, Deen J, Dee SA. 2009. *Vet Microbiol* 136:1–7.
Plana Duran J, Vayreda M, Vilarrasa J, et al. 1992. *Vet Microbiol* 33:203–211.
Pol JM, van-Dijk JE, Wensvoort G, et al. 1991. *Vet Q* 13:137–143.
Prather RS, Rowland RRR, Ewen CL, et al. 2013. *J Virol* 87:9538–9546.
Prickett JR, Zimmerman JJ. 2010. *Anim Health Res Rev* 11:207–216.
Prieto C, Suarez P, Sanchez R, et al. 1994. Semen changes in boars after experimental infection with porcine epidemic abortion and respiratory syndrome (PEARS) virus. Proceedings of the 13th International Pig Veterinary Society Congress, p. 98.
Prieto C, Sanchez R, Martin-Rillo S, et al. 1996a. *Vet Rec* 138:536–539.
Prieto C, Suarez P, Martin-Rillo S, et al. 1996b. *Theriogenology* 46:687–693.
Qiao S, Feng L, Bao D, et al. 2011. *Vet Microbiol* 149:213–220.
Ramos N, Mirazo S, Castro G, et al., 2018. *Transbound Emerg Dis* 65:352–356.
Robinson SR, Li J, Nelson EA, et al. 2015. *Virus Res* 203:56–65.
Robinson SR, Rahe MC, Gray DK, et al. 2018. *Virus Res* 248:13–23.
Rosenfeld P, Turner PV, MacInnes JI, et al. 2009. *Can J Vet Res* 73:313–318.
Rossow KD, Bautista EM, Goyal SM, et al. 1994. *J Vet Diagn Invest* 6:3–12.
Rossow KD, Collins JE, Goyal SM, et al. 1995. *Vet Pathol* 32:361–373.
Rossow KD, Laube KL, Goyal SM, et al. 1996. *Vet Pathol* 33:95–99.
Rossow KD, Shivers JL, Yeske PE, et al. 1999. *Vet Rec* 144:444–448.
Rotolo ML, Sun Y, Wang C, et al. 2017. *Vet Microbiol* 209:20–29.

Rotolo ML, Giménez-Lirola L, Ji J, et al. 2018. *Vet Microbiol* 214:13–20.

Rovira A, Abrahante J, Murtaugh M, et al. 2009. *J Vet Diagn Invest* 21:350–354.

Rowland RRR. 2010. *Virus Res* 154:114–122.

Rowland RR, Yoo D. 2003. *Virus Res* 95:23–33.

Rowland RR, Steffen M, Ackerman T, et al. 1999. *Virology* 259:262–266.

Rowland RRR, Lunney J, Dekkers J. 2012. *Front Genet* 3:260.

Sanford E. 1992. Porcine epidemic abortion and respiratory syndrome (PEARS): Establishment and spread between 1987 and 1992 in Ontario, Canada. Proceedings of the 12th International Pig Veterinary Society Congress, p. 117.

Sattler T, Wodak E, Revilla-Fernndez S, et al. 2014. *BMC Vet Res* 10:300.

Sattler T, Wodak E, Schmoll F. 2015. *BMC Vet Res* 11:70.

Schneider PT, Zhang J, Ramirez A, et al. 2015. *J Swine Health Prod* 23:306–316.

Schroyen M, Eisley C, Koltes JE, et al. 2016. *BMC Gen* 17:196

Shi M, Lam TT, Hon CC, et al. 2010a. *J Virol* 84:8460–8469.

Shi M, Lam TT, Hon CC, et al. 2010b. *Vet Microbiol* 154:7–17.

Shibata I, Mori M, Uruno K. 1998. *J Vet Med Sci* 60:1285–1291.

Shin J-H, Kang Y-B, Kim Y-J, et al. 1993. *RDA J Agri Sci* 35:572–576.

Shirai J, Kanno T, Tsuchiya Y, et al. 2000. *J Vet Med Sci* 62:85–92.

Silva-Campa E, Flores-Mendoza L, Resendiz M, et al. 2009. *Virology* 387:373–379.

Sirinarumitr T, Zhang Y, Kluge J, et al. 1998. *J Gen Virol* 79:2989–2995.

Snijder EJ, Meulenberg JM. 2001. Arteriviruses. In Knipe DM, Howley PM, Griffin DE, et al., eds, *Fields Virology*, 4th ed. Philadelphia, PA: Lippincott Williams and Wilkins, pp. 1205–1220.

Spilman MS, Welbon C, Nelson E, et al. 2009. *J Gen Virol* 90:527–535.

Spronk G, Otake S, Dee S. 2010. *Vet Rec* 166:758–759.

Stadejek T, Oleksiewicz MB, Potapchuk D, et al. 2006. *J Gen Virol* 87:1835–1841.

Stadejek T, Stankevicius A, Murtaugh MP, et al. 2013. *Vet Microbiol* 165: 21–28.

Stankevicius A, Buitkuviene J, Deltuvytiene J, et al. 2014. Five years seroprevalence study of PRRSV in Lithuanian pig and wild boar populations. *Bull Vet Inst Pulawy* 58:379–383.

Stevenson GW, Van Alstine WG, Kanitz CL, et al. 1993. *J Vet Diagn Invest* 5:432–434.

Stockhofe-Zurwieden N, Navarro Camarro JA, Grosse-Beilage E, et al. 1993. *Zentralbl Veterinarmed B* 40:261–271.

Sur J-H, Cooper VL, Galeota JA, et al. 1996. *J Clin Microbiol* 34:2280–2286.

Sur J-H, Doster AB, Christian JS, et al. 1997. *J Virol* 71:9170–9179.

Sur J, Doster A, Osorio F. 1998. *Vet Pathol* 35:506–514.

Suradhat S, Thanawongnuwech R. 2003. *J Gen Virol* 84:2755–2760.

Swenson SL, Hill HT, Zimmerman JJ, et al. 1994a. *J Am Vet Med Assoc* 204:1943–1948.

Swenson SL, Hill HT, Zimmerman JJ, et al. 1994b. Artificial insemination of gilts with porcine reproductive and respiratory syndrome (PRRS) virus-contaminated semen. *Swine Health Prod* 2(6):19–23.

Terpstra C, Wensvoort G, Pol JMA. 1991a. *Vet Q* 13:131–136.

Terpstra C, Wensvoort G, Ter Laak EA. 1991b. The "new" pig disease: laboratory investigations. In: The new pig disease. Porcine reproductive and respiratory syndrome. A report on the seminar/workshop held in Brussels on 29–30 April 1991 and organized by the European Commission Directorate General for Agriculture, pp. 36–45.

Thanawongnuwech R, Thacker EL. 2003. *Viral Immunol* 16:357–367.

Thanawongnuwech R, Halbur PG, Andrews JJ. 1997a. *J Vet Diagn Invest* 9:334–337.

Thanawongnuwech R, Thacker E, Halbur P. 1997b. *Vet Immunol Immunopath* 59:323–335.

Thanawongnuwech R, Halbur P, Ackermann M, et al. 1998a. *Vet Pathol* 35:398–406.

Thanawongnuwech R, Thacker E, Halbur P. 1998b. *Vet Microbiol* 63:177–187.

Thanawongnuwech R, Brown G, Halbur P, et al. 2000a. *Vet Pathol* 37:143–152.

Thanawongnuwech R, Halbur PG, Thacker EL. 2000b. *Anim Health Res Rev* 1:95–102.

Thanawongnuwech R, Thacker B, Halbur P, et al. 2004. *Clin Diagn Lab Immunol* 11:901–908.

Tian K, Yu X, Feng Y, et al. 2007. *PLoS One* 2:e526.

Tian D, Wei Z, Zevenhoven-Dobbe JC, et al. 2012. *J Virol* 86:3701–3712.

Tong G, Qiu H. 2003. PRRS in China. In Zimmerman J, Yoon K-J, eds. *The PRRS Compendium*, 2nd ed. Des Moines, IA: National Pork Board, pp. 223–229.

Torremorell M, Henry S, Christianson WT. 2003. Eradication using herd closure. In Zimmerman J, Yoon K-J, eds. *The PRRS Compendium*, 2nd ed. Des Moines, IA: National Pork Board, pp. 157–161.

Torremorell M, Geiger JO, Thompson B, et al. 2004. Evaluation of PRRSV outbreaks in negative herds. *Proc Congr Int Pig Vet Soc* 1:103.

Torrison J, Vannier P, Albina E, et al. 1994. Incidence and clinical effect of PRRS virus in gilts on commercial swine farms. In Proceedings of the 13th International Pig Veterinary Society Congress, p. 511.

Umthun AR, Mengeling WL. 1999. *Am J Vet Res* 60:802–806.

Van Alstine WG, Kanitz CL, Stevenson GW. 1993. *J Vet Diagn Invest* 5:621–622.

Van Alstine WG, Popielarczyk M, Albergts SR. 2002. *J Vet Diagn Invest* 14:504–507.

Van Breedam W, Verbeeck M, Christiaens I, et al. 2013. *J Gen Virol* 94:1955–1960

Van Gorp H, Van Breedam W, Delputte PL, et al. 2008. *J Gen Virol* 89:2943–2953.

van Vugt JJFA, Storgaard T, Oleksiewicz MB, et al. 2001. *J Gen Virol* 82:2615–2620.

Wagstrom EA, Chang CC, Yoon KJ, et al. 2001. *Am J Vet Res* 62:1876–1880.
Wang G, Song T, Yu Y, et al. 2011. *Vet Immun Immunol* 142:170–178.
Wang G, He Y, Tu Y, et al. 2014. *Virology* 302:363–372.
Wang G, Yu Y, Tu Y, et al. 2015a. *PLoS One* 10:e0128292
Wang T, Wang X, Li X, et al. 2015b. *Vet Res* 46:14241.
Wang X, Marthaler D, Rovira A, et al. 2015c. *Virus Res* 210:34–41.
Wei Z, Zhang J, Zhuang J, et al. 2013. *Vaccine* 31:2062–2066.
Weigel RM, Firkins LD, Scherba G. 2000. *Vet Res* 31:87–88.
Wells KD, Bardot R, Whitworth KM, et al. 2017. *J Virol* 91:e01521-16.
Wensvoort G, Terpstra C, Pol JMA, et al. 1991. *Vet Q* 13:121–130.
Wesley RD, Mengeling WL, Lager KM, et al. 1998. *J Diagn Invest* 10:140–144.
White MEC. 1992a. *Pig Vet J* 28:62–68.
White MEC. 1992b. *Pig Vet J* 29:179–187.
Whitworth KM, Rowland RRR, Ewen CL, et al. 2016. *Nature Biotechnol* 34:20–22.
Wills RW, Zimmerman JJ, Yoon K-J, et al. 1997a. *Vet Microbiol* 57:69–81.
Wills RW, Zimmerman JJ, Yoon KJ, et al. 1997b. *Vet Microbiol* 55:231–240.
Wills RW, Osorio FA, Doster AR. 2000a. Susceptibility of selected non-swine species to infection with PRRS virus. In Proceedings of American Association of Swine Practitioner Annual Meeting, pp. 411–413.
Wills R, Gray J, Fedorka-Cray P, et al. 2000b. *Vet Microbiol* 71:177–192.
Wills RW, Doster AR, Galeota JA, et al. 2003. *J Clin Microbiol* 41:58–62.
Wissink EH, Kroese MV, van Wijk HA, et al. 2005. *J Virol* 79:12495–12506.
Wongyanin P, Buranapraditkun S, Chokeshai-Usaha K, et al. 2010. *Vet Immunol Immunopathol* 133:170–182.
Wu WH, Fang Y, Rowland RR, et al. 2005. *Virus Res* 114:177–181.
Wyckoff AC, Henke SE, Campbell TA, et al. 2009. *J Wildl Dis* 45:422–429.
Xiao Z, Batista L, Dee S, et al. 2004. *J Virol* 78:5923–5933.
Xu M, Wang S, Li L, et al. 2010. *Virol J* 7:184.
Yaeger M. 2002. *J Vet Diagn Invest* 14:15–19.
Yaeger MJ, Prieve T, Collins J, et al. 1993. Evidence for the transmission of porcine reproductive and respiratory syndrome (PRRS) virus in boar semen. *Swine Health Prod* 1(5):7–9.
Yang KS, Park PK, Kim JH. 2003. PRRS in the Republic of Korea. In Zimmerman J, Yoon K-J, eds. *The PRRS Compendium*, 2nd ed. Des Moines, IA: National Pork Board, pp. 253–255.
Yim-Im W, Huang H, Park J, et al. 2017. Comparison of PRRSV isolation from clinical samples using MARC-145 and ZMAC cell lines. In Conference of Research Workers in Animal Diseases, p 105.
Yoo D, Wootton SK, Li G, et al. 2003. *J Virol* 77:12173–12183.
Yoon K-J, Zimmerman JJ, Chang C-C, et al. 1999. *Vet Res* 30:629–638.
Yoon KJ, Christopher-Hennings J, Nelson EA. 2003. Diagnosis. In Zimmerman J, Yoon K-J, eds. *The PRRS Compendium*, 2nd ed. Des Moines, IA: National Pork Board, pp. 59–74.
Yuan S, Murtaugh MP, Faaberg KS. 2000. *Virology* 275:158–169.
Yuan S, Murtaugh MP, Schumann FA, et al. 2004. *Virus Res* 105:75–87.
Zejda JE, Barber E, Dosman JA, et al. 1994. *J Occup Med* 36:49–56.
Zhang H, Kono H. 2012. *Trop Agric Res* 23:152–159.
Zhang J, Zheng Y, Xia XQ, et al. 2017. *J Vet Diagn Invest* 29:41–50.
Zhao K, Ye C, Chang XB, et al. 2015. *J Virol* 89:10712–10716.
Zhou YJ, Hao XF, Tian ZJ, et al. 2008. *Transbound Emerg Dis* 55:152–164.
Zhou L, Zhang J, Zeng J, et al. 2009a. *J Virol* 83:5156–5167.
Zhou L, Chen S, Zhang J, et al. 2009b.*Virus Res* 145:97–105.
Zimmerman J, Sanderson T, Eernisse KA, et al. 1992. Transmission of SIRS virus from convalescent animals to commingled penmates under experimental conditions. *Am Assoc Swine Pract News Lett* 4(4):25.
Zimmerman J, Yoon K-J, Wills RW, et al. 1997a. *Vet Microbiol* 55:187–196.
Zimmerman JJ, Yoon K-J, Pirtle EC, et al. 1997b. *Vet Microbiol* 55:329–336.

42

Swinepox Virus

Tânia Rosária Pereira Freitas

Revelance

Swinepox occurs sporadically in pigs worldwide and is generally associated with poor sanitation. This is a skin disease and typically produces pustular (pox) skin lesions in localized areas, with more severe forms in young pigs (<3–4 months). The causative agent is swinepox virus (SwPV) and swine are the only susceptible species. It is noteworthy that pustular skin disease caused by vaccinia virus (VACV) was observed in pigs during the successful campaign to eradicate smallpox from humans using VACV-based vaccines (Delhon 2017). However, the recent emergence of VACV in bovine and buffalo (Medaglia et al. 2009; Singh et al. 2007) suggests the need to determine whether these VACV can spread to pigs.

Etiology

Poxviruses belong to the family *Poxviridae*, which includes two subfamilies: *Chordopoxvirinae* (members infecting vertebrates) and *Entomopoxvirinae* (members infecting insects) (Damon 2013). SwPV is the sole member of the genus *Suipoxvirus* in the subfamily *Chordopoxvirinae*. Genus *Orthopoxvirus* in this subfamily includes important poxviruses, such as variola virus, VACV, cowpox virus, monkeypox virus, and others.

The structure and the composition of the SwPV virion resemble those of the VACV, and large brick-shaped membrane-bound virus particles measuring approximately 240 × 310 nm may be observed by electron microscopy (Figure 42.1) (Condit et al. 2006; Conroy and Meyer 1971; Moussatche and Condit 2015).

Poxviruses contain a DNA genome and are the only known family of DNA viruses replicating and assembling in the cytoplasm of the host cell. The SwPV genome is a linear double-stranded DNA molecule of 146 kb containing a conserved central coding region with inverted terminal repeat sequences at both ends. The virus genome is predicted to contain 150 genes, of which 146 conserved genes encode proteins involved in basic replicative functions, viral virulence, host range, and immune evasion similar with other poxviruses (Afonso et al. 2002). Poxvirus-infected cells produce two forms of progeny virions: mature virions (MVs) and extracellular enveloped virions (EEVs) (Traktman 1996). As shown in Figure 42.2, viral genome DNA and nucleoprotein are organized as a nucleosome within the central biconcave core, and the core membrane has regular subunit structure. The virion core and lateral bodies are enclosed within the outer membrane (lipoprotein) to form the MV, which may acquire an additional envelope composed of host cellular membrane and virus-specific proteins to form the EEV (Figure 42.2). In infected cells, the majority of progeny virions are MVs that are released by budding or after cell lysis, whereas some virions (EEVs) exit the cell by exocytosis (Delhon 2017). MVs are very stable, whereas EEVs are relatively fragile (Delhon 2017).

SwPV appears to be antigenically distinct from other poxviruses. SwPV antibodies do not cross-react with vaccinia, cowpox, or fowlpox viruses in precipitation tests and do not cross-neutralize other poxviruses (de Boer 1975; Meyer and Conroy 1972; Ouchi et al. 1992). SwPV strains from various geographic regions have shown high nucleotide identity (96–100%) when compared to the genomic sequences of a SwPV prototype strain (SwPV-Nebraska) (Afonso et al. 2002; Borst et al. 1990; Medaglia et al. 2011; Riyesh et al. 2016), suggesting that there is little genetic variability among SwPV strains.

Successful SwPV virus isolation has been reported in primary cultures of pig kidney cells (de Boer 1975; Kasza et al. 1960; Paton et al. 1990) and/or continuous porcine kidney cell lines such as PK-15 (Garg and Meyer 1972; Paton et al. 1990; Riyesh et al. 2016). Cytopathic effects, characterized by intranuclear vacuoles, cytoplasmic inclusion bodies, and destruction of most of the cells, are observed starting from the second to fourth blind passages and in subsequent passages usually within 3–5 days

Diseases of Swine, Tenth Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson.

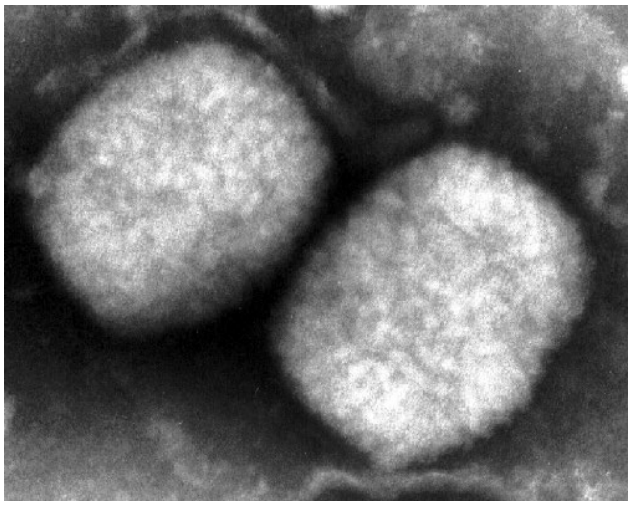


Figure 42.1 Negative stain of swinepox virus particles with characteristic brick shape and complex woven pattern of surface filaments. *Source:* Delhon et al. (2012). Reproduced with permission of John Wiley and Sons.

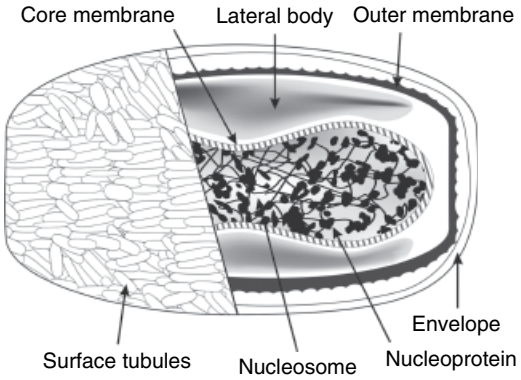


Figure 42.2 Schematic structure of a poxvirus virion.

post inoculation (de Boer 1975; Kasza et al. 1960; Riyesh et al. 2016). Attempts to isolate or grow SwPV in non-swine cell cultures or on chicken chorioallantoic membranes have been unsuccessful (Garg and Meyer 1972; Kasza et al. 1960; Meyer and Conroy 1972; Paton et al. 1990).

The restricted host range of SwPV and its ability to induce solid immune responses have stimulated interests in using SwPV as a vaccine vector (Foley et al. 1991; Tripathy 1999). SwPV has been used for expressing proteins of some swine pathogens, such as pseudorabies virus (van der Leek et al. 1994), classical swine fever virus (Hahn et al. 2001), swine influenza virus (Xu et al. 2013), *Streptococcus suis* (Huang et al. 2012), porcine circovirus 2 (Lin et al. 2012), porcine reproductive and respiratory syndrome virus (Lin et al. 2017), transmissible gastroenteritis virus, and porcine epidemic diarrhea virus (Yuan et al. 2015, 2017), as well as for expressing antigens of some non-swine pathogens (Winslow et al. 2003, 2005).

Public health

SwPV is not zoonotic and there is no evidence that SwPV is a threat in public health.

Epidemiology

Swinepox was first reported in North America in 1929 (McNutt et al. 1929) and thereafter in many regions of the world (Neufeld 1981; Shope 1940), including Europe (Borst et al. 1990; de Boer 1975; Moorkamp et al. 2008; Paton et al. 1990), Brazil (Medaglia et al. 2011), India (Manickam and Mohan 1987), and elsewhere.

The pig is the only natural host of SwPV. SwPV can infect pigs of all ages, but swinepox disease only occurs sporadically, and clinical disease is most commonly seen in young pigs. Under experimental conditions, SwPV failed to cause lesions in the scarified skin of rabbits and guinea pigs nor infected kidney cells from cattle and rabbits (Kasza et al. 1960).

SwPV is transmitted most commonly between swine by the bite of the pig louse, *Haematopinus suis*, whose prevalence in pig populations is variable and geographically dependent, for example, 96.1–100% in Kenya and 2.5% in Germany (Damriyasa et al. 2004; Kagira et al. 2013). Poor sanitation can increase susceptibility to lice infestation (Damriyasa et al. 2004). Flies (*Stomoxys calcitrans*) and mosquitoes may also be mechanical vectors for SwPV transmission (Fenner 1996). SwPV may also be horizontally transmitted between pigs by direct contact of nasal and oral secretions and by desquamated scabs coming into contact with skin abrasions. Vertical (transplacental) SwPV transmission is possible, as evidenced by sporadic cases of congenital infections (Borst et al. 1990; Neufeld 1981; Paton et al. 1990).

Smallpox eradication in humans using VACV-based vaccines was associated with the spread of VACV to pigs and the appearance of pustular skin lesions (Delhon 2017). Subsequent to successful smallpox eradication, swinepox is only associated with SwPV. However, since 1999 VACV has reappeared in some regions and is endemic in cattle (Medaglia et al. 2009) and in Asian buffalo herds (Singh et al. 2007). Differentiation of SwPV from VACV becomes relevant for evaluating whether VACV infection can spread to pigs.

Pathogenesis

SwPV enters the host through a break in the skin and then replicates in the cytoplasm of keratinocytes of the stratum spinosum, causing hydropic degeneration (Cheville 1966a; Meyer and Conroy 1972). Lymphadenitis of regional lymph nodes may occur, but virus has not

been isolated from lymph nodes (Kasza and Griesemer 1962). Infectious virus can be isolated from the skin lesion materials as early as 3 days post intradermal inoculation (Kasza and Griesemer 1962). It remains unclear how virus spreads from the primary site of replication to secondary sites; viremia has been proposed to account for this process, but substantiating evidence is needed. It has been suggested that congenital infections may result from viremic sows, but virus has not been isolated from blood samples from infected animals (Borst et al. 1990; Kasza and Griesemer 1962; Paton et al. 1990; Shope 1940).

Clinical signs

The clinical signs of SwPV infection are age dependent. Pigs of all ages are susceptible to SwPV, but the disease is most severe in piglets and young pigs <3–4 months of age. In contrast, SwPV infection in adults is usually mild and self-limiting. In young pigs that are affected, morbidity may be high, but the mortality is usually very low. In congenital infections, the affected piglets may be stillborn or die within a few days after birth, although the sows may appear clinically normal.

SwPV-induced cutaneous lesions are commonly found on the flanks, ventral abdomen, legs, inguinal areas, and ears and less frequently on the face near the eyes (Figure 42.3) (Delhon et al. 2012). These areas often coincide with insect vectors' preferred feeding areas. Lesions can also be found on the teats of sows and on skin surface, bulbs of the heels, coronets, face, lips, tongue, and oral mucosa of suckling piglets (Olufemi et al. 1981). In congenital SwPV infections, lesions may be observed over the entire body of the newborn piglets (Borst et al. 1990; Neufeld 1981; Paton et al. 1990).

The incubation period of SwPV infection may be 4–14 days under field conditions (de Boer 1975; McNutt et al. 1929) and 3–6 days in experimental inoculations (Kasza and Griesemer 1962; Paton et al. 1990). Lesions evolve from maculae, papulae, and vesicles to umbilicated lesions with pustular content, followed by crusting. At the macular stage, lesions are pale, flat, round spots 1–5 mm in diameter. In about 48 hours, these progress to

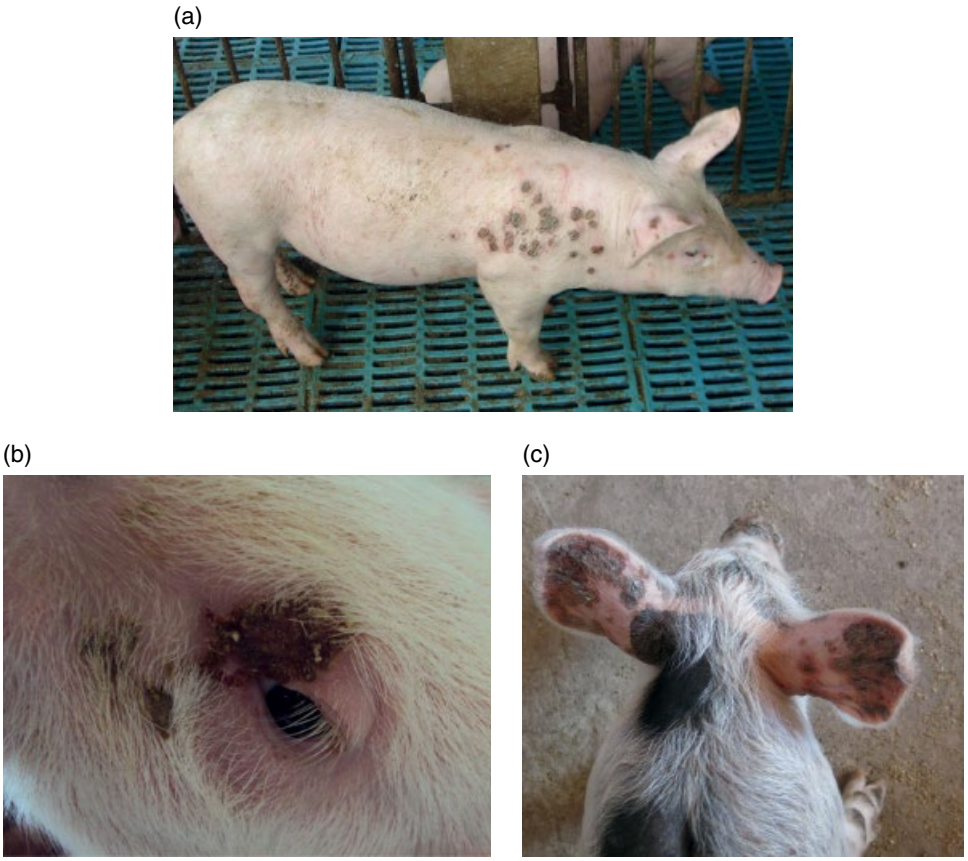


Figure 42.3 Swinepox lesions on skin. The pustular lesions in the dorsal and the lateral flank of young pig (a). The crust formation near the palpebrae (b) and the ears (c). *Source:* Images (a) and (b): Courtesy of Pereira, Adriana Cassia (Centro de Pesquisa e Patologia Animal, Paulinia, Sao Paulo, Brazil.); image (c): Courtesy of Robério G. Olinda (Centro de Saúde e Tecnologia Rural, Universidade Federal de Campina Grande, Campus de Patos, Patos, Paraíba, Brasil).

papulae 1–2 mm in height and 1–2 cm in diameter. In young animals, the appearance of papulae may be accompanied by slight elevation of body temperature and loss of appetite (Kasza and Griesemer 1962; Kasza et al. 1960). A true vesicular stage may be absent or inapparent (Borst et al. 1990; Kasza and Griesemer 1962). The lesions usually become umbilicated and then are replaced by crusts in about 1 week. Eventually crusts fall off, leaving spots on the skin. The recovery of swinepox could be delayed if secondary bacterial and/or parasitic infections occur (Fenner 1996).

Lesions

The classic histological alteration of SwPV infection is the hydropic degeneration of the stratum spinosum keratinocytes of the epidermis, occurring in the papular stage of lesions (Figure 42.4a). As a result, thickening of the epidermis due to the fluid accumulation within the cells can be observed. The epidermal hyperplasia caused by SwPV may be less marked when compared with other poxvirus infections (McNutt et al. 1929; Schwarte and Biester 1941). Another typical histopathological observation is inclusion bodies in the enlarged cytoplasm of infected cells (Figure 42.4b) (Cheville 1966a,b). Hydropic degeneration and inclusion bodies can also be observed in the outer root sheaths of the hair follicles (Kasza and Griesemer 1962; Meyer and Conroy 1972). Concomitantly, margination of chromatin and a large central vacuole (central nuclear clearing) can be seen in the nucleus (Cheville 1966a; Kasza and Griesemer 1962; Meyer and Conroy 1972).

The rupture and coalescence of epidermal cells may form a few small vesicles. Subsequently, infiltration of inflammatory cells (lymphocytes, neutrophils, eosinophils, and histiocytes) into the underlying dermis converts vesicles to pustules. The basal layer undergoes extensive necrosis with infiltration of leukocytes during the pustular stage. Prior to initiating regeneration of the epidermis, the necrosis of apical keratinocytes and crust formation are predominant. At this stage, the regional lymph nodes become edematous, hyperemic, and hyperplastic, but virus antigens may be detected in some cells (Cheville 1966b). In the crusting stage, the lesions mature and heal; the crusts desquamate and leave regenerated epithelium and a little keratinization as a white macula.

Diagnosis

A presumptive diagnosis of swinepox is based on the observation of typical pox lesions on the skin of affected animals, especially under poor sanitary conditions and with lice and/or fly infestations (Cheville 1966b). The differential diagnosis includes vesicular diseases (foot-and-mouth disease, vesicular exanthema of swine, vesicular stomatitis, swine vesicular disease, Seneca Valley virus), skin lesions associated with classical swine fever virus, erysipelas, pityriasis rosea, streptococcal dermatitis, vegetative dermatitis, parasitic skin disease, allergic skin lesion, nutritional disorders, and sunburn.

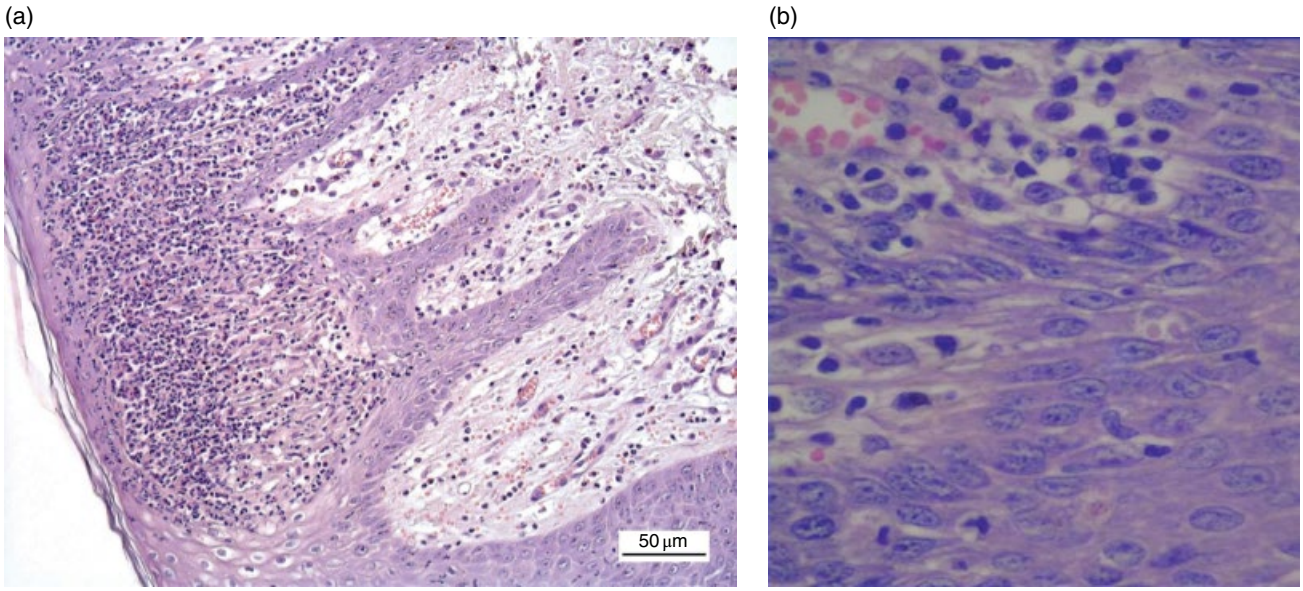


Figure 42.4 Histopathological changes caused by swinepox virus. (a) The severe hydropic degeneration on stratum spinosum of the epidermis. (b) Eosinophilic inclusion body in the cytoplasm of keratinocytes of the epidermis. Hematoxylin and eosin. *Source:* Courtesy of Robério G. Olinda (Centro de Saúde e Tecnologia Rural, Universidade Federal de Campina Grande, Campus de Patos, Patos, Paraíba, Brasil).

Examinations by electron microscopy and histopathology may confirm involvement of SwPV. SwPV infection has some pathognomonic histopathological changes in the epidermis: ballooned stratum spinosum keratinocytes containing cytoplasmic eosinophilic inclusion bodies and a "vacuolated" nucleus. Virus isolation in primary swine kidney cells or PK-15 cell line followed by immunofluorescence staining or virus neutralization using SwPV-specific antibodies is definite confirmation (Borst et al. 1990). Immunocytochemistry on skin samples or infected cell cultures can be a confirmatory method as well. Papular/pustular exudates or crusted materials are appropriate clinical samples for virus isolation. Multiple blind passages should be attempted before considering the sample negative by virus isolation test.

Serological assays to detect SwPV antibody include precipitation antibody tests, immunoelectroosmophoresis (IEOP), serum virus neutralization, and ELISA (Deng et al. 2013). Swine do not develop high levels of neutralizing antibody (Kasza et al. 1960; Shope 1940); therefore, negative neutralizing antibody results should be interpreted with caution.

SwPV-specific polymerase chain reaction (PCR) assays provide a rapid and sensitive method to reliably detect SwPV DNA in samples. This also allows for differentiation from VACV, when needed. In several countries, VACV is endemic in cattle or buffaloes, and there is a risk of VACV spreading to pigs. Differentiation of SwPV from VACV can be achieved using multiplex PCRs (Medaglia et al. 2011, 2015; Singh et al. 2007).

Immunity

Like other viruses, SwPV infection of pigs may elicit innate, humoral, and cell-mediated immune responses. However, SwPV can also potentially modulate the host immune responses (Afonso et al. 2002; Shchelkunov 2012). Limited information concerning the SwPV immune response is available. Convalescent swine are resistant to SwPV challenge, indicating that SwPV infection induces protective immunity (de Boer 1975; Garg and Meyer 1972; Kasza et al. 1960; Schwarte and Biester 1941; Shope 1940). SwPV neutralizing antibody may be detected in swine sera as early as 7 days post inoculation, but antibody titers are low and may become undetected at 50 days post inoculation (Kasza et al. 1960; Meyer and Conroy 1972; Shope 1940; Williams et al. 1989). Maternal antibody may protect suckling piglets (Nelson 1932), but high neonatal mortality and piglets born with skin lesions have also been reported (Borst et al. 1990; Neufeld 1981; Olufemi et al. 1981), indicating that passive protection may not be adequate.

Prevention and control

There is no specific treatment for swinepox. Antibiotic treatment is recommended if it is necessary to control secondary bacterial infections. Swinepox is of relatively low economic impact, and no vaccine has been developed. The best prevention and control is good animal husbandry, including ectoparasite control.

References

Afonso CL, Tulman ER, Lu Z, et al. 2002. *J Virol* 76:783–790.

de Boer GF. 1975. *Arch Virol* 49:141–150.

Borst GH, Kimman TG, Gielkens AL, et al. 1990. *Vet Rec* 127:61–63.

Cheville NF. 1966a. *Am J Pathol* 49:339–352.

Cheville NF. 1966b. *Pathol Vet* 3:556–564.

Condit RC, Moussatche N, Traktman P. 2006. *Adv Virus Res* 66:31–124.

Conroy JD, Meyer RC. 1971. *Am J Vet Res* 32:2021–2032.

Damon IK. 2013. Poxviruses. In Knipe DM, Howley PM, eds. *Fields Virology*, 6th ed. Philadelphia: Wolters Kluwer-Lippincott Williams & Wilkins, pp. 2160–2184.

Damriyasa IM, Failing K, Volmer R, et al. 2004. *Med Vet Entomol* 18:361–367.

Delhon GA. 2017. Poxviridae. In MacLachlan NJ, Dubovi EJ, eds. *Fenner's Veterinary Virology*, 5th ed. Cambridge, Amsterdam: Academic Press, Elsevier Inc., pp. 157–174.

Delhon G, Tulman ER, Afonso CL, et al. 2012. Swinepox virus. In Zimmerman JJ, Karriker LA, Ramirez A, et al., eds. *Diseases of Swine*, 10th ed. Hoboken: Wiley, pp. 456–460.

Deng S, Jiang X, Leng C, et al. 2013. *J Vet Sci* 10:1488–1492.

Fenner F. 1996. Poxviruses. In Knipe DM, Howley PM, eds. *Fields Virology*, 3rd ed. Philadelphia: Wolters Kluwer-Lippincott Williams & Wilkins, pp. 2673–2702.

Foley PL, Paul PS, Levings RL et al. 1991. *Ann N Y Acad Sci* 646:220–222.

Garg SK, Meyer RC. 1972. *Appl Microbiol* 23:180–182.

Hahn J, Park SH, Song JY, et al. 2001. *J Virol Methods* 93:49–56.

Huang D, Zhu H, Lin H, et al. 2012. First insights into the protective effects of a recombinant swinepox virus expressing truncated MRP of Streptococcus suis type 2 in mice. *Berl Munch Tierarztl Wochenschr* 125:144–152.

Kagira JM, Kanyari PN, Maingi N, et al. 2013. Relationship between the prevalence of ectoparasites and associated

risk factors in free-range pigs in Kenya. *ISRN Vet Sci* 2013:650890.
Kasza L, Griesemer RA. 1962. *Am J Vet Res* 23:443–450.
Kasza L, Bohl EH, Jones DO. 1960. *Am J Vet Res* 21:269–273.
van der Leek ML, Feller JA, Sorensen G, et al. 1994. *Vet Rec* 134:13–18.
Lin HX, Ma Z, Fan HJ, et al. 2012. *Vaccine* 30:6307–6313.
Lin H, Ma Z, Hou X, et al. 2017. *Sci Rep* 7:43990.
Manickam R, Mohan M. 1987. *Indian J Vet Med* 7:71–72.
McNutt SH, Murray C, Purwin P. 1929. *J Am Vet Med Assoc* 74:752.
Medaglia ML, Pessoa LC, Sales ERC, et al. 2009. *Emerg Infect Dis* 15:1142–1143.
Medaglia ML, Pereira AC, Freitas TRP, et al. 2011. *Emerg Infect Dis* 17:1976–1978.
Medaglia ML, Sá NM, Correa IA, et al. 2015. *J Virol Methods* 219:10–13.
Meyer RC, Conroy JD. 1972. *Res Vet Sci* 13:334–338.
Moorkamp L, Beineke A, Kaim U, et al. 2008. Swinepox–skin disease with sporadic occurrence. *Dtsch Tierarztl Wochenschr* 115:162–166.
Moussatche N, Condit RC. 2015. *Virology* 15:204–218.
Nelson JB. 1932. *J Exp Med* 56:835–840.
Neufeld JL. 1981. *Can Vet J.* 22:156–158.
Olufemi BE, Ayoade GO, Ikede BO, et al. 1981. *Vet Rec* 109:278–280.
Ouchi M, Fujiwara M, Hatano Y, et al. 1992. *J Vet Med Sci* 54:731–737.
Paton DJ, Brown IH, Fitton J, et al. 1990. *Vet Rec* 127:204.
Riyesh T, Barua S, Kumar N, et al. 2016. *Comp Immunol Microbiol Infect Dis* 46:60–65.
Schwarte LH, Biester HE. 1941. *Am J Vet Res* 2:136–140.
Shchelkunov SN. 2012. *Adv Virol.* 2012:524743.
Shope RE. 1940. Swine pox. *Arch Gesamte Virusforsch* 1:457–467.
Singh RK, Hosamani M, Balamurugan V, et al. 2007. *Anim Health Res Rev* 8:105–114.
Traktman P. 1996. Poxvirus DNA replication: cold Spring Harbor monographs. *DNA Replication Eukaryot Cells* 31:775–798.
Tripathy DN. 1999. *Adv Vet Med* 41:463–480.
Williams PP, Hall MR, McFarland MD. 1989. *Vet Immunol Immunopathol* 23:149–159.
Winslow BJ, Cochran MD, Holzenburg A, et al. 2003. *Virus Res* 98:1–15.
Winslow BJ, Kalabat DY, Brown SM, et al. 2005. *Vet Microbiol* 111:1–13.
Xu J, Huang D, Xu J, et al. 2013. *Vet Microbiol* 162:259–264.
Yuan X, Lin H, Fan H. 2015. *Vaccine* 33:3900–3906.
Yuan X, Lin H, Li B, et al. 2017. *Arch Virol* 162:3779–3789.

43

Reoviruses (Rotaviruses and Reoviruses)

Frances K. Shepherd, Matthew J. Freeman, Marie R. Culhane, and Douglas G. Marthaler

Overview

Viruses in the family *Reoviridae* are non-enveloped, icosahedral viruses with a capsid approximately 75 nm in diameter composed of a triple protein layer. The family *Reoviridae* includes two subfamilies, *Sedoreovirinae* and *Spinareovirinae*, of six and nine genera, respectively. Individual viruses within the family infect a variety of hosts, including mammals, birds, reptiles, amphibians, fish, mollusks, crustaceans, insects, plants, and fungi (Mertens et al. 2005). Viruses in this family infecting pigs are rotaviruses (RVs) in the genus *Rotavirus* in the subfamily *Sedoreovirinae* and reoviruses in the genus *Orthoreovirus* in the subfamily *Spinareovirinae*. The RV and reovirus genomes are composed of 11 and 10 segments of double-stranded (ds) RNA (dsRNA), respectively. Reassortment is common within reoviruses and each RV species due to their segmented genome.

RVs and reoviruses are ubiquitous. RVs are a major cause of diarrhea in neonatal and young pigs. However, the association of reoviruses with pig disease is unclear since reoviruses have been found in both healthy pigs and those with respiratory, enteric, and reproductive disease.

Porcine rotavirus

Relevance

RVs are a major cause of diarrhea in humans and animals, including pigs. RV infections can be found in both clinical and subclinical pigs (Amimo et al. 2015; Theuns et al. 2016b). RV infections can also occur in combination with enteric bacteria, leading to increased disease severity, including dehydration, diarrhea, and death (Theuns et al. 2014). Among the 10 RV groups (rotavirus A [RVA] to rotavirus J [RVJ]), RVA, rotavirus B (RVB), rotavirus C (RVC), rotavirus E (RVE), and rotavirus H (RVH) have been reported in pigs (Vlasova et al. 2017).

Porcine RVA was discovered in 1975 (Rodger et al. 1975). Atypical swine RV (non-RVA) strains were discovered in the 1980s and classified into groups B, C, and E (RVB, RVC, RVE) (Bridger 1980; Chasey and Davies 1984; Chasey et al. 1986; Pedley et al. 1986). In 2011, RVH was identified in pigs from Japan and then found in Brazil, the United States, and South Africa (Molinari et al. 2015; Nyaga et al. 2016; Wakuda et al. 2011). Historically, RVA was considered the most prevalent and pathogenic in pigs, but RVC has been identified as a significant cause of enteritis in neonatal pigs (Marthaler et al. 2013; Theuns et al. 2016b), and RVB has been associated with infections in older animals. Whether RVE and RVH are significant pathogens has not been established.

Etiology

The RV genome consists of 11 segments of dsRNA encoding viral proteins (VP) 1–4, 6, and 7 and nonstructural proteins (nsp) 1–5. Each gene segment encodes for a single protein, except for segment 11, which encodes for nsp5 and nsp6 proteins in some RV species. RVs are unique in the sense that the nsp4 produces an enterotoxin that contributes to viral pathogenesis (Estes and Greenberg 2013). The VP1, VP2, and VP3 proteins form the inner capsid of the virion. The middle capsid layer is made up of the VP6 protein while the outer capsid is composed of the VP7 and VP4 proteins. The VP4 is proteolytically cleaved into VP5 and VP8 proteins forming the spike that is used for host attachment and infectivity (Estes and Greenberg 2013; Prasad et al. 1988). The complete three-layered RV particles (inner, middle, and outer capsid layers) resemble a wheel with a smooth surface when visualized by electron microscopy (Figure 43.1a). The outer capsid layer of VP7 and VP4 can be removed by various chemical and enzymatic treatments, and the remaining double-layered particles without the outer capsid have rough outlines (Figure 43.1b).

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

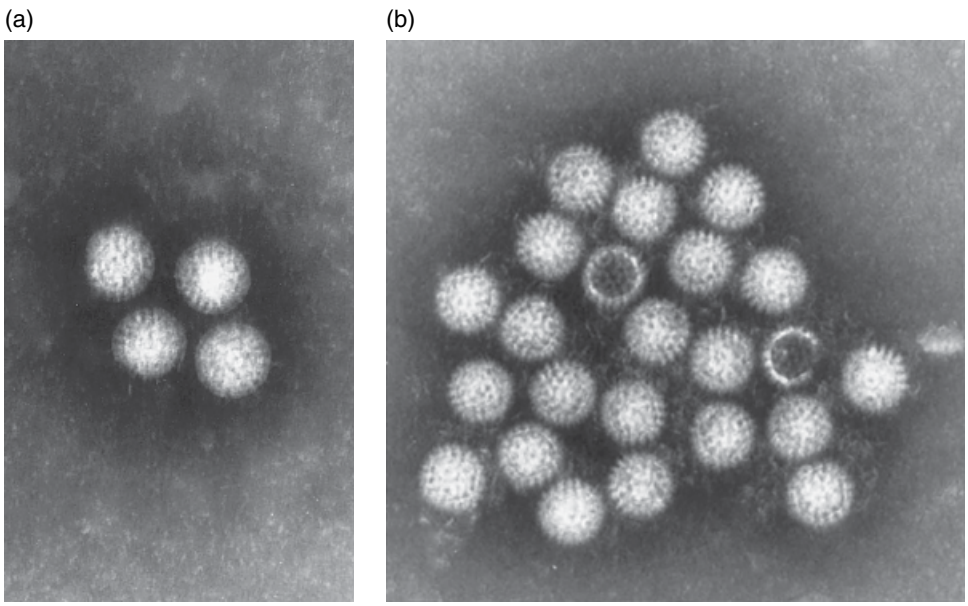


Figure 43.1 Rotavirus particles in feces viewed by negative-staining EM (×130,000). (a) Triple-layered virus particles with intact outer capsids have characteristic smooth outlines. (b) Double-layered particles lack outer capsids and have spiked outlines. *Source:* Chang et al. (2012). Reproduced with permission of John Wiley and Sons.

Historically, RVs were grouped based on banding patterns of the dsRNA in an electropherogram and cross-neutralization capabilities in plaque reduction assays (Estes and Greenberg 2013). Today, RV species are classified based on sequencing of the VP6 using a 53% nucleotide identity cutoff value (Matthijnssens et al. 2008b, 2012), resulting in 10 RV species *Rotavirus A* to *Rotavirus J* (corresponding to viruses RVA-RVJ) (Bányai et al. 2016; Vlasova et al. 2017). A genotype constellation scheme for the 11 RVA gene segments has been developed using nucleotide percent identity cutoffs (Matthijnssens et al. 2008b). Similar genotype schemes have been developed for some genes of the other RV species as well. A binary classification system is used to describe RV strains based on VP7 or G genotype (for the glycoprotein VP7) and VP4 or P genotype (for the protease-sensitive VP4). A standardized naming system for RV strains reported in GenBank® has been established by the Rotavirus Classification Working Group, which includes information on each RV species, host species and wild type or cell culture adapted, country of origin, strain name, year of identification, and G and P genotypes (Matthijnssens et al. 2011).

RVs grow in culture with varying success. Trypsin or pancreatin aids in the growth of RV in cell culture, facilitating cleavage of the outer capsid protein VP4. *In vitro*, visualization of cytopathic effects, such as a rounding phenotype of the cells, is indicative of RV growth.

Rotavirus A

RVA has been propagated in cultures of rhesus monkey kidney (MA104) cells (Saif et al. 1988a). HepG2 liver cells, pancreatic islet cells, colon adenocarcinoma, or other kidney cell lines are also permissive for RVA growth (Arnold et al. 2009; Coulson et al. 2002; Ramig and Galle 1990). The development of polarized small intestinal cell lines is a promising model system for representing *in vivo* growth and replication (Porta et al. 2016). While most RVA strains tend to grow well in culture, some RVA strains remain difficult to propagate.

Through the use of neutralization assays, 27 G serotypes have been assigned to RVA strains (Estes and Greenberg 2013). Given the ease of sequencing, neutralization assays are rarely performed. Genotypic analysis of RVA VP7 uses an 80% nucleotide identity cutoff value to designate different G genotypes (Matthijnssens et al. 2008b). To date, all known G genotypes and serotypes are identical. There have been 18 RVA P serotypes identified (Estes and Greenberg 2013). Unlike G serotype and genotype classifications, P serotypes and genotypes do not necessarily correspond; thus the P genotype is reported in brackets ([X]), while the serotype is not. It is difficult to obtain the necessary reagents to determine the P serotype by neutralization assays. Therefore, only the P genotype is known for many RVA strains. The RVA P genotypes are also defined by an 80% VP4 gene nucleotide identity cutoff value (Matthijnssens et al. 2008a). The genotypes for VP6, VP1, VP2, VP3, nsp1, nsp2, nsp3, nsp4, and nsp5, designated I, R, C, M, A, N, T, E, and H,

Table 43.1 Rotavirus A (RVA) gene segments, number of genotypes, and nucleotide identity cutoff values.

Gene segment number	Gene segment	Name of genotypes (abbreviation)	Nucleotide identity cutoff values (%) for RVA	RVA genotypes found in pigs
9	VP7	Glycosylated (G)	80	G1–G6, G8–G12, and G26
4	VP4	Protease sensitive (P)	80	P[1], P[5]–P[8], P[11], P[13], P[14], P[19], P[23], P[26], P[27], P[32], and P[34]
6	VP6	Inner capsid (I)	85	I1, I2, and I5
1	VP1	RNA-dependent RNA polymerase (R)	83	R1
2	VP2	Core protein (C)	84	C1 and C2
3	VP3	Methyltransferase (M)	81	M1
5	nsp1	Interferon antagonist (A)	79	A1 and A8
8	nsp2	NTPase (N)	85	N1
7	nsp3	Translation enhancer (T)	85	T1
10	nsp4	Enterotoxin (E)	85	E1 and E9
11	nsp5	Phosphoprotein (H)	91	H1

are classified using an 85, 83, 84, 81, 79, 85, 85, 85, and 91% nucleotide identity cutoff value, respectively (Table 43.1).

Rotavirus B

Reports of successful RVB propagation are rare. Porcine kidney cells were used to culture a single strain of RVB (SKA-1) in 1996 using pancreatin, but the strain was later determined by sequencing to belong to a RVH strain (Sanekata et al. 1996). Due to the challenges with growing RVB, serotyping is uncommon, and information regarding circulating serotypes is unknown. Instead, RVB strains are classified using an 80% nucleotide identity cutoff value for VP7, resulting in 21 G genotypes (Lahon et al. 2014; Marthaler et al. 2012).

Rotavirus C

A limited number of RVC strains have been cultured in MA104 and primary porcine kidney cells, with high pancreatin or trypsin concentrations (Saif et al. 1988b; Terrett and Saif 1987; Tsunemitsu et al. 1991). The RVC G genotypes are classified using an 85% nucleotide identity cutoff value while P genotypes use an 83% nucleotide identity cutoff value (Jeong et al. 2015; Marthaler et al. 2013). Thirteen G genotypes and eight P genotypes have been identified for RVC to date (Jeong et al. 2015; Marthaler et al. 2013; Moutelíková et al. 2015; Niira et al. 2016). Like RVB, RVC serotypes are not commonly determined due to difficulties in propagating the strains; thus only G and P genotypes are reported.

Public health

Direct transmission of RVs between animals and humans has not been shown. The detection of animal–human reassortant RVA strains in human patients has suggested a zoonotic potential for RVA (Chitambar et al. 2009; Degiuseppe et al. 2013; Hwang et al. 2012; Midgley et al. 2012; Than et al. 2013; Wilhelm et al. 2015). However, host-specific genotypes for RVB, RVC, and RVH suggest a lack of transmission between swine and humans for these RV species (Gabbay et al. 2008).

Epidemiology

RVs are ubiquitous, and every pig will experience RV infection within its lifetime, if not multiple times. Mixed infections with multiple RV strains have been reported as pigs grow older, indicating a temporal distribution of RV infectivity over a pig's lifetime (Homwong et al. 2016). The age of the pig is one of the most important factors for determining the prevalence of infection and severity of disease. Younger pigs without established immunity and piglets that have not received proper passive immunity from the sow are more susceptible to RV infections (Lecce et al. 1978). Neonatal susceptibility combined with the fact that sows shed RV while farrowing increases the chance of infection in young piglets (Benfield et al. 1982).

RV particles are shed in the feces of infected animals (Debouck and Pensaert 1983). They are highly infectious, and as few as 90 particles are sufficient to induce viral shedding and diarrhea in colostrum-deprived piglets. It is estimated each gram of fecal material contains 1×10^{10} infectious RV particles, further increasing the risk of transmission (Payment and Morin 1990). Transmission occurs by fecal–oral contact with RV-contaminated material, resulting in rapid spread within swine herds (Fu and Hampson 1989).

RV is commonly found in the environment, on fomites, and even in drinking water (Fongaro et al. 2015;

Gratacap-Cavallier et al. 2000; Lachapelle et al. 2014; Meleg et al. 2008). RV is highly stable in the environment, and complete drying will not inactivate all virions (Ward et al. 1991). Halogen (chlorine-based) or phenolic disinfectants may be helpful to control RV spread on a farm, if used consistently (Chandler-Bostock and Mellits 2015; Nemoto et al. 2014). Chlorine and glutaraldehyde are the most effective disinfectants for enteric viruses on clothing (Yeargin et al. 2016).

Since reassortment is common within each RV species, numerous G and P genotypes circulate in pig populations, and the dominant global G and P genotypes are unknown. In addition, coinfections with multiple RV strains are common in diagnostic investigations, but comprehensive studies regarding coinfections are lacking. A brief epidemiological synopsis for each RV species follows.

Rotavirus A

RVA infects the widest range of hosts, including humans (Zhang et al. 2017), cows (Komoto et al. 2016), bats (Sasaki et al. 2016), broiler chickens (Ter Veen et al. 2017), turkeys (Fujii et al. 2016), horses (Matthijnssens et al. 2015), cats (Otto et al. 2015), canines (Hoshino et al. 1982), rats (Tonietti Pde et al. 2013a), and more.

RVA has been found in pig populations on every continent. Recent epidemiological studies indicated that 4.1–89% of swine clinical samples contained RVA (Amimo et al. 2013; Chandler-Bostock et al. 2015; Lachapelle et al. 2014; Monini et al. 2015; Otto et al. 2015; Theuns et al. 2016b; Tonietti et al. 2013b). In the United States, RVA detection is most common in pigs between 21 and 55 days old, with neonatal infection occurring slightly less commonly (Homwong et al. 2016; Miyazaki et al. 2013). Risk factors associated with clinical RVA include larger herd sizes, early weaning, and "all-in/all-out" production (Dewey et al. 2003). Presumably, continuous-flow nurseries had a lower incidence of RV infection because sows were more highly exposed to RVs and, therefore, provided higher levels of maternal immunity to their piglets.

Within swine, 12 G genotypes/serotypes (G1–G6, G8–G12, and G26) and 14 P genotypes (P[1],P[5], P[6], P[7], P[8], P[11], P[13], P[14], P[19], P[23], P[26], P[27], P[32], and P[34]) of RVA have been described (Matthijnssens et al. 2011; Papp et al. 2013; Pham et al. 2014). Although P serotyping is not routinely performed or reported for swine RVA, P1A, P2A–P2C, P7, P9, P12, and P14 have been identified in pigs, and more serotypes may exist (Estes and Greenberg 2013).

Rotavirus B

RVB has been detected in cows (Tsunemitsu et al. 1999), humans (Alam et al. 2013), goats (Munoz et al. 1996), lambs (Theil et al. 1995), rats (Vonderfecht et al. 1994), and others. Of the 21 RVB G genotypes, G4 and G6–G21 have been identified in pigs (Lahon et al. 2014; Marthaler et al. 2012). Currently, information on RVB P genotypes and serotypes circulating in pig populations is lacking. While a few reports have indicated RVB as a cause of neonatal diarrhea, a lower prevalence of RVB (1.6, 4.9, and 47%) has been reported, and infections are generally associated with pigs >3 weeks old (Lahon et al. 2014; Marthaler et al. 2012, 2014a; Otto et al. 2015).

Rotavirus C

In addition to pigs, RVC can infect humans (Kumazaki and Usuku 2014), cows (Soma et al. 2013), ferrets (Wise et al. 2009), cats (Otto et al. 2015), and dogs (Marton et al. 2015), among others. In pigs, 11 RVC G genotypes (G1, G3, G5–G13) and 6 P genotypes (P[1], P[4]–P[8]) have been identified (Jeong et al. 2015; Moutelíková et al. 2015; Niira et al. 2016; Suzuki et al. 2015). Prevalence of RVC was reported as 19.5–34% in recent studies (Amimo et al. 2013; Marthaler et al. 2013; Moutelíková et al. 2014; Otto et al. 2015; Theuns et al. 2016b). In the United States, RVC is an important cause of diarrhea in neonatal pigs, especially in pigs <3 days of age (Marthaler et al. 2013, 2014a). Single infections of RVC more commonly occur in this age group, with RV coinfections occurring as pigs grow older (Homwong et al. 2016; Marthaler et al. 2014a). This age-dependent RVC pattern has yet to be identified in other countries.

Rotaviruses E and H

There is only one report of RVE in pigs (Chasey et al. 1986), and studies confirming the original observations are needed. More recently, RVH has been detected in swine (Marthaler et al. 2014b) and humans (Yang et al. 1998). In the United States and Brazil, 15 and 18%, respectively, of diarrheic samples from pigs were positive for RVH (Marthaler et al. 2014b; Molinari et al. 2016). Coinfections with other RV species are far more common and should be investigated prior to conclusively diagnosing RVH as the causative agent.

Pathogenesis

RV primarily replicates in the epithelial cells of small intestinal jejunal and ileal tissues. Replication occurs mainly in small intestinal proximal villi, resulting in villous blunting and atrophy. The main pathological lesions are likewise observed in the small intestine. RV antigen can be detected transiently in other body tissues, such as the lung, liver, spleen, or choroid plexus (Azevedo et al. 2005; Kim et al. 2013; Shaw et al. 1989; Zijlstra et al. 1997), but there is no evidence of extraintestinal replication, and lesions in extraintestinal tissues are rare.

The VP5 and VP8 subunits of the VP4 outer capsid protein bind to permissive cells by interacting with sialic

acid (Haselhorst et al. 2009; Zárate et al. 2000). There are no known universal mechanisms for cellular entry, although clathrin-mediated endocytosis appears to be important for some strains of porcine RVA (Gutierrez et al. 2010).

The widely accepted mechanism of RV-induced diarrhea is the loss of villi, leading to a deficiency in fluid absorption and subsequent malabsorptive diarrhea (Osborne et al. 1988; Ramig 2004). Other mechanisms could contribute to RV-induced diarrhea as well. After entry into permissive cells, nsp4 disrupts intracellular calcium (Ca^{2+}) regulation, resulting in increased levels of cytoplasmic Ca^{2+} (Hyser et al. 2010; Michelangeli et al. 1991; Ruiz et al. 2000). The Ca^{2+} can then act on chloride (Cl^-) secretion pathways to increase the release of Cl^- into the lumen, ultimately leading to diarrhea (Dong et al. 1997; Estes and Greenberg 2013; Ruiz et al. 2000). Diarrhea may also be caused by nsp4-mediated activation of the enteric nervous system (Lundgren et al. 2000).

Clinical signs

RV infection ranges from subclinical to severe clinical disease, depending on the RV strain, the age of the pig, immune status, overall herd health, and the presence of secondary bacterial or viral infections. The incubation period is reportedly 17–48 hours in gnotobiotic piglets and 1–4 days in conventional piglets (Torres-Medina and Underdahl 1980; Tzipori and Williams 1978). Clinical signs include profuse watery diarrhea, lethargy, vomiting, and anorexia accompanied by rapid weight loss. Feces become lighter colored or yellow, and undigested milk can be seen in fecal matter and intestines at necropsy (Bohl et al. 1982; Janke et al. 1988; Lecce et al. 1978; Lecce and King 1978; Pearson and McNulty 1977; Torres-Medina and Underdahl 1980; Tzipori and Williams 1978).

Studies involving conventionally reared piglets have reported mortality rates below 20%, but higher mortality rates have been reported in diagnostic cases (Bohl et al. 1978; Lecce and King 1978; Tzipori and Williams 1978). Mortality rates can be very high (86–100%) in gnotobiotic piglets (Bohl et al. 1982; Janke et al. 1988; Tzipori and Williams 1978). Diarrhea in pigs <7 days of age persists for 1–10 days, and pigs that recover usually rapidly return to normal body weight (Crouch and Woode 1978; Tzipori and Williams 1978). In piglets >5 days of age, less severe clinical signs and shorter disease duration are generally observed. Death is much less common in these older piglets as well (Bohl et al. 1982; Lecce et al. 1982). Although disease can be severe, subclinical RV infections have been detected, especially in pigs older than 55 days (Amimo et al. 2015; Collins et al. 2008, 2010; Steyer et al. 2008; Theuns et al. 2016a).

The severity of clinical signs can be exacerbated by several factors, including diet and coinfection with other enteric pathogens. Malnutrition correlated with longer periods of diarrhea and less complete restoration of intestinal crypt depths (Zijlstra et al. 1997). In addition to general malnutrition, vitamin A status has been proposed as an important determinant of RV infection recovery. Pigs deficient in vitamin A experienced longer episodes of more severe diarrhea, higher viral titers in feces, and more intestinal damage compared with pigs with sufficient vitamin A levels (Vlasova et al. 2013). Vitamin A deficiency may also alter B-cell immune responses after vaccination, leading to poorer immunologic protection and lower immunoglobulin levels (Kandasamy et al. 2014). Finally, coinfections with enterotoxigenic *Escherichia coli* or *Clostridium perfringens* type A can cause more severe diarrhea in piglets (Neog et al. 2011; Tzipori et al. 1980a).

Lesions

Lesions from RVA, RVB, and RVC are similar (Bohl et al. 1982; Janke et al. 1988; Marthaler et al. 2012, 2013). Small and large intestines of infected pigs are commonly dilated with excessive, watery, yellow, or gray contents. Intestinal walls are thin compared with those of noninfected individuals. The stomach can be swollen, containing undigested milk (Janke et al. 1988; Neog et al. 2011; Pearson and McNulty 1977).

Histological examination of intestinal tissues early in the course of infection reveals swelling of jejunal and ileal epithelial cells, which becomes more pronounced as infection progresses. Beginning approximately 24 hours of postinfection, epithelial cells in the ileum and jejunum slough into the intestinal lumen, leading to thinner and shorter villi (Figure 43.2). Villi are then covered by abnormal cuboidal epithelial cells (Crouch and Woode 1978; Marthaler et al. 2013; Narita et al. 1982a, b). Shortening of villi is accompanied by elongated intestinal crypts with cellular hyperplasia (Bohl et al. 1982). At 2–5 days of post inoculation, intestinal morphology begins to improve with villi returning to normal length. Despite the intestinal tissue repair, decreased villus-to-crypt length ratios were still present at 3 weeks of postinfection in inoculated pigs compared with uninfected pigs (Crouch and Woode 1978; Narita et al. 1982a).

A study of G9 genotype RVA strains reported extraintestinal lesions in the lung, liver, and nervous system, including interstitial pneumonia, hepatocyte necrosis, and degeneration of the choroid plexus, respectively (Kim et al. 2013). This highlights the significance of systemic spread and damage that occurs after infection with some RVA genotypes. However, whether this damage was due to actual viral replication was not conclusive. In RVC infections, extraintestinal RVC RNA was detected

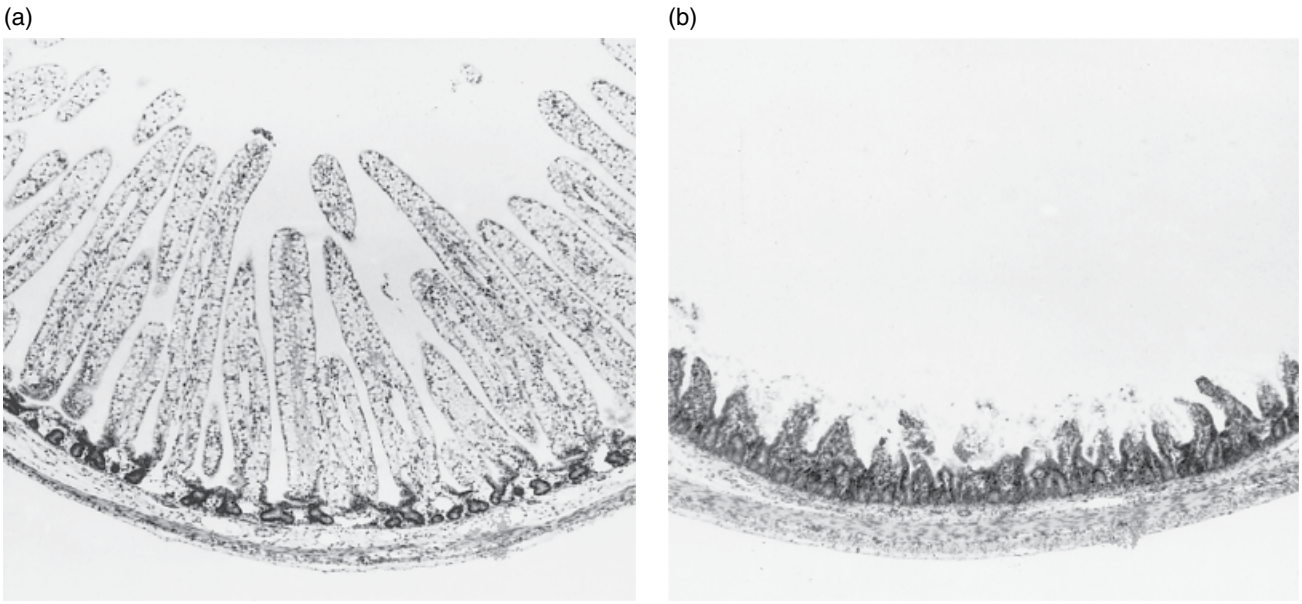


Figure 43.2 The ileum from 3-day-old gnotobiotic pigs (hematoxylin and eosin [H&E]; ×35). (a) Normal villi in an uninoculated control pig. (b) Severe villous atrophy present 18 hours of post inoculation. *Source:* Chang et al. (2012). Reproduced with permission of John Wiley and Sons.

in lung tissues, but lesions were not present and viral replication in these tissues could not be confirmed (Marthaler et al. 2013).

Diagnosis

RV primarily affects neonatal piglets, for which reason it should be included among the differentials when diagnosing enteric disease in animals of this age group. Clinical signs of RV are similar to other enteric pathogens, and, therefore, a diagnosis cannot be made without laboratory testing. However, the fact that RV can also produce subclinical infections should be a consideration when establishing a diagnosis.

Fecal or stomach content samples can be used to detect RV dsRNA and antigens. RT-PCR is the most common diagnostic method used to detect RV and, if quantitative, can determine viral concentrations within a sample (Costantini et al. 2007). The development of multiplex RT-PCR tests containing primers for several enteric viruses allows for the simultaneous detection of multiple diarrhea-causing pathogens in a clinical sample (Masuda et al. 2016; Ogawa et al. 2009; Song et al. 2006; Zhao et al. 2013). Multiplex real-time RT-PCR (rRT-PCR) methods can distinguish between RVA, RVB, and RVC (Marthaler et al. 2014a; Zhou et al. 2016). RT-PCR can also be used to identify different G and P genotypes using primers for the VP7 and VP4 gene segments (Chandler-Bostock et al. 2015; Marthaler et al. 2012; Suzuki et al. 2015; Theuns et al. 2016b).

Other methods to identify RV infection in clinical samples include immunochromatography strip tests (Kang et al. 2007), commercial ELISA kits (Brown et al. 1987; Sharma et al. 2013; Yolken et al. 1988; Zhu et al. 2013), hemagglutination inhibition tests (Eiguchi et al. 1987), cell culture immunofluorescence (Bohl et al. 1984; Terrett et al. 1987), and polyacrylamide gel electrophoresis (Markowska-Daniel et al. 1996). Postmortem diagnosis can be enhanced by the use of immunohistochemistry, *in situ* hybridization, or electron microscopy. Intestinal tissues can be stained and examined for characteristic lesions, and the use of antibodies allows visualization of virus particles within cells (Bohl et al. 1982; Crouch and Woode 1978; Zhao et al. 2013).

ELISA, enzyme-linked immunospot (ELISpot), plaque reduction neutralization (PRN), or fluorescent focus neutralization (FFN) assays are useful for testing the RV immune status of piglets or sows. Assessing levels of neutralizing antibodies against VP7 or VP4 is best done using PRN or FFN (Bohl et al. 1984; Hoshino et al. 1987; To et al. 1998). However, these assays require cultured RV, which is a challenge for some strains. ELISpot assays are useful for identifying responses of antibody-secreting cells in RV-vaccinated and RV-challenged pigs (Yuan et al. 1996, 2001). The anti-RV IgA, IgG, or IgM is quantified using ELISA with isotype-specific monoclonal antibodies (Azevedo et al. 2004).

Immunity

RV infections stimulate both systemic and local immunity, but virulent RV strains elicit stronger immune responses than attenuated strains. The immune response

produces antibodies against VP7, VP4, VP6, nsp3, and nsp4, although the strength and type of immune responses against each of these proteins vary (Chang et al. 2001; Iosef et al. 2002). While antibody-secreting cell responses and immunoglobulin production can be detected against all five proteins, only VP7 and VP4 elicit neutralizing antibody. However, serum neutralizing antibody levels are poor indicators of protective immunity (To et al. 1998).

Increased levels of RV-specific IgA in intestines and serum have been found after vaccination or exposure to virulent RV, and IgA presence is often used as a correlate of protection (Azevedo et al. 2004; Hoshino et al. 1988; To et al. 1998; Yuan and Saif 2002). Numbers of intestinal IgA antibody-secreting cells have been proposed as an even better indication of protective immunity (Chang et al. 2001; Saif et al. 1997; Yuan et al. 1996, 2001).

While there is no cross-protection between different RV species, evidence of heterotypic immunity or cross-protection against multiple genotypes of the same RV species is scarce in pigs. The best protection occurs when VP7 and VP4 segments of the vaccine strain are closely related to the challenge strain (Bishop et al. 1986; Gaul et al. 1982; Hoshino et al. 1988). In addition, RV strains belonging to different lineages within a genotype do not cross-neutralize effectively, making it difficult to conclude whether cross-protection occurs consistently (Hoshino et al. 2004). However, repeated exposure or vaccination increases the likelihood of cross-protection (Chiba et al. 1993; Gorrell and Bishop 1999). Therefore, vaccines should include the most prevalent RV genotypes in a geographic region to provide the best range of protection.

Passive immunity from sows is currently the primary method to protect piglets from RV infections. Sows provide IgG and IgA to their piglets in colostrum and milk (Gellberg et al. 1991; Ward et al. 1996). IgA is the longest-lasting immunoglobulin present in lactating sows, but antibodies taper off after farrowing and are short lived in the piglet. Levels of maternal IgG and IgM in piglets also decline over time, following the trend in the sow, until active immunity in the piglets is induced and levels of anti-RV neutralizing antibodies increase (Hess and Bachmann 1981, Tzipori et al. 1980b). Cytokines in the sow's colostrum and milk could be important in modulating immune responses, especially to activate Th2 cells and antibody production (Nguyen et al. 2007). Early weaning and lack of colostrum lead to severe RV diarrhea in piglets, demonstrating the importance of maternal antibodies in protecting piglet health (Lecce et al. 1978).

Studies in mice (Franco and Greenberg 1995; McNeal et al. 1995) and a few studies in gnotobiotic piglets (Wen et al. 2016) have looked into the relative roles of B- and T-cell immune responses against RV infection after vaccination. In the absence of B cells, T-cell populations are still able to clear RV infections, although the duration of clinical signs is prolonged. Depleting CD8 T cells in B-cell-deficient gnotobiotic pigs resulted in greater virus shedding after vaccination. However, upon challenge it appeared that CD4 T cells were able to compensate for this depletion and confer protection similar to CD8-sufficient animals (Wen et al. 2016). Not surprisingly, long-term immunity is not present in these animals, showing the importance of B-cell responses in developing immune memory.

Prevention and control

RV infections are endemic in swine populations, and elimination and eradication from swine herds is not practical. Instead, the aim is to minimize the impact of RV infections by reducing mortality and the incidence of diarrhea. Maintaining maternal immunity is key to achieving these aims. Exposure of females to infectious RV is commonly used to stimulate maternal immunity but can result in the contamination of farrowing facilities with infectious RV. Even sows with subclinical RV infections shed high amounts of virus into the environment.

Commercial vaccines are available for RVA, but not for RVB or RVC. In general, killed vaccines are not as effective as modified live or attenuated viral strains (Welter and Welter 1990). Also, maternally acquired antibodies in piglets may interfere with live RV vaccines (Hodgins et al. 1999; Parreño et al. 1999).

The most promising vaccine research involves the VP6 protein due to its strong immunogenicity and induction of high levels of both antibodies and antibody-secreting cells (Azevedo et al. 2010; Chang et al. 2001; Yuan et al. 2000, 2001). However, VP6 alone does not stimulate protective immunity. Virus-like particles containing VP6 and VP2 can be delivered intranasally and used in conjunction with attenuated RV strains to induce better immunity and protection in gnotobiotic piglets. Other potential avenues of research in vaccine development include a bicistronic plasmid vaccine system using *S. typhimurium* (Cui et al. 2013) or the use of *Lactobacillus acidophilus* as a more potent adjuvant (Wen et al. 2009; Zhang et al. 2008).

Palliative measures for RV-affected animals include maintaining adequate ambient temperature (35 °C, 95 °F) and feeding a high energy diet to weaned pigs (Steel and Torres-Medina 1984; Tzipori et al. 1980b). Fluid administration with electrolytes or L-glutamine to prevent dehydration from diarrhea and vomiting, as well as antibiotic therapy for cases with bacterial coinfections, can be helpful (Bywater and Woode 1980; Bywater 1983; Rhoads et al. 1991). Mucosal repair can be assisted by TGF-alpha administration (Rhoads et al. 1995). Dietary supplements may help treat or prevent RV infections. Extract of *Glycyrrhiza uralensis*, a plant native to Asia,

reduced levels of inflammatory cytokines in piglets with RV diarrhea, and a dose-dependent repair of histological lesions was observed (Alfajaro et al. 2012). Leucine supplementation was able to combat a decrease in mucin production in the piglet jejunal mucosa (Mao et al. 2015). *In vitro* studies suggested vitamin D3 supplementation can attenuate RV replication by modulating the RIG-I signaling pathway, which recognizes dsRNA (Zhao et al. 2015). Treating piglets with *Bifidobacteria* and *Lactobacillus* spp. probiotics may improve mucosal immune responses and reduce the severity of RV-induced disease (Kandasamy et al. 2014; Shu et al. 2001; Vlasova et al. 2016; Wen et al. 2015).

Porcine reovirus

Relevance

Porcine reovirus was discovered in pigs in 1951, but reoviruses have since been found in a large number of mammalian, avian, and reptilian species. The term "reo" is an acronym for "respiratory and enteric orphan" because the virus was thought to lack clinical significance. As reovirus research continued, its clinical significance was established, although the role of reoviruses in causing disease in pigs is still unclear.

Etiology

Mammalian reoviruses are members of the genus *Orthoreovirus*, which also includes avian reoviruses, reptilian reoviruses, etc. Reoviruses have a non-enveloped, icosahedral/spheroidal double-layered capsid 65–80 nm in diameter (Figure 43.3) and encase 10 dsRNA segments, which are separated into three categories: lambda (L), mu (M), and sigma (S). The lambda and mu categories consist of 3 segments (L1–L3 and M1–M3), while the sigma category is composed of 4 segments (S1–S4).

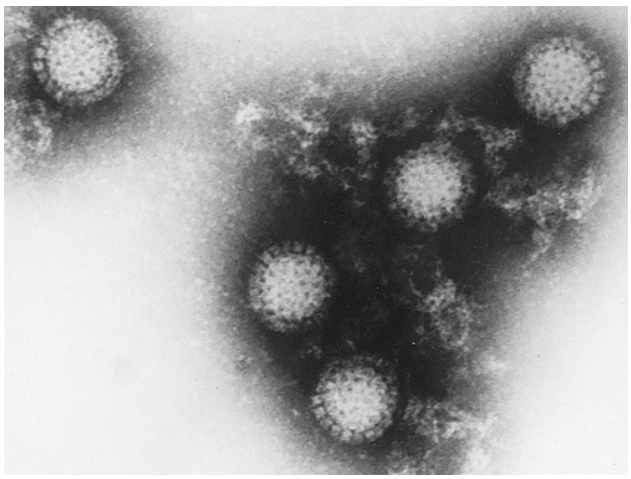


Figure 43.3 Electron micrograph of reovirus particles viewed by negative-staining EM (×115,000). *Source:* Chang et al. (2012). Reproduced with permission of John Wiley and Sons.

Nearly all mammalian species, as well as some reptilian and avian species, are susceptible to reoviruses. Unidentified reoviruses are likely present in many mammalian species but remain undetected because they do not produce clinical disease. Mammalian and avian reoviruses have specific antigen groups that distinguish them from each other. Mammalian reoviruses are currently identified by a common serotyping scheme that consists of three types (1, 2, and 3). This replaced the previous classification scheme of Lang, Jones, and Abney or Dearing (Ramig et al. 1977). A fourth (Ndelle) serotype, associated with human infection, has been proposed (Day 2009). The serotyping classification correlates to a sequenced-based subtyping of the Sigma1 (S1) segment, which encodes for the Sigma1 outer glycoprotein.

Reoviruses are stable at acidic conditions (pH 3) and resistant to many disinfectants, including ether, chloroform, and trypsin. However, the virus is sensitive to heat at 50 °C (122 °F) for 1 hour and 0.1% sodium deoxycholate (Hirahara et al. 1988). Reoviruses are resistant to extreme environmental conditions, surviving in wastewater for extended periods of time. Reoviruses are susceptible to disinfection through extended UV light exposure in a wastewater environment; however, not many chemical disinfectants have been extensively tested (Sharp et al. 1975).

Reoviruses can be propagated in multiple cells lines, including BHK-21, HEK293, MCT, HEF, Vero, and PaKiT03 (Mok et al. 2015). Mouse fibroblast L929 cells are most commonly used to isolate reoviruses and also for purification and plaque assays (Tyler 2001). In addition, reoviruses have been cultured in eggs (Narayanappa et al. 2015). While the cytopathic effects of reoviruses vary, infected cells become rounded, granular, and detached from the surface in culture. After staining with May–Grünwald–Giemsa, eosinophilic inclusion bodies can be seen in the cell cytoplasm under light microscopy (Paul and Stevenson 1999).

Public health

Reoviruses cause a wide variety of human diseases, and zoonotic transmission has been discussed, especially between bats and humans (Steyer et al. 2013). However, direct evidence of zoonotic transmission is lacking.

Epidemiology

Type 1–3 reoviruses have been found in swine populations in the United States, Europe, and China (Fukutomi et al. 1996; Tyler 2001; Yang et al. 1976). Reoviruses are transmitted via respiratory and fecal–oral routes. Immunity to reovirus infection persists for approximately

11 weeks, after which time pigs are susceptible to reinfection (Watt 1978).

Pathogenesis

While reoviruses replicate in the respiratory and intestinal systems, details on pathogenesis are lacking. Initially, cell attachment and entry into the respiratory epithelium was solely attributed to the S1 glycoprotein. A recent study suggested that S1 may not be required for cell entry, but plays a role in systemic dissemination of the virus and viremia. Once a pig is infected via the respiratory track, the virus can infect the gastrointestinal tract, the neural system, and the leukocytes (Boehme et al. 2009). Reoviruses can be detected in nasal secretions and feces as soon as 24 hours of post inoculation, and shedding may continue for 6–14 days. Hemagglutination-inhibiting antibody may be detected at 7 days of postinfection and peak at 11–21 days of postinfection (Hirahara et al. 1988).

Clinical signs

Reovirus infections (types 1–3) have been found in the respiratory, gastrointestinal, and nervous systems of healthy and diseased pigs (Elazhary et al. 1978; Kasza 1970; McFerran and Connor 1970; Robl et al. 1971). Healthy swine are commonly infected with type 2 reovirus (Fukutomi et al. 1996). Many experimental infection studies were able to produce transient febrile reactions, but not clinical disease in swine (Baskerville et al. 1971; Kasza 1970; McFerran and Connor 1970; McFerran et al. 1971; Watt 1978). In addition, reoviruses have been found in aborted fetuses (Kirkbride and McAdaragh 1978). A recent study with type 3 reovirus was able to produce gross lesions of catarrhal enteritis and intussusception in piglets with clinical signs of diarrhea, lethargy, and weight loss (Narayanappa et al. 2015), and a single report detected type 3 reoviruses in diarrheic pigs from Europe (Lelli et al. 2016). In another study, Cesarean-derived colostrum-deprived pigs and conventional pigs inoculated intranasally or exposed via aerosol to reovirus type 1 developed mild respiratory disease (pyrexia, sneezing, inappetence, and listlessness) (Hirahara et al. 1988). Reovirus was also isolated from fecal tissues and sow placenta after inoculation of reovirus via intravenous or intramuscular routes in seronegative sows between 40 and 85 days, yielding litters of mummified, stillborn, weak live-born, and normal pigs (Paul and Stevenson 1999).

Lesions

Gross lesions are rarely associated with reovirus infections in swine. Histologically, lesions of the small intestine include villous epithelial syncytia, villous blunting and fusion, multifocal necrosis of the mucous epithelium, granular cytoplasm, and vacuolation. Oral inoculation with an enteric reovirus in 1-week-old, colostrum-deprived pigs resulted in focal villous atrophy in the jejunum and ileum (Elazhary et al. 1978). Aerosol inoculation of type 1 reoviruses in 4-week-old specific-pathogen-free (SPF) pigs resulted in microscopic lung (alveoli and alveolar septa) lesions characterized by infiltration with lymphocytes and macrophages and mild peribronchiolar nodular lymphocytic hyperplasia (Baskerville et al. 1971). Intranasally inoculated respiratory type 3 reovirus in 70 kg SPF pigs yielded vesicular emphysema, lobular atelectasis, and peribronchiolar nodular lymphocytic hyperplasia, with varying intensity between lobules (Paul and Stevenson 1999). Sows inoculated via the intravenous or intramuscular routes between 40 and 85 days of gestation yielded reproductive deficits (see "Clinical Signs"), but no specific gross or histopathological lesions were observed.

Diagnosis

Since the role of reoviruses as a primary pathogen is debatable, the diagnostic investigation should assess the contributions of other respiratory and intestinal pathogens to the clinical picture. Reovirus detection relies on RT-PCR or viral isolation. Commercial antibody assays are not available for reoviruses, but viral typing can be done via virus neutralization and hemagglutination inhibition tests using reference antisera to the three reovirus types (Paul and Stevenson 1999). In addition, typing can be done by sequencing the S1 gene. More recently, whole genome sequencing of the 10 dsRNA segments has been used to subtype the virus (Narayanappa et al. 2015).

Immunity, prevention, and control

Given the lack of consistent clinical data and few clinical signs in pigs, very little information is available to understand reovirus immunity in swine. A recent study found that 5C6 and 9BG5 antibodies block reovirus binding to JAM-A, as expressed in Chinese hamster ovary cells (Dietrich et al. 2017). In addition, little emphasis is placed on prevention and control of reovirus infections.

References

Alam MM, Pun SB, Gauchan P, et al. 2013. *Trop Med Health* 41:129–134.

Alfajaro MM, Kim HJ, Park JG, et al. 2012. *Virol J* 9:310–320.

Amimo JO, Vlasova AN, Saif LJ. 2013. *J Clin Microbiol* 51:1142–1151.

Amimo JO, Junga JO, Ogara WO, et al. 2015. *Vet Microbiol* 175:195–210.

Arnold M, Patton JT, McDonald SM. 2009. Culturing, storage, and quantification of rotaviruses. *Curr Protoc Microbiol* Chapter 15:Unit 15C.3. doi: https://doi.org/10.1002/9780471729259.mc15c03s15.
Azevedo MSP, Yuan L, Iosef C, et al. 2004. *Clin Vaccine Immunol* 11:12–20.
Azevedo MSP, Yuan L, Jeong K-I, et al. 2005. *J Virol* 79:5428–5436.
Azevedo MSP, Gonzalez AM, Yuan L, et al. 2010. *Clin Vaccine Immunol* 17:420–428.
Bányai K, Kemenesi G, Budinski I, et al. 2016. *Infect Genet Evol* 48:19–26.
Baskerville A, McFerran JB, Connor T. 1971. *Res Vet Sci* 12:172–174.
Benfield DA, Stotz I, Moore R, et al. 1982. *J Clin Microbiol* 16:186–190.
Bishop RF, Tzipori SR, Coulson BS, et al. 1986. *J Clin Microbiol* 24:1023–1028.
Boehme KW, Guglielmi KM, Dermody TS. 2009. *Proc Natl Acad Sci* 106:19986–19991.
Bohl EH, Kohler EM, Saif LJ, et al. 1978. *J Am Vet Med Assoc* 172:458–463.
Bohl EH, Saif LJ, Theil KW, et al. 1982. *J Clin Microbiol* 15:312–319.
Bohl EH, Theil KW, Saif LJ. 1984. *J Clin Microbiol* 19:105–111.
Bridger JC. 1980. *Vet Rec* 107:532–533.
Brown DW, Beards GM, Chen GM, et al. 1987. *J Clin Microbiol* 25:316–319.
Bywater RJ. 1983. *Ann Rech Vet* 14:556–560.
Bywater RJ, Woode GN. 1980. *Vet Rec* 106:75–78.
Chandler-Bostock R, Mellits KH. 2015. *Lett Appl Microbiol* 61:538–543.
Chandler-Bostock R, Hancox LR, Payne H, et al. 2015. *Vet Microbiol* 180:205–211.
Chang KO, Vandal OH, Yuan L, et al. 2001. *J Clin Microbiol* 39:2807–2813.
Chang KO, Saif LJ, Kim Y. 2012. Reoviruses (Rotaviruses and Reoviruses). In Zimmerman JJ, Karriker LA, Ramirez A, et al., eds. *Diseases of Swine*, 10th ed. Hoboken: Wiley, pp. 621–634.
Chasey D, Davies P. 1984. *Vet Rec* 114:16–17.
Chasey D, Bridger JC, McCrae MA. 1986. *Arch Virol* 89:235–243.
Chiba S, Nakata S, Ukae S, et al. 1993. *Clin Infect Dis* 16:S117–S121.
Chitambar SD, Arora R, Chhabra P. 2009. *J Med Microbiol* 58:1611–1615.
Collins PJ, Martella V, O'Shea H. 2008. *J Clin Microbiol* 46:2973–2979.
Collins PJ, Martella V, Sleator RD, et al. 2010. *Arch Virol* 155:1247–1259.
Costantini VP, Azevedo AC, Li X, et al. 2007. *Appl Environ Microbiol* 73:5284–5291.
Coulson BS, Witterick PD, Tan Y, et al. 2002. *J Virol* 76:9537–9544.
Crouch CF, Woode GN. 1978. *J Med Microbiol* 11:325–334.
Cui T, Xiong J, Wang Y, et al. 2013. *Onderstepoort J Vet Res* 80:498.
Day JM. 2009. *Infect Genet Evol* 9:390–400.
Debouck P, Pensaert M. 1983. *Ann Rech Vet* 14:447–448.
Degiuseppe JI, Beltramino JC, Millán A, et al. 2013. *Clin Microbiol Infect* 19:367–371.
Dewey C, Carman S, Pasma T, et al. 2003. *Can Vet J* 44:649–653.
Dietrich MH, Ogden KM, Katen SP, et al. 2017. *J Virol* 91:e01621-16.
Dong Y, Zeng CQ-Y, Ball JM, et al. 1997. *Proc Natl Acad Sci U S A* 94:3960–3965.
Eiguchi Y, Yamagishi H, Fukusho A, et al. 1987. Hemagglutination and hemagglutination-inhibition tests with porcine rotavirus. *Kitasato Arch Exp Med* 60:167–172.
Elazhary MA, Morin M, Derbyshire JB, et al. 1978. *Res Vet Sci* 25:16–20.
Estes MK, Greenberg HB. 2013. Rotaviruses. In Fields BN, Knipe DM, Howley PM, eds. *Fields Virology*, 6th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins, pp. 1347–1401.
Fongaro G, Padilha J, Schissi CD, et al. 2015. *Environ Sci Pollut Res* 22:20060–20066.
Franco MA, Greenberg HB. 1995. *J Virol* 69:7800–7806.
Fu ZF, Hampson DJ. 1989. *Res Vet Sci* 46:312–317.
Fujii Y, Mitake H, Yamada D, et al. 2016. *Genome Announc* 4:e01565–15
Fukutomi T, Sanekata T, Akashi H. 1996. *J Vet Med Sci* 58:555–557.
Gabbay YB, Borges AA, Oliveira DS, et al. 2008. *J Med Virol* 80:1666–1674.
Gaul SK, Simpson TF, Woode GN, et al. 1982. *J Clin Microbiol* 16:495–503.
Gellberg HB, Patterson JW, Woode GN. 1991. *Vet Microbiol* 28:231–242.
Gorrell RJ, Bishop RF. 1999. *J Med Virol* 57:204–211.
Gratacap-Cavallier B, Genoulaz O, Brengel-Pesce K, et al. 2000. *Appl Environ Microbiol* 66:2690–2692.
Gutierrez M, Isa P, Sanchez-San Martin C, et al. 2010. *J Virol* 84:9161–9169.
Haselhorst T, Fleming FE, Dyason JC, et al. 2009. *Nat Chem Biol* 5:91–93.
Hess RG, Bachmann PA. 1981. *Am J Vet Res* 42:1149–1152.
Hirahara T, Yasuhara H, Matsui O, et al. 1988. Characteristics of reovirus type 1 from the respiratory tract of pigs in Japan. *Nihon Juigaku Zasshi* 50:353–361.
Hodgins DC, Kang SY, deArriba L, et al. 1999. *J Virol* 73:186–197.
Homwong N, Diaz A, Rossow S, et al. 2016. *PLoS One* 11:e0154734.
Hoshino Y, Wyatt RG, Scott FW, et al. 1982. *Arch Virol* 72:113–125.
Hoshino Y, Sereno MM, Midthun K, et al. 1987. *J Clin Microbiol* 25:290–294.
Hoshino Y, Saif LJ, Sereno MM, et al. 1988. *J Virol* 62:744–748.
Hoshino Y, Jones RW, Ross J, et al. 2004. *J Virol* 78:7795–7802.

Hwang KP, Wu FT, Bányai K, et al. 2012. *J Med Microbiol* 61:990–997.
Hyser JM, Collinson-Pautz MR, Utama B, et al. 2010. *MBio* 1:e00265-10.
Iosef C, Chang KO, Azevedo MSP, et al. 2002. *J Med Virol* 68:119–128.
Janke BH, Morehouse LG, Solorzano RF. 1988. *Can J Vet Res* 52:364.
Jeong YJ, Matthijnssens J, Kim DS, et al. 2015. *Vet Microbiol* 176:61–69.
Kandasamy S, Chattha KS, Vlasova AN, et al. 2014. *Gut Microbes* 5:639–651.
Kang JH, Kwon DH, Chung TW, et al. 2007. *J Virol Methods* 146:74–79.
Kasza L. 1970. *Vet Rec* 87:681–686.
Kim HH, Park JG, Matthijnssens J, et al. 2013. *Vet Microbiol* 166:123–137.
Kirkbride CA, McAdaragh JP. 1978. *J Am Vet Med Assoc* 172:480–483.
Komoto S, Pongsuwanna Y, Tacharoenmuang R, et al. 2016. *Vet Microbiol* 195:37–57.
Kumazaki M, Usuku S. 2014. *Arch Virol* 159:761–771.
Lachapelle V, Sohal JS, Lambert MC, et al. 2014. *Arch Virol* 159:1771–1779.
Lahon A, Ingle VC, Birade HS, et al. 2014. *Vet Microbiol* 174:342–352.
Lecce JG, King MW. 1978. *J Clin Microbiol* 8:454–458.
Lecce JG, King MW, Dorsey WE. 1978. *Science* 199:776–779.
Lecce JG, Balsbaugh RK, Clare DA, et al. 1982. *J Clin Microbiol* 16:715–723.
Lelli D, Beato MS, Cavicchio L, et al. 2016. *Virol J* 13:139–144.
Lundgren O, Peregrin AT, Persson K, et al. 2000. *Science* 287:491–495.
Mao X, Liu M, Tang J, et al. 2015. *PLoS One* 10:e0137380.
Markowska-Daniel I, Winiarczyk S, Gradzki Z, et al. 1996. *Comp Immunol Microbiol Infect Dis* 19:219–232.
Marthaler D, Rossow K, Gramer M, et al. 2012. *Virology* 433:85–96.
Marthaler D, Rossow K, Culhane M, et al. 2013. *Virology* 446:189–198.
Marthaler D, Homwong N, Rossow K, et al. 2014a. *J Virol Methods* 209:30–34.
Marthaler D, Rossow K, Culhane M, et al. 2014b. *Emerg Infect Dis* 20:1203–1206.
Marton S, Mihalov-Kovács E, Dóró R, et al. 2015. *J Gen Virol* 96:3059–3071.
Masuda T, Tsuchiaka S, Ashiba T, et al. 2016. *Jpn J Vet Res* 64:5–14.
Matthijnssens J, Ciarlet M, Rahman M, et al. 2008a. *Arch Virol* 153:1621–1629.
Matthijnssens J, Ciarlet M, Heiman E, et al. 2008b. *J Virol* 82:3204–3219.
Matthijnssens J, Ciarlet M, McDonald SM, et al. 2011. *Arch Virol* 156:1397–1413.
Matthijnssens J, Otto PH, Ciarlet M, et al. 2012. *Arch Virol* 157:1177–1182.
Matthijnssens J, Ons E, De Coster S, et al. 2015. *Vet Microbiol* 176:179–185.
McFerran JB, Connor T. 1970. *Res Vet Sci* 11:388–390.
McFerran JB, Baskerville A, Connor T. 1971. *Res Vet Sci* 12:174–175.
McNeal MM, Barone KS, Rae MN, et al. 1995. *Virology* 214:387–397.
Meleg E, Bányai K, Martella V, et al. 2008. *Appl Environ Microbiol* 74:3394–3399.
Mertens PPC, Attoui H, Duncan R, et al. 2005. Reoviridae. In Eighth Report of the International Committee on Taxonomy of Viruses, pp. 447–454.
Michelangeli F, Ruiz MC, del Castillo JR, et al. 1991. *Virology* 181:520–527.
Midgley SE, Bányai K, Buesa J, et al. 2012. *Vet Microbiol* 156:238–245.
Miyazaki A, Kuga K, Suzuki T, et al. 2013. *Vet Microbiol* 163:162–166.
Mok L, Wynne JW, Grimley S, et al. 2015. *J Gen Virol* 96:1787–1794.
Molinari BLD, Alfieri AF, Alfieri AA. 2015. *Virus Res* 197:48–53.
Molinari BLD, Possatti P, Lorenzetti E, et al. 2016. *Vet Microbiol* 193:125–132.
Monini M, Di Bartolo I, Ianiro G, et al. 2015. *Arch Virol* 160:2547–2556.
Moutelíková R, Prodělalová J, Dufková L. 2014. *Arch Virol* 159:1163–1167.
Moutelíková R, Prodělalová J, Dufková L. 2015. *Arch Virol* 160:1715–1727.
Munoz M, Alvarez M, Lanza I, et al. 1996. *Epidemiol Infect* 117:203–211.
Narayanappa AT, Sooryanarain H, Deventhiran J, et al. 2015. *MBio* 6:e00593-15.
Narita M, Fukusho A, Shimizu Y. 1982a. *J Comp Pathol* 92:589–597.
Narita M, Fukusho A, Konno S, et al. 1982b. Intestinal changes in gnotobiotic piglets experimentally inoculated with porcine rotavirus. *Natl Inst Anim Health Q Tokyo* 22:54–60.
Nemoto M, Bannai H, Tsujimura K, et al. 2014. *J Vet Med Sci* 76:1061–1063.
Neog BK, Barman NN, Bora DP, et al. 2011. *Vet Ital* 47:117–128.
Nguyen TV, Yuan L, Azevedo MSP, et al. 2007. *Vet Immunol Immunopathol* 117:236–248.
Niira K, Ito M, Masuda T, et al. 2016. *Infect Genet Evol* 44:106–113.
Nyaga MM, Peenze I, Potgieter CA, et al. 2016. *Infect Genet Evol* 38:1–7.
Ogawa H, Taira O, Hirai T, et al. 2009. *J Virol Methods* 160:210–214.
Osborne MP, Haddon SJ, Spencer AJ, et al. 1988. *J Pediatr Gastroenterol Nutr* 7:236–248.
Otto PH, Rosenhain S, Elschner MC, et al. 2015. *Vet Microbiol* 179:168–176.
Papp H, László B, Jakab F, et al. 2013. *Vet Microbiol* 165:190–199.

Parreño V, Hodgins DC, de Arriba L, et al. 1999. *J Gen Virol* 80:1417–1428.
Paul P, Stevenson G. 1999. Rotavirus and Reovirus. In Straw BE, D'Allaire S, Mengeling WL, et al., eds. *Diseases of Swine*, 8th ed. Ames, IA: Iowa State University Press, pp. 255–275.
Payment P, Morin E. 1990. *Arch Virol* 112:277–282.
Pearson GR, McNulty MS. 1977. *J Comp Pathol* 87:363–375.
Pedley S, Bridger JC, Chasey D, et al. 1986. *J Gen Virol* 67:131–137.
Pham HA, Carrique-Mas JJ, Nguyen VC, et al. 2014. *Vet Microbiol* 170:258–265.
Porta DC, López S, Arias CF, et al. 2016. *Virology* 499:65–71.
Prasad BVV, Wang GJ, Clerx JPM, et al. 1988. *J Mol Biol* 199:269–275.
Ramig RF. 2004. *J Virol* 78:10213–10220.
Ramig RF, Galle KL. 1990. *J Virol* 64:1044–1049.
Ramig RF, Cross RK, Fields BN. 1977. *J Virol* 22:726–733.
Rhoads JM, Keku EO, Quinn J, et al. 1991. *Gastroenterology* 100:683–691.
Rhoads JM, Ulshen MH, Keku EO, et al. 1995. *Pediatr Res* 38:173–181.
Robl MG, McAdaragh JP, Phillips CS, et al. 1971. *Vet Med Small Anim Clin* 66:903–909.
Rodger SM, Craven JA, Williams I. 1975. *Aust Vet J* 51:536.
Ruiz MC, Cohen J, Michelangeli F. 2000. *Cell Calcium* 28:137–149.
Saif LJ, Rosen BI, Kang SY, et al. 1988a. *J Tissue Cult Methods* 11:147–156.
Saif LJ, Terrett LA, Miller KL, et al. 1988b. *J Clin Microbiol* 26:1277–1282.
Saif L, Yuan L, Ward L, To T. 1997. *Adv Exp Med Biol* 412:397–403.
Sanekata T, Kuwamoto Y, Akamatsu S, et al. 1996. *J Clin Microbiol* 34:759–761.
Sasaki M, Orba Y, Sasaki S, et al. 2016. *J Gen Virol* 97:2488–2493.
Sharma R, Bora DP, Chakraborty P, et al. 2013. *Indian J Virol* 24:250–255.
Sharp DG, Floyd R, Johnson JD. 1975. *Appl Microbiol* 29:94–101.
Shaw DP, Morehouse LG, Solorzano RF. 1989. *Am J Vet Res* 50:1966–1970.
Shu Q, Qu F, Gill HS. 2001. *J Pediatr Gastroenterol Nutr* 33:171–177.
Soma J, Tsunemitsu H, Miyamoto T, et al. 2013. *J Gen Virol* 94:128–135.
Song DS, Kang BK, Oh JS, et al. 2006. *J Vet Diagn Invest* 18:278–281.
Steel RB, Torres-Medina A. 1984. *Infect Immun* 43:906–911.
Steyer A, Poljsak-Prijatelj M, Barlic-Maganja D, et al. 2008. *J Gen Virol* 89:1690–1698.
Steyer A, Gutiérrez-Aguire I, Kolenc M, et al. 2013. *J Clin Microbiol* 51:3818–3825.
Suzuki T, Hasebe A, Miyazaki A, et al. 2015. *Virus Res* 197:26–34.
Ter Veen C, de Bruijn ND, Dijkman R, et al. 2017. *Avian Pathol* 46:95–105.
Terrett LA, Saif LJ. 1987. *J Clin Microbiol* 25:1316–1319.
Terrett LA, Saif LJ, Theil KW, et al. 1987. *J Clin Microbiol* 25:268–272.
Than VT, Park JH, Chung IS, et al. 2013. *Arch Virol* 158:2385–2393.
Theil KW, Grooms DL, McCloskey CM, et al. 1995. *J Vet Diagn Investig* 7:148–150.
Theuns S, Desmarets LMB, Heylen E, et al. 2014. *Vet Microbiol* 172:23–34.
Theuns S, Vyt P, Desmarets LMB, et al. 2016a. *Virus Res* 213:172–183.
Theuns S, Conceição-Neto N, Zeller M, et al. 2016b. *Infect Genet Evol* 43:135–145.
To TL, Ward LA, Yuan L, et al. 1998. *J Gen Virol* 79:2661–2672.
Tonietti Pde O, da Hora AS, Silva FD, et al. 2013a. *Scientific World Journal*, article ID 648406.
Tonietti PO, Hora AS, Silva FDF, et al. 2013b. *J Clin Microbiol* 51:2750–2753.
Torres-Medina A, Underdahl NR. 1980. *Can J Comp Med* 44:403–411.
Tsunemitsu H, Saif LJ, Jiang BM, et al. 1991. *J Clin Microbiol* 29:2609–2613.
Tsunemitsu H, Morita D, Takaku H, et al. 1999. *Arch Virol* 144:805–815.
Tyler KL. 2001. Mammalian Reoviruses. In Fields BN, Knipe DM, Howley PM, et al., eds. *Fields Virology*, 4th ed. Philadelphia: Lippincott Williams & Wilkins, pp. 1729–1745.
Tzipori S, Williams IH. 1978. *Aust Vet J* 54:188–192.
Tzipori S, Chandler D, Makin T, et al. 1980a. *Aust Vet J* 56:279–284.
Tzipori S, Chandler D, Smith M, et al. 1980b. *Aust Vet J* 56:274–278.
Vlasova AN, Chattha KS, Kandasamy S, et al. 2013. *J Immunol* 190:4742–4753.
Vlasova AN, Kandasamy S, Chattha KS, et al. 2016. *Vet Immunol Immunopathol* 172:72–84.
Vlasova AN, Amimo JO, Saif LJ. 2017. *Viruses* 9:48.
Vonderfecht SL, Lindsay DA, Eiden JJ. 1994. *J Clin Microbiol* 32:1107–1108.
Wakuda M, Ide T, Sasaki J, et al. 2011. *Emerg Infect Dis* 17:1491–1493.
Ward RL, Bernstein DI, Knowlton DR, et al. 1991. *J Clin Microbiol* 29:1991–1996.
Ward LA, Rich ED, Besser TE. 1996. *J Infect Dis* 174:276–282.
Watt RG. 1978. *Res Vet Sci* 24:147–153.
Welter MW, Welter CJ. 1990. *Vet Microbiol* 22:179–186.
Wen K, Azevedo MSP, Gonzalez A, et al. 2009. *Vet Immunol Immunopathol* 127:304–315.
Wen K, Liu F, Li G, et al. 2015. *J Pediatr Gastroenterol Nutr* 60:834–843.
Wen K, Bui T, Weiss M, et al. 2016. *Viral Immunol* 29:112–127.
Wilhelm B, Waddell L, Greig J, et al. 2015. *Prev Vet Med* 119:61–79.

Wise AG, Smedley RC, Kiupel M, et al. 2009. *Vet Pathol* 46:985–991.
Yang YF, Yang SC, Tai FH. 1976. Reovirus antibodies among animals in Taiwan. *Zhonghua Min Guo Wei Sheng Wu Xue Za Zhi* 9:1–4.
Yang H, Chen S, Ji S. 1998. A novel rotavirus causing large scale of adult diarrhea in Shi Jiazhuang. *Zhonghua Liu Xing Bing Xue Za Zhi* 19:336–338.
Yeargin T, Buckley D, Fraser A, Jiang X. 2016. *Am J Infect Control* 44:1365–1373.
Yolken R, Wee SB, Eiden J, et al. 1988. *Am Soc Microbiol* 26:1853–1858.
Yuan L, Saif LJ. 2002. *Vet Immunol Immunopathol* 87:147–160.
Yuan L, Ward LA, Rosen BI, et al. 1996. *J Virol* 70:3075–3083.
Yuan L, Geyer A, Hodgins DC, et al. 2000. *J Virol* 74:8843–8853.
Yuan L, Iosef C, Azevedo MSP, et al. 2001. *J Virol* 75:9229–9238.
Zárate S, Espinosa R, Romero P, et al. 2000. *J Virol* 74:593–599.
Zhang W, Azevedo MSP, Wen K, et al. 2008. *Vaccine* 26:3655–3661.
Zhang S, Yin J, Yang J, et al. 2017. *J Med Virol* 89:71–78.
Zhao J, Shi B, Huang X, et al. 2013. *J Virol Methods* 194:107–112.
Zhao Y, Yu B, Mao X, et al. 2015. *Arch Anim Nutr* 69:227–235.
Zhou W, Ullman K, Chowdry V, et al. 2016. *Vet Microbiol* 182:75–81.
Zhu J, Yang Q, Cao L, et al. 2013. *Virol J* 10:91.
Zijlstra RT, Donovan SM, Odle J, et al. 1997. *J Nutr* 127:1118–1127.

44

Retroviruses

Joachim Denner

Relevance

Retroviral particles were first described in immortalized and tumor porcine cell lines (Armstrong et al. 1971; Bouilant and Greig 1975; Breese 1970; Suzuka et al. 1985). These viruses have been identified as porcine endogenous retroviruses (PERVs). It is still unknown whether PERVs cause clinical disease in swine. Rather, PERVs are important in the context of xenotransplantation. Pigs are appropriate tissue and organ donors for several reasons, including their physiological similarity to humans, the similar size of organs, the short gestation time (100 days), the high number of progeny, and the ease with which to clone and genetically modify pigs. Multiple genetic modifications have been introduced into pigs intended for xenotransplantation to prevent immunological rejection of the xenotransplant and to overcome physiological incompatibility (Niemann and Petersen 2016). Thus, swine transplants could potentially address the shortage of available human transplants (Ekser et al. 2015). However, the prospect of transplanting live pig cells, tissues, and organs into immunosuppressed humans led to careful consideration of the potential for the transmission of infectious agents from pig tissues to humans (Fishman et al. 2012; Tucker and Scobie 2012). The discovery that pig retroviruses were capable of infecting human cells *in vitro* (Patience et al. 1997) provided further motivation to study PERV and to develop strategies to prevent PERV transmission via xenotransplants (Scobie and Takeuchi 2009; Tucker and Scobie 2012; Wilson 2008).

Although endogenous retroviruses have been shown to play an important role in placentogenesis in many species including humans (Denner 2016a), it is still unknown whether PERVs play such a role in pigs (Keller et al. 2014).

Etiology

PERVs are RNA viruses in the genus *Gammaretrovirus* in the family *Retroviridae*. Retroviruses have a lipid envelope and two copies of their RNA genome. On the surface they carry "knobs" composed of trimers of the surface and transmembrane envelope protein (Figure 44.1).

Retroviruses encode for reverse transcriptase, an enzyme that transcribes the viral RNA genome into a DNA copy that will be integrated into the DNA of the host's cells (Weiss 2006). A high portion (approximately 8%) of mammalian genomic DNA is believed to be retroviral in origin (Kurth and Bannert 2009), likely resulting from the integration of DNA copies (proviruses) of ancient exogenous retroviruses into the genomes of host ancestors. PERVs are closely related to murine leukemia viruses (MuLV), gibbon ape leukemia viruses (GaLV), feline leukemia viruses (FeLV), and koala retroviruses (KoRV) (Patience et al. 2001). PERVs probably originated from a murine retrovirus that infected the ancestors of present-day pigs. At most, this occurred 7.6 million years ago, a time coincident with the separation of pigs (Suidae, *Sus scrofa*) from their closest relatives, the peccaries (Tayassuidae, *Pecari tajacu*) approximately 7.4 million years ago (Niebert and Tönjes 2005; Tönjes and Niebert 2003). In addition to the PERV sequences characterized by repeats in the long terminal repeat (LTR), proviruses without such repeats were detected and determined to have evolved approximately 3.4 million years ago, which is a phylogenetically younger structure.

The full PERV genome contains the typical retroviral coding regions of gag (group-specific antigen, encoding for the core proteins), pol (polymerase, encoding for reverse transcriptase, integrase, and protease), and env (envelope, encoding for the surface and transmembrane envelope proteins) (Figure 44.2).

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

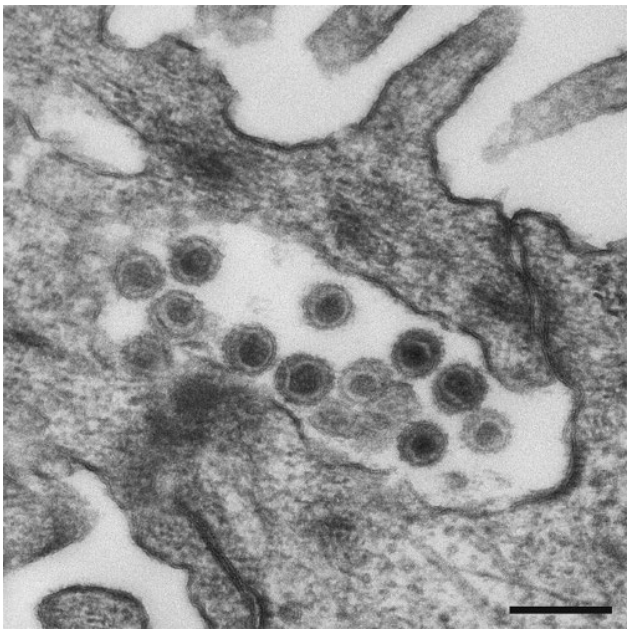


Figure 44.1 Electron microscopic presentation of PERV produced by a porcine cell line derived from a pig melanoma. *Source:* Dieckhoff et al. (2007a). Reproduced with permission of Elsevier. Bar: 200 nm. G. Holland, Dr. M. Laue, Robert Koch Institute.

Several subtypes of PERVs have been identified: PERV-A, PERV-B, and PERV-C (Takeuchi et al. 1998). PERV-A and PERV-B are present in the genome of all pigs and are able to infect human cells, as well as cells of other species (polytropic viruses), whereas PERV-C is present in most pigs and only infects pig cells (ecotropic virus). The receptors for PERV-A are known (human PERV-A receptor [HuPAR]-1 and HuPAR-2) (Ericsson et al. 2003); however, receptors for PERV-B and PERV-C are currently unknown.

Recombination between PERV-A and PERV-C has been observed; the recombinants designated PERV-A/C acquired the receptor-binding site for the receptor of PERV-A and, therefore, were able to infect human cells (Bartosch et al. 2004; Martin et al. 2006; Wilson et al. 2000; Wood et al. 2004). Interestingly, rapid passaging of a PERV-A/C on human cells was associated with an increase of such repeats in the LTR and an elevated titer of PERV in the supernatant (Denner et al. 2003). The recombinants PERV-A/C act like exogenous retroviruses, and de novo integrations into the genome of certain somatic cells were observed; however, these viruses were not found in the germ line (Denner 2008a). The recombinants PERV-A/C are characterized by a high replication rate and specific mutations in the genome (Denner et al. 2003; Harrison et al. 2004). First attempts to infect pigs with PERV-A/C failed (Kaulitz et al. 2011).

At present, the number of integrated proviruses reported in different pig breeds ranges from 1 to 114 (Denner 2016b). There are also marked differences in regard to the extent of the expression of PERVs in different pig breeds, as well as among different organs of one animal (Bittmann et al. 2012; Clemenceau et al. 1999; Dieckhoff et al. 2009; Martin et al. 1998; Moon et al. 2009). Treatment of porcine peripheral blood mononuclear cells (PBMCs) with mitogens and other agents increased the expression of PERV (Cunningham et al. 2004; Tacke et al. 2003; Wilson et al. 1998). PERVs have been isolated from pig plasma (infectious virus) and factor VII preparations (genomic RNA only) that were used for the treatment of hemophilia (Takefman et al. 2001).

Public health

There is no evidence that PERVs can be transmitted to humans by food or contact with pigs (Hermida-Prieto et al. 2007; Tacke et al. 2001). Furthermore, no PERV

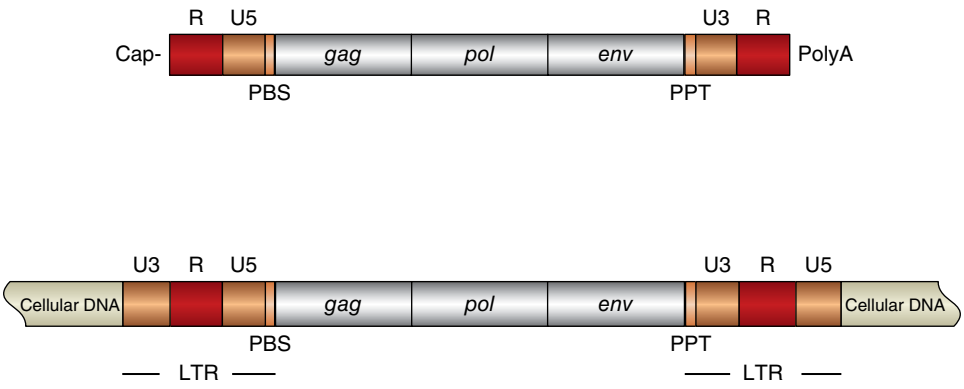


Figure 44.2 Schematic presentation of a genome and an integrated provirus of PERV. (a) The genome consists of 10Kb of RNA with a 5′ cap and a 3′ polyA tail. The viral RNA is structured as follows: R, a 5′ and 3′ direct repeat; U5 and U3, noncoding unique regions; PBS, a primer binding site; open reading frames for gag, pol, and env genes; PPT, a polypurine tract. (b) The DNA provirus with two long terminal repeats (LTRs).

transmission was observed in pig-to-small animal or pig-to-nonhuman primate experiments, either with or without pharmaceutical immunosuppression (Denner 2008b; Denner and Töenjes 2012; Specke et al. 2009). The same was true for the first clinical trials in humans (Paradis et al. 1999; Valdes-Gonzalez et al. 2010). In two more recent clinical trials, transplanting encapsulated pig islet cells for the treatment of diabetes provided a clinical benefit for the patients, and no PERV transmission was observed (Cooper et al. 2016; Matsumoto et al. 2014; Morozov et al. 2017; Wynyard et al. 2014). However, until now, no large vascularized organs have been transplanted into severely immunosuppressed patients, and, therefore, the risk posed by PERVs in the case of organ transplantation is still not fully defined.

In this context it is important to note that genetic modification of source pigs to enhance xenotransplant compatibility, either by including transgenes encoding for cell surface human complement regulatory proteins or by removing galactose-alpha-1,3-galactose epitopes recognized by human natural antibodies, resulted in released PERV particles that were protected from components of the recipient's innate immune system (Takefman et al. 2002; Weiss 1998). Since humans also contain endogenous retroviruses in their genome, it was shown that recombination or co-packaging between PERV and human endogenous retroviruses, as well as other human retroviruses (e.g. human immunodeficiency viruses), is unlikely to happen (Suling et al. 2003). Guidelines and regulations have been developed to prevent PERV transmission in future clinical xenotransplantations (Cozzi et al. 2016).

Epidemiology

Although there is evidence that PERVs may exist as exogenous retroviruses and amplify (replication and de novo integration) inside the pig (Denner 2016b), there are no data showing transmission of PERV from one pig to another. Attempts to infect pigs with a high titer PERV-A/C (Kaulitz et al. 2011) or to infect PERV-C-negative animals with PERV-C (Denner and Tönjes 2012) have failed.

Pathogenesis, clinical signs, and lesions

Retroviruses are known to induce leukemia, as in the case for the PERV-related viruses MuLV, KoRV, GaLV, and FeLV. Furthermore, retroviruses are known to induce severe immunodeficiencies. This is true for the human immunodeficiency virus 1 (HIV-1) and also for MuLV, FeLV, and KoRV (Denner 2014). There is evidence that endogenous retroviruses in mice, cats, and koalas are also associated with tumor development, partially after recombination with other endogenous sequences (Denner 2010). It is unclear whether PERVs can induce similar outcomes in pigs.

Although PERVs were expressed at higher levels in melanomas of diseased pigs compared with normal tissues and despite the fact that PERV particles were isolated from melanoma-derived cell lines (Dieckhoff et al. 2007a) (Figure 44.1), there is no evidence that these melanomas were caused by PERV. PERVs were also isolated from lymphoma cells (Moennig et al. 1974; Suzuka et al. 1985) and from radiation-induced leukemia cells (Frazier 1985); however, there is no evidence for an association with the disease.

However, a correlation was observed between PERV expression and the health status of pigs. Pigs housed in production units experiencing higher mortality and more endemic diseases showed higher levels of PERV RNA in their plasma (Harrison et al. 2010; Pal et al. 2011; Tucker and Scobie 2006). This was due to a higher PERV expression in lymphoid organs as the result of a more vigorous immune response against the endemic infections. As reported above, stimulation of pig PBMCs with mitogens (simulating an antigen response) was associated with a higher PERV expression and release (Dieckhoff et al. 2009; Tacke et al. 2003; Wilson et al. 1998).

Like most retroviruses, including HIV-1, PERVs are potentially immunosuppressive (Denner 1998). PERV contains a so-called immunosuppressive domain in the transmembrane envelope protein (Denner 2014). This domain is highly conserved in all retroviruses. It had been shown that a synthetic peptide corresponding to the immunosuppressive domain of PERV and purified PERV particles inhibited mitogen-induced proliferation and modulated cytokine release by human PBMCs (Morozov et al. 2013; Tacke et al. 2000), suggesting that PERV is immunosuppressive.

Diagnosis

Reviews describing methods for detecting PERV in donor pigs and transplant recipients are available (Denner 2011; Denner and Töenjes 2012; Godehardt et al. 2015; Herring et al. 2001). The prevalence of integrated PERV proviruses can be measured by PCR-based methods (PCR, nested PCR, and real-time PCR) or Southern blot analysis. The digital droplet PCR allows exact measurement of the copy number of PERV in the pig genome (Yang et al. 2015). PERV expression at the mRNA level can be measured by different RT-PCR methods.

Virus expression at the protein level can be measured using immunoperoxidase assays, immunofluorescence assays, Western blot analysis, immunohistochemistry, and immunogold electron microscopy. Specific antibodies against viral proteins are required for these methods.

Virus particles can be analyzed by detection of the enzyme reverse transcriptase and by scanning and transmission electron microscopy. The infectivity of the particles can be measured using infection assays. The readout of these assays may be provirus integration, mRNA or protein expression, or virus release. One of the methods to determine the release of infectious virus is co-cultivation of pig cells with human target cell lines (for testing human-tropic PERV release) or pig target cell lines (for testing ecotropic PERV release). This assay relies on the fact that retrovirus infection is more efficient via cell-to-cell contact.

Detecting PERV infections in transplant recipients requires exclusion of the presence of pig cells (so-called microchimerism) to avoid false-positive results. To detect microchimerism, PCR screening for pig genes, especially mitochondrial genes that are abundantly present, can be used (Paradis et al. 1999).

Measurement of PERV expression, including PERV release after stimulation of PBMCs with mitogens and other agents, has been used to classify pigs in high producers and low producers (Dieckhoff et al. 2009; Tacke et al. 2003). However, at present it is unclear whether this method can be used to define the expression of PERV in organs and tissues required for transplantation, since replication and de novo integrations of PERVs were observed in some, but not all organs of pigs (Denner 2016b).

No anti-PERV antibodies have been reported in pigs, because they are tolerant to their endogenous retroviruses (Keller et al. 2014). However, detection of antibodies is an efficient tool to screen for PERV infection in transplant recipients. Sensitive and specific ELISA and Western blot assays have been developed using synthetic peptides or recombinant proteins corresponding to the main core and envelope proteins of PERV (Denner and Tönjes 2012 and the references therein). Highly purified virus particles can also be used as antigens for the detection of PERV-specific antibodies (Morozov et al. 2017; Tacke et al. 2001).

Prevention and control

Unlike most infectious agents, such as herpesviruses and hepatitis E virus, PERVs cannot be eliminated by treatment, vaccination, Cesarean delivery, colostrum deprivation, early weaning, or embryo transfer because they are integrated into the genome of every pig (Denner 2015a,b; Denner and Mueller 2015; Denner and Töenjes 2012). Because PERVs were not shown to be transmitted from pig to pig or to produce clinical disease in pigs, strategies to prevent or control PERV transmission have focused on preventing transmission to humans via xenotransplantation: (1) the selection of PERV-C-negative animals to avoid recombination, (2) the selection of pigs with a low copy number and a low expression of PERV-A and PERV-B, (3) the development of transgenic pigs carrying and expressing a PERV-specific small interfering RNA to reduce the expression of PERV in all cells of the pig (Dieckhoff et al. 2007b, 2008; Karlas et al. 2004; Ramsoondar et al. 2009; Semaan et al. 2012), and (4) the concept of inactivating PERVs in pigs using gene editing techniques (Niu et al. 2017; Yang et al. 2015). If this strategy of gene editing could be used to inactivate all PERVs in viable pigs, the risk PERVs pose for xenotransplantation would be completely eliminated (Denner 2015b, 2017a). Furthermore, other tools are available to prevent transmission of PERV to the recipients such as the use of antiretroviral drugs (for review see Denner [2017b]) and vaccines based on neutralizing antibodies against the envelope proteins of PERV (Fiebig et al. 2003; Waechter and Denner 2014).

References

Armstrong JA, Porterfield JS, de Madrid AT. 1971. *J Gen Virol* 10:195–198.

Bartosch B, Stefanidis D, Myers R, et al. 2004. *J Virol* 78:13380–13390.

Bittmann I, Mihica D, Plesker R, et al. 2012. *Virology* 433:329–336.

Bouillant AM, Greig AS. 1975. *J Gen Virol* 27:173–180.

Breese SS, Jr. 1970. Virus-like particles occurring in cultures of stable pig kidney cell lines. Brief report. *Arch Gesamte Virusforsch* 30:401–404.

Clemenceau B, Lalain S, Martignat L, et al. 1999. *Diabetes Metab* 25:518–525.

Cooper DK, Matsumoto S, Abalovich A, et al. 2016. *Transplantation* 100:2301–2308.

Cozzi E, Tönjes RR, Gianello P, et al. 2016. *Xenotransplantation* 23:14–24.

Cunningham DA, Dos Santos Cruz GJ, Fernandez-Suarez XM, et al. 2004. *Transplantation* 77:1071–1079.

Denner J. 1998. *Ann N Y Acad Sci* 862:75–86.

Denner J. 2008a. *Arch Virol* 153:1421–1426.

Denner J. 2008b. *Transplant Proc* 40:587–589.

Denner J. 2010. Endogenous retroviruses. In Kurth R, Bannert N, eds. *Retroviruses: Molecular Biology, Genomics and Pathogenesis*. Hethersett, Norwich: Caister Academic Press, pp. 35–69.

Denner J. 2011. *Xenotransplantation* 18:151–157.

Denner J. 2014. *AIDS* 28:1081–1090.

Denner J. 2015a. *Xenotransplantation* 22:329–335.
Denner J. 2015b. *Xenotransplantation* 22:411–412.
Denner J. 2016a. *APMIS* 124:31–43.
Denner J. 2016b. *Viruses* 3:8.
Denner J. 2017a. *N Engl J Med* 377:1891–1893.
Denner J. 2017b. *Viruses* 9(8):213.
Denner J, Mueller NJ. 2015. *Int J Surg* 23:306–311.
Denner J, Tönjes RR. 2012. *Clin Microbiol Rev* 25:318–343.
Denner J, Specke V, Thiesen U, et al. 2003. *Virology* 314:125–133.
Dieckhoff B, Puhlmann J, Buscher K, et al. 2007a. *Vet Microbiol* 123:53–68.
Dieckhoff B, Karlas A, Hofmann A, et al. 2007b. *Arch Virol* 152:629–634.
Dieckhoff B, Petersen B, Kues WA, et al. 2008. *Xenotransplantation* 15:36–45.
Dieckhoff B, Kessler B, Jobst D, et al. 2009. *Xenotransplantation* 16:64–73.
Ekser B, Tector AJ, Cooper DK. 2015. *Int J Surg* 23:197–198.
Ericsson TA, Takeuchi Y, Templin C, et al. 2003. *Proc Natl Acad Sci U S A* 100:6759–6764.
Fiebig U, Stephan O, Kurth R, et al. 2003. *Virology* 307:406–413.
Fishman JA, Scobie L, Takeuchi Y. 2012. *Xenotransplantation* 19:72–81.
Frazier ME. 1985. *Arch Virol* 83:83–97.
Godehardt AW, Rodrigues Costa M, Tönjes RR. 2015. *Xenotransplantation* 22:95–100.
Harrison I, Takeuchi Y, Bartosch B, et al. 2004. *J Virol* 78:13871–13879.
Harrison S, Baker R, Pal N, et al. 2010. Detection of porcine endogenous retrovirus (PERV) A/C recombinant in U.S. pigs by quantitative real-time RT-PCR. In Proceedings, of International Pig Veterinary Society Congress, p. 894.
Hermida-Prieto M, Domenech N, Moscoso I, et al. 2007. *Transplantation* 84:548–550.
Herring C, Cunningham DA, Whittam AJ, et al. 2001. *Clin Biochem* 34:23–27.
Karlas A, Kurth R, Denner J. 2004. *Virology* 325:18–23.
Kaulitz D, Mihica D, Plesker R, et al. 2011. *Arch Virol* 156:707–710.
Keller M, Petersen B, Niemann H, et al. 2014. *J Gen Virol* 295:1827–1831.
Kurth R, Bannert N. 2009. *Int J Cancer* 126:306–314.
Martin U, Kiessig V, Blusch JH, et al. 1998. *Lancet* 352:692–694.
Martin SI, Wilkinson R, Fishman JA. 2006. *Virol J* 3:91.
Matsumoto S, Tan P, Baker J, et al. 2014. *Transplant Proc* 46:1992–1995.
Moennig V, Frank H, Hunsmann G, et al. 1974. *Virology* 57:179–188.
Moon HJ, Kim HK, Park SJ, et al. 2009. *J Vet Sci* 10:317–322.
Morozov VA, Dao Thi VL, Denner J. 2013. *PLoS One* 8:e70399.
Morozov VA, Wynyard S, Matsumoto S, et al. 2017. *Virus Res* 227:34–40.
Niebert M, Tonjes RR. 2005. *J Virol* 79:649–654.
Niemann H, Petersen B. 2016. *Transgenic Res* 25:361–374.
Niu D, Wei HJ, Lin L, et al. 2017. *Science* 357:1303–1307.
Pal N, Baker R, Schalk S, et al. 2011. *Transbound Emerg Dis* 58:344–351.
Paradis K, Langford G, Long Z, et al. 1999. *Science* 285:1236–1241.
Patience C, Takeuchi Y, Weiss RA. 1997. *Nat Med* 3:282–286.
Patience C, Switzer WM, Takeuchi Y, et al. 2001. *J Virol* 75:2771–2775.
Ramsoondar J, Vaught T, Ball S, et al. 2009. *Xenotransplantation* 16:164–180.
Scobie L, Takeuchi Y. 2009. *Curr Opin Organ Transplant* 14:175–179.
Semaan M, Kaulitz D, Petersen B, et al. 2012. *Xenotransplantation* 19:112–121.
Specke V, Plesker R, Wood J, et al. 2009. *Xenotransplantation* 16:34–44.
Suling K, Quinn G, Wood J, et al. 2003. *Virology* 312:330–336.
Suzuka I, Sekiguchi K, Kodama M. 1985. *FEBS Lett* 183:124–128.
Tacke SJ, Kurth R, Denner J. 2000. *Virology* 268(1):87–93.
Tacke SJ, Bodusch K, Berg A, et al. 2001. *Xenotransplantation* 8:125–135.
Tacke SJ, Specke V, Denner J. 2003. *Intervirology* 46:17–24.
Takefman DM, Wong S, Maudru T, et al. 2001. *J Virol* 75:4551–4557.
Takefman DM, Spear GT, Saifuddin M, et al. 2002. *J Virol* 76:1999–2002.
Takeuchi Y, Patience C, Magre S, et al. 1998. *J Virol* 72:9986–9991.
Tönjes RR, Niebert M. 2003. *J Virol* 77:12363–12368.
Tucker AW, Scobie L. 2006. *Vet Rec* 159:367–368.
Tucker AW, Scobie L. 2012. Retroviruses. In Zimmerman JJ, Karriker LA, Ramirez A, et al., eds. *Diseases of Swine*, 10th ed. Hoboken, NJ: Wiley-Blackwell, pp. 635–638.
Valdes-Gonzalez R, Dorantes LM, Bracho-Blanchet E, et al. 2010. *J Med Virol* 82:331–334.
Waechter A, Denner J. 2014. *Immunol Res* 58:9–19.
Weiss RA. 1998. *Nat Med* 4:391–392.
Weiss RA. 2006. *Retrovirology* 3:67.
Wilson CA. 2008. *Cell Mol Life Sci* 65:3399–3412.
Wilson CA, Wong S, Muller J, et al. 1998. *J Virol* 72:3082–3087.
Wilson CA, Wong S, VanBrocklin M, et al. 2000. *J Virol* 74:49–56.
Wood JC, Quinn G, Suling KM, et al. 2004. *J Virol* 78:2494–2501.
Wynyard S, Nathu D, Garkavenko O, et al. 2014. *Xenotransplantation* 21:309–323.
Yang L, Güell M, Niu D, et al. 2015. *Science* 350:1101–1104.

45

Rhabdoviruses

Sabrina L. Swenson, Daniel G. Mead, and David R. Kinker

Overview

Vesicular stomatitis viruses (VSVs) and rabies virus are members of the family *Rhabdoviridae*. VSVs belong to the genus *Vesiculovirus* and rabies virus belongs to the genus *Lyssavirus*. In general, virions are 100–180 nm in length and 45–100 nm in diameter and contain a single molecule of linear, negative-sense ssRNA (Knudson 1973; Sokol and Loprowski 1975). They are bullet shaped or cone shaped. The viruses generally have five structural polypeptides designated as L, G, N, P, and M (Dietzgen et al. 2012; Howatson 1970). Virions are composed of a host-derived plasma membrane, a phospholipid envelope, and an internal ribonucleoprotein core. After cell attachment, penetration, and uncoating, virus replication occurs in the cytoplasm of infected cells. The viral RNA-dependent RNA polymerase transcribes viral mRNAs from the viral genome (Wagner and Rose 1996). Virions are composed of between 15 and 25% lipids (McSharry and Wagner 1971a; Dietzgen et al. 2012). Generally phospholipids represent about 55–60%, and sterols and glycolipids about 35–40% of the total lipids (Dietzgen et al. 2012; Knudson 1973; McSharry and Wagner 1971b). The virions are composed of about 3% carbohydrate by weight (McSharry and Wagner 1971a). Viral RNA has a sedimentation coefficient of 42–45 Svedberg units and a buoyant density of 1.66 g/cm^3 (Hilfenhaus et al. 1979). Virion buoyant density in CsCl is 1.18–1.22 g/cm^3 and 1.17–1.19 g/cm^3 in sucrose (Fenner 1975).

Vesicular stomatitis

Relevance

Historical reports of vesicular stomatitis (VS) date back to the 1800s (Hanson 1952), but VSV was first isolated in 1925 in New Jersey and in Indiana the following year (Cotton 1927). VS in cattle and pigs resembles foot-and-mouth disease (FMD) and in pigs swine vesicular disease (SVD) or Seneca Valley virus (SVV). The presence of VS in livestock results in export restrictions on livestock from VS-affected to VS-free zones. VSV is a zoonotic virus, and appropriate personal protective equipment (PPE) should be utilized to reduce transmission from affected animals to people.

Etiology

Genetically related, but distinct, VSVs (Wunner et al. 1995) cause VS. Isolates found to be serologically distinct are classified as different serotypes: vesicular stomatitis New Jersey virus (VSNJV) and vesicular stomatitis Indiana virus (VSIV) (Cartwright and Brown 1972). Both VSNJV and VSIV serotypes are pathogenic in domestic livestock, but only VSNJV has been associated with disease in swine. Two VSIV subtypes, Cocal virus and Alagoas virus, have caused disease in livestock in South America. Virus detection is based on cell culture cultivation and/or molecular technologies. VSV may be propagated in many cell culture types, including African green monkey kidney (Vero) and baby hamster kidney (BHK-21) continuous cell lines (Swenson and Allende 2015).

Public health

Humans are susceptible to VSV and may become infected by direct contact with virus on fomites or shed by animals or by aerosolization of virus. Clinical signs in people include influenza-like symptoms and blister-like lesions appearing 1–2 days after exposure. When working with live virus, handling diagnostic specimens potentially containing VSV, or handling animals suspected of having VS, appropriate measures should be in place to prevent exposure to VSV.

Diseases of Swine, Tenth Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson.

Epidemiology

VS affects domestic livestock including cattle, horses, and swine and is the most common vesicular disease of livestock in the Americas. Antibodies to the VSVs have been found in a wide variety of wild animals, but naturally occurring clinical disease has not been reported in wildlife (Jenney et al. 1970; Tesh et al. 1970).

VS is not known to occur outside of the Americas (Swenson and Allende 2015). Within the Americas, VS, primarily caused by VSNJV and VSIV, occurs in endemic cycles in southern Mexico, Central America, and northern South America (Colombia, Venezuela, Ecuador, and Peru).

Both endemic and epidemic patterns of VS historically occurred in the United States. In the Southeastern United States, VSNJV was detected in domestic livestock almost yearly from the early 1900s to the mid- to late 1970s and in wildlife on Ossabaw Island, Georgia, from 1956 to 2007 (Killmaster et al. 2011). Based on recent negative serologic results from white-tailed deer and feral swine and the failure to isolate VSNJV from the endemic sand fly vector, *Lutzomyia shannoni*, it is possible that VSNJV is no longer present at this site (Killmaster et al. 2011). In the Western United States, epidemics of VSNJV (and VSIV to a lesser extent) generally occurred in cattle and horses at 5–10-year intervals (Rodriguez 2002). VSNJV epidemics generally affect a larger number of animals than VSIV epidemics. Naturally occurring VSNJV in domestic swine has not been reported in the United States since 1968 (Jenney and Brown 1972), and VSIV infection in swine has never been reported.

VSNJV can be transmitted via several routes, including direct animal-to-animal contact, and biologically or mechanically by insect vectors. VSNJV was efficiently transmitted among domestic swine via contact transmission (Stallknecht et al. 2001). In experimental settings, the development of vesicles in inoculated animals facilitated contact transmission. Infection in contacts ranged from subclinical to clinical.

Radeleff (1949) proposed transmission of VSNJV by arthropod vectors, but the concept remained controversial because it was not clear how vectors could acquire the infection since vertebrate hosts do not develop a detectable VSV viremia. However, Mead et al. (2000, 2004) demonstrated that (1) hematophagous insects could become infected with VSNJV when they fed on or near virus-rich vesicular lesions; (2) VSNJV could be transmitted from VSNJV-infected to uninfected black flies (*Simulium vittatum*) during the process of co-feeding on the same vertebrate host; and (3) VSNJV-infected insects transmitted the virus to livestock.

In clinically affected animals, VSV is primarily localized to areas where lesions are present. Virus can be isolated in high concentrations from vesicular fluid, throat swabs, tonsils, saliva, and the epithelium of clinically affected animals for up to 10 days post infection (range of 1–10 days), depending on the species.

Virus infectivity is stable in the pH range of 5–10 (Crimmins et al. 1983; Gaudin et al. 1993) and is rapidly inactivated by heat treatment at 56 °C (133 °F), following ultraviolet (UV) or X-ray irradiation or exposure to lipid solvents and oxidizing agents including detergents, formalin, and many common disinfectants, such as household bleach (Larghi and Nebel 1980; Zimmer et al. 2013). Under natural conditions, VSNJV can remain viable in contaminated saliva on pails or food buckets for 3–4 days (Hanson 1952) and can be recovered from plant surfaces up to 24 hours after surface inoculation at room temperature (Drolet et al. 2009).

Pathogenesis

VSV infection is localized to the site of inoculation, with occasional spread to local draining lymph nodes. Virus is not found in other tissues. Primary replication seems to occur in keratinocytes (Scherer et al. 2007).

Virulence in swine is dependent on virus strain (VSNJV is more virulent than VSIV), route of inoculation, and dose. In domestic swine, experimental inoculation of the snout with $1 \times 10^{0.7}$ median tissue culture infectious dose per ml ($TCID_{50}$) of VSNJV resulted in seroconversion without clinical disease, whereas inoculation of the snout with doses $\geq 1 \times 10^{3.3}$ $TCID_{50}$ resulted in clinical disease followed by seroconversion. VSNJV produces large vesicular lesions at the inoculation site in 1–2 days, and pigs shed high concentrations of virus for up to 7–8 days post infection. Inoculation of the oral mucosa (including tongue), snout, and coronary bands results in the development of vesicular lesions in the majority of animals. In contrast, inoculation of haired skin results only in seroconversion and subclinical disease (Howerth et al. 2006; Scherer et al. 2007; Stallknecht et al. 1999).

Naturally occurring VS in domestic swine due to VSIV has not been reported, although swine are susceptible to VSIV and develop clinical signs when the inoculation dose is $\geq 1 \times 10^{3}$ $TCID_{50}$ (Stallknecht et al. 2004). Inoculation of VSIV at doses $\leq 1 \times 10^{2}$ $TCID_{50}$ did not result in clinical signs or a detectable antibody response. Compared with VSNJV, lesions are much smaller (if present), and virus is shed at lower concentrations for 3–4 days.

The role of host factors (e.g. age and breed) in clinical disease has not been investigated, but field studies suggest that age may be associated with the outcome of infection.

Clinical signs

VS is characterized by fever, if present, from 24 to 72 hours post infection (hpi). Excessive salivation due to lesions in the mouth is usually the first sign and may be

the only sign observed. Pain associated with oral lesions can lead to anorexia and weight loss. Foot lesions and subsequent lameness are seen most frequently in swine but occur in cattle and horses as well.

The VS incubation period in domestic livestock ranges from 1 to 3 days. Viremia has never been reported in naturally infected livestock and only once under experimental conditions. In that instance, Cotton (1927) inoculated the blood of horses and cows experimentally infected with VSNJV into guinea pigs. The subsequent development of vesicular lesions in the guinea pigs at the site of inoculation (footpads) was interpreted as evidence of viremia in the horses and cows. This outcome has not been reproduced despite attempts by several investigators.

Mortality due to VSV infection is low, but morbidity rates during epidemics can be as high as 90%. VS is a self-limiting disease, and animals typically recover in 2–3 weeks if there are no complications from secondary bacterial infections (Hanson 1952).

Lesions

Noticeable vesicle formation can occur in the oral mucosa, snout, teats, and coronary bands between 24 and 72 hpi. Multiple lesions on or near the same site, or on different sites, can occur on the same animal. Lesions typically begin at the site of infection as small, in some cases pinpoint, blanched areas with raised borders. These quickly develop into 2–4 cm (0.75–1.5 in.) grayish-red vesicles. If multiple lesions develop in the same area, they may coalesce and appear as a single vesicle. Vesicles usually rupture 1–2 days after formation, releasing straw-colored, virus-rich exudates (Howerth et al. 1997). There may be extensive epidermal erosion and ulceration followed by scabbing. In severe cases, the complete tongue epithelium may slough, and if lesions develop along a coronary band, the claw may separate. Lesions typically begin to reepithelialize soon after eruption and, unless complicated by secondary infection, will completely heal in 1–2 weeks. Microscopic lesions are observed in the stratum spinosum where the virus replicates. Epithelial necrosis occurs with pleocellular inflammatory reaction in the mucosa and lamina propria (Uzal et al. 2016).

Diagnosis

Clinically, VS in swine is indistinguishable from FMD, SVD, vesicular exanthema of swine (VES), or SVV. Therefore, it is imperative to collect and submit diagnostic samples for laboratory evaluation. Rule-outs for infectious causes of vesicular disease in swine include FMDV, SVDV, VESV, SVV, porcine parvovirus, enterovirus infection, and swinepox virus. Noninfectious causes include trauma (e.g. chemical or thermal burns), course feed, toxins, plant awns, and photosensitization.

Clinical specimens for virus detection include vesicular fluid, tissue tags from ruptured vesicles, biopsies of affected areas, and swabs. To maintain virus viability, place diagnostic materials in a small volume of medium, chill to 4 °C (39 °F), and transport materials to the laboratory on ice packs. Do not freeze specimens as this can adversely affect virus detection.

Virus isolation, complement fixation (CF), antigen-capture ELISA, and reverse transcription polymerase chain reaction are used for detection of virus or viral nucleic acid (Swenson and Allende 2015). Virus isolation can be done in continuous cell lines, embryonated chicken eggs, and mice. If present in high titers, VSV can be detected in <24 hours after inoculation and within 7 days of inoculation at lower titers.

Serum antibody can be detected by virus neutralization (VN), CF, and ELISA. VN-detectable serum antibody persisted for years in experimentally infected cattle (Sorensen et al. 1958) and in naturally infected horses by the VN test and a competitive ELISA. The antibody response detected by CF is usually of shorter duration, generally months. In experimentally infected calves and ponies, the competitive ELISA detected antibody before it was detectable by CF or VN (Katz et al. 1997). Because of the long duration of antibody, a fourfold change in antibody titer (CF or VN) in paired sera collected approximately 7 days apart is required to establish VSV infection.

Immunity

The immune response to VSV infection can vary with the virus serotype, the route of exposure, method of exposure, and serologic assay used to test samples (Katz et al. 1997; Stallknecht et al. 1999). Seroconversion can be detected as early as 5 days following exposure to VSV. Maternal antibodies have been detected in offspring born to infected dams. In an experimental study, maternal antibody was demonstrated at 3 months of age, but not at 7 months of age (Sorensen et al. 1958).

The development of antibodies is associated with a decreased ability to detect viral shedding. In one study, virus was detected in 82 samples from experimentally infected pigs prior to seroconversion and in one sample following seroconversion (Stallknecht et al. 1999). However, the degree or duration of protective immunity is not fully characterized. In one study, animals reexposed to homologous virus 49–77 days after initial exposure did not shed virus and did not show a change in antibody response. In contrast, animals reexposed to a heterologous virus shed virus and responded serologically (Katz et al. 1997). All the same, field studies suggested that animals were not protected when reexposed

to homologous virus, even in the presence of neutralizing antibodies (Rodriguez et al. 1990).

Prevention and control

When vesicular disease is observed in swine, steps should be taken to stop the movement of animals and materials onto or off the premises until a diagnosis is made. The appropriate animal health authorities should be notified immediately.

As with most viral infections, treatment of VSV-infected animals is largely ineffective, except for palliative care such as feeding soft feed and providing padding for hard surfaces. Antibiotics may be useful to prevent or treat secondary bacterial infections. Treatment of vesicular lesions with topical antiseptics may promote faster healing and reduce the risk of secondary infections.

VSNJV is easily transmitted among swine by animal-to-animal contact, so affected animals and animals that may have had recent contact with them should be isolated. Minimize animal-to-animal contact to reduce transmission between animals. Disinfection of equipment, transport, and facilities occupied by infected animals is important.

Preventive measures include insect control, housing animals indoors during peak insect feeding times, and implementation of biosecurity procedures designed to avoid the introduction of VSV from affected premises via equipment, personnel, or animals. Insect repellents such as permethrin or other repellents approved for use on swine should also be considered. Vaccination with inactivated vaccines is practiced in some countries where VS is endemic.

Rabies virus

Relevance

The potential for rabies in swine is present wherever contact with wildlife or canine reservoirs is possible. The predominate rabies reservoirs are foxes in Europe; dogs and foxes in Asia; dogs, jackals, and mongooses in Africa; dogs and vampire bats in South America; and foxes, raccoons, skunks, and bats in North America. Feral and non-vaccinated cats may contract rabies from wildlife and can pose a public health risk due to their close proximity to humans.

Compared with cattle, the spillover of rabies into swine is low and probably reflects the separation between wildlife and swine that occurs with confinement operations. The United States averages about one case per year in domestic swine. In Europe, spillover to wild boars is an infrequent occurrence.

Etiology

Rabies virus is a member of the genus *Lyssavirus* in the family *Rhabdoviridae*. It is an enveloped neurotropic single-stranded RNA virus. The genus *Lyssavirus* included a number of other related viruses, including Lagos bat virus, Mokola virus, Duvenhage virus, European bat lyssaviruses, and Australian bat lyssaviruses (Gould et al. 1998).

Rabies virus is susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, 70% ethanol, and formaldehyde. It is inactivated by UV radiation, heat (1 hour at 50 °C/122 °F), and lipid solvents. The virus is rapidly inactivated by sunlight and does not retain infectivity for long out of the host.

Public health

Rabies is an important zoonotic disease because of its high mortality rate in humans. However, there is little documented evidence of swine as a source of rabies for humans. Steele and Fernandez (1991) reported that of 521 people exposed to presumed rabid swine in the subcontinent of India, Pakistan, and Bangladesh from 1908 to 1972, none developed rabies. Notably, each exposed person received rabies prophylaxis.

Epidemiology

Rabies exists worldwide, except for certain islands and countries with strong geographical barriers, such as Norway and Sweden. Australia is free of rabies but does have Australian bat lyssavirus. All warm-blooded mammals are susceptible to rabies, although some appear to be more susceptible than others. The susceptibility of animals to rabies is influenced by the quantity of the virus introduced, the site of the bite, the age of the animal, and the virus strain involved. Strain differences play an important part in species susceptibility. Epidemiologic investigations may identify events that support the possible exposure of pigs to rabies-infected wildlife, such as the presence of a skunk in an outside pen.

Pathogenesis

Transmission occurs via the bite of an infected animal that has virus in its saliva. The amount and duration of virus in saliva varies significantly and is dependent on the amount of virus inoculated. In dogs, animals that received a high dose of virus died quickly with little virus in the saliva, while dogs that received lower doses of inoculums had longer incubation periods and more viruses in the saliva (Fekadu 1991). The duration of virus shedding was from 14 days before the onset of clinical signs to 4 days after onset. Studies have not been

conducted to determine the amount and duration of virus shedding in swine, but it should be assumed that virus is present when determining treatment options following human exposure.

Upon entering the body through a bite wound, rabies virus quickly enters an eclipse phase where it remains undetectable by fluorescent antibody staining or virus isolation. Murphy et al. (1973) suggested that rabies virus replicates in muscle fibers prior to invading the nervous system. They postulated that replication of the virus in muscle fibers may be a necessary amplification step prior to infection of the peripheral nervous system and may account for the long and variable incubation period of the disease. Once the virus enters the axoplasm, it travels to the dorsal root ganglia and then to the spinal cord and brain (Baer et al. 1965). As the virus spreads through the central nervous system (CNS), there is simultaneous centrifugal movement of the virus in peripheral nerves to nonnervous tissue, including the epidermis, cornea, epithelium of the mouth, nasal mucosa, intestine, lacrimal glands, pancreas, muscle fibers, myocardium, lungs, kidneys, adrenal medulla, and salivary glands.

Clinical signs

Rabies has classically been divided into furious and dumb forms. The clinical course of the disease is divided into the prodromal, excitement, and paralytic periods. During the prodromal period, there may be slight changes in temperament with a slight rise in temperature, dilation of pupils, and impaired corneal reflexes. The prominence of the excitement phase is what differentiates the furious form from the dumb form. Aggressive behavior, muscular tremors, incoordination, loss of balance, and increased salivation are common. The paralytic period, the final stage of the disease, is characterized by ascending paralysis, coma, and death.

Published reports on the clinical signs of rabies in swine are limited and, as in other species, not consistent. Sudden unexplained mortality with few clinical signs was reported in feeder pigs (Hazlett and Koller 1986). Morehouse et al. (1968) reported twitching, prostration, excessive salivation, and clonic muscle spasms. Other signs reported include uneasiness, incoordination, rapid chewing, fever, increased grunting, anorexia, marked thirst, and head and face rubbing (Dhillon and Dhingra 1973; DuVernoy et al. 2008; Merriman 1966; Morehouse et al. 1968; Yates et al. 1983).

As in other species, the incubation period in swine is variable, ranging from 17 days in a pig bitten by a skunk to 132 days in a potbellied pig bitten by a raccoon (DuVernoy et al. 2008). Baer and Olson (1972) reported the recovery of pigs from rabies. Specifically, four of six pigs developed clinical signs, including progressive paralysis, beginning 32–47 days after having been bitten by a rabid skunk. The signs subsided in 1–2 weeks with the pigs developing high antibody titers to rabies virus.

Lesions

Gross lesions, other than those caused by self-mutilation, do not occur with rabies. Microscopic changes in the CNS in swine range from mild vasculitis and focal gliosis in the brain to extensive meningoencephalitis and neuronal degeneration in the brain and spinal cord (Morehouse et al. 1968).

Diagnosis

The differential diagnosis of rabies in swine would include any disease that can mimic the neurological clinical signs associated with rabies, including pseudorabies (Aujeszky's disease), classical swine fever, Teschen disease, toxicosis, vitamin deficiencies, and congenital tremors.

The fluorescent antibody technique (FAT) for the detection of viral antigen in the brain is the preferred diagnostic test in animals, including swine, because of its speed and accuracy (Goldwasser and Kissling 1958). Antigen may also be detected in corneal impressions and in biopsies of tactile hairs in *antemortem* samples (Blenden et al. 1983; Schneider 1969).

Immunochemical (Lembo et al. 2006) and rapid immunodiagnostic (Kang et al. 2007) tests have been developed for rabies, but the results need to be confirmed by FAT. RT-PCR assays have also been developed for rabies virus, but their use is not currently recommended for routine post mortem rabies testing. Virus isolation in cell culture and the mouse inoculation test can be used to assess the infectivity of a suspension of emulsified brain tissue from a rabies suspect animal, but they are not routinely used for rabies diagnosis.

Serological methods exist, such as the rapid fluorescent focus inhibition test (RFFIT) (Velleca and Forrester 1981), but are primarily used to ensure protective vaccination titers in humans exposed or at risk of exposure to rabies virus.

Serology is less frequently used in animals for similar purposes. Serology is also not generally used for diagnosis because of the rapid progression of the disease once clinical signs are observed.

Immunity

Survival of pigs following exposure to rabies virus is common and is dependent on the site of the bite (bites around the face and neck are more likely to be fatal), the quantity of the virus introduced, the age of the animal (young animals are more susceptible), and the virus strain involved. Both humoral immunity and cell-mediated immunity are

necessary to prevent fatal infections. Once clinical signs develop, rabies is usually fatal.

Prevention and control

Because of the expense of conducting duration-of-immunity efficacy tests and the limited market, there are no licensed rabies vaccines for use in swine. Off-label use of inactivated vaccines may be justified in expensive breeding stock in rabies-endemic areas, but vaccine efficacy has not been demonstrated. Overall, the best way to prevent rabies is to control the disease in wildlife reservoirs and use physical barriers to protect swine from wildlife.

References

Baer GM, Olson HR. 1972. *J Am Vet Med Assoc* 160:1127–1128

Baer GM, Shanthaveerappa TR, Bourne GH. 1965. *Bull World Health Organ* 33:783–794.

Blenden DC, Bell JF, Tsao AT, et al. 1983. *J Clin Microbiol* 18:631–636.

Cartwright B, Brown F. 1972. *J Gen Virol* 16:391–398.

Cotton WE. 1927. Vesicular stomatitis. *Vet Med* 22:169–175.

Crimmins DL, Mehard WB, Schlesinger S. 1983. *Biochemistry* 22:5790–5796.

Dhillon SS, Dhingra PN. 1973. *Vet Med Small Anim Clin* 68:1044.

Dietzgen RG, Calisher CH, Kurath G, et al. 2012. Rhabdoviridae. In King AMQ, Adams MJ, Carstens EB, et al., eds. *Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses*. San Diego: Elsevier, pp. 686–713.

Drolet BS, Stuart MA, Derner JD. 2009. *Appl Environ Microbiol* 75:3029–3033.

DuVernoy TS, Mitchell KC, Myers RA, et al. 2008. *Zoonoses Public Health* 55:431–435.

Fekadu M. 1991. Canine rabies. In Baer GM, ed. *The Natural History of Rabies*, 2nd ed. Boca Raton: CRC Press, pp. 367–378.

Fenner F. 1975. The classification and nomenclature of viruses. *Intervirology* 6:1–12.

Gaudin Y, Ruigrok RWH, Knossow M, et al. 1993. *J Virol* 67:1365–1372.

Goldwasser RA, Kissling RE. 1958. *Proc Soc Exptl Biol Med* 98:219–223.

Gould RA, Hyatt AD, Lunt R, et al. 1998. *Virus Res* 54:165–187.

Hanson RP. 1952. *Bacteriol Rev* 16:179–204.

Hazlett MH, Koller MA. 1986. *Can Vet J* 27:116–118.

Hilfenhaus J, Köhler R, Moser H, et al. 1979. Large-scale concentration and purification of various animal viruses using isopycnic banding in a continuous-flow ultra-centrifuge. In Peeters H, ed. *Separation of Cells and Subcellular Elements*. New York: Pergamon Press Inc., pp. 103–106.

Howatson, AF. 1970. Vesicular stomatitis and related viruses. In Smith KM, Lauffer MA, Barg EB, eds. *Advances in Virus Research*. New York: Academic Press, pp. 195–256.

Howerth EW, Stallknecht DE, Dorminy M, et al. 1997. *J Vet Diagn Invest* 9:136–142.

Howerth EW, Mead DG, Mueller PO, et al. 2006. *Vet Pathol* 43:943–955.

Jenney EW, Brown CL. 1972. Surveillance for vesicular stomatitis in the United States January 1968 through July 1972. Proc U.S. *Anim Health Assoc* 76:183–193.

Jenney EW, Hayes FA, Brown CL. 1970. *J Wildl Dis* 6:488–493.

Kang B, Oh J, Lee C, et al. 2007. *J Virol Methods* 145:30–36.

Katz JB, Eernisse KA, Landgraf JG, et al. 1997. *J Vet Diagn Invest* 9:329–331.

Killmaster LF, Stallknecht DE, Howerth EW, et al. 2011. *Vector Borne Zoonotic Dis* 11:559–565.

Knudson DL. 1973. *J Gen Virol* 20:105–130.

Larghi OP, Nebel AE. 1980. *J Clin Microbiol* 11:120–122.

Lembo T, Niezgoda M, Velasco-Villa A, et al. 2006. *Emerg Infect Dis* 12:310–313.

McSharry JJ, Wagner RR. 1971a. *J Virol* 7:59–70.

McSharry JJ, Wagner RR. 1971b. *J Virol* 7:412–415.

Mead DG, Ramberg FB, Besselsen DG, et al. 2000. *Science* 287:485–487.

Mead DG, Gray EW, Noblet R, et al. 2004. *J Med Entomol* 41:78–82.

Merriman GM. 1966. *J Am Vet Med Assoc* 148:809–811.

Morehouse LG, Kintner LD, Nelson SL. 1968. *J Am Vet Med Assoc* 153:57–64.

Murphy FA, Bauer SP, Harrison AK, et al. 1973. *Lab Invest* 28:361–376.

Radeleff RD. 1949. Clinical encephalitis occurring during an outbreak of vesicular stomatitis in horses. *Vet Med* 44:494–96.

Rodriguez LL. 2002. *Virus Res* 85:211–219.

Rodriguez LL, Vernon S, Morales AI, et al. 1990. *Am J Trop Med Hyg* 42:272–281.

Scherer CFC, O'Donnell V, Golde WT, et al. 2007. *Vet Res* 38:375–390.

Schneider LG. 1969. *Zentralbl Veterinarmed* 16:24–31.

Sokol F, Loprowski H. 1975. *Proc Nat Acad Sci* 72:933–936.

Sorensen DK, Chow TL, Kowalczyk T, et al. 1958. *Am J Vet Res* 19:74–77.

Stallknecht DE, Howerth EW, Reeves CL, et al. 1999. *Am J Vet Res* 60:43–48.

Stallknecht DE, Perzak DE, Bauer LD, et al. 2001. *Am J Vet Res* 62:516–520.

Stallknecht DE, Greer JB, Murphy MD, et al. 2004. *Am J Vet Res* 65:1233–1239.

Steele JH, Fernandez PJ. 1991. History of rabies and global aspects. In Baer GM, ed. *The Natural History of Rabies*, 2nd ed. Boca Raton: CRC Press, pp. 1–24.

Swenson SL, Allende R. 2015. Vesicular stomatitis. In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015 (on-line English version), Part 1. Paris, France: Office International des Epizooties, World Organisation for Animal Health.

Tesh RB, Peralta PH, Johnson KM. 1970. *Am J Epidemiol* 91:216–224.

Uzal FA, Plattner BL, Hostetter JM. 2016. Alimentary System. In Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, 6th ed., Vol. 2. China: Elsevier, pp. 1–257.

Velleca WM, Forrester FT. 1981. In Laboratory methods for detecting rabies. United States Department of Health and Human Services, Centers for Disease Control, Atlanta, pp. 1–153.

Wagner RR, Rose JK. 1996. Rhabdoviridae: The viruses and their replication. In Fields BN, Knipe DM, Howlet PM, eds. *Fundamental Virology*, 3rd ed. Philadelphia: Lippincott-Raven, pp. 561–575.

Wunner WH, Calisher CH, Dietzgen RG, et al. 1995. *Arch Virol* 140(Suppl 10):275–288.

Yates WDG, Rehmtulla AJ, McIntosch DW. 1983. *Can Vet J* 24:162–163.

Zimmer B, Summermatter K, Zimmer G. 2013. *Vet Microbiol* 162:78–84.

46

Togaviruses

Fun-In Wang, Chia-Yi Chang, and Chin-Cheng Huang

Overview

The family *Togaviridae* comprises two genera: *Alphavirus* and *Rubivirus*. The mostly arthropod-borne genus *Alphavirus* includes about 30 recognized members that can cause a variety of human and animal diseases. The genus *Rubivirus* is composed of a single member (rubella virus) pathogenic to humans. The virions are spherical, 70 nm in diameter, and consist of an envelope with fine peplomers surrounding an icosahedral nucleocapsid 40 nm in diameter. The peplomers are formed by 80 trimers of the envelope proteins E1–E2 heterodimer.

The genome is a linear positive-sense, single-stranded RNA 9.7–11.8 kb in size, arranged as 5′ m_7G-nsP1-nsP2-nsP3-nsP4 - C-E3-E2-6 K-E1-$(A)_n$ 3′. Genomic RNA serves as mRNA to translate into a polyprotein, which is processed into mature nonstructural proteins (nsP). The structural proteins are translated from a subgenomic 26S mRNA and cotranslationally processed into mature proteins (Powers et al. 2012). The E2 protein contains most neutralizing epitopes, while the E1 protein contains more conserved, cross-reactive epitopes.

Alphaviruses are stable at pH 7–8, but rapidly inactivated by very acidic pH. Virions have a half-life of 7 hours at 37 °C (98.6 °F) in culture medium, and most are rapidly inactivated at 58 °C (136 °F) with a half-life of minutes. Generally, they are sensitive to organic solvents and detergents, which solubilize their lipoprotein envelopes.

Based on serological cross-reactivity, alphaviruses are grouped into eight antigenic complexes (nine including the fish alphaviruses). Eastern equine encephalitis virus (EEEV) is in the EEEV complex, and Getah virus (GETV), Sagiyama virus (SAGV), and Ross River virus (RRV) are in the Semliki Forest complex.

Eastern equine encephalitis virus

EEEV is endemic in eastern Canada, the United States east of the Mississippi River, the Caribbean islands, and Central and South America. Ornithophilic mosquitoes (e.g. *Culiseta melanura*) maintain endemic cycles. Epidemic cycles are mediated by arthropod vectors (e.g. *Aedes* spp., *Anopheles* spp., and in some settings *Coquillettidia* spp.) that feed on both birds and mammals.

EEEV is zoonotic and causes encephalitis, fever, drowsiness, and nuchal rigidity in humans. Symptoms may progress to confusion, paralysis, convulsion, and coma.

In pigs, response to EEEV infection is age dependent. Nursing pigs are most severely affected, and infection in pigs older than 2 months is inapparent. Clinical signs include incoordination, depression, seizure, vomiting, low fever, and mortality. Growth is usually retarded in survivors. Predisposing factors, such as environmental conditions and concurrent disease(s), may contribute to higher mortality.

In 1958, serology on pigs in the states of Georgia, Massachusetts, and Wisconsin of the United States showed that prevalence ranged from 17 to 26%. In Georgia, up to 20% of domestic herds and 16% of feral swine were seropositive, with virus neutralization (VN) titers of 1 : 4–1 : 128 (Elvinger et al. 1996). VN antibody titers in domestic swine were generally lower than feral swine, and pigs from sale barns and stockyards had seropositive rates of 7%. However, a natural EEEV outbreak was not reported until 1972 in 3-week-old pigs (Pursell et al. 1972) and in 1991 in pigs under 2 weeks of age, with 80% mortality (Elvinger et al. 1994). Thus, EEEV infection in pigs is not uncommon, but clinical episodes are rare, as witnessed by no apparent increase in reports of EEEV episodes in pigs, despite the global climate extremes experienced in recent years.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

The incubation period in pigs is unknown, since most infections are subclinical, but ranged from 1 to 3 days in experimental inoculations (Elvinger and Baldwin 2006). EEEV initially replicates in regional lymph nodes and then invades extraneural tissues, resulting in high virus titers and secondary viremia. Viremia is the key to invasion of the central nervous system (CNS). Viremia lasts for 7 days post inoculation (DPI). EEEV is recoverable from oropharyngeal and rectal swabs for 4 DPI and from tonsils for 20 DPI. Potentially, infected young pigs could transmit EEEV to contacts and serve as a source of virus for vectors (Elvinger and Baldwin 2006).

Gross lesions are unremarkable. Microscopic lesions include meningoencephalitis with necrosis, microabscesses, perivascular cuffing of neutrophils (early) and lymphocytes (later), and myocardial necrosis. Pigs that die in the acute phase may not have CNS lesions (Elvinger et al. 1994).

Diagnosis by viral isolation can be done using CNS tissues and/or the specimens specified above in Vero cells or other cell culture systems, followed by identification by immunofluorescence or polymerase chain reaction (PCR) (Ostlund 2012).

There is no treatment for clinically affected pigs. Prevention is achieved by vaccination of animals at risk and/or control of the vector population. Vaccines are available for humans and horses. Vaccination may be economically justifiable in severe outbreaks or for the protection of valuable pigs. Vaccination of sows will provide protective maternal antibody to piglets.

Getah virus

GETV is present throughout Asia as far north as Russia and south to Sarawak in Malaysia. It is primarily a pathogen of horses, but the virus has been isolated from a variety of mosquito species in the vicinity of swine. *Aedes vexans nipponii, Culex tritaeniorhynchus, Culex gelidus,* and *Culex fuscocephala* are considered the main vectors, depending on the location.

GETV was first reported in swine in 1987 and in newborn piglets exhibiting depression, tremors, yellowish-brown diarrhea, and 100% case mortality (Yago et al. 1987). Subclinically infected piglets showed a transient depression in growth rate, whereas sows were unaffected. GETV has also been recovered from late-term dead fetuses from a naturally infected sow (Shibata et al. 1991). Sows inoculated before 26–28 days of gestation farrowed dead fetuses, with virus recoverable from the placenta, amniotic fluid, and fetal organs (Izumida et al. 1988), likely attributed to the presence of virus in the blood and various hematolymphoid organs (Kumanomido et al. 1988). GETV and Japanese encephalitis virus (JEV) share vector species (e.g. *C. tritaeniorhynchus*), habitats, and transmission cycles and produce similar clinical signs in pigs, and infection with GETV may be masked by coinfection with JEV (Tajima et al. 2014).

In Japan where GETV is endemic in some districts, serum antibody rates were 3–19% in domestic pigs (Hohdatsu et al. 1990) and averaged 48% in wild boar, with higher seropositive rates in adult pigs (Sugiyama et al. 2009). In areas of Sri Lanka where there is abundant rainfall and small-scale pig herds are surrounded by rice fields, there is subclinical infection with a seroprevalence of 41% compared with only 0.6% in humans (Peiris et al. 1994).

Diagnosis is done by viral isolation on rabbit kidney (RK-13) or Vero cells or by detection of viral RNA by PCR (Ogawa et al. 2009). GETV has been co-isolated with JEV, grows faster than JEV in successive passages, and produces clear plaques resembling those of alphavirus (Tajima et al. 2014). ELISA or hemagglutination inhibition (HI) is available for use in paired sera. An inactivated vaccine has been used successfully in racehorses.

Sagiyama virus

SAGV is considered a variant of GETV, but can be differentiated from GETV by complement fixation due to the presence of the amino acid leucine in the SAGV capsid protein (versus proline in GETV) (Wekesa et al. 2001). *C. tritaeniorhynchus* and *Aedes vexans* are believed to be the major vectors.

Natural subclinical infection in pigs was first reported in the 1960s, albeit the VN antibody rate in pigs was 67 versus 18% in humans living in the vicinity of infected pigs (Scherer et al. 1962). These antibody rates are higher than those reported for GETV and raise the question as to whether the presence of leucine on its capsid protein might indicate selection for more efficient transmission.

The first SAGV outbreak was reported in 2006 in 9-week-old pigs exhibiting growth retardation, panting, abdominal breathing, and arthritis (Chang et al. 2006). The clinical picture may have been affected by concurrent infection with JEV and porcine circovirus 2 (PCV2); thus, subsequent intramuscular inoculation of nursery pigs produced no specific clinical signs.

A low level of SAGV viremia occurs at 2–4 DPI, during which time virus can be recovered from the spleen, tonsil, lymph nodes, and kidney, but not from oral and nasal swabs. VN antibody can be detected by 4 DPI, and viremia is resolved by 7 DPI. SAGV is considered nonpathogenic to pigs, but concurrent infections may interact synergistically to cause more severe clinical signs.

SAGV causes CPE in swine testis (ST) (ATCC CRL-1746™, Rockville, MD), porcine kidney (PK-15),

Vero, RK-13, and baby hamster kidney (BHK-21) cells. Virus isolation is definitive, but has little diagnostic value due to the transient nature of viremia. Antibody detection is indicative of SAGV infection, in which VN antibody titers >1:48 or a seropositive rate >50% is suggestive of repeated exposures to SAGV.

There is neither treatment nor vaccine for SAGV infection, leaving vector control as the most rational form of prevention and control.

Ross river virus

RRV is endemic to Australia, Papua New Guinea, and Irian Jaya, Indonesia. Several RRV epidemics occurred in south Pacific islands in 1979–1980, but it did not become permanently established there. The main vectors are *Aedes vigilax*, *Aedes camptorhynchus*, *Aedes polynesiensis*, and *Culex annulirostris*.

RRV is regularly transmitted via a human–mosquito–macropod marsupials–mosquito–human cycle. However, in epidemics in densely populated areas, a direct human–mosquito–human route is believed to occur because of the high levels of viremia reached in humans. However, the threat may be low for RRV to emerge in areas where the macropod population and the density of major vector species are not abundant (Tompkins and Slaney 2014).

RRV is not known to cause disease in pigs. Experimental infection of juvenile domestic and feral pigs resulted in no detectable virus or only moderate viremia at 0–5 DPI (Harley et al. 2001). During epidemics, the seroprevalence in domestic pigs was 43 and 77% by HI and VN, respectively (Rosen et al. 1981). During inter-epidemic intervals, seroprevalence was 15% in feral pigs by HI (Gard et al. 1976).

In humans, RRV infection may cause symmetrical epidemic polyarthritis, predominantly involving peripheral joints, fever, skin rash, and constitutional effects like myalgia, fatigue, and malaise. Seroconversion of immunoglobulin G in paired sera as demonstrated by ELISA is indicative of a recent infection. Detection of immunoglobulin M is diagnostically unreliable, particularly in endemic areas. PCR assays have been described for human and equine sera (Sellner et al. 1995; Studdert et al. 2003) and should be used in conjunction with serology. Nonsteroidal anti-inflammatory drugs (NSAID) provide the best and most effective symptomatic relief. No vaccine is available for human use.

References

Chang CY, Huang CC, Huang TS, et al. 2006. *J Vet Diagn Invest* 18:156–161.

Elvinger F, Baldwin CA. 2006. Eastern equine encephalomyelitis virus. In Straw BE, Zimmerman J, D'Allaire S, et al., eds. *Diseases of Swine*, 9th ed. Ames, IA: Blackwell Publishing Company, pp. 554–557.

Elvinger F, Liggett AD, Tang KN, et al. 1994. *J Am Vet Med Assoc* 205:1014–1016.

Elvinger F, Baldwin CA, Liggett AD, et al. 1996. *J Vet Diagn Invest* 8:481–484.

Gard GP, Giles JR, Dwyer-Gray RJ, et al. 1976. *Aust J Exp Biol Med Sci* 54:297–302.

Harley D, Sleigh A, Ritchie S. 2001. *Clin Microbiol Rev* 14:909–932.

Hohdatsu T, Ide S, Yamagishi H, et al. 1990. *Jpn J Vet Sci* 52:835–837.

Izumida A, Takuma H, Inagaki S, et al. 1988. *Jpn J Vet Sci* 50:679–684.

Kumanomido T, Wada R, Kanemaru T, et al. 1988. *Vet Microbiol* 16:295–301.

Ogawa H, Taira O, Hirai T, et al. 2009. *J Virol Methods* 160:210–214.

Ostlund EN. 2012. Chapter 2.5.5: Equine encephalomyelitis (Eastern and Western). In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Vol. 2, 7th ed. Paris, France: OIE, World Organisation for Animal Health.

Peiris JSM, Amerasinghe PH, Amerasinghe FP, et al. 1994. *Am J Trop Med Hyg* 51:154–161.

Powers A, Huang H, Roehrig J, et al. 2012. Togaviridae. In King AMQ, Adams MJ, Carstens EB, et al., eds. *Virus Taxonomy: Classification and Nomenclature of Viruses: The Ninth Report of the International Committee on Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press, pp. 1103–1110.

Pursell AR, Peckham JC, Cole JR, et al. 1972. *J Am Vet Med Assoc* 161:1143–1147.

Rosen L, Gubler DJ, Bennett PH. 1981. *Am J Trop Med Hyg* 30:1294–1302.

Scherer WF, Funkenbusch M, Buescher EL, et al. 1962. *Am J Trop Med Hyg* 11:255–268.

Sellner LN, Coelen RJ, Mackenzie JS. 1995. *Clin Diagn Virol* 4:257–267.

Shibata I, Hatano Y, Nishimura M, et al. 1991. *Vet Microbiol* 27:385–391.

Studdert MJ, Azuolas JK, Vasey JR, et al. 2003. *Aust Vet J* 81:76–80.

Sugiyama I, Shimizu E, Nogami S, et al. 2009. *J Vet Med Sci* 71:1059–1061.

Tajima S, Kotaki A, Yagasaki K, et al. 2014. *Arch Virol* 159:2969–2975.

Tompkins DM, Slaney D. 2014. *Vector Borne Zoonotic Dis* 14:141–148.

Wekesa SN, Inoshima Y, Murakami K, et al. 2001. *Vet Microbiol* 83:137–146.

Yago K, Hagiwara S, Kawamura H, et al. 1987. *Jpn J Vet Sci* 49:989–994.

Section IV

Bacterial Diseases

47

Overview of Bacteria

Karen W. Post

Diseases caused by bacteria including mycoplasmas and chlamydias are all considered in this chapter. While viral diseases such as porcine reproductive and respiratory virus (PRRSV) and porcine circovirus remain as some of the greatest challenges to worldwide pork production, bacterial diseases continue to have a significant impact on the industry. Concurrent bacterial infection with lung pathogens such as *Bordetella bronchiseptica* and *Mycoplasma hyopneumoniae* has been shown to increase the severity and duration of PRRSV-induced pneumonia (Brockmeier et al. 2000; Thacker et al. 1999). Bacterial respiratory and enteric infections are among the most common and economically significant diseases facing swine producers today. A major pneumonia complex is *M. hyopneumoniae* with secondary bacterial bronchopneumonia (Straw et al. 1989). *Escherichia coli* is among the most important cause of neonatal and post weaning diarrhea in pigs (Fairbrother and Gyles 2006).

Phenotypic classification of bacteria places them into groups based upon readily identifiable characteristics, which include Gram stain reaction, microscopic cellular morphology, oxygen requirements, and ability to form endospores (Table 47.1).

The Gram stain reaction divides bacteria into gram-positive or gram-negative forms based upon differences in the composition and thickness of their cell wall. Although most of the swine bacterial pathogens are able to be Gram stained, there are notable exceptions. Even though mycoplasmas are of gram-positive lineage, their permanent lack of a cell wall precludes them from retaining the crystal violet stain. Treponemes and leptospires cannot be satisfactorily stained and observed microscopically by this method. Additionally the high lipid and mycolic acid content in the cell walls of *Mycobacterium* species may render them difficult to Gram stain.

Bacterial cellular morphology comprises cocci (spheres), bacilli (rods), and spirochetes (curved rods or spiral forms). Within these three morphologic groups, there may be considerable variability in shape and size. Cocci may be found in clusters (*Staphylococcus* species) or in long or short chains (*Enterococcus* and *Streptococcus* species). While many bacilli are considered to be regular rods (members of the genera *Escherichia, Salmonella,* and *Listeria*), others may appear coccobacillary (*Pasteurella*), pleomorphic (*Trueperella* [*Arcanobacterium*] *pyogenes* and *Actinobacillus* species), or filamentous in shape (*Haemophilus parasuis* and *Erysipelothrix rhusiopathiae*). Among the spirochetes there are those that are loosely or tightly coiled (*Brachyspira, Treponema,* and *Leptospira* species) or curved to seagull shaped (*Campylobacter* species and *Lawsonia intracellularis*). The size of the individual bacterial cell may vary depending upon its growth phase and the type of medium the organism was cultivated in or on. As a general rule, most spirochetes, *Bacillus* species, and clostridia are regarded as large. Medium-sized organisms include pseudomonads and members of the family *Enterobacteriaceae* (salmonellae and *E. coli*). *Brucella*, *Haemophilus,* and *Pasteurella* species are small in size, while mycoplasmas and chlamydias are extremely small.

Bacteria may be further categorized by their ability to utilize or tolerate oxygen. Bacteria with an absolute requirement for oxygen are called obligate aerobes. There are no genera of swine bacterial pathogens that fall into this category. Facultatively anaerobic bacteria can survive in the presence or absence of oxygen, and this category represents the majority of the swine bacterial pathogens. Microaerophilic bacteria require trace amounts of oxygen for growth but may be killed by normal atmospheric concentrations. Members of the genus *Campylobacter* best represent the microaerophiles. Obligate anaerobes are killed by even trace amounts of oxygen. Many clostridia are obligate anaerobes.

Another classification of bacteria, primarily gram-positive bacilli, is based on their ability to produce spores. Spore-forming bacteria produce a unique resting cell called the endospore when vegetative cells are deprived of a necessary growth factor or requirement.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 47.1 Classification of the principal bacterial pathogens of swine.

Classification	Genus/genera
Gram-positive aerobic to facultatively anaerobic cocci	*Enterococcus, Staphylococcus, Streptococcus*
Gram-positive aerobic to microaerophilic non-spore-forming bacilli	*Trueperella, Erysipelothrix, Listeria, Mycobacterium, Rhodococcus*
Gram-positive aerobic spore-forming bacilli	*Bacillus*
Gram-positive anaerobic spore-forming bacilli	*Clostridium*
Gram-positive anaerobic non-spore-forming bacilli	*Actinobaculum*
Gram-negative aerobic to facultatively anaerobic bacilli	*Actinobacillus, Bordetella, Brucella, Burkholderia, Escherichia, Haemophilus, Pasteurella, Salmonella, Yersinia*
Gram-negative microaerophilic to anaerobic curved to spiral-shaped bacilli	*Brachyspira, Campylobacter, Lawsonia, Leptospira, Treponema*
Bacteria without cell walls and obligately intracellular bacteria	*Mycoplasma, Chlamydophila*

Spores are extremely resistant to harsh environmental conditions and disinfectants. The two genera of sporeformers with veterinary medical importance are *Bacillus*, the members of which are aerobic sporeformers, and *Clostridium*, whose species are anaerobic sporeformers. Notable non-spore-forming bacilli that represent swine pathogens include *Actinobaculum suis, E. rhusiopathiae,* and *T.* (*A.*) *pyogenes*.

Knowledge of the characteristics of a given bacterium is useful to the practitioner. The Gram stain reaction of a suspected pathogen can provide information regarding which antimicrobial agent to prescribe for empiric therapy while the veterinarian is awaiting the results of culture and susceptibility testing. When carrying out primary observations on stained clinical materials, it is important to know the Gram stain and cellular morphology of the common pathogens that might be present in the specimen. Knowledge of a bacterium's oxygen requirements is an important consideration when collecting and transporting diagnostic specimens to the laboratory. Organisms that produce spores, such as *Clostridium difficile* and *Clostridium perfringens*, may be extremely difficult to eliminate from the farrowing house environment because the spores are resistant to most disinfectants, heat, and ultraviolet light. This is an important consideration for practitioners faced with the challenge of managing these infections in neonates.

The majority of swine bacterial pathogens are able to be grown on artificial culture media that provide necessary nutrients. Of these, many may form discrete colonies on plated media within 24–72 hours of inoculation. Other bacteria like *Leptospira* and *Mycobacterium* species may require several months for growth to occur in culture media. Some obligate intracellular parasites such as chlamydias and *L. intracellularis* can only be cultivated *in vivo* or in cell culture systems because they are unable to produce energy in amounts required to sustain their metabolism outside a living host.

Swine bacteria may be considered as normal flora or commensals, opportunists, or frank pathogens. Commensals live on or in the host harmlessly. Most of the aerobic and anaerobic bacteria in the porcine alimentary tract are commensals. Opportunistic pathogens cause disease when the host's innate or acquired immunity is compromised in some way. *T. (A.) pyogenes* represents one of the most common opportunists. Frank pathogens are consistently able to infect and cause disease in a percentage of healthy hosts by evading various defense mechanisms. *Bacillus anthracis* is an example of a frank pathogen.

Bacterial pathogenicity is a multifactorial process. Bacteria initially must establish an infection. This process involves bacterial attachment or other means of gaining entry into the host, evading host defenses, multiplying to significant numbers, causing damage to the host either directly or indirectly, and concluding with transmission of the agent to another susceptible host (Gyles and Prescott 2004). Host immune factors, the numbers of organisms present in the initial exposure, and the virulence attributes of the bacterium all play a role in the development of disease.

As a general rule, bacteria cause disease by two primary mechanisms: tissue invasion and toxin production (Songer and Post 2005). To invade host tissues, bacteria employ methods for adhering to and/or penetrating cells, producing extracellular substances to facilitate the process of invasion, and overcoming host defenses. Adhesins are surface proteins that cause adhesion of organisms to host cells. Many strains of pathogenic *E. coli* possess surface adhesive organelles called pili that mediate cellular attachment. *Listeria monocytogenes* and *Yersinia* species are examples of facultatively intracellular bacteria that employ invasins to gain entry into host cells (Niemann et al. 2004). Certain bacteria produce extracellular enzymes like coagulase produced by *Staphylococcus hyicus* and streptokinase produced by

beta-hemolytic streptococci that enable these agents to spread widely within host tissue. For those bacteria that cause disease through toxin production, exotoxin and endotoxin are the major types. Exotoxins are proteins released primarily by gram-positive bacteria into their extracellular environment. They vary greatly in their potency, ranging from the highly toxic botulinum toxin to the weakly toxic product released by *T. (A.) pyogenes*. Other swine pathogens that may produce exotoxins are *Clostridium perfringens*, enteropathogenic strains of *E. coli*, *Pasteurella multocida*, and *S. hyicus*. Endotoxins are the lipopolysaccharides found in the cell wall of gram-negative bacteria. These may be released from both actively growing bacterial cells and those that have been lysed as a result of the effect of certain antibiotics or through successful host defense mechanisms like lysozyme production. The release of endotoxins is a very important component of the toxicity of gram-negative bacteria and is directly responsible for many of the clinical signs produced by these pathogens including fever, shock, and disseminated intravascular coagulopathy.

Many swine bacterial diseases may be recognized by their specific clinical signs, gross postmortem lesions, or epidemiological features (Tables 47.2 and 47.3). Direct contact, close contact with infected droplets or feces, and mechanical transfer by fomites or vectors are the ways in which bacteria are commonly spread. Succeeding chapters provide in-depth coverage of bacterial diseases including relevance, potential public health significance, epidemiology, pathogenesis, clinical signs, lesions, diagnosis, immunity, and prevention and control.

Table 47.2 Gram-positive bacteria and associated swine disease(s) and/or clinical signs.

Bacterium	Disease(s) and/or clinical signs
Actinobaculum suis	Cystitis, pyelonephritis
Actinomyces hyovaginalis	Sporadic abortion, embolic pneumonia
Bacillus anthracis	Anthrax
Clostridium botulinum	Botulism
Clostridium chauvoei	Blackleg
Clostridium difficile	Neonatal colitis
Clostridium perfringens	Neonatal enteritis, pseudomembranous colitis
Clostridium novyi	Sudden death, hepatitis
Clostridium septicum	Malignant edema
Clostridium tetani	Tetanus
Enterococcus species	Enteritis
Erysipelothrix rhusiopathiae	Erysipelas
Listeria monocytogenes	Listeriosis, abortion, encephalitis, septicemia
Mycobacterium species	Tuberculosis
Mycoplasma hyopneumoniae	Enzootic pneumonia
Mycoplasma hyorhinis	Arthritis, otitis, polyserositis
Mycoplasma hyosynoviae	Arthritis
Mycoplasma suis	Anemia, infertility, decreased growth rate, pericarditis, unthriftiness
Rhodococcus equi	Granulomatous lymphadenitis
Staphylococcus aureus	Abscesses, arthritis, enteritis, mastitis, metritis, neonatal septicemia, vaginitis
Staphylococcus hyicus	Exudative epidermitis
Streptococcus dysgalactiae subsp. equisimilis	Arthritis, endocarditis, meningitis, septicemia
Streptococcus porcinus	Cervical lymphadenitis
Streptococcus suis	Septicemia, arthritis, endocarditis, polyserositis
Trueperella abortisuis	Sporadic abortion
Trueperella pyogenes	Abscesses, arthritis, endocarditis, mastitis, osteomyelitis, pneumonia

Table 47.3 Gram-negative bacteria and associated swine diseases and/or clinical signs.

Bacterium	Disease(s) and/or clinical Signs
Actinobacillus pleuropneumoniae	Pleuropneumonia
Actinobacillus suis	Pneumonia, septicemia
Bordetella bronchiseptica	Nonprogressive atrophic rhinitis, pneumonia
Burkholderia pseudomallei	Melioidosis, internal abscesses, lymph node abscesses
Brachyspira hyodysenteriae, Brachyspira hampsonii, or Brachyspira suanatina	Swine dysentery
Brachyspira pilosicoli	Porcine intestinal/colonic spirochetosis
Brachyspira murdochii or Brachyspira intermedia	Mild colitis, loose stools
Brucella suis	Brucellosis, abortion, arthritis, infertility
Campylobacter coli/Campylobacter jejuni	Subclinical enterocolitis
Chlamydia suis	Conjunctivitis, neonatal diarrhea, pneumonia, possibly abortion
Chlamydia abortus	Early embryonic death, abortion
Escherichia coli	Colibacillosis, edema disease, cystitis, enteritis, mastitis, neonatal septicemia
Haemophilus parasuis	Glässer's disease, arthritis, polyserositis
Klebsiella pneumoniae	Piglet septicemia
Lawsonia intracellularis	Porcine proliferative enteropathy
Leptospira species	Leptospirosis, infertility, stillbirths, weak piglets
Pasteurella multocida	Progressive atrophic rhinitis, pneumonia
Salmonella enterica	Enteric and septicemic salmonellosis
Treponema pedis	Cutaneous spirochetosis
Yersinia enterocolitica	Subclinical enterocolitis
Yersinia pseudotuberculosis	Diarrhea, enterocolitis

References

Brockmeier SL, Palmer MV, Bolin SR. 2000. *Am J Vet Res* 61:892–899.

Fairbrother JM, Gyles CL. 2006. *Escherichia coli* infections. In Straw BE, Zimmerman JJ, D'Allaire S, et al., eds. *Diseases of Swine*, 9th ed. Ames, IA: Blackwell Publishing, pp. 639–679.

Gyles CL, Prescott JF. 2004. Themes in bacterial pathogenic mechanisms. In Gyles CL, Prescott JF, Songer JG, eds. *Pathogenesis of Bacterial Infections in Animals*, 3rd ed. Ames, IA: Blackwell Publishing, pp. 3–12.

Niemann HH, Wolf-Dieter S, Heinz DW. 2004. *Microbes Infect* 6:101–112.

Songer JG, Post KW. 2005. Origin and evolution of virulence. In *Veterinary Microbiology: Bacterial and Fungal Agents of Animal Disease*. St. Louis, MO: Elsevier Saunders, pp. 3–9.

Straw B, Tuovinen VK, Bigras-Poulin M. 1989. *J Am Vet Med Assoc* 195:1702–1706.

Thacker EL, Halbur PG, Ross RF, et al. 1999. *J Clin Microbiol* 37:620–627.

48

Actinobacillosis

Marcelo Gottschalk and André Broes

Actinobacillus pleuropneumoniae

Relevance

Actinobacillus pleuropneumoniae (App) (originally named as *Haemophilus pleuropneumoniae*) is the etiologic agent of porcine pleuropneumonia. An organism also isolated from porcine pleuropneumonia, originally known as "*Pasteurella haemolytica*-like," was later determined to be a nicotinamide adenine dinucleotide (NAD)-independent biotype of App (Pohl et al. 1983) and is known as App biotype II (see below).

App is one of the most important swine bacterial respiratory pathogens and is found worldwide. The most virulent strains can induce rapidly fatal fibrinohemorrhagic and necrotizing pleuropneumonia in swine of all ages. Survivors often have devitalized bacteria-laden sequestra in their lungs. The economic importance of App is mainly due to the mortality, reduced growth, veterinary costs (antimicrobials, vaccinations), and condemnations at the slaughterhouse. However, in chronically infected herds, results from studies investigating impact on average daily gain have been controversial (Andreasen et al. 2001; Hartley et al. 1988; Hunneman 1986).

Virulence of App strains varies remarkably; some strains produce high mortality, others are avirulent, and yet others are intermediate in virulence. High mortality outbreaks are now rather infrequent in the United States and Canada, but remain a problem in Latin American, Asian, and European countries (Gottschalk et al. 2003a). Certain App strains can also act as secondary pathogens contributing to increased mortality in conjunction with other viral and bacterial pathogens as a component of the porcine respiratory disease complex (PRDC).

App infections can be frustrating for swine producers and veterinarians. Many herds are infected with several strains. High virulence strains may be present in a herd for extended periods of time without evidence of clinical signs or even lesions at the slaughterhouse. The organism resides mainly in the tonsils, and carrier pigs can be difficult to detect. Outbreaks may suddenly appear in the presence of concomitant diseases or concurrent with changes in management or other significant stressors such as overcrowding. There are not readily available laboratory tests to differentiate highly virulent strains from strains of lesser virulence.

Both the organism and the disease have been studied extensively. Knowledge gained has allowed for better diagnostic tests and strategies, vaccines, and relatively effective eradication strategies. However, App remains a significant cause of economic loss to the swine industry, and there remains opportunity for improvement in control and eradication of this agent.

Etiology

App is a small, gram-negative encapsulated rod with typical coccobacillary morphology. App isolates are classified on the basis of the NAD requirement for *in vitro* growth into biotype I (NAD dependent) and biotype II (NAD independent) (Pohl et al. 1983). Biotype I strains do not grow on blood agar unless it contains NAD or unless NAD is supplied by a staphylococcal nurse streak where colonies of App grow in immediate proximity as "satellites." App forms colonies 0.5–1 mm after 24 hours of incubation and is usually beta-hemolytic. In fact, App produces an increased zone of hemolysis within the zone of partial lysis surrounding a beta-toxigenic *Staphylococcus aureus* or *Staphylococcus epidermidis* (the CAMP phenomenon) (Nicolet 1970). This CAMP phenomenon is due to secretion of combinations of the cytolysins ApxI, ApxII, and ApxIII by various strains of App (see below) (Frey et al. 1994). Additional detailed morphologic and biochemical characteristics may be found in original reports (Kilian et al. 1978). Biotype I strains should be differentiated from other NAD-dependent actinobacilli present in the upper respiratory

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

tract of swine such as *Actinobacillus minor*, *Actinobacillus porcinus*, or *Actinobacillus porcitonsillarum* (Gottschalk et al. 2003b; Kielstein et al. 2001). Definitive identification may require species-specific polymerase chain reaction (PCR) testing (Schaller et al. 2001; Tonpitak et al. 2007). Biotype II strains grow easily on blood agar plates without the presence of NAD; colonies must be differenced from those of *Actinobacillus suis* using a panel of biochemical tests or by PCR (Serrano-Rubio et al. 2008). This is especially important because *A. suis* may induce pleuropneumonia similar to App (MacInnes and Desrosiers 1999; MacInnes et al. 2008; Yaeger 1995) but has different implications for control.

App biotype I has been divided into 14 serotypes (1–12, 15, and 16) and biotype II into 2 serotypes (13–14) for a total of 16 serotypes (Blackall et al. 2002; Kamp et al. 1987; Kilian et al. 1978; Nielsen 1985a,b, 1986b; Nielsen et al. 1997; Nielsen and O'Connor 1984; Rosendal and Boyd 1982; Sárközi et al. 2015). Serotype 5 is subdivided into subtypes 5a and 5b (Nielsen 1986a); however, this subdivision has neither epidemiological nor pathological significance, and most laboratories do not perform the subtyping. The above association of serotypes and biotypes is predominant but not exclusive. Biotype II strains belonging to serotypes 2, 4, 7, 9, and 11 (normally found among biotype I strains) have been reported (Beck et al. 1994; Maldonado et al. 2009). Additionally, biotype I serotype 13 strains that are antigenically different from the reference serotype 13 biotype II strain have been isolated from the United States and Canada (Perry et al. 2012). Untypable biotype I or biotype II strains are sometimes isolated in both North America and Europe, and new serotypes will probably be described soon (Gottschalk et al. 2003a; Bossé J, unpublished observations).

Serotype specificity is considered to be conferred by the capsular polysaccharides (CPS) (Dubreuil et al. 2000). CPS are usually associated with particular cell wall lipopolysaccharides (LPS), i.e. CPS type 1 is usually associated with LPS type 1. However, some capsular serotype groups share identical O-chain LPS, thus explaining the cross-reactions observed in serological assays using LPS as antigen between serotypes 1, 9, and 11; serotypes 3, 6, 8, and 15; and serotypes 4 and 7 (Dubreuil et al. 2000; Gottschalk 2015; Perry et al. 1990). Yet, isolates with a given capsular type and a different LPS type have been sporadically reported. For example, strains of CPS/LPS serotype 1/7 and 2/7 have been reported in North America and Europe, respectively (Gottschalk et al. 2000; Nielsen et al. 1996), whereas a CPS/LPS 12/3 strain has been recently described in Japan (Ito and Matsumoto 2015). The biotype I serotype 13 strains recovered in North America are antigenically different from the biotype II serotype 13 reference strain (isolated in Europe) in that they have an LPS that cross-reacts with that from App serotype 10 (Perry et al. 2012). These strains can cause false-positive serotype 10 serologic reactions in LPS-based serologic tests (see below). It has been suggested that serotypes of App should be more rigorously defined by specifying both capsular (K) and LPS (O) antigens (Perry et al. 1990), but this nomenclature has not been widely adopted (Dubreuil et al. 2000).

Public health

App does usually not infect humans and poses no public health risk. However, necrosis from needlestick injury with live App vaccine (non-virulent strain for pigs) and the isolation of an App serotype 5 strain from a poorly healing wound of a swine producer after being bitten by a boar have been reported (Rycroft et al. 2011; R. Higgins, personal communication).

Epidemiology

Distribution of App

App is widely distributed and only infects pigs, although a recent report suggested that an App strain was able to infect poultry (Pérez Márquez et al. 2014). The primary reservoir is domestic swine, but various studies in wild boar populations in Europe, North America, and Australia showed that this species is frequently infected by App (Hälli et al. 2014; McGregor et al. 2015; Pearson et al. 2014; Reiner et al. 2010). In Canada, these populations are serologically positive for serotype 14, a capsular type not found so far in domestic pigs in North America (McGregor et al. 2015).

Outbreaks of pleuropneumonia have been reported in domestic swine from practically all European countries and from different parts of the United States, Mexico, South America, Japan, Korea, Taiwan, and Australia as well as in many other countries. Interestingly, there has been a simultaneous increase in clinical App in recent years in many European countries as reflected in increased chronic pleuritic adhesions in slaughterhouses (Hoeltig et al. 2009; Sjölund and Wallgren 2010). This is possibly due to older weaning ages mandated by new welfare laws, since App is known to be a late colonizer in piglets and earlier weaning dramatically reduces carriage rates in weaned pigs.

The distribution of serotypes involved in acute outbreaks and chronic infections in different regions of the world is radically different. Moreover, strains of a given serotype may be highly virulent in one region, and yet strains of the same serotype may be of low virulence in another region. Therefore, it is critical when testing imported pigs for App prior to introduction into a closed herd to select diagnostic tests for the most important virulent serotypes present in their region of origin.

Unfortunately, only a few reports on the distribution of serotypes recovered from diseased animals have been published during the last 25 years (Gottschalk 2015). Moreover, the relative prevalence of serotypes may dramatically change during the years as described in Korea and Canada (Gottschalk and Lacouture 2015; Kim et al. 2016). In some regions, one or more virulent serotypes predominate and cause most outbreaks – serotypes 5 and 7 in North America, serotypes 2 and 9 in many European and Asian countries, and serotype 15 in Australia, to name a few. Serotype 2 strains are highly virulent in European and Asian countries owing to secretion of two cytotoxins, ApxII and ApxIII, but serotype 2 strains in North America secrete only ApxII and are of low virulence (Gottschalk et al. 2003a). Serotype 4 is one of the most frequent virulent serotypes in Spain, but is uncommon in most other countries (Maldonado et al. 2009). This serotype was isolated once from asymptomatic animals in Canada (Lebrun et al. 1999), but there are no other reports of serotype 4 strains in North America. Serotype 15, originally described and the predominant serotype in Australia (Blackall et al. 2002), has also occasionally been reported in North and South America (Gottschalk and Lacouture 2014; Gottschalk et al. 2003a) as well as in Japan (Koyama et al. 2007). Serotype 8 is by far the predominant serotype in the United Kingdom and occasionally isolated from diseased pigs in North America and Japan (Gottschalk and Lacouture 2014; Koyama et al. 2007; Li et al. 2016).

Biotype II strains are more commonly isolated in some European countries; however, these strains are occasionally reported in North America (Frank et al. 1992; Gottschalk et al. 2003a). Biotype II strains have traditionally been considered of low virulence; however, there are reports of their isolation in several cases of fatal pleuropneumonia (Gambade and Morvan 2001). Maldonado et al. (2009) reported that 25% of isolates recovered from diseased pigs in Spain belonged to biotype II of which serotypes 7, 2, 4, and 11 were the most common.

Most conventional herds are infected with one or more serotypes of App, and although these strains are often of low virulence, high virulence strains may also be present (Gottschalk et al. 2003a). Most prevalent serotypes (detected by serology) are often different from those recovered from diseased pigs (Gottschalk 2015). In a Spanish report, almost 90% of studied herds were serologically positive for App, many of them presenting pleuritic lesions at slaughterhouse, although the distribution of involved serotypes was not studied (Fraile et al. 2010). In a Canadian study, 78% of herds were App positive based on PCR detection in the upper respiratory tract of piglets (MacInnes et al. 2008). In the same study, 70% of the herds were seropositive by LPS-ELISA with distribution of serotypes as follows: 26% were positive for serotype 7/4, 17% for 12, 15% for 3/6/8/15, 6% for 5, 4% for 2, and 2% for 1/9/11. Although serotypes 2, 12, 3, 6, 8, and 15 are usually considered as of low/intermediate virulence (Gottschalk 2015; Gottschalk et al. 2003a), serotypes 7 and 5 are currently the most common serotypes recovered from diseased pigs in Canada (Gottschalk and Lacouture 2015). Interestingly, most herds included in the study did not present clinical signs of pleuropneumonia. The somewhat paradoxical trend of high seroprevalence combined with low disease prevalence highlights a concept important in the epidemiology of App. Low virulence strains tend to be highly prevalent in herds, resulting in high seroprevalence; however, highly virulent strains are carried by a much lower proportion of animals, resulting in lower seroprevalence. This has to be taken into account when determining the number of animals to be tested in serological surveillance programs.

Strains belonging to the same serotype have been genotypically compared. Although a given genotype profile cannot be clearly related to a given serotype (Gottschalk 2015), several studies have shown a relative homogeneity within a particular serotype with one or few clones represented and this, depending on the technique used (Chatellier et al. 1999; Fussing 1998; Møller et al. 1992). Further rapid subtyping methods are needed, and a combination of different methods will have to be used to reach conclusions about epidemiological relationships (Rossi et al. 2013). Complete genome sequencing has also been used to compare different strains belonging to the same serotype (Pereira et al. 2015).

Routes and duration of shedding and transmission

With few exceptions of systemic disease (Jensen et al. 1999; Madsen et al. 2001; Ohba et al. 2008, 2010), App is essentially a swine respiratory pathogen. In peracute and acute infections, it can be found not only in pneumonic lung but also in large numbers in nasal discharges. Survivors may remain carriers for several months (Desrosiers 2004) mainly in chronic lung lesions and tonsils. Some exposed animals remain healthy and become subclinical carriers mainly in the tonsils. Less frequently, carrier animals may harbor the organism in the nasal cavity (Chiers et al. 2010). Subclinical carriage of App in tonsils occurs not only for low virulence strains but also for high virulence strains that can sometimes be present in well-managed herds for long periods without clinical signs or lesions (Gottschalk 2015). Environmental stressors or concurrent respiratory pathogens can result in sudden clinical outbreaks.

Transmission between herds occurs mainly through the introduction of carrier animals. The main route of spread is by direct nose-to-nose contact or by droplets within short distances. Moving and mixing pigs increases the risk of spread.

In acute outbreaks, infection may not occur in every pen, suggesting the possible role of aerosols and air movement in the transmission of the disease over longer

distances within buildings, or the indirect transmission of contaminated exudates from acutely infected pigs by farm personnel. Studies confirm that App may be transmitted by aerosol over short distances (Desrosiers 2004; Jobert et al. 2000). Kristensen et al. (2004b) reported that airborne transmission between closely located pig units is possible, but uncommon. In contrast, Zhuang et al. (2007) suggested that local spread of App serotype 2 from neighboring herds was a predominant factor in the contamination of Danish specific-pathogen-free (SPF) genetic herds. Introduction of the disease by artificial insemination or embryo transfer is highly unlikely, since the genital tract is not a common site of infection and antimicrobials in the semen diluent or the embryo washing procedures could possibly prevent the persistence of the organism. Birds and small rodents are unlikely to transfer App and are not considered significant sources of the infection. The role of App-contaminated fomites in the transmission of the infection is suspected but has not been studied.

In endemically infected herds, App is transmitted by infected sows to their offspring via close contact. The frequency of transmission is likely related to the amount of bacteria shed by the sow, although more quantitative studies are needed. A recent study showed that although most sows were colonized, only some litters of piglets (and some piglets within a litter) were infected (Tobias et al. 2014). It has been suggested that the level of antitoxin maternal antibodies does not influence the colonization of piglets (Tobias et al. 2014). The level of maternal antibodies against the LPS, however, seems to be positively correlated with a late colonization (unpublished results). The persistence of colostral antibody in piglets ranges from 2 weeks to 2 months of age, depending on the initial level of acquired colostral antibodies, the antigen used in the test, and the serological test used (Vigre et al. 2003; unpublished observations). Usually only a few piglets are infected from their dams during the late suckling period; then lateral spread occurs after weaning as decay of maternal immunity renders more pigs susceptible (Vigre et al. 2002) or if mixed with naïve pigs.

Survival of the organism in the environment is of short duration, especially in warm, dry conditions. However, when protected with mucus or other organic matter, it can survive for several days (even weeks), and it can survive in clean water for periods of up to 30 days at 39 °F (4 °C). Common disinfectants are effective against App when organic matter is first removed by thorough washing (Gutierrez et al. 1995).

Pathogenesis

Virulence factors and pathogenesis of the infection

The incubation period can be quite variable. Inoculation of pigs with large numbers of virulent App can lead to fatal pleuropneumonia in as few as 6 hours. Following exposure by oronasal contact or inhalation, App first colonizes the epithelial cells on the surface and later in the crypts of the palatine tonsil (Chiers et al. 1999). Colonized epithelial cells vacuolate and desquamate and, along with transmigrating neutrophils, distend tonsillar crypts. In contrast, App does not bind well to the ciliated epithelium of the trachea or bronchi (Bossé et al. 2002); an exception may be the trachea of newborns (Auger et al. 2009). When able to reach the lower respiratory tract, App is able to adhere to pneumocytes that line alveoli (Bossé et al. 2002; Van Overbeke et al. 2002).

Colonization is dependent upon bacterial-to-cell adhesion that seems to be mediated by polysaccharides and proteins (Van Overbeke et al. 2002). Evidence from other pathogen–host cell interactions suggests that adherence to host cells is a complex and multifactorial process (Bossé et al. 2002). The oligosaccharide core of LPS seems to play an important role in adherence of App to swine cells (Chiers et al. 2010). The presence of proteinaceous fimbriae and fimbrial subunits on the surface of App has been demonstrated (Zhang et al. 2000). However, their eventual role in adhesion remains to be elucidated.

Once in the respiratory tract environment, certain bacterial nutrients, particularly iron, are scarce. App expresses a number of factors that are involved in the acquisition and uptake of iron (Chiers et al. 2010; Jacques 2004). Among other mechanisms, App is capable of utilizing porcine transferrin and heme compounds including free heme, hemin, hematin, and hemoglobin as well as a siderophore (Bossé et al. 2002; Chiers et al. 2010; Jacques 2004).

In pigs that are tonsillar carriers of virulent strains of App, the mucociliary apparatus normally clears any stray bacterial cells that are inhaled. This prevents App access to and subsequent replication in alveoli, the essential step to development of pleuropneumonia. Factors that overwhelm or reduce the function of the mucociliary apparatus are necessary to deliver sufficient App to alveoli for subsequent disease development. High inhaled doses of App in finely atomized particles can result in App reaching alveoli. This is most likely during acute outbreaks when sick pigs are shedding large amounts of App. Alternatively, cilia can be damaged by colonizing *Mycoplasma hyopneumoniae* or by replication of pseudorabies (Aujeszky's) virus or influenza virus in tracheal and bronchial epithelial cells. Marois et al. (2009) showed that experimental inoculation of 10-week-old pigs with a serotype 9 strain of App produced disease only in pigs previously exposed to *M. hyopneumoniae* and not in non-exposed controls. Other factors may also impair ciliary function, like chilling or high environmental ammonia levels. It has been suggested that outbreaks of App on endemic farms could be caused by a trigger inducing pneumonia in already infected pigs (Klinkenberg et al. 2014).

Once in the alveoli, a battle ensues between host innate and acquired immune mechanisms in the alveoli and virulence mechanisms of App to overcome them, the outcome determining whether App is killed or causes pleuropneumonia. Initially LPS on the surface of App acts a potent attractor of macrophages and neutrophils as well as stimulates host alveolar macrophages to secrete inflammatory cytokines. It has been recently reported (Brogaard et al. 2015) that a local expression of proinflammatory cytokines during App infection would play an important role in the pathogenesis of swine pleuropneumonia. In fact, these cytokines would activate macrophages and increase vascular permeability, flooding alveoli with important antibacterial serum proteins including complement and anti-App IgG antibodies (maternally derived, vaccinated, or previously exposed animals). App has several strategies to resist these host responses. The polysaccharide bacterial capsule of App inhibits engulfment by phagocytes as has been demonstrated by the characterization of non-virulent isogenic non-capsulated mutants (Rioux et al. 2000). Both macrophages and neutrophils can phagocytose App only in the presence of convalescent pig serum owing to the opsonic activity of anti-App IgG (Bossé et al. 2002). App is also resistant to the action of complement (Rioux et al. 2000; Ward and Inzana 1994).

The most important factors involved in impairment of the phagocytic function of both macrophages and neutrophils are the secreted protein RTX toxins ApxI, ApxII, and ApxIII (Frey 2003). ApxI is strongly hemolytic and cytotoxic, ApxII is weakly hemolytic and moderately cytotoxic, and ApxIII is nonhemolytic but strongly cytotoxic (Frey 2003). ApxI toxin has been shown to induce apoptosis in porcine alveolar macrophages (Chien et al. 2009), whereas ApxIII is highly toxic for peripheral blood mononuclear cells. In general, strains of serotypes 1, 5, 9, and 11 produce ApxI and ApxII; strains of serotypes 2, 3, 4, 6, 8, and 15 produce ApxII and ApxIII; strains of serotypes 7, 12, and 13 produce only ApxII; and strains of serotypes 10, 14, and 16 produce only ApxI (Gottschalk et al. 2003a; Sárközi et al. 2015). It seems that strains of serotype 3 secrete low levels of ApxII. A fourth toxin (ApxIV) is produced (*in vivo* only) by all serotypes (Schaller et al. 1999). The possible role of this toxin on phagocyte damage remains to be elucidated (Frey 2003). Using an ApxIV knockout mutant, it has been reported that ApxIV would be essential for expression of full virulence of App, although more studies are needed to confirm such hypothesis.

Finally, other virulence factors have also been suggested to play important roles in the pathogenesis of the infection, such as outer membrane proteins, proteases, and many products coded by genes that are clearly upregulated during infection, although in some cases their exact function is not yet known (Chiers et al. 2010). The capacity of App to form biofilms with a certain implication during App chronic infection and/or colonization has also been suggested as being part of the pathogenesis of the infection (Roberts et al. 2015). Finally, genome sequencing and preliminary analysis of strains belonging to serotypes 2, 3, 5, 6, 7, and 8 have been reported (Foote et al. 2008; Gouré et al. 2009; Li et al. 2016; Pereira et al. 2015; Xu et al. 2008; Zhan et al. 2010). The analysis of the functions of the encoded proteins will extend current knowledge on the metabolic and virulence characteristics of this pathogen.

Tissue damage in the lung is extensive and due principally to the combined effects of the Apx cytotoxins on a variety of lung cells (Frey 2003) and the App LPS-stimulated host inflammatory response. Host phagocytes are attracted by LPS- and Apx-induced chemokines. Macrophages are activated and secrete toxic oxygen metabolites, and macrophages as well as neutrophils are killed by Apx toxins and release lysosomal enzymes, which together further damage lung cells. Damage to endothelial cells results in activation of the coagulation pathway, microthrombus formation, and localized ischemic necrosis (Bossé et al. 2002). In most fatal cases of peracute pleuropneumonia, death is caused by endotoxic shock arising from absorption of copious amounts of App-derived LPS.

Factors affecting severity of disease

Differences in virulence between serotypes or even within the same serotype have often been observed. In the field, strains of serotypes 1, 5, and 7 in North America and of serotypes 2 and 9/11 (the latter being extremely difficult to be differentiated) in Europe are generally found to be more virulent than those of other serotypes. It is suggested that such differences are due to their combination of Apx toxins/hemolysins (Frey 2003), capsular structure (Jacques et al. 1988), and LPS composition (Jensen and Bertram 1986). In addition to production of ApxIV by all strains, virulent strains seem to produce 2 (rather than 1) of either Apx I, II, or III. Interestingly, atypical strains that are either more or less virulent than others in their respective serotype have often been shown to have either added (more virulent) or lost (less virulent) an Apx toxin (Beck et al. 1994; Gottschalk et al. 2003a; Maldonado et al. 2009). However, not all differences in virulence are explained by capsule, LPS, and Apx toxin profiles as exemplified by some low virulence serotype 1 strains that have no atypical CPS, LPS, or toxin profiles (Gottschalk et al. 2003a). Attempts have been made using molecular genetic techniques directed toward Apx toxin and other gene targets to differentiate degrees of virulence in strains of App (Chatellier et al. 1999; Møller et al. 1992); however, there are currently no methods to definitively differentiate virulence levels of App strains apart from controlled animal inoculation experiments.

Outcome of infection by App and severity of outbreaks as determined by increased morbidity and mortality can be impacted by several factors. As already mentioned, strain virulence and the presence of other pathogens like *M. hyopneumoniae*, pseudorabies virus, and, likely, swine influenza virus significantly impact disease. Interestingly, serotypes considered as low pathogenic (such as serotypes 6, 8, 12, and 15, at least in North America) can sometimes induce necrotizing bronchopneumonia or pleuropneumonia, especially in the presence of these pathogens. In fact, the same strain may either behave as completely avirulent or induce serious clinical disease in the absence or presence of a *M. hyopneumoniae* coinfection (Marois et al. 2009). A synergy between PRRSV and App is controversial. Early experimental studies showed that a coinfection does not result in more severe App-induced disease (Pol et al. 1997). This is in agreement with field observations in areas of endemic PRRSV infection (such as certain regions of Canada) where the incidence of clinical pleuropneumonia has not significantly increased. Even an antiviral effect of App antigens has been reported (Lévesque et al. 2014). However, a certain effect of the coinfection has been suggested for acute and chronic (pleuritic) infections (Fablet et al. 2012; van Dixhoorn et al. 2016). Factors such as crowding and adverse environmental conditions such as rapid changes in temperature and high relative humidity coupled with insufficient ventilation also promote the development and spread of the disease and, consequently, affect morbidity and mortality. It is therefore not surprising that the highest incidence of outbreaks is observed in growing and finishing pigs, mainly in seasons with adverse weather conditions, and in herds where pigs from numerous sources are mixed.

Immune status of the animals relative to the infecting serotype of App is also important, whether passive, convalescent, or vaccinal. Animals from conventional herds that might have been in contact with low virulent serotypes of App or with *A. suis* may be more resistant to the infection from a specific strain than SPF animals that are negative to all serotypes of App (unpublished observations). However, this may depend on the ability of the previous colonizing serotypes to induce antibodies against the toxins produced by the newly infecting strain.

Immunity

Experimental or natural infections stimulate an immune response, and circulating antibodies can be detected approximately 10–14 days postinfection, depending on the serotype and the serological assay. These antibodies reach a maximum level within 4–6 weeks postinfection and may persist for many months (Desrosiers 2004). In some cases, subclinically infected animals may present low levels or absence of antibodies against toxins (Chiers et al. 2010). In fact, ELISA tests that detect antibodies against the ApxIV toxin seem to present a lower sensitivity than those detecting antibodies against LPS, especially in the absence of clinical signs (Gottschalk 2015; Opriessnig et al. 2013). However, more studies are needed to confirm this observation. Immune sows confer passive immunity to their offspring. Such colostral antibodies may persist for about 5–12 weeks (Vigre et al. 2003), but this may depend on the sensitivity of the test used to detect the antibodies and on the initial level of acquired colostral antibodies. Protection may last for as little as 3 weeks in some cases (Nielsen 1975), but this may be due to the use of a very low sensitivity test such as the complement fixation test (CFT). The antibodies are directed against a wide range of bacterial structures and products, including capsule, LPS antigens, toxins (which can be neutralized), outer membrane proteins, superoxide dismutase, and iron-binding proteins. Both local IgA antibodies and serum IgG antibodies are produced. Immunity resulting from infection is directed against the corresponding serotypes; natural cross-protection between serotypes sharing common LPS antigens has not been extensively studied.

Clinical signs

Clinical signs vary with the age of the animals, their state of immunity, the environmental conditions, and the degree of exposure to the infectious agent. The clinical course can be peracute, acute, or chronic (Gottschalk 2015). All stages of disease – from intermediate to fatal, subacute, or chronic – may develop within an affected group.

In the peracute form, one or more pigs in the same or different pens suddenly become sick with high fever to 106.7 °F (41.5 °C), apathy, and anorexia. Some pigs may vomit. The affected animals lie on the floor without distinct respiratory signs, heart rate is increased, and cardiovascular function fails. The skin on the nose, ears, legs, and later the whole body becomes cyanotic. In the terminal phase, there is a severe dyspnea with mouth breathing, animals remain in a sitting posture, and rectal temperatures drop. Shortly before death, there is usually a copious, foamy, blood-tinged discharge through the mouth and nostrils. In the peracute form, it is also common to find one or more animals dead without any premonitory signs and with typical foamy, blood-tinged nasal discharge (Figure 48.1). Experimental studies have shown that the course of the disease may be as little as 6 hours from infection to death.

In the acute form, many pigs in the same or different pens are affected. Body temperature rises to 105–106 °F (40.5–41 °C), the skin may be reddened, and the animals are depressed, are reluctant to rise, refuse food, and are reluctant to drink. Severe respiratory clinical signs with

Figure 48.1 Animal that died from peracute pleuropneumonia caused by *Actinobacillus pleuropneumoniae* presenting with typical foamy blood-tinged nasal discharge. *Source:* Courtesy of Dr. Enric Marco.

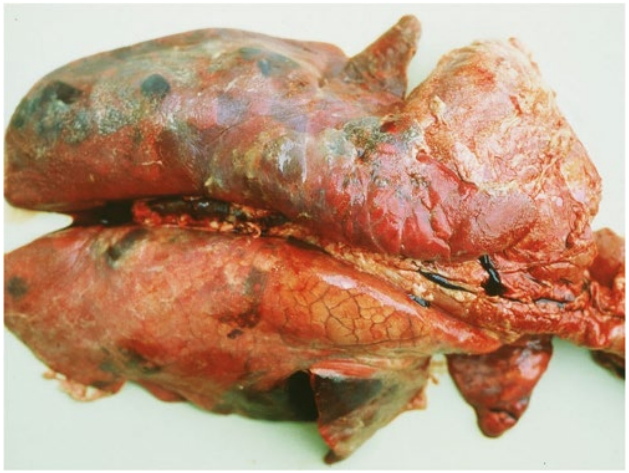


Figure 48.2 Severe diffuse fibrinonecrotic pleuropneumonia caused by *Actinobacillus pleuropneumoniae*. The lungs are rubbery and non-collapsing with florid diffuse fibrinous pleuritis, multifocal hemorrhages, and hemorrhagic interlobular edema.

dyspnea, cough, and sometimes mouth breathing are evident. The course of the disease differs from animal to animal, depending on the extent of the lung lesions and the time of initiation of therapy.

The chronic form develops after the disappearance of acute signs or when treatment or preventive measures are not able to completely control the infection and/or infections due to intermediate virulence serotypes. There is little or no fever, and a spontaneous or intermittent cough of varying intensity develops. Appetite may be reduced, and this may contribute to decreased rate of gain. The clinical signs may be exacerbated by other respiratory infections (bacterial or viral). Atypical mild respiratory signs with low mortality resembling influenza have also been reported (Tobias et al. 2009).

Less common manifestations may be seen in some outbreaks. Fatal septicemia may rarely be observed in neonatal pigs. In primary epidemics involving pregnant females, abortions (probably due to fever episodes) may be observed (Wilson and Kierstead 1976), especially when naïve sows/gilts from SPF herds are infected. Head tilt and drooping of one or both ears may be observed owing to middle ear infection associated with App infection (Duff et al. 1996).

Lesions

The gross pathological lesions are located mainly in the lungs and vary according to clinical course of disease (Sibila et al. 2014). Pneumonia can be unilateral, bilateral, lobar, diffuse or multifocal. In acute cases there is consolidation that is dark red to black, interlobular edema, and mild to severe fibrinous pleuritis (Figure 48.2).

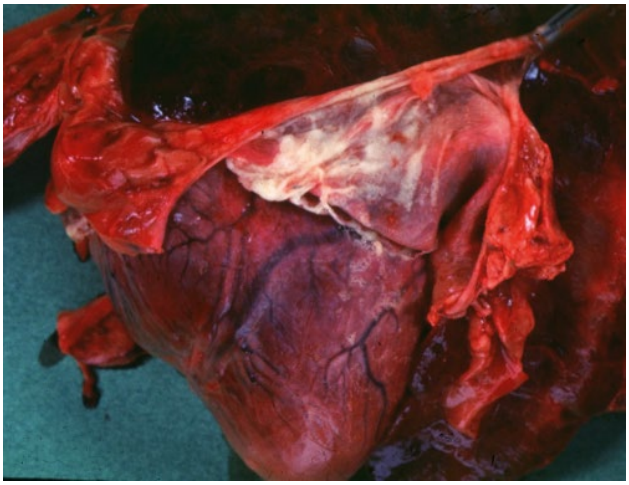


Figure 48.3 Fibrinous epicarditis and pericarditis in a pig with severe pleuropneumonia caused by *Actinobacillus pleuropneumonia*. *Source:* Courtesy of Dr. Greg Stevenson.

In peracute cases the trachea and bronchi are filled with a foamy, blood-tinged mucus exudate, and there may be few other gross changes. In slightly later peracute cases, the pneumonic areas appear dark red purple and slightly to moderately firm yet resilient with little or no fibrinous pleurisy. On cut surface there is diffuse hemorrhage, and areas of necrosis are friable.

In acute cases, layers of fibrin are obvious on the pleural surface and infrequently on the epicardium and pericardium (Figure 48.3) in animals that live for at least 24 hours after infection. The thoracic cavity usually contains a blood-tinged fluid. Affected areas of the lung are firm, rubbery, and mottled dark red purple to lighter white in areas that contain abundant fibrin. On cut surface, the parenchyma is heterogeneous (Figure 48.4).

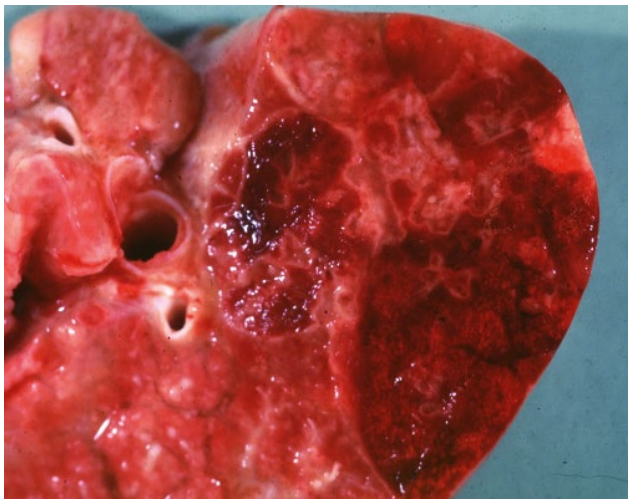


Figure 48.4 Cut surface of a lung with severe fibrinohemorragic and necrotizing pneumonia caused by *Actinobacillus pleuropneumonia*. Note the typical irregular light and dark areas that are surrounded by white lines composed of white blood cells and fibrin. *Source:* Courtesy of Dr. Greg Stevenson.

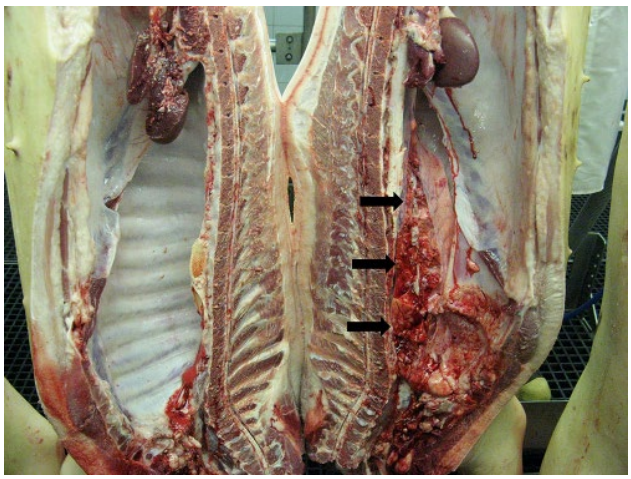


Figure 48.5 Split carcass hanging vertically. Note that the right lung remains adhered in the right thoracic cavity (arrows), whereas the left lung is removed, revealing the empty left thoracic cavity devoid of fibrous adhesions. *Source:* Courtesy of Dr. Scott Hurd.

There are areas of hemorrhage and other areas that are necrotic and friable. White lines composed of fibrin and white blood cells are observed surrounding necrotic areas and distending interlobular septa. In some areas, interlobular septa may be instead distended with hemorrhagic red fluid.

In chronic cases, fibrosis of the previously fibrinous pleuritis results in firm adhesions between visceral and parietal pleura. These areas often result in tearing of the lungs during removal at necropsy or in the slaughterhouse, leaving portions of the lung adhered to the thoracic wall (Figure 48.5). Resolution of non-necrotic areas from acute infection results in remaining cavitated necrotic foci that are surrounded by scar tissue known as sequestra. In many less severe cases, lesions in the lung parenchyma resolve, leaving only fibrous pleural adhesions. It has been demonstrated that a high prevalence of dorsocaudal pleuritis at slaughter is very suggestive of previous App pleuropneumonia (Merialdi et al. 2012; Meyns et al. 2011).

Diagnosis

Confirmation of App pleuropneumonia and typing of strains

App pleuropneumonia should be suspected when typical clinical signs and gross lesions are observed. Differentials in peracute cases where lungs are dark red and edematous should include other diseases that may produce similar lesions such as classical swine fever (Chapter 39), influenza (Chapter 36), pseudorabies (Chapter 35), septicemic salmonellosis (Chapter 59), and erysipelas (Chapter 53). In acute cases with typical fibrinohemorrhagic pleuropneumonia, *A. suis* (see below) (MacInnes and Desrosiers 1999; MacInnes et al. 2008; Yaeger 1995) and pleuritic strains of *Pasteurella multocida* (Chapter 57) should also be considered. Diagnosis is confirmed by culture, identification, and often typing of App. Lung samples for culture should come from lesioned areas of lungs collected from peracutely or acutely affected untreated animals. In chronic cases, where sequestra are in the lungs and/or fibrous pleuritis is observed at necropsy or in the slaughterhouse, App culture is usually negative. Surveillance of the herd using serology can be used to determine herd status (see below).

It is relatively easy to demonstrate App in pneumonic lesions from freshly dead animals. However, in some cases App can be overgrown by other bacteria such as *P. multocida*. Primary isolation of App from tissues and secretions may be carried out on 5% sheep blood agar with a cross-streak of *S. aureus* or *S. epidermidis*. After aerobic incubation overnight (usually in the presence of 5% CO_2), small colonies appear in the neighborhood of the streak (NAD requirement) surrounded by a clear zone of complete hemolysis. This allows a rapid presumptive bacteriologic diagnosis. For some serotypes (such as serotypes 7 and 12), the zone of hemolysis is usually less intense. Altered blood agars ("chocolate agar") or PPLO agar supplemented with NAD or yeast extract allow the growth of the organism, but colonies are less distinctive on these media. Presumptive biochemical identification can be carried out by demonstrating the CAMP phenomenon and urease activity. Usually, molecular testing will help confirm the identity of App (see below).

Most isolates recovered from acutely infected animals are typical App, and its identification is relatively

straightforward. However, when biochemically atypical isolates (for example, urease-negative isolates) (Blanchard et al. 1993), unexpected isolates recovered from the lungs without typical lesions, and/or untypable isolates are identified, the use of species-specific PCR should be used to confirm the identity of the App (Schaller et al. 2001). Biotype II (NAD-independent) isolates have been recovered more frequently in recent years (Gambade and Morvan 2001; Gottschalk et al. 2003a; Maldonado et al. 2009). These isolates might be misidentified as *A. suis*. In these cases, complete biochemical identification along with species-specific PCR tests must be done before sending the isolate for serological serotyping. *A. suis* isolates can present strong cross-reactions with rabbit sera against serotypes 3–6–8 of App, and without a correct identification, these isolates can be serotyped and reported as App (unpublished observations).

Serotyping is still of major interest for App since different serotypes have different virulence potential. In addition, serotyping is essential for choosing the most appropriate bacterins when they are used in a preventive strategy. Only biochemically (typical) or genetically (atypical) confirmed isolates should be serotyped. Although serotyping using antibodies with a variety of classical serological tests, such as agglutination, coagglutination, ring precipitation, immunodiffusion, and indirect hemagglutination, can be used, there is a need for high titer and highly specific antisera (Dubreuil et al. 2000). In addition, the presence of cross-reactions is a problem with many serotypes, and the use of two to three types of tests is usually required to identify these serotypes. In fact, serotyping by the use of polyclonal antibodies can be seriously questioned (Gottschalk 2015). Indeed, some serotypes (such as serotypes 3, 6, 8, and 15) are highly difficult to differentiate. Using serological tests the prevalence of serotype 8 has been underestimated in both the United Kingdom and North America (Gottschalk and Lacouture 2014; Li et al. 2016), since most strains had originally been typed as serotype 3 or 6 by serological methods. Serotypes 4 and 7 are also difficult to be differentiated using polyclonal antibodies (Lebrun et al. 1999). The use of monoclonal antibodies for serotyping has also been reported (Lacouture et al. 1997; Lebrun et al. 1999). These antibodies, especially those directed against the capsular epitopes, can differentiate, for example, serotype 1 from serotypes 9 and 11 and serotype 7 from serotype 4. However, monoclonal antibodies against the LPS will cross-react between serotypes sharing common LPS epitopes (Lacouture et al. 1997; Lebrun et al. 1999). Some of these monoclonal antibodies even showed that LPS epitopes are common between App serotype 7 and *Actinobacillus lignieresii* (Lebrun et al. 1999). Serotypes 9 and 11 cannot be differentiated by serological tests.

To overcome the problems of serological serotyping, several molecular techniques (mostly PCR) have been developed to serotype App from pure cultures. For a recent review on the available techniques and their advantages/disadvantages, see Gottschalk (2015). The most reliable PCR tests are based on *cps* locus genes; as it is the case with antibodies, it is important to note that serotypes 9 and 11 cannot yet be differentiated by the available PCR. Although PCR tests based on Apx toxin genes have been suggested to differentiate most serotypes, its use showed poor correlation when strains of known serotypes were tested (unpublished observations). It is important to mention that using PCR tests (many of them based on *cps* locus genes) will not identify atypical strains with a combination of a given CPS serotype and a different LPS serotype as being described in North America, Europe, and Japan (Gottschalk et al. 2000; Ito and Matsumoto 2015; Nielsen et al. 1996). Hence, there is still a need for reference laboratories using well-standardized serological techniques to confirm the identity of such atypical strains. Since PCR tests can be performed by any laboratory with minimal infrastructure and without the need of serotype-specific antisera, data on serotype distribution of isolates recovered from diseased animals in different countries will probably be available during the next years.

Although not highly useful for serotyping, PCR toxin typing (Frey et al. 1995) can be used to determine which Apx toxin genes are carried by a certain isolate, which may help to anticipate its virulence. For example, a nonvirulent serotype 1 strain recovered from healthy animals from a herd free of App-related disease was shown to produce ApxI only (Gottschalk et al. 2003b). Similarly, low virulent serotype 2 strains isolated in North America produces ApxII only, whereas highly virulent European strains produce both ApxII and ApxIII (Gottschalk et al. 2003a).

Bacteriological diagnosis of chronic disease is more complex, because it is difficult to culture App from such lungs, especially when lesions are old. In the lungs that contain sequestra, direct detection of App antigens in lung tissues can be performed by extracting antigens and testing by ring precipitation, coagglutination, latex agglutination, ELISA, and/or counterimmunoelectrophoresis (Bunka et al. 1989; Dubreuil et al. 2000). However, results should be interpreted with caution, since most of these tests have not been validated in the field. Bacterial presence can also be observed in tissues by using either a fluorescent or an immunoperoxidase antibody test (Bunka et al. 1989; Gutierrez et al. 1993). However, these tests are serotype specific, and they will not detect all App strains. Nucleic acid from App (species specific) may also be detected by labeled DNA probes in tissue (Cho and Chae 2003). Direct confirmation by PCR of the presence of App in lung tissue using

App-specific PCR (Schaller et al. 2001) is not yet routinely used, although it has been used with success in a few cases (unpublished observations). When only fibrous pleuritic lesions are present, App antigen/DNA may not be detected in the lung. Therefore, serology (see below) along with disease history in the herd of origin may be used to determine herd status.

Detection of tonsillar carriers

The detection of App from clinically healthy carrier animals is even more complex (Gottschalk 2015). This may be required in cases of equivocal serological results of seedstock introduction into negative herds or in eradication programs. Bacteria are usually localized in tonsils and, less frequently, in the nasal cavities where they must be cultured in the presence of other commensal bacteria, including several other NAD-dependent bacterial species (Gottschalk et al. 2003b; Kielstein et al. 2001). Selective media have been described (Jacobsen and Nielsen 1995), although their sensitivity is very low due to typical heavy contamination of plates. To overcome the presence of a highly contaminating flora, an immunomagnetic separation technique for the selective isolation of a given serotype of App from tonsils has been developed and applied to serotype 1 (in Canada) (Gagné et al. 1998) and serotype 2 (in Denmark) (Angen et al. 2001). Additional field studies confirmed its better sensitivity when compared with the classical bacterial isolation method (Fittipaldi et al. 2003; Gottschalk 2015). Any specific serotype can be isolated using the magnetic beads by only changing the specificity of the antibody (unpublished data). Although this test presents good sensitivity, it is time consuming and expensive. On the other hand, it allows the isolation of live App for further use in antimicrobial testing or in autogenous vaccine production. It is important to mention that the species identity of any App-like isolate recovered from a tonsil must be confirmed by PCR (Schaller et al. 2001). Due to antigenic similarities, *A. porcitonsillarum* has already been isolated using magnetic beads coated with anti-serotype 1 immunoglobulins (Gottschalk et al. 2003b).

Molecular techniques are presently used for the detection of App from tonsils. Several PCR techniques that amplify well-defined sequences of the genome have been described for rapid and affordable detection of App, and ready-to-use PCR kits have been commercialized (Gottschalk 2015). PCR tests can be directly used with swine tonsil homogenates (direct PCR to detect live and killed bacteria) or after culture of tonsils (after-culture PCR to detect only live bacteria) (Fittipaldi et al. 2003). After-culture PCR is considered to have a higher sensitivity. Although tonsil biopsies can be used, the sensitivity of this type of sample is lower than that of whole tonsils (Fittipaldi et al. 2003). Most PCR tests are specific for the species App and cannot differentiate among serotypes (Gottschalk 2015). Since most conventional herds are infected with several low virulent serotypes, the significance of a positive result is difficult to interpret. To overcome this problem, serotype-specific PCR tests have more recently been described (see above); however, most of these tests have been validated with pure cultures (with a general goal of serotyping) rather than detection of carrier animals (Gottschalk 2015). Some of these serotype-specific PCR with after-culture methods have been successfully used (unpublished observations). Finally, when detection of App is performed from tonsils collected at slaughter, results have to be interpreted with caution. Although Fittipaldi et al. (2003) did not show any cross-contamination at the slaughterhouse, another study suggested that cross-contamination principally by scald water during the slaughter process can occur (Marois et al. 2008). Finally, oral fluid has been shown to be a very low sensitive method to detect App after an experimental infection (Costa et al. 2012). An even much lower sensitivity is expected in samples from subclinically and chronically infected animals.

Serology

Serology is the preferred and most cost-effective method for App surveillance. Serological testing has been used widely for the diagnosis, management, and eradication of virulent serotypes of App. In fact, serology is the most effective tool used to detect subclinical App infections (Broes et al. 2007). For a recent review, see Gottschalk (2015). Some countries, such as Canada and Denmark, use serology for epidemiological surveillance of breeding herds on a routine basis. There are basically two types of serological tests: (1) those that detect all serotypes of App (without discrimination among serotypes) and (2) those that are serotype/serogroup specific. Commercial kits exist for both types of ELISA. There is only one test that can be considered App specific, since the antigen is produced by all App strains (with rare exceptions) and is not produced by any other bacterial species: the ApxIV ELISA test (Dreyfus et al. 2004). However, few controlled and comprehensive studies regarding its use are available. It has been recently shown that the test presents an excellent specificity but, under the conditions tested, a relatively low sensitivity, since it correctly identified less than 13% of samples from App experimentally infected pigs (Opriessnig et al. 2013), a conclusion also reached in a previous study (Eamens et al. 2008). Interestingly, an increasing detection rate was observed when animals became older (Opriessnig et al. 2013). Recently, a four-plex fluorescent microbead-based immunoassay was developed for the simultaneous detection of antibodies against ApxI, ApxII, ApxIII, and ApxIV (Giménez-Lirola et al. 2014). Using this method, and although false-positive results probably due to cross-reactions with antibodies against ApxI and ApxII were observed, a high sensitivity

was obtained with the ApxIV antigen (Giménez-Lirola et al. 2014). Interestingly, many false-negative sera previously tested by ELISA by Opriessnig et al. (2013) were positive when tested by this new highly sensitive test. Finally, interpretation of the ApxIV test must take into account that subclinically infected herds with animals harboring bacteria in tonsils only may not induce high levels of antibodies against App toxins (Chiers et al. 2002). Moreover, some strains possess insertion sequences in their genome that abolish ApxIV production. Animals infected with such strains do not produce antibodies against this toxin (Tegetmeyer et al. 2008).

Concerning the serotype/serogroup-specific tests, the CFT has been largely used in Denmark and is still used in a few US laboratories (Gottschalk 2015); in fact, this test has been clearly shown to possess a very low sensitivity (Gottschalk 2015). Amazingly, some countries (such as China and Russia) still require a negative App CFT tests for imported swine even though the low sensitivity and propensity for false positives of the CFT are well known (Gottschalk 2015). The most commonly used test in the last 20 years has been the ELISA, and among serotype/serogroup-specific antigens, purified O-chain LPS antigen (LPS-ELISA) has been the most successful (Dubreuil et al. 2000; Gottschalk 2015; Gottschalk et al. 1994; Grøndahl-Hansen et al. 2003; Klausen et al. 2007). A well-characterized O-chain LPS as a diagnostic antigen in a standardized ELISA for App was first reported in Canada in 1994 (Gottschalk et al. 1994). Serological tests for additional serotypes have subsequently been set up using similar antigens, individually or by mixing different serotypes (Gottschalk 2015; Grøndahl-Hansen et al. 2003). These ELISA tests may identify animals infected with the following serotypes/serogroups based on antigenic similarities of the O-chain LPS: 1/9/11, 2, 3/6/8/15, 4/7, 5, 10, 12, 13, and 14. Atypical biotype I North American App strains of serotype 13 present cross-reactions with serotype 10 due to a similar LPS composition (Perry et al. 2012). In fact, when the diagnosis is directed toward specific serotypes/serogroups, the LPS-ELISA seems to be the test of choice, as previously reported (Costa et al. 2011; Opriessnig et al. 2013). O-chain LPS antigens are also used in blocking/inhibition ELISA tests using polyclonal or monoclonal antibodies (Andresen et al. 2002; Stenbaek et al. 1997). It has been shown that the LPS-ELISA for serotype 1/9/11 showed low levels of cross-reactions with animals experimentally infected with high doses of *A. porcitonsillarum* (Gottschalk et al. 2003b). In addition, animals infected with one atypical strain (without O-chain LPS) did not induce antibodies detected by this ELISA (Jacques et al. 2005). However, the latter two situations are exceptional and have been observed only once (unpublished data). As for the ApxIV test, multiplex fluorescent microbead-based immunoassays for the identification of several serotypes (but identifying which ones are reacting) based on LPS and/or capsular antigens have been recently developed (Berger et al. 2014; Broes et al. 2016).

Tests detecting antibodies against specific serotype/serogroups and those detecting all serotypes of App are both useful, but they should be used appropriately (Gottschalk 2015). Although false-positive results have been exceptionally described (Broes et al. 2007), high health status herds already known to be free of all serotypes of App may decide to use the ApxIV-based test as a surveillance tool. It may be hypothesized that even though the ApxIV ELISA seems to present lower sensitivity than the LPS-ELISA (Opriessnig et al. 2013), an App strain that would have been introduced in a completely naïve herd would induce seroconversion and, depending on the strain, a relatively high seroprevalence. Under these circumstances, the ApxIV ELISA should significantly increase its sensitivity, although this remains to be demonstrated. If positive reactions are observed in herds, serological testing using antigens of different serotypes of App should be used. Otherwise, it would be extremely difficult to evaluate the level of risk of disease without knowing the serotype involved. In those cases, it is advisable to test all samples (positive and negative) with serotype/serogroup-specific ELISA to confirm the diagnosis.

Most conventional herds are infected with one or more serotypes (Gottschalk 2015). It was shown that 70% of the Canadian herds tested had antibodies against App (MacInnes et al. 2008). In some herds, sows may be infected by more than one serotype, but only one of them is consistently transmitted to offspring and found in grower–finisher animals (Broes et al. 2007). The use of the ApxIV test would not be useful in these cases, since it is not able to evaluate the risk. As mentioned above, it is important for a country to have reliable updated data regarding the most important serotypes clinically affecting animals in order to determine which serotypes (or serogroups) should be tested serologically.

Sera are still the main sample used for serology of App. LPS-ELISA tests have been used experimentally with fluids collected from muscles of pigs as well as colostrum (Desrosiers 2004; Gottschalk 2015). Oral fluid samples have not been appropriately evaluated so far. Pooling sera from different individuals may significantly reduce the sensitivity of the test, especially in subclinically infected animals, and this practice is not recommended (unpublished observations).

Even though serologic tests are valuable to identify subclinically infected herds and animals, serological testing occasionally generates ambiguous results. In such cases, molecular detection and/or bacterial isolation from tonsils could be carried out to clarify the herd status. In fact, tonsil colonization without induction of antibody response has been demonstrated (Chiers et al.

2010). App is a very dynamic pathogen, and sometimes diagnosticians and practitioners must face atypical situations. Such cases as well as diagnostic approaches have been presented by Broes et al. (2007).

Treatment

Although sporadic reports indicate a certain degree of resistance, in general, recent results of a global study across Europe showed that App is rarely resistant *in vitro* to amoxicillin (with or without clavulanic acid), cefquinome, ceftiofur, danofloxacin, enrofloxacin, marbofloxacin, florfenicol, tulathromycin, tiamulin, tilmicosin, tylosin, lincomycin, trimethoprim/sulfamethoxazole, and spectinomycin (El Garch et al. 2016). Similar high susceptibility has been observed by other studies in Europe (Hendriksen et al. 2008; Matter et al. 2007). Resistance to tetracyclines and to a lesser extent trimethoprim/sulfonamide seems to have increased in the last years (El Garch et al. 2016; Gutiérrez-Martín et al. 2006; Hendriksen et al. 2008). Recent data from North America showed high susceptibility to ceftiofur, florfenicol, enrofloxacin, erythromycin, clindamycin, trimethoprim/sulfamethoxazole, and tilmicosin. A low level of resistance was observed toward tiamulin, penicillin, and ampicillin as well as danofloxacin. On the other hand, a high level of resistance to chlortetracycline (88.4%) and oxytetracycline (90.7%) was observed (Archambault et al. 2012). Sporadic data showed a relatively high number of resistant App isolates to the beta-lactam family (penicillin, ampicillin, amoxicillin) (Dayao et al. 2016). Response of pigs with clinical App to penicillin treatment may be inconsistent (Sjölund et al. 2009). It is generally accepted that there is no clear correlation between the distribution of the antibiotic resistances and the serotypes of App (Matter et al. 2007).

The choice of the first-line antimicrobial should be based on its minimal inhibitory concentration (MIC) as well as its pharmacokinetic and pharmacodynamic (PK/PD) properties. Sjölund et al. (2009) showed that antibiotics with minimal MIC in their categories significantly varied in their capacity to control acute infection. Enrofloxacin has been shown to be particularly effective after experimental challenge (Sjölund et al. 2009). Satisfactory results in the field have been reported with tiamulin (Anderson and Williams 1990) and a combination of lincomycin and spectinomycin (Hsu 1990). Tilmicosin has also been reported as effective (Paradis et al. 2004). One study indicates that using experimentally infected animals, tulathromycin administered as a single dose at either 2.5 or 5 mg/kg body weight was at least as effective as three daily doses of ceftiofur regarding percentage of lung lesions, daily weight gain, days with clinical disease, and rectal temperature (Hart et al. 2006).

Antibiotic therapy is effective only in the initial phase of the disease, when it can reduce mortality (Desrosiers 2004). Interestingly, the success rate of an antibiotic treatment may influence the immune response of animals. In fact, highly effective antibiotics may prevent a strong antibody response, leaving animals susceptible for a later reinfection (Sjölund et al. 2009). On the other hand, the nature of the lesions means that delay in treatment can result in a degree of infarction and chronic damage, which will leave the animal as a respiratory cripple even if it recovers. Antibiotics should be given parenterally (subcutaneously or intramuscularly) and in high dosage, as affected animals may not eat or drink (Desrosiers 2004).

To ensure effective and durable blood concentrations, repeated injections may be required, depending on the pharmacokinetic properties of the antibiotic used. The success of therapy depends mainly on early detection of clinical signs and on rapid therapeutic intervention. Water treatment may be used to treat members of the affected group that are still able to drink. Feed medicated with any of the above antimicrobials may be used successfully if all pigs have a normal food and water intake. In countries where still allowed, feed and water medication can be used as prophylactic antimicrobial therapy to prevent acute outbreaks in highly infected herds. Continuous medication or pulse dosing may be practiced, but neither should be used for long, and the antimicrobial sensitivity of the organism should be regularly monitored. Where allowed, strategic medication may be targeted at periods of risk, which can be identified by routine postmortem examinations, clinical examinations, and herd antibody profiles. A combination of parenteral and oral medication in a recent outbreak often yields the best results. In spite of apparent clinical success, it must be remembered that antibiotic therapy does not eliminate infection in a herd and carrier animal may persist for a long time (Angen et al. 2008; Desrosiers 2004; Fittipaldi et al. 2005). These animals are an important source of infection for other animals. Severely affected animals may not recover even after treatment and should be euthanized.

Prevention and control

When herds are free of App, strict biosecurity measures should be applied to prevent introduction of the organism. The greatest risk is presented by introduction of potentially infected (clinically healthy) pigs. Seedstock should be purchased from herds with a history of absence of clinical sign, lesions, and negative serological testing for App. Ideally, incoming animals should also be quarantined and serologically tested as negative before introduction. If the herd where the animals will be introduced is already positive for certain serotypes known to be of

low virulence, similar precautions to introduction of serotypes known to be generally more virulent should be practiced. Introduction of animal carriers of low virulent strains of App in an SPF App-negative herd should be avoided. Indeed, most strains recovered from clinical cases and belonging to serotypes of lower virulence in Canada (serotypes 6, 12, and 15) are usually isolated in high health status herds, which were previously free of all serotypes (unpublished observations). In herds that are already infected by virulent serotypes, App-negative seedstock should be vaccinated with products appropriate for the infecting virulent serotypes according to label directions, and time should allow for development of immunity before introduction. During outbreaks of pleuropneumonia on App-infected farms, the first priority must be to control mortality by the treatment of affected individuals, usually including all contact animals in the affected pen. Treatment of animals in surrounding pens should be evaluated upon the extension of clinical disease (Desrosiers 2004); in fact, animals should be allowed to develop natural immunity (Desrosiers 2004). Generally, good environmental management minimizes outbreaks in infected herds and includes maintaining appropriate environmental temperature with minimal fluctuations, seasonally appropriate ventilation, use of solid partitions between pens, all-in/all-out movement of pigs, appropriate stocking densities, and younger weaning ages (<21 days).

A wide range of vaccines have been developed for this disease (Ramjeet et al. 2008). These vaccines can basically reduce clinical signs and lung lesions (Del Pozo Sacristán et al. 2014), but they are poorly effective in eliminating the carrier stage. Commercial vaccines fall into two main groups: killed organisms (bacterins) and subunit toxin-based vaccines. Lately, a combination of these types of vaccines (bacterins + toxins) appeared in the market (Thevenon et al. 2014). Vaccination with bacterins is serotype specific (Ramjeet et al. 2008); possible cross-immunity with cross-reacting serotypes has been suggested, although it still should be confirmed (Nielsen 1985c). The use of a bacterin should be done only in farms where the involved serotype has been identified, although animals vaccinated with bacterins will produce antibodies that might cross-react with ELISA tests that use O-LPS as antigens. However, this is not always the case: diagnostic tests have been developed to detect infected animals using highly purified antigens. Vaccination with bacterins may induce high levels of antibodies against a variety of surface antigens (including the CPS). In some cases, the level of antibodies against the O-chain LPS in vaccinated animals may not be high enough to be detected in the ELISA test: this is not an indication of failure of the vaccine on its capacity to induce antibodies. Many commercial bacterins induce protection even if they do not induce such cross-reacting antibodies detectable by the O-chain ELISA kit (unpublished observations). Finally, in some countries, autogenous vaccines are used: this approach may only be useful when serotypes involved in clinical cases are not included in available commercial bacterins. In the latter case, another option may also be the use of toxin-based vaccines that would provide protection against all serotypes.

Subunit vaccines, composed of the purified three major RTX exotoxins (ApxI, ApxII, and ApxIII) with or without a 42 kDa outer membrane protein of App, or in suspensions mixed with bacterins, have been developed and shown to give protection against all major serotypes under experimental conditions as well as in field trials (Thevenon et al. 2014; van den Bosch and Frey 2003). A potential protection conferred by the ApxIV toxin, produced by all strains *in vivo*, seems to be controversial (Liu et al. 2009; Wang et al. 2009), and it has not yet been incorporated in any commercial vaccine. Keeping in mind the very complex pathogenesis of porcine pleuropneumonia, inclusion of other bacterial virulence factors in vaccines might also be of value. A wide range of antigens administered by either parenteral, aerosol, or oral routes have been found to be experimentally protective, but none of them have been validated in the field (Ramjeet et al. 2008). Finally, live vaccines using laboratory obtained non-virulent mutants have also been developed and showed to protect against homologous and heterologous serotypes (Park et al. 2009; Ramjeet et al. 2008). Some of these live vaccines even use the differentiating infected from vaccinated animals (DIVA) concept (Liu et al. 2009). Vaccines may provide high levels of protection against morbidity in experiments, reduce mortality, reduce the number of treatments required, increase daily live weight gain, and improve feed conversion efficiency. The quality of the carcass is also improved, with fewer condemnations for pneumonia and lower slaughtering costs through reductions in pleurisy and pericarditis (Del Pozo Sacristán et al. 2014). The decision to vaccinate should be carefully evaluated; the costs of mortality alone should not be the sole consideration, because the other effects on productivity listed above contribute to the benefits of vaccination. In some cases, individual and intensified medical treatments of affected pigs may be needed to reduce the impact of App (Sjölund and Wallgren 2010). Vaccination of piglets is usually advised; animals should not receive the first dose during the first weeks of age to avoid interference with maternal antibodies. Sows can also be vaccinated without adverse effects (Kristensen et al. 2004a), and replacement animals can be vaccinated before their introduction in an infected herd. Vaccination of sows with serotype-specific bacterins may reduce/delay colonization of piglets, which may lead to a reduction, under certain circumstances, of clinical signs in

grower–finisher pigs (unpublished observations). Since antibodies against somatic antigens are needed, this effect would not be possible with exclusively toxin-based vaccines. Finally, the presence of antibodies (either natural or vaccine induced) will not eliminate the carrier state of animals at the tonsil level (Gottschalk 2015).

Eradication and regional control

Control of pleuropneumonia in a region or breeding pyramid involves health schemes aimed at pleuropneumonia-free breeding and multiplying herds, serologic monitoring, monitoring at slaughter and postmortem examination of casualties, control of management, and controlled pig traffic (serologic testing, quarantine). For herds infected with App intending to join such a scheme, an eradication program is the method of choice but requires careful evaluation of the economic consequences. Depopulation and restocking with pigs originating from certified App-free herds can be used; however, this method is expensive and may lead to the loss of important bloodlines.

Other methods that have succeeded in the past include off-site segregated medicated early weaning, at the same time supported by a program of vaccination, medication, and culling and repopulation with disease-free gilts (Larsen et al. 1990). Age of weaning and level of maternal antibodies may have an important influence on the colonization of piglets by App (Vigre et al. 2002). There are several herds that successfully eradicated App (depending on the serotype) using a medicated early weaning program (unpublished observations). Some serotypes, such as serotypes 12 and 8, are highly infective (even in the absence of clinical signs), and transmission of the infection from sows to piglets occurs relatively early and fast, preventing in some cases the success of the eradication.

Small one-site breeding herds (up to 400 sows) with a relatively low percentage of seropositive animals (up to 30%) have used the "test and removal" of seropositive sows under medication (Nielsen et al. 1976). However, the successful outcome of this method is mainly based on the serological test used: a test with low sensitivity will not eliminate all carrier sows, and a test with low specificity will eliminate healthy non-carrier animals, which could increase the cost of the program; the success of such an approach is deemed not guaranteed. A successful elimination of certain serotypes of App with partial depopulation and antibiotic treatment has been suggested (Andersen and Gram 2004). However, it has also demonstrated that antibiotic treatment cannot eliminate the pathogen from all carrier animals (Angen et al. 2008; Fittipaldi et al. 2005). So far, there is no solid proof that partial depopulation can eradicate all serotypes of App. Before an eradication program is carried out, all aspects of biosecurity and the characteristics of the farm should be taken into consideration to prevent recontamination (Zhuang et al. 2007).

Actinobacillus suis

The gram-negative bacterium *A. suis* is an ubiquitous opportunistic pathogen that colonizes the upper respiratory tract of pigs (MacInnes and Desrosiers 1999). In a Canadian survey, MacInnes et al. (2008) showed that as many as 94% of the tested herds were probably infected by *A. suis*, although no clinical cases were observed. Although originally reported as causing septicemia and death in suckling and recently weaned pigs only, disease can also be observed in fattening pigs and even adult animals, especially in high health status herds (Yaeger 1995, 1996). Prevalence of disease may be greater in new populated herds, before animals develop immunity (Wilson and McOrist 2000).

A. suis produces septicemia and localized infections and has been associated with a variety of clinical signs including sudden death, dyspnea, cough, lameness, fever, weakness, wasting, abscesses, neurological signs, abortion, cyanosis, and diffuse hyperemia. One of the most suggestive gross lesions is the presence of petechial to ecchymotic hemorrhages in the lungs, kidneys, heart, liver, spleen, skin, or intestines and petechial hemorrhages on the ears, abdomen, and skin, as well as cutaneous erysipelas-like lesions (MacInnes and Desrosiers 1999).

Yaeger (1995) reported three common forms of *A. suis* disease. First is an acute fulminant septicemic form occurring mainly in suckling and recently weaned pigs. Very often, pigs are simply found dead. Consistent gross lesions include petechial to ecchymotic hemorrhages in multiple organs and serous to serofibrinous exudates in the thoracic and abdominal cavities. Pleuritis, pericarditis, arthritis, and miliary abscesses in a variety of organs may be observed. Histologic lesions consist of foci of necrosis in multiple organs associated with bacterial thromboemboli. Differential diagnosis should include other agents of septicemia such as *Streptococcus suis* and *Klebsiella pneumoniae*.

A second form is respiratory disease mainly affecting grow–finish pigs, most commonly in high health status herds. Pigs may exhibit a cough and fever, and as with young pigs, sudden death may also be the sole sign. However, in these cases, the most remarkable gross lesions are generally a multifocal or diffuse hemorrhagic and necrotizing pneumonia or pleuropneumonia, petechial hemorrhages on the serosal surfaces of abdominal and thoracic viscera, and, in some cases, a fibrinous peritoneal exudate. Differential diagnosis should include mainly porcine pleuropneumonia caused by App (Yaeger 1995, 1996).

The third form of *A. suis* disease is acute septicemia in adult animals, also most commonly observed in high health status herds. Animals may exhibit lethargy, anorexia, fever, and red, rhomboid skin lesions resembling erysipelas. Abortions may occur and animals may also die of septicemia. Gross lesions in these cases consist of multifocal petechial hemorrhages, serofibrinous exudates in the thoracic and abdominal cavities, and occasionally small foci of hepatic necrosis. This form, particularly when skin lesions (as described above) are present, may be confused with erysipelas.

Actinobacillus suis isolates from both healthy and diseased pigs are very similar based on biochemical analysis, restriction endonuclease fingerprinting, slide agglutination, and toxin typing (MacInnes and Desrosiers 1999). The organism has genes that encode toxins that are very similar to ApxI and ApxII of App. These toxins likely contribute to the virulence of this organism. However, *A. suis* produces lower levels of Apx toxins than App, which may explain why *A. suis* is generally less virulent (MacInnes and Desrosiers 1999). On the other hand, *A. suis* is able to resist bile and serum, suggesting that it has additional, but as yet unidentified, virulence factors when compared with App. It has been shown that different serologically distinct groups of cell surface antigens exist, such as Ol/Kl, O1/K2, and O2/K2, presenting some variation in virulence (Slavic et al. 2000). However, a clear classification of serotypes and their association to virulence are not used routinely. Critical virulence factors of *A. suis* are unknown; however, an outer membrane protein and a type IVb pilus were shown to be important adhesins for swine cells (Bujold and MacInnes 2016; Ojha et al. 2010). During the last years, at least two *A. suis* strains have been sequenced, which may help, in a near future, to better understand the role of these organisms in health and disease in swine (MacInnes et al. 2012).

Preliminary diagnosis is based on typical clinical signs and gross lesions. Diagnosis is confirmed by culture and identification of *A. suis* from tissues with typical microscopic lesions. A strain-specific ELISA test has been shown to be useful to follow up maternal and active antibodies in an infected herd (Lapointe et al. 2001).

Antibiotic treatment should be applied early in the appearance of signs; ceftiofur, gentamicin, and trimethoprim/sulfadiazine seem to be the antibiotics of choice, followed by ampicillin, sulfadimethoxine, and tiamulin (MacInnes and Desrosiers 1999). There are no reports in the literature suggesting resistance of a large number of *A. suis* strains to different antibiotics. Vaccination has been implemented in some herds using autogenous vaccines, with variable results. In one of these herds, gilts and sows were vaccinated, although these animals already presented high titers of antibodies before vaccination (Lapointe et al. 2001).

Other *actinobacillus species*

Actinobacillus equuli, a primary infectious agent of horses, has been suggested as being involved in several swine infections in Europe many years ago (Ramos-Vara et al. 2008). However, most of these infections were probably caused by *A. suis*, since both pathogens are phenotypically and genetically very similar. A revised definition of actinobacilli recovered from animals (from a diagnostic point of view) has been published (Christensen and Bisgaard 2004). On the other hand, swine infection by real and confirmed *A. equuli* has been recently reported in the United States and Canada (Ramos-Vara et al. 2008; Gottschalk, unpublished observations) with, in some cases, high morbidity and mortality that required the use of autogenous vaccines. The source of the infection in most herds remains unknown, but diagnostic laboratories must be aware of the potential of *A. equuli* in pigs and clearly differentiate from *A. suis* infections (Christensen and Bisgaard 2004). A case of human infection by *A. suis* or *A. equuli* has been reported recently in Italy (Montagnani et al. 2015).

Many species of *Actinobacillus* other than App can be found in swine tonsils, such as *A. minor, A. porcinus, A. rossii*, Bisgaard Taxon 10, and *A. porcitonsillarum* (Lowe et al. 2010). As mentioned in the App section, the latter species may be easily misidentified as App. Although usually considered as non-virulent, *A. porcitonsillarum* has been recently isolated from diseased animals presenting a variety of pathologies (Martínez and Maldonado 2006; Ohba et al. 2007), and it seems to present a higher antibiotic resistance than App (Matter et al. 2007). The virulence potential of other actinobacilli has not been demonstrated (Mayor et al. 2006).

References

Andersen L, Gram S. 2004. A successful elimination of *Actinobacillus pleuropneumoniae* (serotype 2), *Mycoplasma hyponeumoniae* and PRRS (European and vaccine-strain) by partial depopulation, early weaning and Tilmicosin (Pulmotil, Elanco) treatment. *Proc Int Congr Pig Vet Soc* 18:179.

Anderson M, Williams J. 1990. Effects of tiamulin base administered intramuscularly to pigs for treatment of pneumonia associated with *Actinobacillus pleuropneumoniae*. *Proc Int Congr Pig Vet Soc* 11:15.

Andreasen M, Mousing J, Krogsgaard Thomsen L. 2001. *Prev Vet Med* 49:19–28.
Andresen L, Klausen J, Barfod K, et al. 2002. *Vet Microbiol* 89:61–67.
Angen Ø, Heegaard P, Lavritsen D, et al. 2001. *Vet Microbiol* 79:19–29.
Angen Ø, Andreasen M, Nielsen E, et al. 2008. *Vet Rec* 163:445–447.
Archambault M, Harel J, Gouré J, et al. 2012. *Microb Drug Resist* 18:198–206.
Auger E, Deslandes V, Ramjeet M, et al. 2009. *Infect Immun* 77:1426–1441.
Beck M, van den Bosch J, Jongenelen I, et al. 1994. *J Clin Microbiol* 32:2749–2754.
Berger S, Boas U, Andresen L, et al. 2014. A multiplexed immunoassay for detection of antibodies to Actinobacillus pleuropneumoniae (App) in pigs. In Proceedings of the European Association of Veterinary Laboratory Diagnosticians Congress, vol. 3, p. 88.
Blackall J, Klaasen H, van den Bosch H, et al. 2002. *Vet Microbiol* 84:47–52.
Blanchard PC, Walker RL, Gardner I. 1993. *J Vet Diagn Invest* 5:279–282.
Bossé J, Janson H, Sheehan B, et al. 2002. *Microb Infect* 4:225–235.
Broes A, Martineau GP, Gottschalk M. 2007. *J Swine Health Prod* 15:264–269.
Broes A, Caya I, Bertrand M. 2016. Multiplex testing for APP 1-9-11, 2, 3-6-8-15, 4-7, 5, 10, and 12 using a multiplexed Fluorometric immunoassay (MFIA). *Proc Int Congr Pig Vet Soc* 24:361.
Brogaard L, Klitgaard K, Heegaard PM, et al. 2015. *BMC Genomics* 16:417.
Bujold AR, MacInnes JI. 2016. *Infect Immun* 84:2944–2952.
Bunka S, Müller E, Petzoldt K. 1989. *Dtsch Tierarztl Wochenschr* 96:371–373.
Chatellier S, Harel J, Dugourd D, et al. 1999. *Can J Vet Res* 63:170–176.
Chien M, Chan Y, Chen Z, et al. 2009. *Vet Microbiol* 135:327–333.
Chiers K, Haesebrouck F, van Overbeke I, et al. 1999. *Vet Microbiol* 31:301–306.
Chiers K, Donné E, van Overbeke I, et al. 2002. *Vet Microbiol* 88:385–392.
Chiers K, De Waele T, Pasmans F, et al. 2010. *Vet Res* 41:65.
Cho W, Chae C. 2003. *Lett Appl Microbiol* 37:56–60.
Christensen H, Bisgaard M. 2004. *Vet Microbiol* 99:13–30.
Costa G, Oliveira S, Torrison J, et al. 2011. *Vet Microbiol* 148:246–251.
Costa G, Oliveira S, Torrison J. 2012. *J Swine Health Prod* 20:78–81.
Dayao D, Gibson JS, Blackall PJ, et al. 2016. *Aust Vet J* 94:227–231.
Del Pozo Sacristán R, Michiels A, et al. 2014. *Vet Rec* 174:302.
Desrosiers R. 2004. Epidemiology, diagnosis and control of swine diseases: Howard Dunne memorial lecture. *Proc Am Assn Swine Vet* 9:37.
van Dixhoorn ID, Reimert I, Middelkoop J, et al. 2016. *PLoS One* 11(9):e0161832.
Dreyfus A, Schaller A, Nivollet S, et al. 2004. *Vet Microbiol* 99:227–238.
Dubreuil D, Jacques M, Mittal K, et al. 2000. *Anim Health Res Rev* 2:73–93.
Duff J, Scott J, Wilkes M, et al. 1996. *Vet Rec* 139:561–563.
Eamens G, Gonsalves J, Whittington A, et al. 2008. *Aust Vet J* 86:465–472.
El Garch F, de Jong A, Simjee S, et al. 2016. *Vet Microbiol* 194:11–22.
Fablet C, Marois-Créhan C, Simon G, et al. 2012. *Vet Microbiol.* 157:152–163.
Fittipaldi N, Broes A, Harel J, et al. 2003. *J Clin Microbiol* 41:5085–5093.
Fittipaldi N, Klopfenstein C, Gottschalk M, et al. 2005. *Can J Vet Res* 69:146–150.
Foote SJ, Bossé JT, Bouevitch AB, et al. 2008. *J Bacteriol* 190:1495–1496.
Fraile L, Alegre A, López-Jiménez R, et al. 2010. *Vet J* 184:326–333.
Frank R, Chengappa M, Oberst R, et al. 1992. *J Vet Diagn Invest* 4:270–278.
Frey J. 2003. *Methods Mol Biol* 216:87–95.
Frey J, Kuhn R, Nicolet J. 1994. *FEMS Microbiol Lett* 124:245–251.
Frey J, Beck M, Van Den B, et al. 1995. *Mol Cell Probes* 9:277–282.
Fussing V. 1998. *Lett App Micriobiol* 27:211–215.
Gagné A, Lacouture S, Broes A, et al. 1998. *J Clin Microbiol* 36:251–254.
Gambade P, Morvan H. 2001. *Bulletin des GTV* 12:19–22.
Giménez-Lirola LG, Gerber PF, Rowland RR, et al. 2014. *Clin Vaccine Immunol* 21:85–95.
Gottschalk M. 2015. *Vet J* 206:30–38.
Gottschalk M, Lacouture S. 2014. *Vet Rec* 174:452.
Gottschalk M, Lacouture S. 2015. *Can Vet J* 56:1093–1094.
Gottschalk M, Altman E, Charland N, et al. 1994. *Vet Micro* 42:91–104.
Gottschalk M, Lebrun A, Lacouture S, et al. 2000. *J Vet Diagn Invest* 12:444–449.
Gottschalk M, Broes A, Fittipaldi N. 2003a. Recent developments on *Actinobacillus pleuropneumoniae*. *Proc Am Assoc Swine Vet* 34:387–393.
Gottschalk M, Broes A, Mittal K, et al. 2003b. *Vet Microbiol* 92:87–101.
Gouré J, Findlay WA, Deslandes V, et al. 2009. *BMC Genomics* 10:88.
Grøndahl-Hansen J, Barfod K, Klausen J, et al. 2003. *Vet Microbiol* 96:41–51.
Gutierrez C, Barbosa J, Suarez J, et al. 1993. *Zentralbl Veterinarmed B* 40:81–88.
Gutierrez C, Barbosa J, Suarez J, et al. 1995. *Am J Vet Res* 56:1025–1029.
Gutiérrez-Martín C, del Blanco N, Blanco M, et al. 2006. *Vet Microbiol* 115:218–222.

Hälli O, Ala-Kurikka E, Wallgren P, et al. 2014. *J Zoo Wildl Med* 45:813–818.
Hart F, Kilgore, R, Meinert T, et al. 2006. *Vet Rec* 158:433–436.
Hartley RW, Wilesmith JW, Bradley R. 1988. *Vet Rec* 123:208.
Hendriksen R, Mevius D, Schroeter A, et al. 2008. *Acta Vet Scand* 50:19.
Hoeltig D, Hennig-Pauka I, Thies K, et al. 2009. *BMC Vet Res* 5:14.
Hsu F. 1990. Evaluation of lincospectin sterile solution and Linco-Spectin 44 premix in the treatment of pleuropneumonia. *Proc Int Congr Pig Vet Soc* 11:15.
Hunneman WA. 1986. *Vet Q* 8:83–87.
Ito H, Matsumoto A. 2015. *J Vet Diagn Invest* 27:102–106.
Jacobsen M, Nielsen JP. 1995. *Vet Micro* 47:191–197.
Jacques M. 2004. *Can J Vet Res* 68:81–85.
Jacques M, Foiry B, Higgins R, et al. 1988. *J Bacteriol* 170:3314–3318.
Jacques M, Labrie J, St Michael F, et al. 2005. *J Clin Microbiol* 43:3522–3555.
Jensen A, Bertram T. 1986. *Infect Immun* 51:419–424.
Jensen TK, Boye M, Hagedorn-Olsen T, et al. 1999. *Vet Pathol* 36:258–261.
Jobert JL, Savoye C, Cariolet R, et al. 2000. *Can J Vet Res* 64:21–26.
Kamp E, Popma J, Leengoed L Van. 1987. *Vet Microbiol* 13:249–257.
Kielstein P, Wuthe H, Angen O, et al. 2001. *Vet Microbiol* 8:243–255.
Kilian M, Nicolet J, Biberstein E. 1978. *Int J Syst Bacteriol* 28:20–26.
Kim B, Hur J, Lee JY, et al. 2016. *Vet Q* 36:137–144.
Klausen J, Ekeroth L, Grøndahl-Hansen J, et al. 2007. *J Vet Diagn Invest* 19:244–249.
Klinkenberg D, Tobias TJ, Bouma A, et al. 2014. *Vet J* 202:99–105.
Koyama T, To H, Nagai S. 2007. *J Vet Med Sci* 69:961–964.
Kristensen C, Andreasen M, Ersbøll A, et al. 2004a. *Can J Vet Res* 68:66–70.
Kristensen C, Angen O, Andreasen M, et al. 2004b. *Vet Microbiol* 98:243–249.
Lacouture S, Mittal K, Jacques M, et al. 1997. *J Vet Diagn Invest* 9:337–341.
Lapointe L, D'Allaire S, Lacouture S, et al. 2001. *Vet Res* 32:175–183.
Larsen H, Hogedahl Jorgensen P, Szancer J. 1990. Eradication of *Actinobacillus pleuropneumoniae* from a breeding herd. *Proc Int Congr Pig Vet Soc* 11:18.
Lebrun A, Lacouture S, Côté D, et al. 1999. *Vet Microbiol* 65:271–282.
Lévesque C, Provost C, Labrie J, et al. 2014. *PLoS One* 9(5):e98434.
Li Y, Bossé JT, Williamson SM, et al. 2016. *Vet Rec* 179:276.
Liu J, Chen X, Tan C, et al. 2009. *Vet Microbiol* 137:282–289.
Lowe B, Marsh T, Isaacs-Cosgrove N, et al. 2010. *Vet Microbiol* 147:346–357.
MacInnes J, Desrosiers R. 1999. *Can J Vet Res* 63:83–89.
MacInnes J, Gottschalk M, Lone A, et al. 2008. *Can J Vet Res* 72:242–248.
MacInnes JI, Mackinnon J, Bujold AR, et al. 2012. *J Bacteriol* 194:6686–6687.
Madsen LW, Boye M, Jensen TK, et al. 2001. *Vet Rec* 149:746–747.
Maldonado J, Valls L, Martínez E, et al. 2009. *J Vet Diagn Invest* 21:854–857.
Marois C, Cariolet R, Morvan H, et al. 2008. *Vet Microbiol* 129:325–332.
Marois C, GottschalK M, Morvan H, et al. 2009. *Vet Microbiol* 135:283–291.
Martínez E, Maldonado J. 2006. *Vet Rec* 159:642–643.
Matter D, Rossano A, Limat S, et al. 2007. *Vet Microbiol* 122:146–156.
Mayor D, Korczak BM, Christensen H, et al. 2006. *Vet Microbiol* 116:194–201.
McGregor GF, Gottschalk M, Godson DL. 2015. *Can Vet J* 56:839–844.
Merialdi G, Dottori M, Bonilauri P, et al. 2012. *Vet J* 193:234–239.
Meyns T, Van Steelant J, Rolly E, et al. 2011. *Vet J* 187:388–392.
Møller K, Nielsen R, Andersen L, et al. 1992. *J Clin Micro* 30:623–627.
Montagnani C, Pecile P, Moriondo M, et al. 2015. *J Clin Microbiol* 53:1990–1992.
Nicolet J. 1970. *Aspects microbiologiques de la pleuropneumonie contagieuse du porc*. Berne: These d'habilitation.
Nielsen R. 1975. *Nord Vet Med* 27:319–328.
Nielsen R. 1985a. *Nord Vet Med* 37:217–227.
Nielsen R. 1985b. *Acta Vet Scand* 26:501–512.
Nielsen R. 1985c. *Acta Vet Scand* 26:581–585.
Nielsen R. 1986a. *Acta Vet Scand* 27:49–58.
Nielsen R. 1986b. *Acta Vet Scand* 27:453–455.
Nielsen R, O'Connor PJ. 1984. *Acta Vet Scand* 25:96–106.
Nielsen R, Thomsen A, Vesterlund S. 1976. *Nord Vet Med* 28:349–352.
Nielsen R, Andersen L, Plambeck P. 1996. *Acta Vet Scand* 37:327–336.
Nielsen R, Andresen L, Plambeck T, et al. 1997. *Vet Microbiol* 54:35–46.
Ohba T, Shibahara T, Kobayashi H, et al. 2007. *J Comp Pathol* 137:82–86.
Ohba T, Shibahara T, Kobayashi H, et al. 2008. *J Comp Pathol* 139:61–66.
Ohba T, Shibahara T, Kobayashi H, et al. 2010. *Can Vet J* 51:733–737.
Ojha S, Lacouture S, Gottschalk M, et al. 2010. *Vet Microbiol* 140:122–130.
Opriessnig T, Hemann M, Johnson JK, et al. 2013. *J Vet Diagn Invest* 25:61–71.
Paradis M, Vessie G, Merrill J, et al. 2004. *Can J Vet Res* 68:7–11.
Park C, Ha Y, Kim S, et al. 2009. *J Vet Med Sci* 71:1317–1323.

Pearson HE, Toribio JA, Hernandez-Jover M, et al. 2014. *Vet Rec* 174:325.
Pereira MF, Rossi CC, de Carvalho FM, et al. 2015. *Genome Announc* 3(2). pii: e01585-14.
Pérez Márquez VM, Ochoa JL, Cruz CV, et al. 2014. *Avian Dis* 58:638–641.
Perry M, Altman E, Brisson J, et al. 1990. *Serodiagn Immunother Infect Dis* 4:299–308.
Perry MB, Angen Ø, MacLean LL, et al. 2012. *Vet Microbiol* 156:403–410.
Pohl S, Bertschinger H, Frederiksen W, et al. 1983. *Inst J Syst Bacteriol* 33:510–514.
Pol J, Leengoed L, Stockhofe N, et al. 1997. *Vet Micro* 55:259–264.
Ramjeet M, Deslandes V, Gouré J, et al. 2008. *Anim Health Res Rev* 9:25–45.
Ramos-Vara J, Wu C, Mitsui I, et al. 2008. *Vet Pathol* 45:495–499.
Reiner G, Fresen C, Bronnert S, et al. 2010. *J Wildl Dis* 46:551–555.
Rioux S, Galarneau C, Harel J, et al. 2000. *Microb Pathog* 28:279–289.
Roberts AE, Kragh KN, Bjarnsholt T, et al. 2015. *J Mol Biol* 427:3646–3661.
Rosendal S, Boyd DA. 1982. *J Clin Microbiol* 16:840–843.
Rossi CR, Vicente AM, Guimarães WV, et al. 2013. *African J Microbiol Res* 7:2916–2924.
Rycroft AN, Assavacheep P, Jacobs M, et al. 2011. *Brit Med J* 343:d6261.
Sárközi R, Makrai L, Fodor L. 2015. *Acta Vet Hung* 63:444–450.
Schaller A, Kuhn R, Kuhnert P, et al. 1999. *Microbiology* 145:2105–2116.
Schaller A, Djordjevic SP, Eamens GJ, et al. 2001. *Vet Microbiol* 79:47–62.
Serrano-Rubio LE, Tenorio-Gutiérrez V, Suárez-Güemes F, et al. 2008. *Mol Cell Probes* 22:305–312.
Sibila M, Aragón V, Fraile L, et al. 2014. *BMC Vet Res* 10:165.
Sjölund M, Wallgren P. 2010. *Acta Vet Scand* 52:23.
Sjölund M, de la Fuente A, Fossum C, et al. 2009. *Vet Rec* 164:550–555.
Slavic D, Toffner T, Monteiro M, et al. 2000. *J Clin Microbiol* 38:3759–3762.
Stenbaek EI, De LaSalle F, Gottschalk M. 1997. *Can J Vet Res* 61:1–7.
Tegetmeyer H, Jones S, Langford P, et al. 2008. *Vet Microbiol* 128:342–353.
Thevenon J, Ivoc M, Rozsnyay Z, et al. 2014. Coglapix, an *Actinobacillus pleuropneumoniae* inactivated vaccine induce high levels of anti-Apx and anti-capsular antibodies. *Proc Eur Symp Porc Health Manag* 6:245.
Tobias T, Raymakers R, van Nes A, et al. 2009. *Vet Rec* 164:402–403.
Tobias TJ, Klinkenberg D, Bouma A, et al. 2014. *Prev Vet Med* 114:223–230.
Tonpitak W, Rohde J, Gerlach GF. 2007. *Vet Microbiol* 122:157–165.
Van den Bosch H, Frey J. 2003. *Vaccine* 21:3601–3607.
Van Overbeke I, Chiers K, Charlier G, et al. 2002. *Vet Microbiol* 88:59–74.
Vigre H, Angen O, Barfod K, et al. 2002. *Vet Microbiol* 89:151–159.
Vigre H, Ersboll A, Sorensen V. 2003. *J Vet Med B Infect Dis Vet Public Health* 50:430–435.
Wang C, Wang Y, Shao M, et al. 2009. *Vaccine* 27:5816–5821.
Ward CK, Inzana TJ. 1994. *J Immunol* 153:2110–2121.
Wilson RW, Kierstead M. 1976. *Can Vet J.* 17:222.
Wilson RJ, McOrist S. 2000. *Aust Vet J* 78:317–319.
Xu Z, Zhou Y, Li L, et al. 2008. *PLoS One* 3(1):e1450.
Yaeger M. 1995. *Swine Health Prod* 3:209–210.
Yaeger MJ. 1996. *J Vet Diagn Invest* 8:381–383.
Zhan B, Angen Ø, Hedegaard J, et al. 2010. *J Bacteriol* 192:5846–5847.
Zhang Y, Tennent JM, Ingham A, et al. 2000. *FEMS Microbiol Lett* 189:15–28.
Zhuang Q, Barfod K, Wachmann H, et al. 2007. *Vet Rec* 160:258–262.

49

Bordetellosis

Susan L. Brockmeier, Karen B. Register, Tracy L. Nicholson, and Crystal L. Loving

Relevance

Bordetella bronchiseptica was first isolated and associated with respiratory disease in dogs in 1910 (Ferry 1910). In the 1940s it was isolated in association with swine pneumonia, and it was being investigated as a cause of atrophic rhinitis of swine by the 1950s (Phillips 1943; Switzer 1956). In swine, *B. bronchiseptica* is widespread and plays multiple roles in respiratory disease. It is the primary etiologic agent of nonprogressive atrophic rhinitis (NPAR), a mild to moderately severe, reversible condition. More importantly, nasal colonization by *B. bronchiseptica* promotes colonization by toxigenic strains of *Pasteurella multocida*, which leads to severe progressive atrophic rhinitis (PAR) (see Chapter 58). In young pigs, *B. bronchiseptica* is a primary cause of necrohemorrhagic bronchopneumonia and in older pigs can be an opportunistic pathogen contributing to the porcine respiratory disease complex (PRDC). *B. bronchiseptica* can also enhance respiratory colonization of *Streptococcus suis* and *Haemophilus parasuis*, promote disease caused by *S. suis*, and interact with porcine reproductive and respiratory syndrome virus (PRRSV) and swine influenza virus (SIV) to increase severity of respiratory disease.

Etiology

The genus *Bordetella* belongs to the class *Beta proteobacteria* and is composed of nine species. *B. bronchiseptica* is the only species of importance in swine and infects a broad range of mammals causing a spectrum of acute to chronic clinical diseases such as kennel cough in dogs, bronchitis in cats, and atrophic rhinitis and bronchopneumonia in pigs (Goodnow 1980; Mattoo and Cherry 2005).

Bordetella bronchiseptica is an aerobic, motile, gram-negative rod or coccobacillus approximately 1.0 × 0.3 mm in size. The bacterium grows slowly, but readily, on blood agar or other nonselective media as well as on MacConkey agar. Convex colonies roughly 1–2 mm in diameter, usually hemolytic on blood agar, develop after 36–48 hours at 37 °C (98.6 °F). *B. bronchiseptica* is nonfermentative but positive for oxidase, catalase, urease, and citrate.

No serotyping methodology useful for discriminating among strains or evaluating population diversity is currently available for *B. bronchiseptica*. Nearly all *B. bronchiseptica* strains express one of two antigenically distinct O-antigen serotypes, O1 or O2, that are not cross-reactive (Buboltz et al. 2009a). However, these antigens are not suitable for typing purposes since they are encoded by separate loci that may recombine (Buboltz et al. 2009a).

Researchers have turned to molecular typing methods to identify and characterize genetic relationships among strains. These include ribotyping, random amplified polymorphic DNA (RAPD) fingerprinting, pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing (MLST) (Khattak and Matthews 1993; Musser et al. 1987; Register et al. 1997). Recent genome sequencing studies have generated numerous genome sequences from many *Bordetella* species, including *B. bronchiseptica* isolated from 11 different hosts (Harvill et al. 2013; Nicholson et al. 2016; Register et al. 2015). *B. bronchiseptica* can be separated into two lineages – complex I strains, which are most commonly isolated from animals, and complex IV strains, which are most commonly isolated from humans (Diavatopoulos et al. 2005).

It is well known that the consequence of colonization by *B. bronchiseptica* can vary from asymptomatic infection to lethal pneumonia (Goodnow 1980; Mattoo and Cherry 2005). Using inbred and specific-pathogen-free mice, the 50% lethal dose (LD_{50}) can differ by up to 100,000-fold between bacterial strains, suggesting that substantial differences in virulence may be due to strain variation alone (Buboltz et al. 2008; Gueirard and Guiso 1993). Recently published reports have demonstrated

Diseases of Swine, Tenth Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson.

that phylogenetic lineages can differ in virulence factor expression and virulence (Buboltz et al. 2008, 2009b). These reports support the overarching idea that the diversity of *B. bronchiseptica*-related disease and broad host range may be due, in part, to the distinct sets of virulence factors used by strains of different phylogenetic lineages (Cummings et al. 2006; Giardina et al. 1995).

Public health

Human illness resulting from *B. bronchiseptica* infection is rare but on the rise (Berkelman 2003; Brady et al. 2014; García-de-la-Fuente et al. 2015; Llombart et al. 2006; Tamion et al. 1996). Most at risk are infants and immunocompromised individuals with exposure to carrier animals; however, disease in immunocompetent adults also occurs. A variety of clinical presentations have been documented including tracheobronchitis, whooping cough, pneumonia, sinusitis, septicemia, meningitis, and peritonitis, occasionally with a fatal outcome. In many cases exposure to domesticated pets, particularly dogs, cats, and rabbits, is the presumed source of infection. No human cases related to transmission from swine have so far been reported. However, one of the few animal isolates among a genetically related group of complex IV *B. bronchiseptica* strains strongly associated with human infections is of swine origin (Diavatopoulos et al. 2005). Whether people as well as animals act as reservoirs for complex IV *B. bronchiseptica* strains, or whether people can act as asymptomatic carriers and transmitters of complex I *B. bronchiseptica* animal strains that can subsequently cause human or animal disease, is plausible but currently unknown.

Epidemiology

Bordetella bronchiseptica has a worldwide distribution and is known to infect poultry and a broad range of wild and domesticated mammalian species (Farrington and Jorgenson 1976; Goodnow 1980; Hammond et al. 2009; Heje et al. 1991; Lacasse and Gamble 2006; Ngom et al. 2006; Staveley et al. 2003). It is highly prevalent among swine and frequently isolated both from pigs with pneumonia or atrophic rhinitis as well as those that are apparently healthy (Backstrom et al. 1988; Giles et al. 1980; Kumar et al. 2014; Kureljušić et al. 2016; Palzer et al. 2008; Rutter et al. 1984; Zhao et al. 2011a).

Transmission of *B. bronchiseptica* occurs primarily by aerosol droplets. It is facilitated by close contact, but airborne transmission over short distances, including within a barn or production unit, is probable (Brockmeier and Lager 2002; Nicholson et al. 2012, 2014; Stehmann et al. 1992). Infectious aerosols generated by sneezing or coughing in pigs with active disease further promote the spread of the agent. Pigs of all ages are susceptible to infection, but many litters are colonized before weaning, most likely due to exposure from nursing sows. Antibody passively acquired by piglets from the colostrum of infected or vaccinated sows protects against turbinate lesions and pneumonia but not against infection (Kobisch and Pennings 1989; Magyar et al. 2002; Riising et al. 2002; Rutter 1981). However, vaccination of sows may delay infection for up to several weeks (Rutter et al. 1984), at which time lesions typically fail to develop or are significantly reduced in severity (de Jong and Akkermans 1986; Giles et al. 1980). *B. bronchiseptica* persists in the nasal cavity for at least several months (Backstrom et al. 1988; Riising et al. 2002; Rutter 1981) and perhaps indefinitely, and the introduction of carrier pigs is a source of infection for older animals in systems where an all-in/all-out approach is not practiced. Spread within a herd is rapid, particularly in immunologically naïve animals (Smith et al. 1982).

Newly purchased breeding stock may be a source of infection. *B. bronchiseptica* has been isolated from rodents, birds, raccoons, opossums, and other animals trapped in close proximity to swine (Farrington and Jorgenson 1976; Le Moine et al. 1987). Although so far undocumented, cross-species transmission to pigs following exposure to infected wild animals or domesticated pets, such as dogs and cats, may occur. It has been suggested that non-porcine strains are of low virulence in pigs (Ross et al. 1967), but it is unclear whether the cultures used were propagated so as to retain their original phenotypes. This conclusion should be reevaluated in light of the current understanding of virulence factor regulation in *B. bronchiseptica* and the importance of maintaining a Bvg^+ phenotype during culture (see "Pathogenesis"). A subsequent study reported no differences in the bacteriological properties of swine isolates as compared with isolates obtained from several other hosts (Bemis et al. 1977). Insect vectors may also be capable of introducing the organism into a herd or production unit (Beatson 1972). Transmission via contaminated fomites has not been evaluated but should not be excluded, given the ability of *B. bronchiseptica* to survive *ex vivo*.

The bacterium remains viable for up to 45 days in soil (Mitscherlich and Marth 1984) and for at least several weeks in lake water or non-nutritive liquid media at temperatures ranging from 50 to 98.6 °F (10–37 °C) (Porter et al. 1991; Porter and Wardlaw 1993). The half-life of aerosolized organisms at ambient temperature and approximately 75% relative humidity is 1–2 hours (Stehmann et al. 1992). *B. bronchiseptica* can be inactivated by sonication (Harris and Switzer 1972), heating to 140 °F (60 °C) (Bemis and Kennedy 1981; Lendvai et al. 1992), or treatment with formaldehyde (Jenkins 1978). It

is sensitive to several chemical disinfectants suitable for farm use (Thomson et al. 2007).

Pathogenesis

The pathogenesis of *B. bronchiseptica* is dependent on the sequential coordinated synthesis of an array of virulence factors including adhesins, toxins, and other bacterial products that may alter host functions, facilitate immune evasion, or otherwise assist in transmission or survival. Expression of most virulence genes requires coexpression of the BvgAS (*Bordetella* virulence genes) system (Beier and Gross 2008). A rise in growth temperature that occurs as the organism moves from an external environment into the tissues of the respiratory tract triggers transcription of *bvgAS* and subsequent expression of the BvgAS-inducible genes (Bvg^+ state). At temperatures of approximately 77 °F (25 °C) or lower, *bvgAS* genes are not expressed, and the resultant Bvg^- state is nonpermissive for the synthesis of toxins, adhesins, and other known or suspected virulence proteins, while there is maximal expression of motility genes, virulence-repressed genes (*vrg* genes), and genes required for the production of urease (Akerley et al. 1992; McMillan et al. 1996). Environmental cues, including a rise in growth temperature that occurs as the organism moves from an external environment into the tissues of the respiratory tract, trigger transcription of *bvgAS* and subsequent expression of the BvgAS-inducible genes (Bvg^+ state). This process, known as phenotypic modulation, is fully reversible and is an important adaptive response of the organism to changes in the environment. Previous studies involving phase-locked and ectopic expression mutants demonstrated that the Bvg^+ phase promotes respiratory tract colonization of *B. bronchiseptica*, while the Bvg^- phase promotes survival under conditions of nutrient deprivation, such as those potentially encountered in an environmental reservoir (Cotter and Miller 1994). In swine, the Bvg^+ phase is all that is required for respiratory infection and pig-to-pig transmission (Nicholson et al. 2012).

The *bvgAS* genes are also subject to phase variation, in which a small proportion of growing cells spontaneously acquire deletions or frameshift mutations that irreversibly abolish expression of all BvgAS-activated genes, regardless of the growth conditions. When subculturing *B. bronchiseptica*, it is important to pick for passage only single, well-isolated Bvg^+ colonies (small, domed, and hemolytic on blood agar) in order to avoid gradual transition of the culture to an irreversible Bvg^- state (colonies are larger, flat, and nonhemolytic on blood agar).

So-called "early" BvgAS-inducible genes, including many whose products are involved in attachment, are among the first to be activated during phenotypic modulation from Bvg^- to Bvg^+. Expression of "late" genes, including several toxins, commences only after accumulation of sufficient levels of the *bvgAS* gene products. The existence of the BvgAS system suggests that precise control of the temporal expression of virulence factors in response to a changing environment is important for optimizing the growth and survival of *B. bronchiseptica* as it cycles through transmission, colonization, growth and spread, immune evasion, and shedding.

In the initial stage of infection, *B. bronchiseptica* attaches to epithelial cells lining the nasal mucosa. Organisms display preferential adherence to ciliated cells (Duncan et al. 1966b; Yokomizo and Shimizu 1979), but attachment to nonciliated epithelia may also occur and may be important in establishing microcolonies or biofilms (Irie and Yuk 2007). Biofilms are an adherent community of microorganisms encased within a complex matrix that protects the community from a variety of environmental stresses such as shear flow forces, antimicrobial compounds, and host immune and clearance mechanisms. Biofilms are increasingly being recognized as important contributors to chronic or persistent *Bordetella* infections (Cattelan et al. 2016).

Several *B. bronchiseptica* adhesins have been characterized. Filamentous hemagglutinin (FHA) is a protein secreted by *B. bronchiseptica* and associated with the bacterial cell surface that is highly immunogenic and required for optimal colonization of the upper respiratory tract (Cotter et al. 1998; Edwards et al. 2005; Hibrand-Saint Oyant et al. 2005; Irie and Yuk 2007; Nicholson et al. 2009). At least four domains with distinct binding specificities have been identified, some of which exert immunomodulatory effects (Hannah et al. 1994; Ishibashi et al. 1994; Melvin et al. 2015; Prasad et al. 1993).

Fimbrial proteins, which form a complex of hairlike strands extending from the bacterial cell surface, possess multiple binding specificities including one that enhances FHA-mediated attachment (Geuijen et al. 1997; Hazenbos et al. 1995). Fimbriae are important for colonization and persistence in the trachea, contribute to biofilm development, and also influence the humoral and cell-mediated immune responses to infection (Edwards et al. 2005; Geuijen et al. 1997; Irie et al. 2004; Mattoo et al. 2000; Scheller et al. 2015).

The outer membrane protein pertactin also contributes to colonization, perhaps as an accessory adhesin, but its precise function remains unclear (Hibrand-Saint Oyant et al. 2005; Nicholson et al. 2009). In contrast, its importance as a protective immunogen is well established (Kobisch and Novotny 1990; Montaraz et al. 1985; Novotny et al. 1985). Sequence heterogeneity in a region comprising an immunodominant protective epitope (Boursaux-Eude and Guiso 2000; Register 2001, 2004)

may alter the specificity of host immune responses, providing a potential mechanism for immune evasion (Hijnen et al. 2007).

An additional BvgAS-regulated virulence factor of *B. bronchiseptica* is a type III secretion system (T3SS), used by the bacterium to translocate or inject protein effectors directly into the cytosol of a eukaryotic cell and whose components have immunomodulatory and cytotoxic effects (Medhekar et al. 2009). In swine, the T3SS contributes to the severity of pneumonic lesions and to the ability of the bacterium to persistently infect the lungs (Nicholson et al. 2014).

Once *B. bronchiseptica* is established within the respiratory tract, expression of toxins contributes to the progression of disease. Of central importance is the dermonecrotic toxin (DNT), a protein toxin that exerts pleiotropic effects including impairment of bone formation, that is essential for development of turbinate atrophy and pneumonia in both mice and pigs (Brockmeier et al. 2002; Horiguchi et al. 1995; Magyar et al. 2000). The DNT is primarily responsible for the sometimes fatal pneumonic lesions in suckling pigs characterized by necrosis, hemorrhage, neutrophil accumulation, and eventually fibrosis (Brockmeier et al. 2002). Swine and murine strains of *B. bronchiseptica* secrete higher levels of DNT than isolates from other host species (Okada et al. 2015), suggesting a host-specific adaptation of the bacterium.

A toxin that has both adenylate cyclase and pore-forming activities, referred to as adenylate cyclase toxin (ACT), also contributes to virulence by disrupting innate immunoprotective functions. Phagocytic cells appear to be a primary target of ACT that, in cooperation with FHA or the T3SS, modulates cytokine production and alters serum and secretory antibody responses in pigs (Harvill et al. 1999; Henderson et al. 2012; Hibrand-Saint Oyant et al. 2005; Skinner et al. 2004).

Tracheal cytotoxin (TCT) is a peptidoglycan breakdown product arising from normal bacterial cell wall remodeling during growth. Unlike most other gram-negative bacteria, *B. bronchiseptica* lacks the capability to recycle TCT (Cookson and Goldman 1987), and it is instead released extracellularly where it interacts synergistically with lipopolysaccharide to cause ciliostasis and extrusion of ciliated cells from the mucosal epithelial lining (Flak et al. 2000). TCT is likely responsible for the impairment of mucociliary clearance that occurs early in the course of infection.

Age and immune status play roles in the pathogenesis of disease with *B. bronchiseptica* as well. In the nonimmune pig, younger animals typically develop more severe disease both in regard to bronchopneumonia and atrophic rhinitis. Pigs with passive or acquired immunity from vaccination or natural infection develop less severe disease, although they may still be colonized.

Coinfection with *B. bronchiseptica* and other pathogens in the respiratory tract also affects disease severity. The ability of *B. bronchiseptica* to predispose to colonization of the upper respiratory tract with *P. multocida* leading to PAR has been well established (de Jong and Nielsen 1990; Harris and Switzer 1968; Pedersen and Barfod 1981; Rutter 1983). *B. bronchiseptica* has also been shown to predispose to disease with *S. suis* (Vecht et al. 1989, 1992). Preinoculation of pigs with *B. bronchiseptica* prior to *S. suis* resulted in increased clinical signs and fever, increased isolation of *S. suis*, increased pneumonia and disseminated lesions due to septicemic *S. suis*, and increased mortality. *B. bronchiseptica* has also been shown to enhance colonization of the nasal cavity with *H. parasuis* as well (Brockmeier 2004).

The means by which *B. bronchiseptica* exacerbates or predisposes to secondary bacterial infections is not known, but damage inflicted to innate protective mechanisms during colonization likely plays a major role. TCT causes necrosis of the ciliated respiratory epithelium that lines the nasal cavity and conducting airways, resulting in significant impairment of the clearance function of the mucociliary apparatus. *B. bronchiseptica* is also cytotoxic for swine alveolar macrophages (Brockmeier and Register 2000; Forde et al. 1999), which may result in decreased phagocytosis and killing of inhaled bacteria. Damage to turbinates by DNT may predispose to colonization by other bacteria by alteration or ablation of the normal microbiota, induction of increased mucus production, exposure of submucosal components to which other bacteria may adhere, and/or increased nutrient availability. However, a DNT-negative mutant of *B. bronchiseptica* was still capable of predisposing to infection with toxigenic *P. multocida*, and the subsequent development of atrophic rhinitis appeared unaffected (Brockmeier and Register 2007). Consequently other factors must also contribute to secondary infection and disease.

Bordetella bronchiseptica can also interact with certain swine viruses to influence the development or severity of respiratory disease. PRRSV predisposes to bronchopneumonia with *B. bronchiseptica* (Brockmeier et al. 2000). Although PRRSV alone has not been shown to enhance infection with *P. multocida*, infection with both *B. bronchiseptica* and PRRSV leads to increased pulmonary infection with *P. multocida* (Brockmeier et al. 2001). By facilitating colonization with other prevalent bacterial pathogens, *B. bronchiseptica*, in combination with PRRSV and its immunosuppressive properties, may leave pigs more susceptible to pulmonary or systemic infection with opportunistic bacteria. Coinfection with *B. bronchiseptica* and SIV or porcine respiratory coronavirus (PRCV) leads to increased severity of pneumonia with an earlier onset and longer resolution when compared with either virus alone (Brockmeier et al. 2008;

Kowalczyk et al. 2014; Loving et al. 2010). Coinfected pigs show a greater and more sustained production of proinflammatory cytokines that may contribute to the enhancement of pulmonary lesions (Brockmeier et al. 2008; Kowalczyk et al. 2014; Loving et al. 2010).

Clinical signs

Bordetella bronchiseptica is highly infectious and transmits rapidly and easily via direct contact or aerosol transmission, resulting in high morbidity but generally low mortality except in very young pigs with *B. bronchiseptica*-induced necrohemorrhagic bronchopneumonia or when complicated by certain coinfections (Brockmeier and Lager 2002). In uncomplicated disease, clinical signs typically appear around 2–3 days after infection when colonization and damage to the respiratory epithelium in the nasal cavity (rhinitis), trachea (tracheitis), and bronchi (bronchitis) leads to sneezing, nasal discharge, ocular discharge, and a dry, repeated cough. More severe signs can occur in neonatal pigs if necrohemorrhagic bronchopneumonia develops, including dyspnea, lethargy, and sometimes death. Clinical signs typically abate after a few weeks, but the respiratory tract remains colonized for months.

When *B. bronchiseptica* interacts with other pathogens to cause or enhance disease, clinical signs may include or be solely those of the other agents such as those for PAR when toxigenic strains of *P. multocida* also colonize nasal turbinates (Chapter 57), for PRRS (Chapter 41), for influenza (Chapter 36), for Glasser's disease caused by *H. parasuis* (Chapter 54), or for the various manifestations of disease caused by *S. suis* (Chapter 61).

Lesions

Bordetella bronchiseptica may colonize and cause injury throughout the entirety of the respiratory tract, but lesions are especially associated with the nasal cavity, consisting of atrophic rhinitis, and the lung, consisting of bronchopneumonia (Duncan et al. 1966a,b). Macroscopic lesions of the nasal cavity include nasal exudate and mild to moderate turbinate atrophy, which are often referred to as NPAR (Figures 49.1 and 49.2). Distortions of the nasal septum, curvature of the snout, and brachygnathia are usually not seen with uncomplicated *B. bronchiseptica* infection, but may occur in mixed infections with toxigenic strains of *P. multocida* that lead to PAR (Chapter 57). Staining or accumulation of exudate around the medial canthus of the eye often not only results from inflammation and blockage of the tear ducts but can also develop when other pathogens that cause rhinitis are

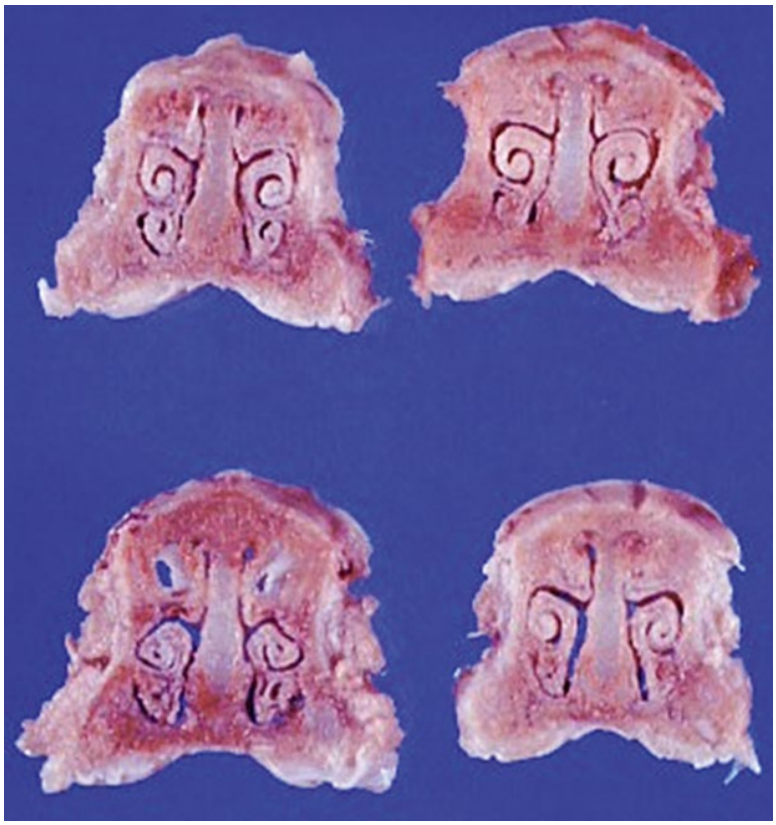


Figure 49.1 Cross sections of the snouts of uninfected 5-week-old pigs showing normal turbinate scrolls.

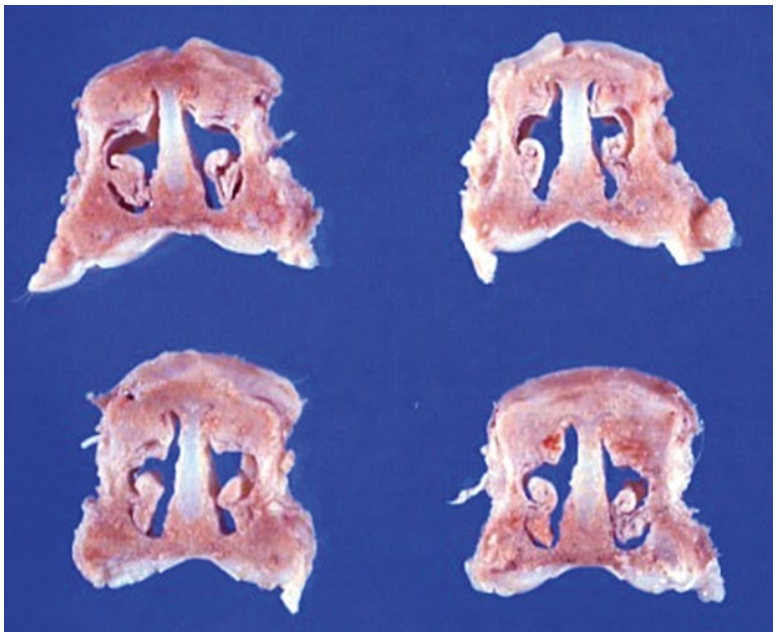


Figure 49.2 Cross sections of the snouts of 5-week-old pigs infected with *B. bronchiseptica* showing moderate turbinate atrophy.

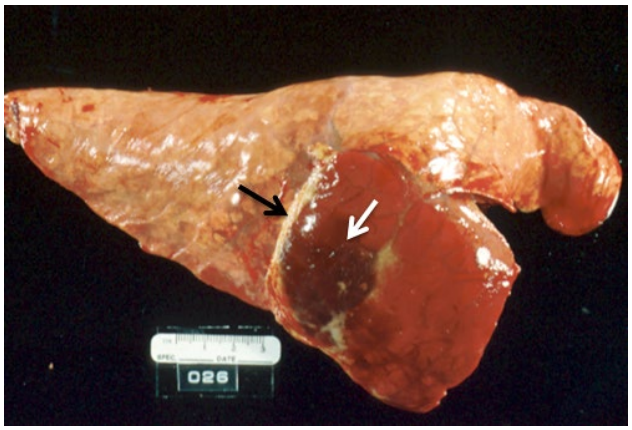


Figure 49.3 Macroscopic lung lesions in a suckling pig infected with *B. bronchiseptica* showing cranial ventral consolidation, hemorrhage (white arrow), and localized fibrinous pleuritis (black arrow). *Source:* Courtesy of Department of Veterinary Pathology, Iowa State University.

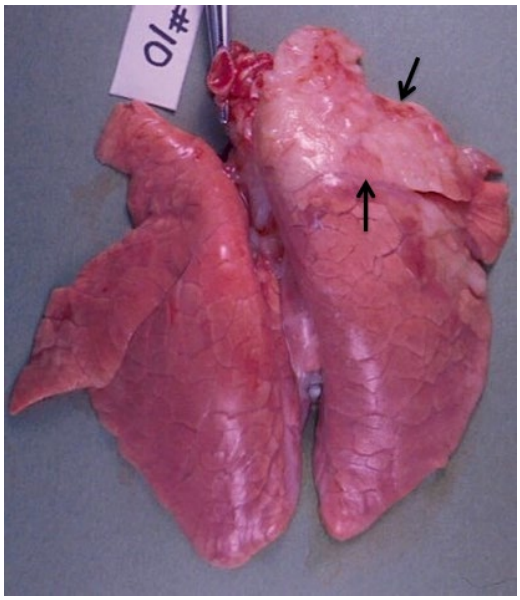


Figure 49.4 Macroscopic lung lesions in a pig infected with *B. bronchiseptica* showing fibrotic lesions in the right cranial lobe (arrows) 4 weeks after infection.

present. In most cases of uncomplicated rhinitis due to *B. bronchiseptica*, regeneration of the turbinates occurs with time, but persistent distortion of the ventral turbinates frequently results (Duncan et al. 1966b). Primary bronchopneumonia in suckling pigs when acute is necrohemorrhagic, characterized by groups of lobules in the cranial ventral lungs that are red and firm, sometimes with mild fibrinous pleuritis overlying only affected lung (Figure 49.3). Subacute lesions are yellow to gray as more leukocytes infiltrate, and chronic lesions are hard, white, and fibrotic (Figure 49.4). Opportunistic bronchopneumonia in older pigs as a component of PRDC is purulent, characterized by plum-to-gray lobular consolidation with purulent exudate in lumens of airways.

Microscopic lesions of the nasal cavity are characterized by epithelial hyperplasia to squamous metaplasia and loss of cilia, and submucosal infiltrates of neutrophils, fewer lymphocytes and macrophages, and occasional microabscesses in the epithelium (Duncan et al. 1966b). Replacement of the bony trabeculae of the turbinates with fibrous connective tissue may also occur. Microscopic lesions of the lung are characterized by hemorrhage, necrosis, and accumulation of inflammatory infiltrates, mostly neutrophils, in the airways and

alveolar spaces (Duncan et al. 1966a). Interlobular and intra-alveolar edema may also occur. Eventually fibroblastic replacement of lung parenchyma with sequestration of necrotic areas develops and may take months to completely resolve. Coinfection with other bacteria and/or viruses may change the character of the lesions, but suppurative bronchopneumonia is nearly always present.

Diagnosis

Many pathogens other than *B. bronchiseptica* often cause pneumonia in pigs. When acting as a primary pathogen, *B. bronchiseptica* alone may be isolated from pneumonic lungs. However, this organism most commonly occurs in mixed infections and is frequently found together with one or more other respiratory disease agents. In such cases, the actual contribution of *B. bronchiseptica* to clinical disease may be difficult to determine. Acute lung lesions in piglets can appear very similar to those caused by *Actinobacillus suis or Actinobacillus pleuropneumoniae*. When investigating the cause of rhinitis in young pigs, porcine cytomegalovirus and *P. multocida* should be considered in a differential diagnosis. Various grading systems for the quantitative assessment of turbinate atrophy have been used to evaluate treatment of PAR. These systems are based on transversely sectioning the snout at the level of the first or second premolar tooth and assigning a score estimating the amount of atrophy of each of the four scrolls of the ventral turbinates.

Detection of infection with *B. bronchiseptica* is typically based on isolation and biochemical testing of suspect colonies cultured from nasal swabs, post mortem lung washings, or biopsy specimens. Mini-tipped swabs are available that facilitate nasal sampling in young pigs. Swabs should be placed in a non-nutritive transport medium, such as PBS, and kept at 39 °F (4 °C) for delivery to a diagnostic laboratory, preferably within 24 hours.

Bordetella bronchiseptica grows readily on blood agar; however use of a selective medium is desirable to prevent overgrowth by other faster-growing and typically more numerous commensal and pathogenic bacteria that may be present. Many laboratories use a modified MacConkey agar with 1% glucose and 20 µg/mL furaltadone (Farrington and Switzer 1977), but a peptone agar formulation (Smith and Baskerville 1979) may be superior when the number of *B. bronchiseptica* is low. In a direct comparison, neither the modified MacConkey agar nor the peptone agar performed as well as blood agar with cephalexin for isolation of *B. bronchiseptica* from nasal swabs (Lariviere et al. 1993). Blood agar plates containing 20 µg/mL penicillin, 10 µg/mL amphotericin B, 10 µg/mL streptomycin, and 10 µg/mL spectinomycin also perform well for isolation from highly contaminated sites such as the nasal cavity (Brockmeier 1999). A blood-containing medium should be utilized for subsequent passage of primary isolates so that it is possible to evaluate and maintain the original Bvg phenotype. Although the required reagents are widely available, conventional identification methods are time consuming and suffer from poor sensitivity. A DNA probe hybridization assay using colony lifts of primary isolation plates has been described that is highly specific and more sensitive than traditional isolation and biochemical testing (Register et al. 1995). More recently a PCR target originally evaluated for use with human specimens (Hozbor et al. 1999) was found to be 100% sensitive and specific for *B. bronchiseptica* when tested with bacterial genera and species commonly found in the swine respiratory tract (Register and DeJong 2006).

Early methods employed for the serologic diagnosis of *B. bronchiseptica* infection were based on the detection of agglutinating serum antibody in assays using either tube (Jenkins 1978) or plate (Shashidhar et al. 1983) formats. ELISAs for detection of antibody in serum (Kono et al. 1994; Venier et al. 1984) or nasal secretions (Kono et al. 1994) were subsequently described. Because these assays depend solely on antibody binding and provide objective endpoints, they provide greater sensitivity and reproducibility than agglutination-based methods. Serology may be useful for monitoring the status of a herd but is rarely used routinely for diagnostic purposes.

Immunity

Studies characterizing the mechanisms of protection against bordetellosis have been carried out primarily in mice and provide useful information for understanding immunity to infection/vaccination. Studies have shown that IgA is important for clearance of organisms from the upper respiratory tract, whereas IgG is critical for clearance from the lungs; thus, vaccines that elicit strong IgA responses are critical for complete respiratory tract clearance (Kirimanjeswara et al. 2003; Wolfe et al. 2007). Infection-induced immunity, as opposed to vaccine-induced immunity, provides significant protection in the upper and lower respiratory tract. Although serum antibody titers are higher following parenteral vaccination, as opposed to mucosal infection, the antibody generated following parenteral vaccination is less effective at providing protection (Gopinathan et al. 2007). A modified live strain of *B. bronchiseptica* delivered by the intranasal route to pigs provided superior protection against challenge when compared with an inactivated vaccine, and protection was associated with mucosal *B. bronchiseptica*-specific IgA titers (Zhang et al. 2013). Together, this information suggests that immune responses elicited at the site of natural infection are likely to be the most

efficacious and live attenuated vaccines delivered intranasally are an obvious approach.

Vaccines that elicit a response to pertactin, a 68 kDa protein on the bacterial surface, have been shown to be highly protective in reducing disease severity (Kobisch and Novotny 1990; Li et al. 1992). Vaccines that induce large amounts of antibody to lipopolysaccharide appear to provide the least protection from disease (Novotny et al. 1985). Pertactin gene heterogeneity has been described (Register 2004) and is an important consideration for creating a vaccine that is efficacious against field strains. Although pertactin appears to be a primary protective antigen, reactivity to additional outer membrane proteins is important for complete protection evidenced by superior protection provided by whole-cell vaccines (Kobisch and Novotny 1990; Novotny et al. 1985). Current commercial vaccines typically contain *B. bronchiseptica* whole-cell bacterin as well as a *P. multocida* toxoid for optimal protection against PAR. Vaccination does not provide sterilizing immunity but does significantly limit or even abolish clinical disease.

Piglets may become infected with *B. bronchiseptica* prior to weaning; thus sow vaccination may be useful for preventing disease in piglets. Although sow vaccination can decrease disease severity and increase piglet performance, it does not eliminate colonization of pathogenic organisms from piglets (Riising et al. 2002). In addition, passive antibody from the sow to piglet can interfere with parenteral vaccination of the piglet (Smith et al. 1982). Vaccination of nonimmune piglets can provide protection from turbinate atrophy as piglets vaccinated with whole-cell bacterin at 1 and 4 weeks of age develop circulating antibody lasting beyond 12 weeks of age (Farrington and Switzer 1979). However, until the piglet mounts a response to the vaccine, it remains susceptible to natural infection.

Prevention and control

Bordetella bronchiseptica is largely susceptible to chlortetracycline, oxytetracycline, and enrofloxacin; all are antibiotics approved for the treatment of swine respiratory disease caused by pathogens that are often associated with *B. bronchiseptica*, such as *P. multocida*, *H. parasuis*, and *S. suis* (Kadlec et al. 2004). Tulathromycin specifically lists swine respiratory disease caused by *B. bronchiseptica* as an indicated use. *Bordetella* is largely resistant to ampicillin and cephalosporins such as ceftiofur, which is approved for treatment of swine respiratory disease of mixed bacterial etiology (Dayao et al. 2014; Pruller et al. 2015; Zhao et al. 2011). Accordingly, ceftiofur is not the optimal choice when treating mixed infections that include *B. bronchiseptica* as a component. Antibiotics may alleviate pneumonia and reduce clinical signs, but total clearance of *Bordetella* from the upper respiratory tract is difficult. Normally the need for treatment of atrophic rhinitis arises when there are signs of PAR in the herd, which almost always involves coinfection with toxigenic strains of *P. multocida*. Use of antibiotics to control cases of atrophic rhinitis includes administration by feeding or parenteral route to sows and piglets around the time of farrowing/weaning in an effort to limit the extent of colonization in young pigs. Tetracyclines and trimethoprim–sulfonamide preparations have historically been successfully used for this purpose, but examining susceptibility profiles of porcine pathogens coming out of diagnostic laboratories indicates there is significant resistance of *B. bronchiseptica* to trimethoprim-sulfonamide combinations (de Jong and Oosterwoud 1977). Treatment of older pigs probably has limited efficacy on the disease progression of atrophic rhinitis.

Most vaccine studies have examined efficacy in terms of prevention of atrophic rhinitis, but since *B. bronchiseptica* predisposes to infection with other respiratory pathogens, vaccination or elimination from a herd may have broader effects on respiratory health. Vaccines for *B. bronchiseptica* largely consist of whole-cell bacterins often in combination with *P. multocida* whole-cell bacterins and/or toxoids for control of atrophic rhinitis, but there are a few attenuated intranasal vaccines available as well. When using vaccination to prevent PAR, the *P. multocida* toxin (PMT) is an important component of the vaccine, and those with added PMT toxoid offer superior protection (Foged et al. 1989; Hsuan et al. 2009; Nielsen et al. 1991; To et al. 2005; Voets et al. 1992). Vaccination of sows around 6 weeks and again around 2 weeks prior to farrowing appears to work well to protect piglets and minimize transmission (Riising et al. 2002). Protection of piglets, whether through antibiotic treatment, passive antibody through sow vaccination, or a combination of both, during the first few weeks of life appears to be an important factor in the control of atrophic rhinitis (Pejsak et al. 1994). Vaccination of piglets around 2 and 4 weeks of age has had mixed results; when less favorable results occur, it is probably due to the occurrence of maternal antibody interference and/or prior colonization of young piglets before an immune response has developed (Farrington and Switzer 1979; Smith et al. 1982). Bacterins provide protection against disease manifestations such as turbinate atrophy but typically fail to protect animals from colonization. Thus vaccinated animals can still serve as subclinical carriers that continue to shed and transmit *B. bronchiseptica* to cohorts.

Attenuated live vaccines are usually given intranasally within a few days of birth in an attempt to prevent colonization with virulent strains of *B. bronchiseptica* by competitive exclusion and to induce a mucosal immune response. Previous studies have shown a resistance to

reinfection in animals naturally infected with *B. bronchiseptica*, indicating a mucosal response may be important for clearance of *B. bronchiseptica* from the respiratory tract (Gopinathan et al. 2007; Harris and Switzer 1969). Results with attenuated vaccines have been mixed, possibly because of maternal antibody interference or due to the nature of the attenuated strain (de Jong 1987; Pejsak et al. 1994). *B. bronchiseptica* attenuated via mutation of the *bvg* locus is avirulent but poorly colonizes the respiratory tract and would not be expected to elicit a robust immune response. Strains defective only in DNT production have been engineered and are greatly attenuated but may not be desirable for vaccine use since, like wild type, they predispose to colonization with *P. multocida* (Brockmeier and Register 2007). Recently an attenuated strain of *B. bronchiseptica* based on deletion of *aroA* that was administered intranasally to pigs provided superior protection against challenge when compared with an inactivated vaccine, and protection was associated with mucosal *B. bronchiseptica*-specific IgA titers (Zhang et al. 2013).

Attempts to control *B. bronchiseptica* infection in swine herds are usually instituted when PAR is severe and *B. bronchiseptica* is confirmed as a component along with toxigenic strains of *P. multocida*. Measures to control or eliminate PAR from a herd include combinations of improved husbandry, such as improved air quality/ventilation and pig flow (all-in/all-out management with proper cleaning and disinfection between groups), medicated early weaning, and vaccination of the breeding herd and/or pigs. Intensive use of these methods has been shown to reduce the bacterial burden of both *B. bronchiseptica* and *P. multocida* and, in combination with nasal swab testing and elimination of positive animals, has even been able to eliminate toxigenic *P. multocida* from herds. *B. bronchiseptica* is a ubiquitous pathogen that is highly infectious. Whether or not *B. bronchiseptica* can be totally eliminated by these methods remains unclear.

References

Akerley, B.J., Monack, D.M., Falkow, S. et al. (1992). *J Bacteriol* 174: 980–990.

Backstrom, L.R., Brim, T.A., and Collins, M.T. (1988). *Can J Vet Res* 52: 23–29.

Beatson, S.H. (1972). *Lancet* 1: 425–427.

Beier, D. and Gross, R. (2008). *Adv Exp Med Biol* 631: 149–160.

Bemis, D.A. and Kennedy, J.R. (1981). *J Infect Dis* 144: 349–357.

Bemis, D.A., Greisen, H.A., and Appel, M.J. (1977). *J Clin Microbiol* 5: 471–480.

Berkelman, R.L. (2003). *Clin Infect Dis* 37: 407–414.

Boursaux-Eude, C. and Guiso, N. (2000). *Infect Immun* 68: 4815–4817.

Brady, C., Ackerman, P., Johnson, M. et al. (2014). *J Cyst Fibros* 13: 43–48.

Brockmeier, S.L. (1999). *FEMS Microbiol Lett* 174: 225–229.

Brockmeier, S.L. (2004). *Vet Microbiol* 99: 75–78.

Brockmeier, S.L. and Lager, K.M. (2002). *Vet Microbiol* 89: 267–275.

Brockmeier, S.L. and Register, K.B. (2000). *Vet Microbiol* 73: 1–12.

Brockmeier, S.L. and Register, K.B. (2007). *Vet Microbiol* 125: 284–289.

Brockmeier, S.L., Palmer, M.V., and Bolin, S.R. (2000). *Am J Vet Res* 61: 892–899.

Brockmeier, S.L., Palmer, M.V., Bolin, S.R. et al. (2001). *Am J Vet Res* 62: 521–525.

Brockmeier, S.L., Register, K.B., Magyar, T. et al. (2002). *Infect Immun* 70: 481–490.

Brockmeier, S.L., Loving, C.L., Nicholson, T.L. et al. (2008). *Vet Microbiol* 128: 36–47.

Buboltz, A.M., Nicholson, T.L., Parette, M.R. et al. (2008). *J Bacteriol* 190: 5502–5511.

Buboltz, A.M., Nicholson, T.L., Karanikas, A.T. et al. (2009a). *Infect Immun* 77: 3249–3257.

Buboltz, A.M., Nicholson, T.L., Weyrich, L.S. et al. (2009b). *Infect Immun* 77: 3969–3977.

Cattelan, N., Dubey, P., Arnal, L. et al. (2016). *Pathog Dis* 74: ftv108.

Cookson, B.T. and Goldman, W.E. (1987). *J Cell Biochem* 11 (suppl. B).

Cotter, P.A. and Miller, J.F. (1994). *Infect Immun.* 62: 3381–3390.

Cotter, P.A., Yuk, M.H., Mattoo, S. et al. (1998). *Infect Immun* 66: 5921–5929.

Cummings, C.A., Bootsma, H.J., Relman, D.A. et al. (2006). *J Bacteriol* 188: 1775–1785.

Dayao, D.A., Gibson, J.S., Blackall, P.J. et al. (2014). *Vet Microbiol* 171: 232–235.

Diavatopoulos, D.A., Cummings, C.A., Schouls, L.M. et al. (2005). *PLoS Pathog* 1: e45.

Duncan, J.R., Ramsey, F.K., and Switzer, W.P. (1966a). *Am J Vet Res* 27: 467–472.

Duncan, J.R., Ross, R.F., Switzer, W.P. et al. (1966b). *Am J Vet Res* 27: 457–466.

Edwards, J.A., Groathouse, N.A., and Boitano, S. (2005). *Infect Immun* 73: 3618–3626.

Farrington, D.O. and Jorgenson, R.D. (1976). *J Wildl Dis* 12: 523–525.

Farrington, D.O. and Switzer, W.P. (1977). *J Am Vet Med Assoc* 170: 34–36.

Farrington, D.O. and Switzer, W.P. (1979). *Am J Vet Res* 40: 1347–1351.

Ferry, N.S. (1910). *Am Vet Rev* 37: 499–504.

Flak, T.A., Heiss, L.N., Engle, J.T. et al. (2000). *Infect Immun* 68: 1235–1242.
Foged, N.T., Nielsen, J.P., and Jorsal, S.E. (1989). *Vet Rec* 125: 7–11.
Forde, C.B., Shi, X., Li, J. et al. (1999). *Infect Immun* 67: 5972–5978.
García-de-la-Fuente, C., Guzmán, L., Cano, M.E. et al. (2015). *Diagn Microbiol Infect Dis* 82: 20–25.
Geuijen, C.A., Willems, R.J., Bongaerts, M. et al. (1997). *Infect Immun* 65: 4222–4228.
Giardina, P.C., Foster, L.A., and Musser, J.M. (1995). *J Bacteriol* 177: 6058–6063.
Giles, C.J., Smith, I.M., Baskerville, A.J. et al. (1980). *Vet Rec* 106: 25–28.
Goodnow, R.A. (1980). *Microbiol Rev* 44: 722–738.
Gopinathan, L., Kirimanjeswara, G.S., Wolfe, D.N. et al. (2007). *Microbes Infect* 9: 442–448.
Gueirard, P. and Guiso, N. (1993). *Infect Immun* 61: 4072–4078.
Hammond, E.E., Sosa, D., Beckerman, R. et al. (2009). *J Zoo Wildl Med* 40: 369–372.
Hannah, J.H., Menozzi, F.D., Renauld, G. et al. (1994). *Infect Immun* 62: 5010–5019.
Harris, D.L. and Switzer, W.P. (1968). *Am J Vet Res* 29: 777–785.
Harris, D.L. and Switzer, W.P. (1969). *Am J Vet Res* 30: 1161–1166.
Harris, D.L. and Switzer, W.P. (1972). *Am J Vet Res* 33: 1975–1984.
Harvill, E.T., Cotter, P.A., Yuk, M.H. et al. (1999). *Infect Immun* 67: 1493–1500.
Harvill ET, Goodfield LL, Ivanov Y, et al. 2013. *Genome Announc* 1(6). pii: e01075-13. doi: https://doi.org/10.1128/genomeA.01075-13.
Hazenbos, W.L., van den Berg, B.M., Geuijen, C.W. et al. (1995). *J Immunol* 155: 3972–3978.
Heje, N.I., Henriksen, P., and Aalbaek, B. (1991). *Acta Vet Scand* 32: 205–210.
Henderson, M.W., Inatsuka, C.S., Sheets, A.J. et al. (2012). *Infect Immun* 80: 2061–2075.
Hibrand-Saint Oyant, L., Bourges, D., Chevaleyre, C. et al. (2005). *Vet Res* 36: 63–77.
Hijnen, M., de Voer, R., Mooi, F.R. et al. (2007). *Vaccine* 25: 5902–5914.
Horiguchi, Y., Okada, T., Sugimoto, N. et al. (1995). *FEMS Immunol Med Microbiol* 12: 29–32.
Hozbor, D., Fouque, F., and Guiso, N. (1999). *Res Microbiol* 150: 333–341.
Hsuan, S.L., Liao, C.M., Huang, C. et al. (2009). *Vaccine* 27: 2923–2929.
Irie, Y. and Yuk, M.H. (2007). *FEMS Microbiol Lett* 275: 191–198.
Irie, Y., Mattoo, S., and Yuk, M.H. (2004). *J Bacteriol* 186: 5692–5698.
Ishibashi, Y., Claus, S., and Relman, D.A. (1994). *J Exp Med* 180: 1225–1233.
Jenkins, E.M. (1978). *Can J Comp Med* 42: 286–292.
de Jong, M.F. (1987). *Vet Q* 9: 123–133.
de Jong, M.F. and Akkermans, J.P. (1986). *Vet Q* 8: 204–214.
de Jong, M.F. and Nielsen, J.P. (1990). Definition of progressive atrophic rhinitis. *Vet Rec* 126: 93.
de Jong, M.F. and Oosterwoud, R.A. (1977). *Tijdschr Diergeneeskd* 102: 66–273.
Kadlec K, Kehrenberg C, Wallmann J, et al. 2004. *Antimicrob Agents Chemother* 48:4903–4906.
Khattak, M.N. and Matthews, R.C. (1993). *Int J Syst Bacteriol* 43: 659–664.
Kirimanjeswara, G.S., Mann, P.B., and Harvill, E.T. (2003). *Infect Immun* 71: 1719–1724.
Kobisch, M. and Novotny, P. (1990). *Infect Immun* 58: 352–357.
Kobisch, M. and Pennings, A. (1989). *Vet Rec* 124: 57–61.
Kono, Y., Suzuki, S., Mukai, T. et al. (1994). *J Vet Med Sci* 56: 249–253.
Kowalczyk, A., Pomorska-Mól, M., Kwit, K. et al. (2014). *Vet Microbiol* 170: 206–212.
Kumar S, Singh BR, Bhardwaj M, et al. 2014. *Int J Microbiol* 2014:Article ID 238575. doi:10.1155/2014/238575.
Kureljušić, B., Weissenbacher-Lang, C., Nedorost, N. et al. (2016). *Vet J* 207: 177–179.
Lacasse, C. and Gamble, K.C. (2006). *J Zoo Wildl Med* 37: 190–192.
Lariviere, S., Leblanc, L., Mittal, K.R. et al. (1993). *J Clin Microbiol* 31: 364–367.
Le Moine, V., Vannier, P., and Jestin, A. (1987). *Prev Vet Med* 4: 399–408.
Lendvai, N., Magyar, T., and Semjen, G. (1992). *Vet Microbiol* 31: 191–196.
Li, J., Fairweather, N.F., Novotny, P. et al. (1992). *J Gen Microbiol* 138: 1697–1705.
Llombart, M., Chiner, E., and Senent, C. (2006). *Arch Bronconeumol* 42: 255–256.
Loving, C.L., Brockmeier, S.L., Vincent, A.L. et al. (2010). *Microb Pathog* 49: 237–245.
Magyar, T., Glavits, R., Pullinger, G.D. et al. (2000). *Acta Vet Hung* 48: 397–406.
Magyar, T., King, V.L., and Kovacs, F. (2002). *Vaccine* 20: 1797–1802.
Mattoo, S. and Cherry, J.D. (2005). *Clin Microbiol Rev* 18: 326–382.
Mattoo, S., Miller, J.F., and Cotter, P.A. (2000). *Infect Immun* 68: 2024–2033.
McMillan, D.J., Shojaei, M., Chhatwal, G.S. et al. (1996). *Microb Pathog* 21: 379–394.
Medhekar, B., Shrivastava, R., Mattoo, S. et al. (2009). *Mol Microbiol* 71: 492–504.
Melvin, J.A., Scheller, E.V., Noël, C.R. et al. (2015). *MBio* 6: e01189–e01115.
Mitscherlich E, Marth EH. 1984. In *Microbial Survival in the Environment: Bacteria and Rickettsiae Important in Human and Animal Health*. Springer-Verlag, pp. 45–47.
Montaraz, J.A., Novotny, P., and Ivanyi, J. (1985). *Infect Immun* 47: 744–751.
Musser, J.M., Bemis, D.A., Ishikawa, H. et al. (1987). *J Bacteriol* 169: 2793–2803.

Ngom, A., Boulanger, D., Ndiaye, T. et al. (2006). *Vector Borne Zoonotic Dis* 6: 179–182.
Nicholson, T.L., Brockmeier, S.L., and Loving, C.L. (2009). *Infect Immun* 77: 2136–2146.
Nicholson, T.L., Brockmeier, S.L., Loving, C.L. et al. (2012). *Infect Immun* 80: 1025–1036.
Nicholson, T.L., Brockmeier, S.L., Loving, C.L. et al. (2014). *Infect Immun* 82: 1092–1103.
Nicholson, T.L., Shore, S.M., Register, K.B. et al. (2016). *Vet Microbiol* 182: 87–94.
Nielsen, J.P., Foged, N.T., Sorensen, V. et al. (1991). *Can J Vet Res* 55: 128–138.
Novotny, P., Kobisch, M., Cownley, K. et al. (1985). *Infect Immun* 50: 190–198.
Okada, K., Abe, H., Ike, F. et al. (2015). *PLoS One* 10: e0116604.
Palzer, A., Ritzmann, M., Wolf, G. et al. (2008). *Vet Rec* 162: 267–271.
Pedersen, K.B. and Barfod, K. (1981). *Nord Vet Med* 33: 513–522.
Pejsak, Z., Wasinska, B., Markowska-Daniel, I. et al. (1994). *Comp Immunol Microbiol Infect Dis* 17: 125–132.
Phillips, C.E. (1943). *Can J Comp Med Vet Sci* 7: 58–59.
Porter, J.F. and Wardlaw, A.C. (1993). *FEMS Microbiol Lett* 110: 33–36.
Porter, J.F., Parton, R., and Wardlaw, A.C. (1991). *Appl Environ Microbiol* 57: 1202–1206.
Prasad, S.M., Yin, Y., Rodzinski, E. et al. (1993). *Infect Immun* 61: 2780–2785.
Pruller, S., Rensch, U., Meemken, D. et al. (2015). *PLoS One* 10 (8): e0135703.
Register, K.B. (2001). *Infect Immun* 69: 1917–1921.
Register, K.B. (2004). *Vaccine* 23: 48–57.
Register, K.B. and DeJong, K.D. (2006). *Vet Microbiol* 117: 201–210.
Register, K.B., Ackermann, M.R., and Dyer, D.W. (1995). *J Clin Microbiol* 33: 2675–2678.
Register, K.B., Boisvert, A., and Ackermann, M.R. (1997). *Int J Syst Bacteriol* 47: 678–683.
Register, K.B., Ivanov, Y.V., Jacobs, N. et al. (2015). *Genome Announc* 3: e00152–e00115.
Riising, H.J., van Empel, P., and Witvliet, M. (2002). *Vet Rec* 150: 569–571.
Ross, R.F., Switzer, W.P., and Duncan, J.R. (1967). *Can J Comp Med Vet Sci* 31: 53–57.
Rutter, J.M. (1981). *Vet Rec* 108: 451–454.
Rutter, J.M. (1983). *Res Vet Sci* 34: 287–295.
Rutter, J.M., Taylor, R.J., Crighton, W.G. et al. (1984). *Vet Rec* 115: 615–619.
Scheller EV, Melvin JA, Sheets AJ, et al. 2015. *MBio* 6:e00500–15.
Shashidhar, B.Y., Underdahl, N.R., and Socha, T.E. (1983). *Am J Vet Res* 44: 1123–1125.
Skinner JA, Reissinger A, Shen H, et al. 2004. *J Immunol* 173:1934–1940.
Smith, I.M. and Baskerville, A.J. (1979). *Res Vet Sci* 27: 187–192.
Smith, I.M., Giles, C.J., and Baskerville, A.J. (1982). *Vet Rec* 110: 488–494.
Staveley, C.M., Register, K.B., Miller, M.A. et al. (2003). *J Vet Diagn Invest* 15: 570–574.
Stehmann, R., Rottmayer, J., Zschaubitz, K. et al. (1992). *Zentralbl Veterinarmed B* 39: 546–552.
Switzer, W.P. (1956). *Am J Vet Res* 17: 478–484.
Tamion, F., Girault, C., Chevron, V. et al. (1996). *Scand J Infect Dis* 28: 197–198.
Thomson, J.R., Bell, N.A., and Rafferty, M. (2007). *The Pig J* 60: 15–25.
To, H., Someno, S., and Nagai, S. (2005). *Am J Vet Res* 66: 113–118.
Vecht, U., Arends, J.P., van der Molen, E.J. et al. (1989). *Am J Vet Res* 50: 1037–1043.
Vecht, U., Wisselink, H.J., van Dijk, J.E. et al. (1992). *Infect Immun* 60: 550–556.
Venier, L., Rothschild, M.F., and Warner, C.M. (1984). *Am J Vet Res* 45: 2634–2636.
Voets, M.T., Klaassen, C.H., Charlier, P. et al. (1992). *Vet Rec* 130: 549–553.
Wolfe, D.N., Kirimanjeswara, G.S., Goebel, E.M. et al. (2007). *Infect Immun* 75: 4416–4422.
Yokomizo, Y. and Shimizu, T. (1979). *Res Vet Sci* 27: 15–21.
Zhang, Q., Hu, R., Hu, J. et al. (2013). *Res Vet Sci* 94: 55–61.
Zhao, Z., Wang, C., Xue, Y. et al. (2011a). *Vet J* 188: 377–340.
Zhao, Z., Xue, Y., Wang, C. et al. (2011b). *J Vet Med Sci* 73: 103–106.

50

Brucellosis

Steven C. Olsen, Paola Boggiatto, Pauline Nol, and Luis Samartino

Relevance

Swine are the reservoir host for *Brucella suis*, the predominant cause of swine brucellosis. However, *Brucella abortus* and *Brucella melitensis* can also infect swine in regions where they are endemic in their respective reservoir hosts (cattle and small ruminants). In swine and other livestock hosts, *Brucella* can produce pregnancy loss, stillbirths, and infertility in females, and epididymitis and orchitis in males after localizing in reproductive tissues of sexually mature animals (Corbel 2006). Uncommon clinical signs can occur in swine due to *B. suis* localization in a variety of tissues, with lameness following localization in joint(s) or bone being most common. *B. suis* is separated into 5 biovars. Biovars 1, 2, and 3 are reported to infect swine, and only biovars 1 and 3 are reported in domestic or feral swine in the United States.

B. suis remains endemic in swine populations in many parts of the world where it causes clinical disease in swine and humans. Human brucellosis, also known as undulant fever, is most commonly contracted through direct or indirect contact with infected animals or their products (Corbel 2006; Franco et al. 2007). Many species of *Brucella* can cause disease in humans, with *B. suis* and *B. melitensis* being the more virulent human pathogens. Brucellosis is the world's most common bacterial zoonosis, with over half a million new cases annually and prevalence rates in some countries exceeding 10 cases per 100,000 population (Pappas et al. 2006). The highest prevalence of human brucellosis occurs in regions where *Brucella* spp. are endemic in reservoir hosts. For example, the highest prevalence of *B. melitensis* infections in humans are in regions with high populations of small ruminants (sheep and goats), especially countries along the Mediterranean Sea and in Central Asia. *B. suis* can be an important cause of human brucellosis in areas where pigs are raised in significant numbers and the disease is present in domestic or wild populations. For example, the high prevalence of *B. suis* (biovar 1) infections in feral swine in Australia is associated with numerous reports of brucellosis in humans and animals (Massey et al. 2011; Mor et al. 2016).

Human brucellosis is the primary basis for control or eradication programs targeting *Brucella* spp. in domestic livestock hosts. Control or eradication programs are justified by data, which indicates that addressing the disease in livestock reservoirs is the most efficient and economical approach for reducing human brucellosis (Jelastopulu et al. 2008; Roth et al. 2003; Zinsstag et al. 2007). Prior to the initiation of the eradication program in the United States in 1959, the number of reported human cases in the United States peaked at 6321 in 1949 with most cases attributed to infections with *B. abortus* and *B. suis* (Pappas et al. 2006). Following a rapid decline in *B. abortus* and *B. suis* in livestock in the United States due to eradication efforts, human infections have been reduced to an average of 159 human cases per year since 1973. These human cases are mostly due to *B. melitensis* imported through the US–Mexican border in infected dairy products (Pappas et al. 2006) or international travel, and occasionally due to *B. suis* contracted by hunters of feral swine.

Due to the success of regulatory programs in many developed countries, increased use of confinement operations, and changes in swine management practices, feral swine or wild boar is the predominant reservoir host for *B. suis* in a number of countries (Australia, United States, Canada, Western and Central Europe, and others). In the United States, the highest disease prevalence is in feral pigs in south central and southeastern states. Not only can feral swine transmit brucellosis to domestic swine raised outside of containment, but people involved in hunting feral swine or wild boar are also at risk for infection (CDC 2009; Garin-Bastuji et al. 2006; Irwin et al. 2009; Lagier et al. 2005; Robson et al. 1993).

In most European countries, brucellosis in domestic swine is almost exclusively in swine raised outdoors and

Diseases of Swine, Tenth Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson.

related to spillover of *B. suis* biovar 2 from wild boars (*Sus scrofa scrofa*) or wild hares (*Lepus europaeus*, *Lepus capensis*) (Cvetnic et al. 2003; Godfroid et al. 2005; Godfroid and Käsbohrer 2002). Venereal transmission is proposed as the main route of transmission of *B. suis* biovar 2 from wild boars to domestic swine, whereas transmission from brown hares is probably through oral consumption. Disease prevalence in Eurasian wild boar is high with estimates ranging from 8 to 32% throughout continental Europe (Al Dahouk et al. 2005; Cvetnic et al. 2003; Garin-Bastuji and Delcueillerie 2001).

In Central and South America, biovar 1 of *B. suis* is predominantly reported in domestic swine in a number of countries including Argentina, Brazil, Columbia, Cuba, Chile, Honduras, Paraguay, and Peru (Luna-Martínez and Mejía-Terán 2002; Poester et al. 2002; Samartino 2002). Although feral swine are present in parts of these regions, prevalence of brucellosis among them is currently unknown.

Biovars 1 and 3 of *B. suis* have also been reported in domestic swine in many parts of Central and Southeast Asia including China, India, Indonesia, the Philippines, Taiwan, French Polynesia, Malaysia, Tonga, and other islands in the Pacific (Fahrion et al. 2014; Olsen and Tatum 2016; Praud et al. 2013; Quance et al. 2016; Tay et al. 2016). There has also been one report of a seropositive wild boar in Japan (Watarai et al. 2006). The economic impact of swine brucellosis in Asia is probably greatest in China due to high levels of swine production, sporadic reports of epidemics of *B. suis* in swine, and reports of zoonotic infections in humans. At least one country in Central Asia, Turkey, has reported human infection with biovar 1 of *B. suis* (Kutlu et al. 2016). Porcine brucellosis is also believed to be widespread across sub-Saharan Africa although data is limited and populations of swine are relatively small (McDermott 2002). However *B. suis* biovar 1 has been isolated from cattle in Egypt and Zimbabwe, suggesting that the disease may be endemic in those countries (McDermott 2002; Menshawy et al. 2014).

Etiology

Brucellosis is an infectious disease caused by bacteria in the genus *Brucella*, with 10 species recognized (*B. abortus*, *B. canis*, *B. ceti*, *B. melitensis*, *B. ovis*, *B. microti*, *B. neotomae*, *B. inopinata*, *B. pinnipedialis*, and *B. suis*) that have different preferred hosts (Table 50.1 [Alton 1990]) and that differ by molecular and microbiological characteristics. In addition several new species are under consideration for inclusion in the genus *Brucella*. For *B. suis*, microbiologic, serologic, and molecular tests have further divided the species into 5 biovars (Table 50.2 [Alton 1990]). Biovars 1, 2, and 3 are associated with infections in swine, biovar 4 is almost exclusively limited to reindeer and wild caribou in subarctic areas, and biovar 5 has only been recovered from rodents in the former USSR (Olsen and Tatum 2016). While domestic swine (*Sus scrofa domesticus*) are primarily reservoir hosts for *B. suis*, swine may also be infected by *B. abortus* and *B. melitensis* in areas where brucellosis is enzootic in cattle and small ruminants, respectively (Lucero et al. 2008; Stoffregen et al. 2007). *B. microti* and the cattle vaccine, *B. abortus* strain 19, have also rarely been recovered from feral swine. The remainder of this chapter will primarily focus on infection of swine by *B. suis* biovars 1, 2, and 3.

Like other species of *Brucella*, *B. suis* organisms are gram-negative coccobacilli measuring 0.6–1.5 μm length and 0.5–0.7 μm width. In nature, *B. suis* almost exclusively occurs in the smooth form that express the O side chain on the lipopolysaccharide (LPS). Colonies of *B. suis* cannot be visually differentiated from other smooth *Brucella* species. Since *Brucella* spp. are potent human pathogens, manipulation of cultures or contaminated material from potentially infected animals should be done under Biosafety Level 3 (BSL-3) laboratory conditions.

Complete genomes of the different *Brucella* species have been fully sequenced with more than 59 *B. suis* genomes currently available in GenBank (NCBI – NIH). With the exception of *B. suis* biovar 3 that has a single chromosome of approximately 3.1 Mbp, all other biovars have two circular chromosomes encoding approximately 2.1 and 1.2 Mbp. *Brucella* spp. have no known natural plasmids with which to transfer genetic material or antibiotic resistance (Bavishi et al. 2010). With the exception of biovar 5, genomes of *B. suis* isolates cluster together. A recent analysis of published *B. suis* genomes identified 16,756 single nucleotide polymorphisms (SNPs) between strains, including biovar-specific SNPs that may have value as diagnostic targets (Sankarasubramanian et al. 2016). Currently available polymerase chain reaction (PCR) assays and pipelines for analysis of whole genome sequencing data can be used to differentiate *Brucella* by species, biovars, and strains and to facilitate understanding of strain lineages and epidemiologic relationships (Di et al. 2016; Duvnjak et al. 2015; García-Yoldi et al. 2007; Garin-Bastuji 2008; Kamath et al. 2016; López-Goñi et al. 2008, 2011; Mayer-Scholl et al. 2010; Sankarasubramanian et al. 2016).

Epidemiology

In general, environmental persistence of *B. suis* is of low epidemiological importance. Maintenance of *B. suis* is in infected populations of domestic or feral swine and requires continued infection of susceptible hosts. Direct

Table 50.1 Differential microbiological characteristics of species of the genus *Brucella*.

			Lysis by phages[a]							
			Tb		Wb	Iz_1	R/C			
Species			Colony morphology[b]		Serum requirement	RTD[c]	10^4RTD			RTD
B. abortus	S	–[d]	+	+	+	+	–	+[e]	+[f]	Cattle and other Bovidae
B. suis	S	–	–	+	+[g]	+[g]	–	+	+[h]	Swine, Wild boar, European hare, reindeer and wild rodents
B. melitensis	S	–	–	–	–[i]	+	–	+	+[j]	Sheep and goats
B. neotomae	S	–	–[k]	+	+	+	–	–	+[h]	Desert wood rat[l]
B. ovis	R	+	–	–	–	–	+	–	–	Rams
B. canis	R	–	–	–	–	–	+	+	+[h]	Dogs
B. ceti	S		–[m]		+[n]	+[n]	–	+	+	Cetaceans
B. pinnipedialis	S		–[m]		+[n]	+[n]	–	+	+	Pinnipeds
B. microti	S/R	–	±		+[o]	+[p]	±[q]	+	+	Common vole, red fox, European wild boar, soil
B. inopinata	S	–	–		+[r]			+	+	Unknown

Source: Modified from OIE (2012)
[a] Phages: Tbilisi (Tb), Weybridge (Wb), Izatnagar1(Iz_1) and R/C.
[b] Normally occurring phase: S, smooth; R, rough.
[c] RTD, routine test dilution.
[d] *Brucella abortus* biovar 2 generally requires serum for growth on primary isolation.
[e] Some African isolates of biovar 3 are negative.
[f] Except reference strain 544 and some field strains.
[g] Some isolates of *B. suis* biovar 2 are not or only partially lysed by phage Wb or Iz_1.
[h] Rapid activity.
[i] Some isolates are lysed by phage Wb.
[j] Slow rate, except some strains that are rapid.
[k] Minute plaques.
[l] *Neotoma lepida*.
[m] But some isolates are lysed by Tb.
[n] Most isolates are lysed by Wb and Iz.
[o] Smooth isolates.
[p] Rough and smooth isolates.
[q] Rough isolates only at $10^4 \times$ RTD.
[r] Incomplete lysis.

or close contact is required for disease transmission. However, *Brucella* can survive in a cold moist environment for up to several months in infected aborted fetuses or fluids. Bacteria may also survive in contaminated materials such as feed, bedding, equipment, and clothing (Olsen et al. 2012).

Ingestion of aborted uterine contents or discharges is probably the main route of transmission of *B. suis*, but infection across the conjunctiva or penetration through breaks in the epidermis may also be routes of infection. Unlike most other brucellae, *B. suis* can also be spread venereally, which appears to be important for transmission within and between herds. Shedding in semen of boars is sporadic and of long duration as a consequence of *B. suis* containing granulomas in male reproductive organs. Transplacental infection can result in infected live-born pigs, and piglets can also be infected through consumption of infected milk. Neonatal infection may lead to a latent carrier state that could result in disease transmission in the absence of clinical and immunological signs of brucellosis (Acha and Szyfres 2003; Olsen et al. 2012).

When first entering a naive herd, *B. suis* can quickly spread from a single infected animal resulting in infection rates of up to 70–80% in the early stages of outbreaks (Beer 1980; Olsen et al. 2012; Szulowski 1999). In

Table 50.2 Differential characteristics of the recognized *Brucella* biovars.

					Growth on dyes[a]		Agglutination with monospecific sera		
Species	**Biovar**	**Preferred hosts**	**CO_2 requirement**	**H_2S production**	**Thionin**	**Basic fuchsin**	**A**	**M**	**R**
B. melitensis	1	Sheep and goats	–	–	+	+	–	+	–
	2		–	–	+	+	+	–	–
	3		–	–	+	+	+	+	–
B. abortus	1	Cattle	+	+	–	+	+	–	–
	2		+	+	–	–	+	–	–
	3		+	+	+	+	+	–	–
	4		+	+	–	+[b]	–	+	–
	5		–	–	+	+	–	+	–
	6		–	–	+	+	+	–	–
	9		±	+	+	+	–	+	–
B. suis	1	Swine	–	+	+	–[c]	+	–	–
	2	Swine, wild boar,European hare	–	–	+	–	+	–	–
	3	Swine	–	–	+	+	+	–	–
	4	Reindeer	–	–	+	–[d]	+	–	–
	5	Wild rodents	–	–	+	–	–	–	–

Source: Modified from OIE (2012).
[a] Dye concentration in serum dextrose medium: 20 g/ml.
[b] Most strains are basic fuchsin resistant. Sensitive strains have been isolated.
[c] Most strains are basic fuchsin sensitive. Resistant strains have been isolated (in South America particularly).
[d] Negative for most stains.

epidemically infected herds, pregnancy loss, increased perinatal mortality, and infertility are common; however, in endemically infected herds, only mild to moderate clinical signs are observed, and infection often goes undetected. Large numbers of *B. suis* can be shed in uterine fluids and fetal tissues following reproductive failure. Although uterine infection and vaginal shedding is cleared in most females by 30–40 days after pregnancy loss, a small percentage of females can maintain a persistent uterine infection. This results in temporary or permanent infertility and vaginal shedding of *B. suis* for up to 36 months (Manthei and Deyoe 1970). Data suggests that the majority of live-born pigs infected *in utero* clear the *B. suis* infection by 6 months of age; however, in one study, 8% of 230 pigs were blood culture positive beyond 3 months of age, and 2.5% were tissue positive at slaughter at 2 years of age (Manthei et al. 1952). If infected after sexual maturity, the course of infection appears to be longer, with greater chronicity associated with males as compared with females. In mature boars, *B. suis* was recovered from tissue samples of 66.7% at 6 months after experimental infection with 50% remaining culture positive at 42 months. In females, approximately 25% of those experimentally infected were culture positive between 6 and 42 months after experimental infection (Deyoe 1972). Infection can persist for up to 3–4 years in male reproductive tissues (Manthei 1964).

The main risks for the introduction of porcine brucellosis into pig herds are through addition of infected animals, via contact with infected feral pigs and/or European hares, and by infected semen introduced through natural service or artificial insemination (Alton 1990). Feeding uncooked kitchen waste or garbage containing uncooked meat to pigs could also potentially spread swine brucellosis. The possibility of transmission of *B. suis* to swine through environmental contamination or infection of other mammalian vectors has been suspected but never documented (Komendy and Nagy 1982; Repina et al. 1993). Other mammals uncommonly infected with *B. suis* include dogs, cattle, sheep, horses, opossums (*Didelphis marsupialis*), red foxes (*Vulpes vulpes*), roe deer (*Capreolus capreolus capreolus*), armadillos (*Chaetophractus villosus*), capybara (*Hydrochaeris hydrochaeris*), and collared peccaries (*Tayassu tajacu*) (Barr et al. 1986; Bhongbhibhat et al. 1970; Cook and Kingston 1988; Corbel 2006; Cutler et al. 2005; Ewalt et al. 1997; Garin-Bastuji and Delcueillerie 2001; Hofer et al. 2010; James et al. 2017; Komendy and Nagy 1982; Lord and Flores 1983; Lord and Lord 1991; Lucero et al. 2008; Mor et al. 2016; Olsen and Tatum 2016; Ramamoorthy et al. 2011).

Throughout continental Europe, *B. suis* biovar 2 prevalence in Eurasian wild boar (*S. scrofa scrofa*) is high with estimates ranging from 2 to 55% (Al Dahouk et al. 2005; Cvetnic et al. 2003; Garin-Bastuji and Delcueillerie 2001; Grégoire et al. 2012; Hubálek et al. 2002; Koppel et al. 2007; Leuenberger et al. 2007; Montagnaro et al. 2010; Muñoz et al. 2010; Pilo et al. 2015; Wu et al. 2011). Studies have shown that *B. suis* biovar 2 strains isolated from wild boar and domestic swine in various parts of Europe are closely related, strongly suggestive of cross-transmission (Duvnjak et al. 2015; Krezinger et al. 2014; Muñoz et al. 2010). At least in France, Portugal, and Italy, transmission from wild boars to domestic swine is believed to be through the venereal route with many premises reporting the birth of striped piglets (Barlozzari et al. 2015). The European hare (*L. europaeus*) is a source of biovar 2 infections in domestic swine in Europe through natural ingestion of dead hares in pastures or via swill feeding containing offal from hunted infected hares (Olsen et al. 2012). Biovar 2 isolates from European hares are genetically unique when compared with isolates from wild boars in Europe, indicating separate lineages in each host and no evidence of cross-species infection (Krezinger et al. 2014; Lavín et al. 2006; Muñoz et al. 2010).

Public health

B. melitensis and *B. suis* are generally more virulent human pathogens than other *Brucella* species (Corbel 2006). *B. suis* biovars 1, 3, and 4 are pathogenic in humans, and current data suggests that biovar 2 of *B. suis* is a very rare human pathogen occurring only in immunocompromised hosts (Garin-Bastuji et al. 2006; Lagier et al. 2005; Paton et al. 2001).

Human brucellosis due to *B. suis* is most frequently a disease of farmworkers, veterinarians, and abattoir employees, but it can also be contracted through hunting or other associations with feral swine (CDC 2009; Robson et al. 1993). Transmission of *B. suis* from swine to humans primarily occurs by direct contact with infected animals or contact with materials associated with abortion. Routes of entry include aerosol, oral, or through breaks in epidermal integrity. Processing of infected swine through an abattoir setting is associated with a high risk for infection of human workers (Hendiricks et al. 1962; Huddleson et al. 1933; Trout et al. 1995), most likely through aerosol exposure. Some data suggests that zoonotic infection with *B. suis* can result from handling or consumption of infected raw or undercooked pork (Hutchings et al. 1951). Swine can also transmit *B. suis* to cattle where it has a predilection for localization in mammary tissues and shedding in milk (Ewalt et al. 1997). A number of reports have documented zoonotic transmission of *B. suis* to groups of people through consumption of unpasteurized bovine milk (Beattie and Rice 1954; Borts et al. 1943). Person-to-person transmission of

human brucellosis is extremely rare and reported primarily for *B. melitensis* (Corbel 2006).

Human brucellosis is also known as "undulant fever" due to the single consistent symptom of fever that waxes and wanes. It is associated with a wide spectrum of nonpathognomonic clinical manifestations depending on which organs or tissues it localizes in, including the more common osteoarticular, nervous, pulmonary, and mucocutaneous tissues (Franco et al. 2007). Clinical disease typically begins as an acute febrile illness with nonspecific flu-like symptoms such as headache, malaise, back pain, myalgia, and generalized aches. Drenching sweats can occur, particularly at night. Acute disease is associated with an incubation period of 2–3 weeks, yet in half of human patients the onset is insidious with symptoms developing over a period of weeks to months after exposure (Corbel 2006). In the absence of specific treatment, infection may persist for weeks or months (Ariza et al. 2007). Relapse of infection after treatment is not uncommon, usually within 6 months, and not usually associated with emergence of antibiotic-resistant strains (Corbel 2006). The duration of human illness and its long convalescence mean that brucellosis is not only a medical problem but also an economic issue related to loss of productivity or inability to work.

Pathogenesis

B. suis generally causes prolonged infections characterized by granulomatous inflammation in a variety of organs. Localization in male and female reproductive organs is responsible for the predominant clinical signs of fetal loss and infertility as well as being the predominant route for shedding and disease transmission. The capacity of *Brucella* spp. to invade, survive, and proliferate within cells, especially macrophages and placental trophoblasts, is important for its pathogenesis.

The minimum infectious dose of *B. suis* is not well characterized, but doses of 10^{3-4} are probably capable of infecting swine (Alton 1990; Teske et al. 2011). Infection depends on strain virulence, dose, and route of infection as well as host susceptibility and immunologic status (Enright 1990). *B. suis* typically enters by penetration of mucous membranes or, less commonly, through breaks in the epithelium. Location of entry is determined by route of exposure (i.e. oral, venereal, or contact with skin). Initial localization and replication is in lymphoreticular tissues draining the site of exposure/infection. Studies with *B. abortus* suggest that penetration of the mucosa occurs preferentially through follicle-associated epithelial cells such as M cells and/or via uptake by intraepithelial phagocytes, which may also aid in transport to submucosal lymphoid patches or regional lymph nodes (Ackermann et al. 1988).

Bacteremia follows initial replication, either due to free bacteria or *B. suis*-laden phagocytes, resulting in systemic dissemination to a variety of lymphoid and nonlymphoid organs. Bacteremia usually lasts an average of 5 weeks but can extend up to 34 months in sows (Deyoe 1967, 1972). Lymph nodes believed to be preferential sites include mandibular, gastrohepatic, internal iliac, suprapharyngeal, and supramammary although localization may be influenced by route of exposure (Deyoe and Manthei 1967). Nonlymphoid tissues commonly infected include mammary gland, placenta, and synovial tissues. Less commonly, seminal vesicles, prostate, epididymides, testes, uterus, oviducts, liver, spleen, bones, tendon bursae, and brain can also be affected (Alton 1990; Rosenbusch 1951).

Once in the host, *Brucella* resists destruction by extracellular and intracellular host mechanisms designed to eliminate bacterial infection. Exact mechanisms by which *Brucella* avoids elimination are only partially understood. The O side chain on the LPS of virulent smooth strains is important for extracellular survival as it has low immunogenicity and is relatively resistant to both complement- and cationic peptide-mediated bactericidal functions (Allen et al. 1998; Lapaque et al. 2005). Additional details of mechanisms used by *Brucella* spp. to inhibit host innate and adaptive immune response will be discussed in the section on immunology below.

After internalization by phagocytic cells, primarily macrophages, *Brucella* spp. adapts to survival within the harsh intracellular environment of the host. The capacity of antibody- and complement-opsonized as well as nonopsonized *Brucella* to modify their intracellular environment and maintain long-term residence within macrophages is the basis for the establishment and maintenance of chronic infections (Baldwin and Goenka 2006; Roop et al. 2003). Non-opsonized *Brucella* entry into cells is mediated by lipid rafts (Naroeni and Porte 2002), a process that does not cause significant activation of host cells and may contribute to their intracellular survival. Once inside the host cell, *Brucella* reside in a compartment termed the *Brucella*-containing vacuole (BCV), which interacts with components of the endocytic and secretory pathways.

The majority of BCV (75–80%) merge with phagolysosomes and the bacteria are killed. The remaining 20–25% of BCV undergo a maturation process and acquire markers of early and late endosomes. As maturation proceeds, acidification of BCV is an important step for *Brucella* survival. A drop in pH induces the expression of genes required for virulence, including the *virB* operon, which encodes a type IV secretion system (T4SS) (Boschiroli et al. 2002). Induction of T4SS inhibits BCV fusion with lysosomes and facilitates fusion with the endoplasmic reticulum (ER), creating the "brucellasome" (Celli 2006). The brucellasome is a favorable intracellular

niche where *Brucella* replicate, accumulate, and ultimately lyse the host cell to repeat the cycle.

Survival of *Brucella* in brucellasomes is enhanced by the use of stationary-phase physiology and siderophores to scavenge iron and by their ability to resist oxidative killing (Roop et al. 2003). Additionally *Brucella* spp. can directly interfere with host cell signaling pathways, including Toll-like receptor (TLR) signaling (Cirl et al. 2008; Salcedo et al. 2008) and programmed cell death pathways (He et al. 2006; Gross et al. 2000; Tolomeo et al. 2003). Interference with these pathways decreases immune recognition and enhances long-term intracellular survival.

Clinical signs

Most swine infected with *B. suis* are clinically normal with normal leukograms during acute and chronic infections. Prior to the onset of sexual maturity, clinical signs are generally limited to occasional swollen joints and lameness. In sexually mature swine, brucellosis is characterized by fetal loss, stillbirth, and infertility in sows and orchitis, epididymitis, and infertility in boars with clinical signs that mimic other bacterial and viral diseases. Although *B. suis* is often considered to be an abortifacient bacterium, abortion is generally a minor component of the clinical presentation under field conditions.

Epidemic infection of naïve swine herds results in rapid transmission throughout the herd and fetal loss at all stages of gestation. Infection of sows by natural breeding or artificial insemination or by other routes near the time of conception results in fetal death by 21–27 days that is usually recognized as irregular returns to estrus at 30–45 days after breeding (Manthei and Deyoe 1970). Infection of sows later in gestation results in abortion of fetuses of various sizes, stillborn pigs, or infected live-born pigs that exhibit increased neonatal mortality rates (Hutchings et al. 1946b). Placental retention, metritis, vaginal discharges, and poor subsequent fertility may be observed in sows that have aborted. Infertility is directly related to the duration of infection and the severity of uterine lesions (Manthei and Deyoe 1970; Thomseon 1934).

In endemically infected herds, only mild to moderate clinical signs are typically observed. Pregnancy rates may be decreased due to chronic uterine infection and consequent infertility in a portion of sows (Manthei and Deyoe 1970; Thomseon 1934). When infected boars are used for natural service, depending on whether testicular or epididymal damage is uni- or bilateral, libido and fertility can be decreased, resulting in reduction in conception rates and litter size (Hutchings and Andrews 1946; Manthei and Deyoe 1970). Irregularity in contour and size of testes and epididymides may be observed or palpated in some affected boars. Venereal transmission to naïve females can also result in early pregnancy loss and irregular returns to estrus. Apart from pregnancy loss in females and reduced libido in boars, most immature and mature pigs will typically appear clinically normal. Swollen joints and tendon sheaths accompanied by lameness and incoordination can occasionally occur in both males and females. Less commonly, posterior paralysis resulting from lumbar abscesses or spondylitis may occur (Enright 1990).

Lesions

In the uterus and uterine tubes, multifocal, miliary, 2–3 mm yellow nodules develop in the mucosa that may contain purulent or caseous exudate. These nodules may coalesce to form plaques and are often associated with thickening of the mucosa. In the uterine tubes, the nodules can lead to obstruction and pyosalpinx. The endometrium may be expanded by lymphoid and plasma cell infiltrates including some hyperplasic lymphocytic nodules. Suppurative infiltrates may be present in superficial endometrial glands and in the uterine lumen. Partial desquamation or squamous metaplasia, including rete pegs and intercellular bridges, may be observed in the endometrial epithelium or superficial glands. Uterine ligaments often contain small and irregular granulomas on their surface (Schlafer 2007).

In the pregnant uterus, miliary lesions are superimposed on a diffuse, mucohemorrhagic endometritis with the catarrhal mucosal exudate containing large numbers of bacteria. Lesions in placenta and aborted fetuses are uncommon, but may include patchy placental thickening caused by necrosuppurative placentitis and mild suppurative fetal bronchopneumonia (Manthei and Deyoe 1970).

In intact males, orchitis caused by *B. suis* results in multiple granulomas accompanied occasionally by fibrinopurulent or hemorrhagic periorchitis. Testes may be enlarged or atrophic. Granulomas in the testes or epididymis have a caseous core surrounded by epithelioid macrophages, multinucleate giant cells, lymphocytes, and plasma cells and may be encapsulated in fibrous connective tissue. Infection of accessory glands may be associated with vesicular gland hypertrophy and microabscesses or granulomas in vesicular glands, prostate, or bulbourethral glands (Foster 2007). Calcified foci may also be in the testes and accessory sexual glands and organs, particularly in the epididymis and seminal vesicles. Fibrosis may result in adhesions between the two layers of the tunica vaginalis (Foster 2007).

Articular lesions are characterized by purulent or fibrinopurulent synovitis affecting the joints of the limbs. Osteomyelitis is typically observed in the lumbar vertebra

and commonly is associated with destruction of the intervertebral cartilages. Bone lesions are usually granulomatous with dry caseous necrosis but can become suppurative, leading to paravertebral abscesses (Thompson 2007). Abscesses and/or granulomas may also develop in other tissues with *B. suis* localization, particularly the lymph nodes, spleen, liver, kidneys, joint capsules, tendon sheaths, bones, mammary gland, urinary bladder, and brain.

Diagnosis

Direct diagnosis

Bacterial culture is the definitive method for confirming brucellosis in swine. Due to the lack of sensitivity and specificity of current serologic tests in swine, bacterial culture from lymph nodes is as likely to detect infection as serologic diagnosis (Alton 1990; Rogers et al. 1989). However, the slow growth of *Brucella in vitro*, the tendency for reduced recovery of isolates from chronically infected swine, high costs, and biosafety concerns make diagnosis by bacterial culture unfeasible in some situations.

A presumptive diagnosis of brucellosis in pigs can be made by microscopic examination of Stamp's stained smears from vaginal swabs, placentas, and/or aborted fetuses. However, this testing lacks sensitivity and specificity and should be supported by direct culture techniques to ensure an accurate diagnosis. For direct culture, preferred samples include vaginal secretions (swabs), milk, semen, fetal membranes, and samples from aborted fetuses including stomach contents, spleen, and lung. At necropsy, spleen, cervical lymph nodes, mammary gland, uterus, testes, epididymis, vesicular glands, prostate, and bulbourethral glands are good samples for direct culture.

Recovery of *B. suis* can be done at 37 °C without a 5–10% CO_2 atmosphere using multipurpose basal culture media without serum. Due to the possible overgrowth of bacterial contaminants, use of selective media (Alton 1990) containing serum is recommended in addition to incubation of plates at 37 °C with 5–10% CO_2. These *in vitro* conditions will also maximize the ability to isolate other species of *Brucella* that might be present. Farrell's and modified Thayer-Martin's media (Marín et al. 1996) should be utilized when *B. suis* biovar 2 may be present due to the greater sensitivity of this biovar to antibiotics contained in common *Brucella* selective medias. *Brucella* isolates are generally slow growing with colonies not being visible until 3–4 days. To maximize recovery cultures should be incubated for at least 8 days.

B. suis biovars 1, 2, and 3 may be presumptively identified by slide agglutination with monospecific antiserum combined with additional bacteriological tests (Alton 1990). The precise differentiation of biovars depends on susceptibility to specific phages, ability to produce hydrogen sulfide (H_2S), and growth in the presence of thionin or basic fuchsin dyes (Table 50.2). Oxidative metabolic tests can be used in identification of biovars but are cumbersome and rarely performed. Interpretation of test results may be subjective, and differentiation of biovars 1, 2, and 3 may require additional testing and should therefore be conducted by a brucellosis reference laboratory. Some automated identification systems may misidentify *B. suis* isolates as *Ochrobactrum* due to the high homology between genera (Bartham et al. 1993; Vila et al. 2016).

A number of PCR-based protocols are reported that allow direct diagnosis of *B. suis* from samples (Bounaadja et al. 2009; Huber et al. 2009; López-Goni et al. 2011; Ratushma et al. 2006). The sensitivity of these methods is currently significantly lower than culture methods for some samples due to the presence of DNA inhibitors that interfere with PCR.

Indirect diagnosis

The diagnostic value of serological tests for brucellosis in swine is only at a herd basis rather than an individual basis. None of the conventional serological tests used for the diagnosis of brucellosis in domestic ruminants have adequate sensitivity and specificity for the diagnosis of swine brucellosis in individual pigs. A recent study found that a panel of serologic tests were only able to identify 52% of naturally infected feral swine as seropositive (Pedersen et al. 2014) and 17% of culture-positive swine were negative on all serologic tests (Ferris et al. 1995). In herds with positive serologic test results, *B. suis* infection should be confirmed using bacteriologic isolation and/or molecular assays that detect *Brucella* DNA.

The major antigen involved in the serological response against *in vivo* infection with smooth strains of *Brucella* is the smooth LPS. Antisera developed against the smooth LPS of *B. abortus* (A dominant) and *B. melitensis* (M dominant) are available for diagnostic use. Although there is some variation between strains, the smooth LPS of *B. suis* is recognized by monoclonal antibodies specific for either the A or M antigens and by antibodies against common LPS epitopes shared by *B. abortus* and *B. melitensis* (Douglas and Palmer 1988). The majority of standard brucellosis serologic tests were developed for detection of *B. abortus* in cattle and use the polysaccharide O side chain of the *B. abortus* smooth LPS as antigen. This may be one explanation for observed reductions in sensitivity and specificity of these tests for detection of *B. suis* in swine as compared with their use to detect *B. abortus* infections in cattle. An explanation for their lower specificity in swine

is the similarity of the structural component of the *B. abortus* LPS O side chain (repeated residues of 4-formamindo-4,6-dideoxymannose linked in α-1,2 or α-1,3 conformation) to the LPS of other bacteria, including *Yersinia enterocolitica* O:9 and *Escherichia coli* O:157 (Bundle et al. 1989). *Y. enterocolitica* O:9 infection in pigs is widespread in many countries and is believed to be the major source of false-positive serological reactions in standard brucellosis serologic tests. Tests using *Brucella* protein extracts free of the LPS O side chain (gel immunodiffusion, indirect enzyme-linked immunosorbent assays (ELISAs), counterimmunoelectrophoresis, and latex agglutination) are less likely to result in false-positive results than tests that depend on the LPS O side chain as antigen (Rose Bengal plate test [RBT], ELISAs, and fluorescence polarization assay [FPA]) (Dieste-Perez et al. 2015a; McGiven et al. 2012).

Sensitivity and specificity estimates for serologic tests are typically related to the "gold standard" of bacterial culture, and *B. suis* is typically cultured from a greater proportion of acute than chronic infections. This may explain the great variation in published estimates of sensitivity and specificity for these tests in swine. Ranges for estimates of sensitivity of standard tests are standard tube (SAT; 51.1–100%), mercaptoethanol (38.5–100%), Rivanol (23.1–100%), complement fixation test (CFT) (49.1–100%), card test (RBT; 20–100%), buffered plate antigen (61–77.1%), and FPA (63–98.9%). Estimates of specificity ranges are serum tube agglutination test (SAT) (62–100%), mercaptoethanol (81.1–100%), Rivanol (74–100%), CFT (86–100%), RBT (76–92%), buffered plate agglutination (90–95.9%), and FPA (55–99.9%) (Ferris et al. 1995; García-Carillo et al. 1971; Hutchings et al. 1946a; Lord et al. 1997; Nielsen et al. 1999; Paulo et al. 2000; Rogers et al. 1989). Some data has suggested higher sensitivity and specificity for detecting pigs infected with *B. suis* by use of competitive ELISAs as compared with indirect ELISA, FPA, and the RBT (Praud et al. 2012, 2013). However, in pigs naturally infected with *B. suis* biovar 2, a competitive ELISA was comparable in performance with the RBT, indirect ELISA, and a blocking ELISA (Muñoz et al. 2012). Others have proposed using a combination of the c-ELISA, indirect ELISA, and FPA for maximizing specificity and sensitivity of serologic detection of brucellosis in swine (Di Febo et al. 2012).

Because swine serum may sometimes contain nonspecific antibodies thought to be IgM, the specificity of the SAT is reduced, and this test should not be used for diagnosis of brucellosis in pigs. In addition, swine complement interacts with guinea pig complement to produce anticomplementary activity that reduces the sensitivity of the CFT test, causing its sensitivity to be significantly lower than that of the RBT (Ferris et al. 1995; Rogers et al. 1989). Despite reduced sensitivity, the CFT has high specificity for identifying true infected herds when interpreted on a collective basis.

The delayed-type hypersensitivity (DTH) reaction based on the use of smooth LPS-free *Brucella* cytoplasmic proteins (widely known as brucellin), extracted from rough *B. melitensis* strain B115 (Bhongbhibhat et al. 1970), is of diagnostic value due to reductions in false-positive responses. The brucellin DTH test is currently the only indirect diagnostic test capable of discriminating between *B. suis* infections and infections caused by cross-reacting bacteria. Although developed for use in ruminants, it has been used in swine in the EU for brucellosis diagnosis at the herd level (Dieste-Perez et al. 2015b; EFSA 2009).

Immunity

The central role of interferon gamma (IFN-γ)

The most extensive characterization of the host immune response to *Brucella* spp. has been performed in the murine model. These studies demonstrate that T helper (T_{H-1}) responses, characterized by production of interferon gamma (IFN-γ) from $CD4^+$ and $CD8^+$ T cells are required for the control of *Brucella* infection (Araya et al. 1989; Fernandes et al. 1996). Production of IFN-γ is a critical component of immunity against intracellular pathogens, including *Brucella* spp., as *in vitro* studies have shown that this cytokine activates infected macrophages to inhibit intracellular replication and mediates intracellular killing (Jiang and Baldwin 1993). This important role of IFN-γ in suppressing *Brucella* infection is confirmed *in vivo*, both experimentally and clinically. Mice deficient in IFN-γ production are unable to control systemic bacterial replication and succumb to *B. abortus* infection (Murphy et al. 2001). In humans, polymorphisms in IFN-γ (and other proinflammatory cytokines) contribute to susceptibility to brucellosis (Karaoglan et al. 2009), and chronicity of infection is associated with reduced production of IFN-γ (Rafiei et al. 2006).

Inhibition of the host immune response by *Brucella*

Brucella have several mechanisms to inhibit or avoid components of the host immune responses, allowing them to evade detection and clearance. The host innate immune response depends on pattern recognition receptors (PRR) found on host cell and endosomal membranes and within the cytosol to detect pathogen-associated molecular patterns (PAMPs). Binding of a specific PAMP to host cell PRR stimulates various cellular antibacterial activities. PRR include TLR, nucleotide-binding oligomerization

domain-like receptors (NLR), C-type lectins, and retinoic acid-inducible gene-I-like (RIG-I-like) receptors. LPS produced by *Brucella* spp. has multiple features that allow it to limit its detection by TLR4, a specific TLR. While the LPS lipid A moiety of other bacterial pathogens contain short fatty acid residues (C12–C16), *Brucella* LPS lipid A is longer (C28), which results in greatly reduced TLR4 agonist responses (Barquero-Calvo et al. 2007; Lapaque et al. 2006). In addition, the O-antigen moiety of LPS is typically a target for complement component C3 deposition. However, *Brucella* LPS resists C3 deposition, resulting in decreased generation of anaphylatoxins C3a and C5a, further dampening proinflammatory responses (Barquero-Calvo et al. 2007; Hoffmann and Houle 1983). The unusual composition of the LPS O chain of smooth *Brucella* strains also resists degradation by macrophages, and the accumulation of undigested O chain directly inhibits antigen presentation by infected macrophages to T cells via the MHC class II pathway (Forestier et al. 2000).

Brucella also suppresses innate immune responses by decreasing overall TLR signaling through production of a protein that contains a Toll/interleukin-1 receptor (TIR) domain. This protein ultimately results in inhibition of both TLR2 and TLR4 signaling (Cirl et al. 2008; Salcedo et al. 2008). Dendritic cell (DC) maturation and production of inflammatory cytokines such as IL-12 and TNF-α are inhibited following *Brucella* infection, mediated by the inhibition of TLR signaling (Salcedo et al. 2008).

Brucella have also developed mechanisms to avoid the adaptive immune response. DC play a critical role in bridging innate and adaptive immunity. Infection and proliferation of brucellae within DC has been reported both *in vitro* and *in vivo* (Archambaud et al. 2010; Salcedo et al. 2008). By infecting DC, *Brucella* not only target a replicative niche, but they also hinder the priming of adaptive immune responses. *In vitro B. abortus* 2308 and *B. suis* 1330 inhibit DC maturation and function via decreased surface expression of major histocompatibility complex (MHC) class II, CD80, and CD86, decreased secretion of IL-12 and TNF-α, and poor antigen presentation to naïve T cells (Billard et al. 2007). Similarly, *B. abortus*-infected macrophages have been shown to have reduced major MHC class I and class II expression (Barrionuevo et al. 2008, 2013), further interfering with antigen presentation to $CD8^+$ and $CD4^+$ T cells.

Adaptive host immune response to *Brucella*

Despite these evasion mechanisms, most smooth strains of *Brucella* induce an immune response after infection. However, the chronic nature of *Brucella* infections indicates that these immune responses are often insufficient to eliminate *Brucella* from their intracellular niche for extended periods of time (Olsen and Hennager 2010).

The LPS and other antigens of *Brucella* are highly immunogenic in eliciting humoral responses in infected swine. The LPS of *Brucella* is a prototypical T-cell-independent antigen because it can directly activate B cells to produce antibody without the aid of helper T cells. Antibodies are beneficial against intracellular pathogens through opsonization, complement activation, antibody-dependent cellular cytotoxicity, and binding to bacterial receptors to prevent adherence of bacteria to host tissues. In swine, the pattern of antibody production following *B. suis* infection has not been properly established. However, it should be similar to that induced by other *Brucella* infections with IgM antibodies predominating in the first 2 weeks after infection and IgG isotypes increasing slowly during the first 3 weeks of infection. However, antibodies are not as important for long-term protection as cellular immunity.

Cell-mediated immune responses to *B. suis* infection in swine have not been fully characterized. Both $CD4^+$ and $CD8^+$ T cells from pigs experimentally infected with *B. suis* produce IFN-γ and proliferate in response to *in vitro* stimulation (Riber and Jungersen 2007). Similarly, vaccination of pigs with *B. suis* strain 353–1 results in peripheral blood mononuclear cell (PBMC) proliferation and IFN-γ production in response to antigen stimulation (Stoffregen et al. 2013). It is known that the release of IFN-γ by *Brucella*-specific $CD4^+$ and $CD8^+$ T cells augments bactericidal function of macrophages and that *Brucella*-specific $CD8^+$ T cells are capable of killing infected macrophages via cell-mediated cytotoxicity. However, studies with the intracellular pathogens *Mycobacterium bovis* and *Leishmania major* have demonstrated that measurement of IFN-γ production is not sufficient to predict protective immunity (Elias et al. 2005; Gicheru et al. 2001). A more ideal assessment of the quality of a cellular immune response is the measurement of antigen-specific polyfunctional phenotype (i.e. the ability to produce multiple cytokines including IL-2, TNF-α, and IFN-γ) (Thakur et al. 2012). Currently, the quality of the cellular immune response as assessed by the polyfunctional effector phenotype in swine brucellosis remains uncharacterized.

Despite the initial presence of a proinflammatory response early in disease, studies of chronic brucellosis in mice have demonstrated that the initial anti-*Brucella* T_H1 proinflammatory environment switches to an immunosuppressive state. Similarly, during chronic human brucellosis, the initial T_H1 response is dampened over time and gains effector functions associated with T_H2 responses such as increased production of IL-13 (Rafiei et al. 2006). It is likely that a suppression of cell-mediated immunity occurs in brucellosis in swine as has been demonstrated in mice and humans, thus explaining the chronic nature of the disease.

Prevention and control

There are currently no commercially available vaccines for protecting domestic or feral swine against *B. suis* infection. Although initial reports were promising, currently available data suggests that the oral *B. suis* strain 2 vaccine developed in China (Deqiu et al. 2002) and the *B. abortus* strain RB51 vaccine (Edmonds et al. 2001) do not adequately protect swine against *B. suis* infection (Stoffregen et al. 2007). Recently a rough *B. suis* strain that functions as a differentiating infected from vaccinated animals (DIVA) vaccine was demonstrated to have efficacy after oral or parenteral delivery to domestic or feral swine (Stoffregen et al. 2013). Commercial development of this vaccine is not eminent.

Currently, the only effective strategy to maintain *B. suis*-free status of a swine herd or system is excellent biosecurity. Principal components must include testing to ensure *B. suis*-free status of any source of purchased semen or seedstock prior to herd entry and installation of adequate fencing when husbandry practices allow potential contact with wildlife reservoirs.

Given the few and nonspecific clinical signs in endemically *B. suis*-infected pig herds and the degree of transport and intermingling of pigs in most countries, introduction of *B. suis*-infected pigs in seedstock farms or boar studs could rapidly disseminate the disease. This is true for swine herds in all regions that have *B. suis*-infected feral reservoirs or *B. suis*-infected domestic swine herds, but even more so for Europe and similar regions having a high simultaneous prevalence of feral reservoirs and open-air swine rearing systems (EFSA 2009).

Since serologic surveillance is best performed in swine on a herd basis, regulatory efforts to control or eradicate *B. suis* in domestic swine should be directed toward herds rather than individual animals (Olsen and Tatum 2016). Proximity to an infected herd is also a risk factor; therefore, elimination of brucellosis from a farm may only be transient if neighboring farms remain infected (Acha and Szyfres 2003) and rigorous biosecurity measures are not in place to prevent reinfection.

Test and removal strategies are not economically feasible under all circumstances, so a number of studies have evaluated the ability of antibiotic treatment to control brucellosis in host species, including swine (Bunnell et al. 1947; Radwan et al. 1993). These studies found that although disease prevalence was reduced, cost of treatment and persistence of infection in some treated animals made long-term antibiotic therapy nonviable as a regulatory strategy. More recently, a small study of naturally infected swine (n = 8/treatment) found that oral treatment with oxytetracycline (20 mg/kg/daily for 21 days) eliminated *B. suis* biovar 2 infection from only 50% of infected swine. In contrast, when oral oxytetracycline therapy was combined with the macrolide antibiotic tildipirosin (4 mg/kg administered IM on days 1 and 10), the authors were unable to recover *B. suis* from treated swine at 21 days (Dieste-Perez et al. 2015c). In a larger field study, oral treatment of naturally infected swine (*B. suis* biovar 2) with oxytetracycline (20 mg/kg/daily) was not sufficient to eradicate brucellosis in infected herds (Dieste-Perez et al. 2016). However, when combined with removal of infected animals as identified by one of three diagnostic tests (brucellin DTH or Rose Bengal and indirect ELISA serologic tests), brucellosis was eradicated from infected herds in approximately 16 months.

With the exception of increasing hunting pressure to reduce population densities, no measures are currently available to reduce or eliminate *B. suis* infection in wildlife reservoirs. The risk of disease transmission from wildlife reservoirs to domestic swine is unlikely to diminish in the near future. Current research in swine evaluating immunocontraception, toxin containing baits, and orally deliverable vaccines may eventually provide needed tools to help in managing the disease in free-ranging feral swine (Snow et al. 2016).

References

Acha PN, Szyfres B. 2003 Pan American Health Organization, Washington, USA.

Ackermann MR, Cheville NF, Deyoe BL. 1988 *Vet Pathol* 25:28–35.

Al Dahouk SK, Nockler H, Tomaso WD, et al. 2005 *J Vet Med Ser B-Infect Dis Vet Public Health* 52:444–455.

Allen CA, Adams LG, Ficht TA. 1998 *Infect Immun* 66:1008–1016.

Alton G. 1990 *Brucella suis*. In Nielsen K, Duncan JR, eds. *Animal Brucellosis*. Boston, MA: CRC Press, pp. 411–422.

Araya LN, Elzer PH, Rowe GE. 1989. *J Immunol* 143:3330–3337.

Archambaud C, Salcedo SP, Lelouard H et al. 2010. *Eur J Immunol* 40:3458–3471.

Ariza JM, Bosilkovski A, Cascio JD. 2007. *PLoS Med* 4:e317.

Baldwin CL, Goenka R. 2006. *Crit Rev Immunol* 26:407–442.

Barlozzari GA, Franco G, Macri S. 2015 *Vet Ital* 51:151–154.

Barquero-Calvo EE, Chaves-Olarte DS, Weiss C. 2007. *PLoS One* 2:e631.

Barr SC, Eilts BE, Roy AF, et al. 1986. *J Am Vet Med Assoc* 189:686–687.

Barrionuevo PJ, Cassataro MV, Delpino A. 2008. *Infect Immun* 76:250–262.

Barrionuevo PM, Delpino V, Pozner RG. 2013 *Cell Microbiol* 15:487–502.
Bartham WB, Church P, Brown JE, et al. 1993 *Clin Infect Dis* 17:1068–1069.
Bavishi A, Abhishek A, Lin L, et al. 2010. *Genome* 53:675–687.
Beattie CP, Rice RM. 1954 *J Am Med Assoc* 102:1670–1673.
Beer J. 1980. *Infektionskrankheiten der Haustiere*. Jena, Germany: VEB Fischer-Verlag.
Bhongbhibhat N, Elberg S, Chen TH. 1970. *J Infect Dis* 122:70–81.
Billard E, Dornand J, Gross A. 2007. *Infect Immun* 75:4980–4989.
Borts I H, Harris DM, Joyant MF et al. 1943. *J Am Med Assoc* 121:319.
Boschiroli ML, Ouahrani-Bettache S, Foulongne V, et al. 2002. *Proc Natl Acad Sci* 99:1544–1549.
Bounaadja LD, Albert B, Chénais S. 2009. *Vet Microbiol* 137:156–164.
Bundle DR, Cherwonogrodzky JW, Gidney MAJ. 1989. *Infect Immun* 57:2829–2836.
Bunnell DE, Hutching LM, Duonham CR. 1947. *Am J Vet Res* 8:367–373.
CDC. 2009. *MMWR* 58:618–622.
Celli J. 2006. *Res Microbiol* 157:93–98.
Cirl C, Wieser A, Yadav M. et al. 2008. *Nat Med* 14:399–406.
Cook DR, Kingston GC. 1988. *Aust Vet J* 65:162–163.
Corbel MJ. 2006. Brucellosis in humans and animals. World Health Organization, ISBN 924 1547138.
Cutler SJ, Whatmore AM, Commander AJ. 2005. *J Appl Microbiol* 98:1270–1281.
Cvetnic Z, Mitak M, Ocepek M. 2003. *Acta Vet Hung* 51:465–473.
Deqiu S, Donglou X, Jiming Y. 2002. *Vet Microbiol* 90:165–182.
Deyoe BL. 1967. *Am J Vet Res* 28:951–957.
Deyoe BL. 1972. *J Am Vet Med Assoc* 160:640–643.
Deyoe BL, Manthei CA. 1967. Sites of localization of Brucella suis in swine. *Proc Annu Meet U S Anim Health Assoc* 71:102–108.
Di DD, Jiang H, Tian LL. 2016. *BMC Genomics* 17:741, doi:https://doi.org/10.1186/s12864-016-3076-5.
Di Febo T, Luciani M, Portanti O. 2012. *Vet Ital* 48:133–156.
Dieste-Perez L, Balsco JM, de Miquel MJ. 2015a. *J Microbiol Methods* 111:57–63.
Dieste-Perez L, Fraile L, de Miguel MJ, et al. 2015b. *J Vet Pharm Ther* 38:357–364.
Dieste-Perez L, Barberán M, Munoz PM, et al. 2015c. *Vet Immunol Immunopathol* 163:77–85.
Dieste-Perez L, Frankenea K, Blasco JM, et al. 2016. *Prev Vet Med* 126:105–110.
Douglas JT, Palmer DA. 1988. *J Clin Microbiol* 26:1353–1356.
Duvnjak S, Racic I, Spicic S. 2015. *Vet Microbiol* 180:146–150.
Edmonds MD, Samartino LE, Hoyt PG. 2001. *Am J Vet Res* 61:1328–1331.
EFSA 2009. *EFSA J* 1144:1–112.
Elias D, Akuffo H, Britton S. 2005. *Trans R Soc Trop Med Hyg* 99:363–368.
Enright F. 1990. The pathogenesis and pathobiology of *Brucella* infection in domestic animals. In Nielsen K, Duncan R, eds. *Animal Brucellosis*. Boston, MA: CRC Press, pp. 301–320.
Ewalt DR, Payeur JB, Rhyan JC, et al. 1997. *J Vet Diagn Invest* 9:417–420.
Fahrion AS, Jamir L, Richa K. 2014. *Int J Environ Res Public Health* 11:403–417.
Fernandes DM, Jiang X, Jung JH, et al. 1996. *FEMS Immunol Med Microbiol* 16:193–203.
Ferris RA, Schoenbaum MA, Crawford RP. 1995. *J Am Vet Med Assoc* 207:1332–1333.
Forestier C, Deleuil F, Lapaque N, et al. 2000. *J Immunol* 165:5202–5210.
Foster RA. 2007. Male Genital System. In Maxie G, ed *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*, Vol. 3, 5th ed. St. Louis, MO: Saunders Elsevier, pp. 565–617.
Franco MP, Mulder M, Gilman RH, et al. 2007. *Lancet Infect Dis* 7:775–786.
García-Carillo C, Cedro VCF, de Benedettí LME. 1971. Evaluación de técnicas serológicas en cerdos con infección reciente de Brucella suis. serie 4, Vol. VIII, No 4. R. A. R. d. I. Agropecuarías, INTA, pp. 99–107.
García-Yoldi D, Le Flèche P, De Miguel MJ. 2007. *J Clin Microbiol* 45:4070–4072.
Garin-Bastuji B. 2008. *J Clin Microbiol* 46:3484–3487.
Garin-Bastuji B, Delcueillerie F. 2001. *Med Mal Infect* 31:202–216.
Garin-Bastuji B, Vaillant V, Albert D. 2006. Is brucellosis due to biovar 2 of Brucella suis an emerging zoonosis in France? Two case reports in wild boar and hare hunters. In Proceedings International Society of Chemotherapy Disease Management Meeting. Proceedings of 1st International Meeting on Treatment of Human Brucellosis, Ioannina, Greece.
Gicheru MM, Olobo JO, Anjili CO. 2001. *Infect Immun* 69:245–251.
Godfroid J, Käsbohrer A. 2002. *Vet Microbiol* 90:135–145.
Godfroid J, Cloeckaert A, Liautard JP, et al. 2005. *Vet Res* 36:313–326.
Grégoire F, Mousset B, Hanrez D, et al. 2012. *BMC Vet Res* 8: 80, doi: https://doi.org/10.1186/1746-6148-8-80.
Gross A, Terraza A, Ouahrani-Bettache S, et al. 2000. *Infect Immun* 68:342–351.
He Y, Reichow S, Ramamoorthy S et al. 2006. *Infect Immun* 74:5035–5046.
Hendricks SL, Borts IH, Heren RH, et al. 1962. *Am J Public Health Nations Health* 52:1166–1178.
Hofer E, Reisp K, Revilla-Fernandez S, et al. 2010. *Tierdrztliche Umschau* 65:229–232.
Hoffmann EM, Houle JJ. 1983. *Vet Immunol Immunopathol* 5:65–76.
Hubálek Z, Treml F, Juricova Z, et al. 2002. *Czech Republic Vet Med* 47:60–66.
Huber B, Scholz HC, Lucero NE, et al. 2009. *Int J Med Microbiol* 299:563–573.
Huddleson F, Johnson HW, Hamann EE. 1933. *J Am Vet Med Assoc* 83:16–30.

Hutchings LM, Andrews FN. 1946. *Am J Vet Res* 7:379–384.
Hutchings LM, Delez AL, Donham CR. 1946a. *Am J Vet Res* 7:388–394.
Hutchings LM, Delez AL, Donham CR. 1946b. *Am J Vet Res* 7:11–20.
Hutchings LM, McCullough NB, Bonham CR et al. 1951. *Public Health Rep* 66:1402–1408.
Irwin MJ, Massey PD, Walker P, et al. 2009. *NSW Public Health Bull* 20:192–194.
James DR, Golovsky G, Thornton JM. 2017. *Aust Vet J* 95:19–25.
Jelastopulu, E, Bikas C, Petropoulos C, et al. 2008. *BMC Public Health* 8:241.
Jiang X, Baldwin CL. 1993. *Infect Immun* 61:124–134.
Kamath PL, Foster JT, Drees KP, et al. 2016. *Nat Commun* 11 (7): 111448.
Karaoglan I, Pehlivan S, Namiduru M, et al. 2009. *New Microbiol* 32:173–178.
Komendy B, Nagy G. 1982. *Acta Vet Acad Sci Hung* 30:1–7.
Koppel C, Knopf L, Ryser MP, et al. 2007. *Eur J Wild Res* 53:212–220.
Krezinger Z, Foster JT, Ronai Z, et al. 2014. Genetic relatedness of Brucella suis biovar 2 isolates fom hares, wild boars and domestic pigs. *Vet Microbiol* 172:492–498.
Kutlu M, Cevahir N, Erdenlig-Gurbilek S, et al. 2016. *J Infect Public Health* 9:675–678.
Lagier A, Brown S, Soualah A, et al. 2005. *Méd Mal Infect* 35:185.
Lapaque N, Moriyon I, Moreno E, et al. 2005. *Curr Opin Microbiol* 8:60–66.
Lapaque N, Forquet F, de Chastellier C, et al. 2006. *Cell Microbiol* 8:197–206.
Lavín S, Blasco JM, Velarde R, et al. 2006. Informacion Veterinaria 10:18–21.
Leuenberger R, Boujon P, Thür B, et al. 2007. *Vet Rec* 160:362–368.
López-Goñi, I, García-Yoldi D, Marín CM, et al. 2008. *J Clin Microbiol* 46:3484–3487.
López-Goni I, Garcia-Yodi D, Marin CM, et al. 2011. *Vet Microbiol* 154:152–155.
Lord VR, Flores R. 1983. *J Wildl Dis* 19:308–314.
Lord VR, Lord RD. 1991. *J Wild Dis* 27:1139–1141.
Lord VR, Cherwonogrodzky JW, Melendez G. 1997. *J Clin Microbiol* 35:295–297.
Lucero NE, Ayala SM, Escobar GI, et al. 2008. *Epidemiol Infect* 136:496–503.
Luna-Martínez JE, Mejía-Terán C. 2002. *Vet Microbiol* 90:19–30.
Manthei CA. 1964. *Brucellosis*. Ames, IA: Iowa State University Press.
Manthei CA, Deyoe BL. 1970. *Brucellosis*. Ames, IA: Iowa State University Press.
Manthei CA, Mingle CK, Carter RW. 1952. *J Am Vet Med Assoc* 12:373–362.
Marín CM, Alabart JL, Blasco JM. 1996. *J Clin Microbiol* 34:426–428.
Massey PD, Polkinghorne BG, Durrheim DN, et al. 2011. *Rural Remote Health* 11:1793.
Mayer-Scholl A, Draeger A, Gollner C, et al. 2010. *J Microbiol Methods* 80:112–114.
McDermott JJ. 2002. *Vet Microbiol* 90:111–134.
McGiven JA, Nicola A, Commander NJ, et al. 2012. *Vet Microbiol* 160:378–386.
Menshawy AM, Perez-Sancho M, Garcia-Seco T, et al. 2014. *Biomed Res Int* 2014:353876.
Montagnaro S, Sasso S, De Martino L, et al. 2010. *J Wildl Dis* 46:316–319.
Mor SM, Wiethoelter AK, Lee A, et al. 2016. *BMC Vet Res* 12:199.
Muñoz PM, Boadella M, Arna M, et al. 2010. *BMC Infect Dis* 10:46
Muñoz PM, Blasco JM, Engel B, et al. 2012. *Vet Immunol Immunopathol* 146:150–158.
Murphy EA, Sathiyaseelan J, Parent MA, et al. 2001. *Immunology* 103:511–518.
Naroeni A, Porte F. 2002. *Infect Immun* 70:1640–1644.
Nielsen K, Gall D, Smith P. 1999. *Vet Microbiol* 68:245–253.
OIE (World Organisation for Animal Health). 2012. Bovine Brucellosis. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Paris, France: OIE, World Organisation of Animal Health, pp. 618–619.
Olsen SC, Hennager SG. 2010. *Clin Vaccine Immunol* 17:1891–1895.
Olsen SC, Tatum RM. 2016. Swine brucellosis: Current perspectives. *Vet Med Res Rep* 8:1–12.
Olsen, SC, Garin-Bastuji B, Blasco JM, et al. 2012. Brucellosis. In Zimmerman JJ, Karriker LA, Ramirez A, et al., eds. *Diseases of Swine*, 10th ed. Ames, IA: Wiley-Blackwell, pp. 697–708.
Pappas G, Papadimitriou P, Akritidis N, et al. 2006. *Lancet Infect Dis* 6:91–99.
Paton NI, Tee NWS, Vu CKF, et al. 2001. *Clin Infect Dis* 32:129–130.
Paulo PS, Vigliocco AM, Ramondina RF, et al. 2000. *Clin Diagn Lab Immunol* 7:828–831.
Pedersen K, Quance CR, Robbe-Austerman S, et al. 2014. *J Wildl Dis* 50:171–179.
Pilo C, Addis G, Deidda M, et al. 2015. *J Wildl Dis* 51:885–888.
Poester FP, Goncalves VSP, Lage AP. 2002. *Vet Microbiol* 90:55–62.
Praud A, Gimenez O, Zanella G, et al. 2012. *Prev Vet Med* 104:94–100.
Praud A, Gimenez O, Zanella G, et al. 2013. *Trop Anim Health Prod* 45:391–393.
Quance C, Robbe-Austerman S, Stuber T, et al. 2016. *Emerg Infect Dis* 22:79–82.
Radwan AI, Bekairi SI, al-Bokmy AM, et al. 1993. *Rev Sci Tech* 12:909–922.
Rafiei A, Ardestani SK, Kariminia A, et al. 2006. *J Infect* 53:315–324.
Ramamoorthy S, Woldemeskel M, Ligett A, et al. 2011. *Emerg Infect Dis* 17:2386–2387.
Ratushma VG, Sturgil CM, Ramamoorthy S, et al. 2006. *BMC Microbiol* 6:13.

Repina LP, Nikulina AI, Kosilov IA. 1993. *Zh Mikrobiol Epidemiol Immunobiol* July to August (4):66–68.
Riber U, Jungersen G. 2007. *Vet Immunol Immunopathol* 116:13–25.
Robson JM, Harrison MW, Tilse RN, et al. 1993. *Med J Aust* 159:153–158.
Rogers RJ, Cook DR, Ketterer PJ. 1989. *Aust Vet J* 66:77–80.
Roop RM, Gee JM, Robertson GT, et al. 2003. *Annu Rev Microbiol* 57:57–76.
Rosenbusch F. 1951. Patologia comparada y patologia de la Brucelosis. *Proc Congreso Argent de la Asoc Med Argent* 1:479–483.
Roth F, Zinsstag J, Orkhon D, et al. 2003. *Bull World Health Org* 81:867–876.
Salcedo SP, Marchesini MI, Lelouard H, et al. 2008. *PLoS Pathog* 4 (2):e21.
Samartino L. 2002. *Vet Microbiol* 90:71–80.
Sankarasubramanian J, Vishnu US, Gunasekaran P, et al. 2016. *Infect Genet Evol* 20:375–385.
Schlafer DH. 2007. Female Genital System. In Maxie G, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*, Vol. 3. 5th ed. St. Louis, MO: Saunders Elsevier, pp. 474–537.
Snow NP, Halseth JM, Lavele MJ, et al. 2016. *PLoS One* 11:e0146712.
Stoffregen WC, Olsen SC, Wheeler J, et al. 2007. *J Vet Diagn Invest* 19:227–237.
Stoffregen WC, Johnson CS, Olsen SC. 2013. *Res Vet Sci* 95:451–458.
Szulowski K. 1999. *Polish J Vet Sci* 2:65–70.
Tay BY, Ahmad N, Hashim R, et al. 2016. *BMC Infect Dis* 15:220.
Teske SS, Huang Y, Tamrakar JB, et al. 2011. *Risk Anal* 31:1576–1596.
Thakur A, Pedersen LE, Jungersen G. 2012). *Vaccine* 30(33):4907–4920.
Thompson K. 2007. Bones and joints. In Maxie G, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*, Vol. 1, 5th ed. St. Louis, MO: Saunders Elsevier, pp. 2–184.
Thomseon A. 1934. *Acta Pathol Microbiol Scand Suppl* 21:115–130.
Tolomeo M, Di Carlo P, Abbadessa V, et al. 2003. *Clin Infect Dis* 36:1533–1538.
Trout D, Gomez TM, Bernard BP, et al. 1995. *J Occup Environ Med* 37:697–703.
Vila A, Pagela H, Vera Bello G, et al. 2016. *Infect Dev Ctries* 10:432–436.
Watarai M, Ito N, Onata Y, et al. 2006. *J Vet Med Sci* 68:1139–1141.
Wu N, Abril C, Hinicacute V, et al. 2011. *J Wildl Dis* 47:868–869.
Zinsstag J, Schelling E, Roth F, et al. 2007. *Emerg Infect Dis* 13:527–531.

51

Clostridial Diseases

Francisco A. Uzal and J. Glenn Songer

Introduction

Members of the genus *Clostridium* are mainly anaerobic, gram-positive spore-forming rods. However, there are a few clostridia that stain gram-negative and others that tolerate oxygen (Rood 2016). *Clostridium perfringens* type C, *Clostridium difficile*, *Clostridium septicum*, *C. perfringens* type A, *Clostridium tetani*, *Clostridium botulinum*, and, rarely, *Clostridium chauvoei*, *Clostridium novyi* type A, and *Clostridium sordellii* are the main clostridial pathogens that affect pigs (Diab 2016; Silva et al. 2016; Songer and Uzal 2005). Broadly, clostridial diseases of pigs and other animal species are classified into enteric, histotoxic, and neurotoxic infections (Table 51.1).

Enteric infections

Clostridial enteric infections in pigs are mainly caused by *C. perfringens* type C *and C. difficile. C. perfringens* is divided into seven toxinotypes (A through G) based on production of six so-called major toxins (i.e. alpha [CPA], beta [CPB], epsilon [ETX], iota [ITX], enterotoxin [CPE] and necrotic enteritis B like toxin [NetB]) (Theoret and McClane 2016; Rood et al. 2018) (Table 51.2). Colonies of most *C. perfringens* strains have a typical appearance in blood agar characterized by a double halo of hemolysis produced by CPA and perfringolysin (Figure 51.1).

Clostridium perfringens type C and *C. difficile* are well-established enteric pathogens of suckling pigs. The role of *C. perfringens* type A in enteric disease in pigs is less clear, there being abundant testimonial association with disease but very little experimental evidence supporting *C. perfringens* type A as a cause of enteric disease in mammals (Uzal 2016; Uzal et al. 2016).

Clostridium perfringens type C enteritis

Infection with *C. perfringens* type C occurs worldwide (Azuma et al. 1983; Barnes and Moon 1964; Diab 2016; Field and Gibson 1955; Hogh 1965; Matthias et al. 1968; Morin et al. 1983; Plaisier 1971; Szent-Ivanyi and Szabo 1955). In swine *C. perfringens* type C causes fatal necrohemorrhagic enteritis mostly in neonates.

Etiology and epidemiology

Clostridium perfringens type C is a primary pathogen that produces CPA and CPB, CPB being the main virulence factor (Fisher et al. 2006; Sayeed et al. 2008). *C. perfringens* type C may be found, albeit rarely, in low numbers as a component of normal swine microflora (Songer and Uzal 2005). Neonatal suckling pigs are infected primarily by exposure to sow feces but may also be infected by lateral transmission from infected littermates (Songer and Uzal 2005; Uzal et al. 2016) or from spores ingested from a contaminated environment. Infected suckling pigs act as "enrichment vessels" where *C. perfringens* type C outcompetes other bacteria, multiplying to high levels and producing disease. This may be strain dependent as there is also a large variation in CPB production and virulence between different strains of *C. perfringens* type C (Fisher et al. 2006). Vegetative bacterial cells are shed in feces in low numbers by healthy sows and in high numbers by diseased pigs where they may sporulate. Spores are resistant to heat, disinfectants, and ultraviolet (UV) light (Diab 2016) and may serve as a source of infection for successive litters if the farrowing environment is not sufficiently cleaned and disinfected.

Disease may appear as early as 12 hours after birth but is most common in 3-day-old piglets. Onset is rare in pigs older than one week (Bergeland et al. 1966; Diab 2016; Matthias et al. 1968; Meszaros and Pesti 1965; Uzal et al. 2016). Type C enteritis may occur as epidemics in non-vaccinated populations (Bergeland et al. 1966) and can reach a prevalence of 100% of litters. As dams

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 51.1 Major clostridial infections and associated syndromes affecting swine.

Type of infection	Syndrome	*Clostridium* species
Enteric	Neonatal hemorrhagic and necrotic enteritis	*C. perfringens* type C
	Necrotizing enteritis (presumptive)	*C. perfringens* type A
	Neonatal necrotic enterotyphlocolitis	*C. difficile*
Histotoxic	Gas gangrene	*C. septicum*, *C. perfringens* type A, *C. novyi* type A, *C. chauvoei*, *C. sordellii*
	Blackleg (presumptive)	*C. chauvoei*
Neurotoxic	Tetanus	*C. tetani*
	Botulism	*C. botulinum*

Table 51.2 Production of so-called major toxins by toxinotypes of *Clostridium perfringens* and associated diseases in humans and animals.

	Major toxins						
Toxinotype	Alpha (CPA)	Beta (CPB)	Epsilon (ETX)	Enterotoxin (CPE)	Iota (ITX)	Necrotic Enteritis like Toxin (NetB)	Major diseases
A	+	–	–	–/+	–	–	Gas gangrene in humans[a] and animals, yellow lamb disease in sheep, necrotic enterocolitis in neonatal pigs (presumptive)
B	+	+	+	–/+	–	–	Lamb dysentery, hemorrhagic enteritis in cattle and possibly horses
C	+	+	–	–/+	–	–	Necrotic and/or hemorrhagic enteritis in neonatal pigs, horses, cattle, sheep, and goats Acute enterotoxemia ("struck") in adult sheep
D	+	–	+	–/+	–	–	Enterotoxemia in sheep, goats, and cattle
E	+	–	–	–/+	+	–	Role in animal disease not fully determined
F	+	–	–	+	–		Food poisoning in humans; role in animal disease not fully determined
G	+	–	–	–/+	–	+	Necrotic enteritis of poultry

[a] Human gas gangrene is mediated by CPA acting synergistically with perfringolysin (PFO).

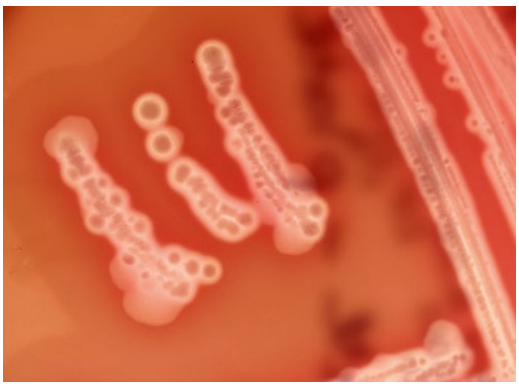


Figure 51.1 *Clostridium perfringens* colonies on blood agar. Observe the double halo of hemolysis surrounding the colonies, characteristic of this microorganism.

develop immunity and provide protective lactogenic immunity to their suckling piglets, disease becomes endemic. When endemic, peracute and acute fatal clinical disease is observed predominantly in litters of nonimmune dams, usually gilts. The case fatality rate varies, but 100% mortality in litters of nonimmune gilts is not unusual. Total herd mortality may be as high as 60% but is usually lower (Bergeland et al. 1966; Hogh 1967). A more protracted chronic form of disease occurs in individual pigs receiving insufficient lactogenic immunity, usually due to insufficient milk intake or in litters of dams providing partially protective lactogenic immunity.

Pathogenesis

Clostridium perfringens has a very short generation time, allowing type C organisms to multiply to 10^8–10^9 cells/g

of intestinal content in only a few hours (Ohnuna et al. 1992). Attachment to intestinal epithelial cells has been suggested (Arbuckle 1972; Walker et al. 1980), but definitive evidence of this is lacking. The CPB toxin has been confirmed as the main virulence factor of *C. perfringens* type C in animal experiments using CPB toxin mutants in rabbits, mice, and goats (Garcia et al. 2012; Sayeed et al. 2008; Uzal et al. 2009). The CPB toxin is exquisitely trypsin sensitive, and low trypsin levels in the intestine of neonatal animals and/or of animals fed trypsin inhibitors are critical in the pathogenesis of type C disease. The high susceptibility of neonatal animals to type C disease is thought to be a consequence of trypsin inhibition by colostrum, a mechanism apparently important for protection of colostral immunoglobulins (Uzal et al. 2016). Experimentally, inoculation of *C. perfringens* type C in combination with a trypsin inhibitor produces acute necrohemorrhagic enteritis and/or enterotoxemia in piglets, guinea pigs, rabbits, lambs, and goats (Garcia et al. 2012; Sayeed et al. 2008; Uzal et al. 2009, 2016).

It is generally assumed that the initial effect of CPB produced in the intestinal contents occurs on enterocytes (Uzal et al. 2016). Recently CPB binding to endothelial cells in the small intestinal mucosa of piglets with type C infection was demonstrated, and it was suggested that CPB-induced endothelial cell damage plays an important role in the early lesion development (Schumacher et al. 2013). However definitive evidence to support this claim is lacking. The CPB toxin has also been detected in peritoneal fluid of affected pigs, suggesting that systemic toxemia along with local intestinal damage and dysfunction causes death. In addition to primary replication in intestine, *C. perfringens* type C can also function as an opportunist, colonizing intestinal mucosal lesions induced by *Isospora suis* (coccidiosis; Chapter 66) and other causes of villous atrophy including rotaviruses (Chapter 43) and coronaviruses (Chapter 31) (Songer and Uzal 2005). *Clostridium perfringens* beta-2 toxin (CPB2) is a minor toxin distinct from CPB that has been suggested to play a role in the pathogenesis of *C. perfringens* type C (and type A)-associated enteric diseases in piglets (Bueschel et al. 2003; Songer and Uzal 2005). However little scientific evidence is available to support these claims.

Clinical signs and lesions

Clinical signs may be peracute, acute, or chronic, varying with immune status and age of affected piglets and perhaps the virulence of strain of *C. perfringens*. Peracutely affected piglets develop hemorrhagic diarrhea that begins 8–22 hours after exposure to *C. perfringens* type C (Songer and Uzal 2005; Uzal et al. 2016). They are weak, are reluctant to move, and become rapidly moribund. Rectal temperature falls to 35 °C (95 °F) or below, and abdominal skin may darken before death. Many piglets are found dead without clinical signs being observed (Songer and Uzal 2005). Acutely affected piglets may survive for 1–2 days after onset of clinical signs. They have reddish-brown diarrhea containing gray shreds of tissue debris and may be dehydrated. Perineal scalding may occur, with adherent reddish feces. Nursing is minimal, and these piglets rapidly become gaunt and weak and then die (Songer and Uzal 2005). Chronically affected pigs usually have intermittent diarrhea for more than a week. Feces are yellow to gray and mucoid, and tail and perineum may be fecal stained. These piglets remain alert but may die after several weeks or be euthanized due to unthriftiness.

Lesions have been well described (Cho et al. 1991; Niilo 1988; Songer and Uzal 2005; Uzal et al. 2016) and are usually segmental in the small intestines, but occasionally may include or be exclusively seen in the spiral colon. The character of lesions is the same regardless of location. Gross lesions in peracute disease include transmural intestinal hemorrhage and emphysema (Figure 51.2) and mucosal fibrinonecrotic exudate. There may also be variable serosanguinous abdominal fluid containing strands of fibrin, fibrinous adhesions between intestinal loops, and reddened mesenteric lymph nodes. Lesions in acute disease may include segments with acute lesions as well as segments of thickened and yellow or grayish intestine with a longitudinal striped appearance, sometimes referred to as "tiger striping," that have mucosa markedly thickened by an adherent fibrinonecrotic diphtheritic membrane (Figures 51.2 and 51.3). Deposition of urate crystals in kidney is common. Gross lesions in chronic disease are limited to segmental mucosal diphtheritic membranes with or without mural thickening and rarely mucosal fibrosis.

Histologically, the hallmark of peracute disease is hemorrhagic necrosis of the intestinal wall that starts in the mucosa but usually progresses to affect most or all layers of the intestine (Cho et al. 1991; Niilo 1988; Songer and Uzal 2005; Uzal et al. 2016). Thick bacilli with square

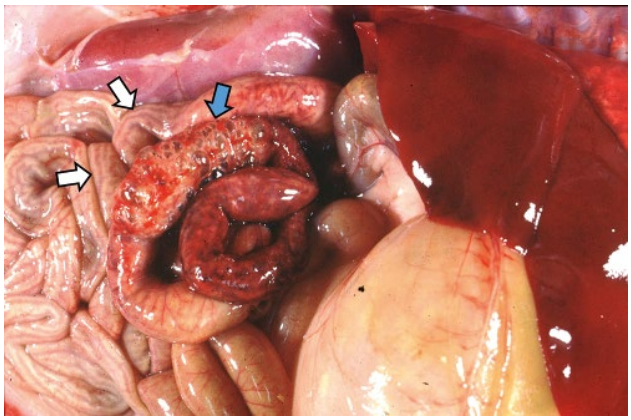


Figure 51.2 Lesions of *C. perfringens* type C enteritis in a neonatal piglet. Peracute mural hemorrhage and emphysema (blue arrow) and acute thickening with longitudinal stripes ("tiger striping") of segments of the jejunum (white arrows) are observed. *Source:* Courtesy of Dr. Mark Anderson.

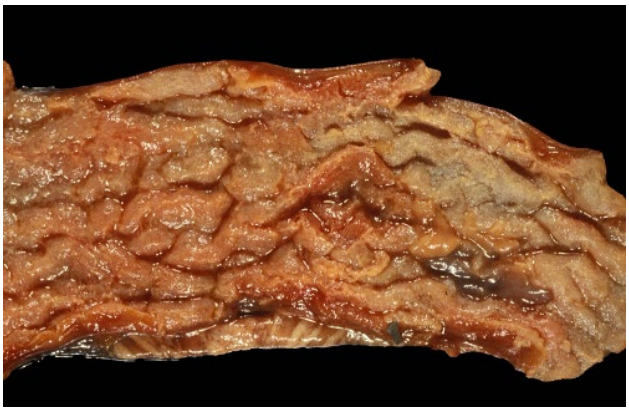


Figure 51.3 Chronic *C. perfringens* type C enteritis in a neonatal piglet. The small intestinal mucosa is covered by a thick fibrinonecrotic diphtheritic membrane. *Source:* Courtesy of Dr. Pat Blanchard.

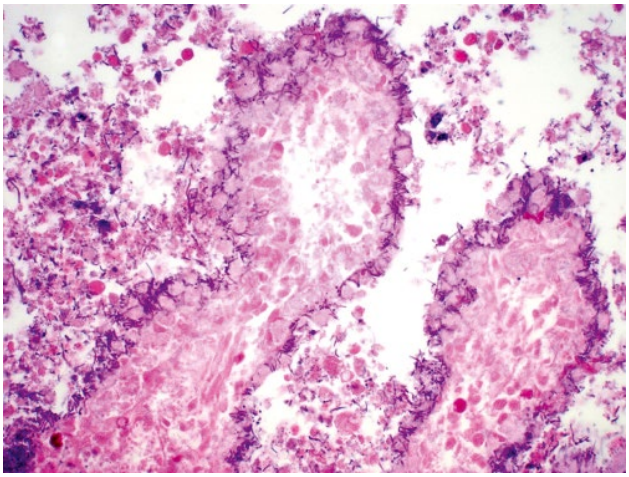


Figure 51.4 Peracute *C. perfringens* type C infection in a neonatal piglet. Association of *C. perfringens*-like organisms with the basement membrane of denuded necrotic villi and accumulation of large numbers of organisms in jejunal lumen. *Source:* Courtesy of Dr. Greg Stevenson.

ends are observed singly or in clusters in intestinal contents or on the luminal interface with necrotic mucosa (Figure 51.4). The superficial mucosa is necrotic, being effaced by necrotic cellular debris, neutrophils, fewer lymphocytes, plasma cells and macrophages, fibrin, and thick bacilli with square ends and rare subterminal spores (Figure 51.5). A few bacilli might also be observed in intestinal crypts and the submucosa. Fibrin thrombi are common in blood and lymphatic vessels of the mucosa and less common in the submucosa. Subserosal vessels are severely congested, and there is diffuse edema in viable layers of the intestine. Specific microscopic changes are not usually observed outside the gastrointestinal (GI) tract, although systemic capillary thrombosis can uncommonly be observed in the lungs, kidney, spleen, and liver as a consequence of terminal disseminated intravascular coagulation (Uzal et al. 2016).

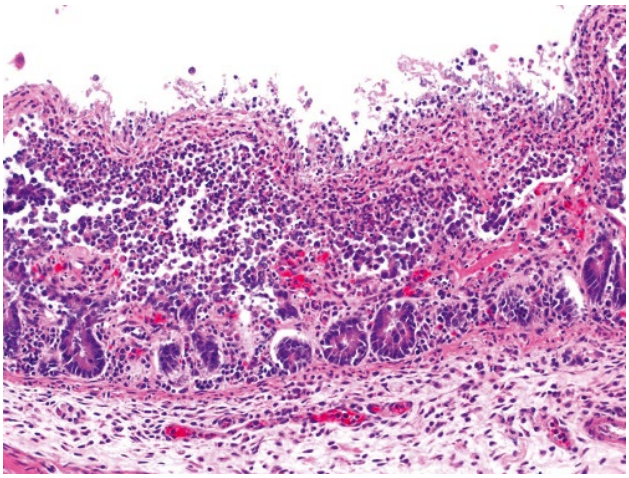


Figure 51.5 Peracute *C. perfringens* type C enteritis in a neonatal piglet. Observe severe mucosal necrosis and pseudomembrane covering the mucosa. HE, 250×.

Lesions are similar in pigs with acute or chronic clinical disease, but the necrosis in intestinal segments extends deeper to encompass the entire mucosa and sometimes the submucosa. Uncommonly, fibrosis will be observed at the interface between viable and necrotic tissue and on the serosal surface.

Diagnosis

Typically, hemorrhagic diarrhea and rapid death in neonatal piglets that have gross lesions of segmental necrohemorrhagic or fibrinonecrotic enteritis are sufficient bases for a presumptive diagnosis of type C enteritis (Songer and Uzal 2005; Uzal et al. 2016). Under field conditions, examination of smears of intestinal mucosa for abundant large gram-positive rods adds confidence to the presumptive diagnosis. Confirmation of the disease relies on detection of CPB in intestinal contents and/or feces (Diab 2016). However, because this toxin is so sensitive to trypsin and it tends to break down soon after being produced in the intestine, failure to detect CPB in intestinal contents does not preclude a diagnosis of *C. perfringens* type C infection. Detection of CPB toxin is most commonly performed by antigen immunoassays that are commercially available. Freezing of and/or adding trypsin inhibitor to intestinal contents or fecal samples prevents proteolysis of CPB for several weeks, allowing shipment to a laboratory for testing (Macias Rioseco et al. 2012).

Culture of *C. perfringens* type C supports a diagnosis of type C disease, but it is not diagnostic since it can be found, rarely and in low numbers in the intestines of healthy pigs (Songer and Uzal 2005). *C. perfringens* is not an efficient sporulator, but ovoid to eccentric spores may be observed. Colonies after 24 hours of incubation on horse or bovine blood agar are usually 3–5 mm in diameter, grayish, and circular. The organism usually produces an inner complete zone of hemolysis, caused by

PFO, and a less complete outer zone caused by CPA (Figure 51.1). Following culture, genotyping of isolates using a multiplexed PCR test to detect genes for the major toxins is the nearly universal method to determine *C. perfringens* type (Buogo et al. 1995; Meer and Songer 1997; Songer and Meer 1996).

Treatment and prevention

Treatment is of little value in animals with clinical signs (Hogh 1967; Szabo and Szent-Ivanyi 1957), and prophylaxis is the preferred approach. In an outbreak, passive immunization with equine-origin antitoxin can protect piglets in litters of nonimmune sows for up to 21 days (Ripley and Gush 1983). Antitoxin should be injected parenterally as soon after birth as possible. Oral antimicrobials such as ampicillin or amoxicillin can also be given prophylactically, beginning immediately after birth and continuing daily for 3 days. There are reports of antimicrobial resistance in *C. perfringens*, and tetracycline resistance plasmids have been identified (Rood et al. 1985). However, the organism remains uniformly susceptible to penicillins. Ceftiofur may be an alternative for treatment of piglets, and bacitracin methylene disalicylate can be administered to sows before and after farrowing to decrease the likelihood of infection of piglets.

Prevention is best achieved by vaccination of sows with type C toxoid at breeding or mid-gestation and at 2–3 weeks before farrowing (Kennedy et al. 1977). Commercial toxoid vaccines are quite effective, and vaccination usually eliminates the disease within one farrowing cycle.

Ten-fold reductions in mortality are common (Ripley and Gush 1983), assuming normal responses of the sow to vaccination (Matishek and McGrinley 1986) and ingestion by piglets of adequate amounts of colostrum. Booster injections of vaccine should be given about 3 weeks before subsequent farrowings.

Clostridium perfringens type A

There is very little information available about the role of *C. perfringens* type A in diarrheal disease of pigs. Although this microorganism has been associated with neonatal diarrhea and many swine practitioners are confident of its role (Chan et al. 2013), disease has not been reproduced in inoculation studies in suckling pigs. This confusion is fueled by the ease with which *C. perfringens* type A is cultured from diarrheic and clinically normal neonatal pigs, since it is well established as normal intestinal microbiota (Diab 2016; Mansson and Smith 1962; Uzal et al. 2016) and by lack of specific and/or consistent associated microscopic lesions (Collins et al. 1989). Lacking clear criteria for definitive diagnosis, attempts have been made to identify other toxins or virulence traits that could be used as diagnostic markers in strains of *C. perfringens* type A cultured from diarrheic pigs that would distinguish them from so-called nonpathogenic strains. These attempts have so far failed. Early studies reported that nearly all type A strains isolated from pigs with enteric disease carried the *cpb2* gene for CPB2 toxin compared with a lower proportion of clinically normal pigs (Bueschel et al. 2003; Waters et al. 2003); however, recent studies found no such correlation (Chan et al. 2012; Farzan et al. 2013). In addition, CPB2-associated enteric diseases of pigs have not been reproduced experimentally.

Etiology and epidemiology

Clostridium perfringens type A resembles type C in culture, but it produces CPA as its sole major toxin. This toxin and *C. perfringens* type A are very frequently found in the intestine of healthy animals and the environment, which complicates significantly the diagnosis of enteric disease associated with this microorganism (see below; Uzal et al. 2016). *C. perfringens* enterotoxin (CPE) that is produced by some type A strains and by type F strains (Rood et al. 2018) is a common cause of food poisoning in humans characterized by abdominal cramping, nausea, and diarrhea. The CPE toxin has also been evaluated as a possible virulence factor for type A-associated diarrhea in pigs. However, presence of CPE-producing strains in animal disease is uncommon (Collins et al. 1989; Estrada Correa and Taylor 1989; Miwa et al. 1997; Uzal et al. 2016; van Damme-Jongsten et al. 1990).

Recently two novel toxins are described, NetB produced by type G strains, and NetF produced by certain type A strains (Mehdizadeh Gohari et al. 2015; Rood et al. 2016). The NetB toxin has been shown to be responsible for cases of necrotic enteritis in poultry (Rood et al. 2016), while some evidence suggests a possible causal relationship between NetF and enteritis of foals and dogs (Mehdizadeh Gohari et al. 2015). It is therefore possible that some strains of *C. perfringens* type A produce one or more yet unidentified virulence factors that are responsible for enteric disease in pigs. This, however, is speculative, and until more is known about type A strains possibly associated with enteric diseases in pigs, no final conclusions can be drawn about the role of this microorganism on such diseases.

Putative type A diarrheal disease in pigs occurs during the first week of life, and sows are the likely source of infection. However, diagnosis is complicated by the fact that differentiation between normal flora and disease-causing strains is not possible, and it may be that under appropriate conditions, any strain of *C. perfringens* type A can cause disease (Uzal et al. 2016). Thus, discussion of the epidemiology of type A enteric infections is highly speculative.

Public health

The CPE produced by some strains of *C. perfringens* type A and by all type F strains is a leading cause of foodborne illness in humans that is characterized by

abdominal cramping, nausea, and diarrhea. It usually occurs when CPE-producing strains that contaminate meats proliferate in meats that are poorly refrigerated or are cooled slowly after cooking. Livestock, including pigs, have been traditionally implicated as the primary source of CPE type F strains through fecal contamination of meat during slaughter. However, the proportion of type F strains in pigs is low (Kanakaraj et al. 1998; Miwa et al. 1996, 1997), and recent evidence also implicates human fecal contamination of meat during handling as a common source (Lindström et al. 2011). Direct contact with swine poses no public health risk associated with *C. perfringens* type A.

Pathogenesis

CPB2 is a so-called minor toxin distinct from CPB that has been suggested to play a role in the pathogenesis of *C. perfringens* type A-associated enteric diseases in piglets (Bueschel et al. 2003; Songer and Uzal 2005). However little scientific evidence is available to support these claims. Recent evidence suggests that the number of *cpb2*-positive *C. perfringens* strains in the intestinal contents is not useful for making a diagnosis of type A enteritis in diarrheic piglets (Farzan et al. 2013).

The pathogenesis of *C. perfringens* type A enteric disease in pigs is poorly established. Association by culture of type A from diarrheic pigs in the absence of other known causes of diarrhea or lesions is often the criteria for diagnosis. This, however, is inadequate since *C. perfringens* type A is normal intestinal flora. No direct information implicates specific toxins in pathogenesis of this condition, including CPB2, CPE, NetB, and NetF (see above) as well as CPA. Studies in ligated gut loops found no consistent changes in loops inoculated with purified CPA when compared with control loops (Johannsen et al. 1993).

Clinical signs and lesions

No consistent and few inconsistent lesions are reported in *C. perfringens* type A-associated diarrheal disease in neonatal pigs. Collins et al. (1989) reported intestinal villi of normal length and morphology with few neutrophil aggregates occasionally observed in the apical lamina propria and large numbers of *C. perfringens*-like bacilli in the lumen. Songer and Uzal (2005) described small intestinal mucosal necrosis with villous atrophy and a fibrinonecrotic pseudomembrane and occasional serositis in low proportion of affected pigs.

Diagnosis

Currently no criteria are available to establish a definitive diagnosis of type A enteritis in pigs. It has been suggested, however, that non-hemorrhagic diarrhea of otherwise unexplained origin and isolation of large numbers of *C. perfringens* type A from affected jejunum or ileum are strongly suggestive of type A disease (Collins et al. 1989). Detection of CPA in gut contents is nondiagnostic because this toxin can be found in the intestinal content of healthy pigs. Currently, no commercial assays are available to detect CPB2, and the significance of finding this toxin in the intestine of pigs is uncertain.

Clostridium difficile-associated disease (CDAD)

Clostridium difficile-associated disease (CDAD) affects numerous animal species with expanded large bowels, including gerbils, guinea pigs, hamsters, horses, rabbits, pigs, and others (Diab et al. 2016). In domesticated animals, CDAD is typically referred to as necrotizing enterotyphlocolitis and, with a few exceptions (notably pigs), is antibiotic associated. In humans, the nature of the typical lesion gives rise to the common name of pseudomembranous colitis. In pigs, CDAD is not antibiotic associated and typically presents at 1–7 days of age as diarrhea and rarely as respiratory distress or sudden death.

Etiology and epidemiology

Clostridium difficile is in cluster XIa (*Peptostreptococcaceae*). It is a gram-positive strict anaerobic rod, and its highly resistant spores are ubiquitous in the environment, being responsible for its long-term survival, transmission, and, to an extent, pathogenesis. *C. difficile* appears as rods, approximately 1.2 µm × 8 µm, that occasionally occur in short chains. Spores are oval and subterminal. *C. difficile* is well known for its widespread contamination of human and animal hospitals and other medical facilities but can be found in surprisingly large numbers in any environment subject to fecal contamination. It is common in the large intestine and feces of domestic animals (particularly herbivores and swine) and, in consequence, can also be found in large numbers in manured soil. It is also common in meats and in vegetables that have been subjected to fecal contamination (Diab et al. 2016; Songer and Uzal 2005).

The vegetative forms of *C. difficile* die readily in an aerobic environment. However, the vegetative cells sporulate readily and the spores are highly resistant to oxygen. In addition, the spores of *C. difficile* are resistant to most common disinfectants, which makes them a sturdy environmental contaminant (Diab et al. 2016).

In pigs, *C. difficile* causes disease mostly between 1 and 7 days of age. The primary source of infection for newborn pigs is spores shed in sow feces or in the contaminated local environment. Route of infection is mostly oral, but spores have also been found in air samples in swine farms, suggesting that inhalation is possible. The prevalence of *C. difficile* is high in neonatal pigs, significantly decreases with age, and is unaffected by antimicrobial use in any age pigs. It can be isolated from

intestinal content of piglets as early as 1 hour after birth with 100% of piglets positive by 3 days postpartum (Songer JG and Post KW, unpublished observations). The prevalence decreases dramatically in nursery-age and older pigs, ranging between 3 and 9%.

Public health

CDAD is the leading cause of antibiotic-associated diarrhea in humans, characterized by syndromes from mild diarrhea to fatal pseudomembranous colitis. Infants are at low risk for disease and can be colonized with either toxigenic or nontoxigenic strains at variable rates worldwide from 4 to 48%, highest among neonates with extended hospitalization. Colonization as an infant can be protective against CDAD later in life. Adults can be colonized without disease. Most cases were previously thought to be nosocomial (i.e. hospital or clinic associated), but with the advent of whole genome sequencing, it is evident that a majority (65%) are community acquired (Warriner et al. 2016). Risk factors for community-acquired CDAD (CA-CDAD) differ from nosocomial CDAD. CA-CDAD occurs in young persons without contact with hospital or clinical environments, but with frequent use of proton inhibitors to reduce stomach acid (Freeman et al. 2010). Source of infection in CA-CDAD is usually unknown. Recent attention has focused on zoonotic or foodborne sources. Toxigenic strains of *C. difficile* are common in pigs, cattle, poultry, dogs, and a variety of other mammals. Contamination of carcasses at slaughter is uncommon and foodborne transmission is unlikely (Warriner et al. 2016). Direct transmission from human carriers or from infected animals has also not been confirmed as a direct cause of CDAD.

Pathogenesis

The pathogenesis of *C. difficile* infections are mediated by toxins A (TcdA) and B (TcdB), both members of the family of large clostridial toxins. A role for a separate ADP-ribosylating toxin (CDTa) also seems likely. The genes for these toxins (*tcdA*, *tcdB*, and *cdtA*) are located chromosomally (Songer et al. 2016). Contrary to CDAD in most animal species, antibiotic administration does not seem to be a significant risk factor in pigs (Diab et al. 2016). Following ingestion spores of *C. difficile* are resistant to the acidity of the stomach, and when they reach the small intestine, bile salts stimulate germination. They subsequently colonize the large intestine, and in some pigs TcdA, TcdB, and CDTa are secreted in amounts sufficient to induce colonic damage and disease.

Clinical signs and lesions

Piglets 1–7 days of age usually present with a history of early-onset scours and rarely with respiratory distress, mild abdominal distension, scrotal edema, or sudden death (Diab et al. 2016).

Figure 51.6 *Clostridium difficile* infection in a neonatal piglet. Edema of the mesocolon. *Source:* Courtesy of Dr. Darin Madson.

Gross lesions are nonspecific. Common lesions include a flaccid cecum and colon filled with fluid or pasty yellow to brown contents and edema of the mesocolon (Figure 51.6). The mucosa of the cecum and colon may be unremarkable or have multifocal-to-coalescing erosions or ulcers with attached fibrinonecrotic exudate. Less commonly, systemic lesions may include ascites, hydropericardium, and hydrothorax and scrotal edema (Bakker et al. 2010; Diab et al. 2016; Hunter et al. 2010; Uzal et al. 2016; Waters et al. 1998; Weese et al. 2010; Yaeger et al. 2002).

Microscopic lesions of CDAD in pigs are limited to the cecum and colon and may be very characteristic, but are not specific. In most piglets there is necrosuppurative or erosive/ulcerative colitis and typhlocolitis. Multifocal erosions of the luminal epithelium are observed in the cecum and colon through which fibrin and neutrophils exude, resembling volcanic eruptions, so-called volcano lesions characteristic of CDAD (Figure 51.7). Similar exudate may be observed in the subjacent lamina propria, and necrosis sometimes extends to involve all layers of the wall in severe cases. The mesocolon may be expanded by clear or pale blue staining fluid and few neutrophils (Songer et al. 2000; Songer and Uzal 2005; Uzal et al. 2016).

Diagnosis

A presumptive diagnosis of CDAD in pigs can be established based on a compatible clinical history, clinical signs, and gross and microscopic findings. Among the latter, mesocolonic edema and necrosuppurative typhlocolitis are highly suggestive of CDAD in piglets. However, a definitive diagnosis of *C. difficile* infection must be based on detection of TcdA and/or TcdB in feces or colonic contents. The reference method is measurement of neutralizable cytotoxicity in monolayers of Chinese hamster ovary or other cells, but most laboratories now use commercially

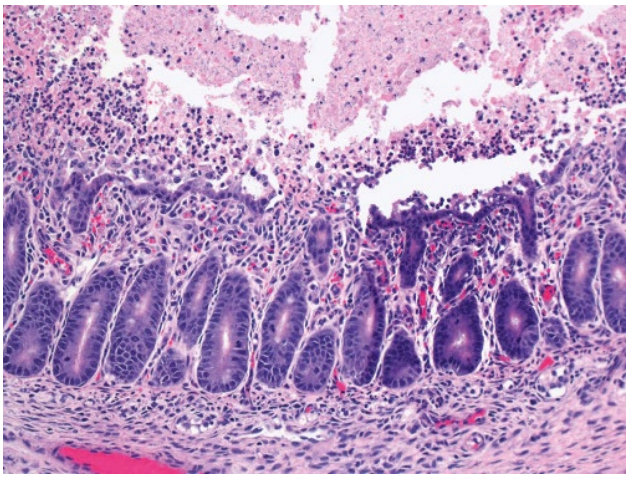


Figure 51.7 *Clostridium difficile* infection in a neonatal piglet. Central erosion of luminal epithelium with fibrinopurulent erupting exudation, so-called volcano lesion; exudate blends with diffuse fibrinosuppurative mucosal exudate.

available enzyme immunoassays (Diab et al. 2016; Songer and Uzal 2005). The finding of toxins must be coupled with compatible clinical signs and postmortem findings because TcdA and/or TcdB have been inconsistently found in the intestinal tract of normal piglets.

Culture of *C. difficile* is of little diagnostic significance because the prevalence of *C. difficile* in the intestinal tract of healthy piglets is relatively high. Culture for *C. difficile* can be somewhat challenging, in that this organism is more strictly anaerobic than some clostridia (Post et al. 2002; Songer et al. 2000). Isolation from feces or other specimens is effective using solid media containing cefoxitin, cycloserine, and fructose, often with taurocholate to initiate spore germination. Colonies are chartreuse under long-wave UV illumination. Most isolates are fully toxigenic, but some produce only TcdB or no toxins at all, and isolation should be followed by toxinotyping of the isolates, usually by PCR.

Treatment and prevention

Immunoprophylaxis of CDAD in domestic animals has not been studied, but precedent in other species suggests that immunity will be antitoxic. Antibodies against TcdA (Allo et al. 1979) and TcdB (Kink and Williams 1998; Viscidi et al. 1983) prevent toxin binding in mouse and hamster models, eliminating secretion, inflammation, and clinical disease. Results of *in vitro* antimicrobial susceptibility testing suggest that tylosin may be effective in treatment of piglets.

Histotoxic infections

Histotoxic clostridial infections are sporadic in swine and are usually exogenous, being associated with wound infections, especially contaminated injection sites, and are collectively known as gas gangrene. However, a small portion caused by *C. chauvoei* (blackleg) or *C. perfringens* type A appear spontaneously and are thought to be endogenous, resulting from oral exposure, where organisms may lie dormant in various tissues until a microenvironment favorable for their growth is generated. Histotoxic clostridial diseases of swine are not zoonotic, hence there is no public health concern.

Gas gangrene

Clostridial wound infections have received different names in the past including gas gangrene, malignant edema, and cellulitis. Because these seem to be variations of the same or similar conditions, in this chapter they will all be dealt here under the generic name of gas gangrene.

Gas gangrene is observed sporadically in pigs and is mostly associated with *C. septicum*, *C. perfringens* type A, *C. novyi* type A, *C. chauvoei*, and *C. sordellii*, acting alone or in combination.

Etiology and epidemiology

C. septicum is the most common etiologic agent of gas gangrene in swine. It is an anaerobic, gram-positive rod that forms oval subterminal spores, is found in soil and feces (Finegold et al. 1983; Kahn 1924; Princewill 1985; Princewill and Oakley 1976; Silva et al. 2016), and is a frequent postmortem invader (MacLennan 1962). Incidence of gas gangrene is particularly high on certain premises that have had large populations of livestock for many years, suggesting that there is a buildup of spore numbers in the environment of these farms. Alpha toxin (ATX), a β-pore-forming member of the aerolysin family, is considered the main virulence factor of *C. septicum* (Silva et al. 2016). The ATX is not related to the alpha toxins (CPA) of *C. perfringens* despite sharing the same name.

Clostridium perfringens type A is among the main etiological agents of gas gangrene in humans. With the use of reverse genetics, it has been demonstrated that CPA and to a lesser extent PFO are the major virulence factors responsible for gas gangrene (Awad et al. 2000). CPA and PFO play local and systemic synergistic roles in myonecrosis produced by *C. perfringens* type A (Awad et al. 2000) as determined from studies in laboratory mice. However, very little is known about the pathogenesis of *C. perfringens* type A-associated gas gangrene in spontaneous infections of animals.

Clostridium novyi type A is a large, strict anaerobic gram-positive rod with subterminal spores. The microorganism produces several toxins, of which alpha toxin (TcnA) is thought to be mainly responsible for the role of this microorganism in gas gangrene (Popoff 2016c). TcnA is not related to ATX of *C. septicum* or CPA of *C. perfringens* type A.

Clostridium chauvoei is a pleomorphic, anaerobic, gram-positive rod that readily forms central of subterminal spores. It causes blackleg (Abreu and Uzal 2016; Burke and Opeskin 1999; Kuhnert et al. 1996), an emphysematous necrotizing myositis that resembles malignant edema mostly in cattle. This microorganism is also a component of the gas gangrene complex in cattle and several other species, including pigs (Silva et al. 2016). Among its several virulence factors, CctA seems to be most important in virulence (Popoff 2016c).

Clostridium sordellii is another pleomorphic, anaerobic, gram-positive rod that readily forms central of subterminal spores. It produces several toxins of which the lethal toxin (TcsL) and hemorrhagic toxin (TcsH) seem to be the most important virulence factors (Popoff 2016c).

Pathogenesis

Most cases of gas gangrene originate from wounds, injection sites being among the most common type, and local tissue damage favors establishment of the infection. Source of the offending *Clostridium* spp. in injection sites can be contaminated syringes, needles, or skin or uncommonly reuse of contaminated injectable products. Lesions are largely the result of the necrotizing effect of ATX of *C. septicum*, CPA of *C. perfringens* type A, TcnA of *C. novyi*, CctA of *C. chauvoei*, and/or TcsL and TcsH of *C. sordellii* (Silva et al. 2016). Hyaluronidase may cause disappearance of the endomysium (Aikat and Dible 1960), which may aid spread of the infection through the muscle. Toxemia is probably the ultimate cause of death.

It has been suggested that as in the case of blackleg, the source of infection in cases of *C. perfringens* type A gas gangrene is in many cases endogenous (i.e. the result of organisms lying dormant in tissues that proliferate when the local environment is favorable), with most cases preceded by localized trauma. However, final evidence to support this claim is lacking. High intraherd incidence of *C. perfringens* type A-associated gas gangrene in young piglets is often a complication that follows injection of iron-containing preparations for prevention of nutritional anemia. Anecdotal evidence supports a view that such injections create a tissue microenvironment that favors growth of *C. perfringens* (Jaartsveld et al. 1962; Taylor and Bergeland 1992) in pure or mixed culture. The case fatality rate approaches 50%.

Clinical signs and lesions

Gas gangrene is nearly always acute and is often fatal in less than 24 hours, although some animals may live longer or survive. Hemorrhage, edema, and necrosis develop as the infection spreads along muscular fascial planes. Common sites include the inguinal and ventral abdominal region, the head and ventral cervical area, and the shoulder. Animals are reluctant to bear weight on affected limbs, and skin overlying the swollen area has a blotchy reddish-purple discoloration (Figure 51.8). Tissues that are initially painful and warm, with pitting edema, become rapidly crepitant and cold. In the terminal stage, affected swine lie in lateral recumbency and commonly groan during forced expiration.

Swelling at the primary infection site overlies subcutaneous edema that may be colorless or tinted with sanguineous fluid. Adjacent skeletal muscle may be edematous, with essentially normal color, or may be black, dry, and crepitant (Figure 51.9). Regional lymph nodes are typically enlarged and hemorrhagic and emphysematous. Acute fibrinohemorrhagic peritonitis is common, the spleen may be slightly enlarged, and there may be moderate pulmonary edema and congestion. Varying amounts of blood-tinged fluid and fibrin may be found in the pleural cavity and pericardial sac. Postmortem accumulation of subcutaneous gas may progress until the subcutis of the entire carcass is emphysematous (Silva et al. 2016). Animals that survive the

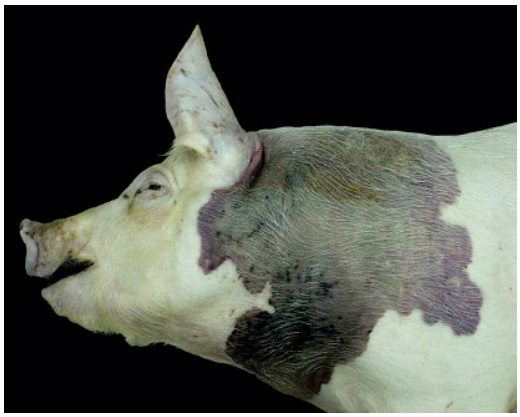


Figure 51.8 *Clostridium septicum* gas gangrene in a pig. Observe clearly demarcated dark discoloration of the skin. *Source:* Courtesy of Dr. Greg Stevenson.

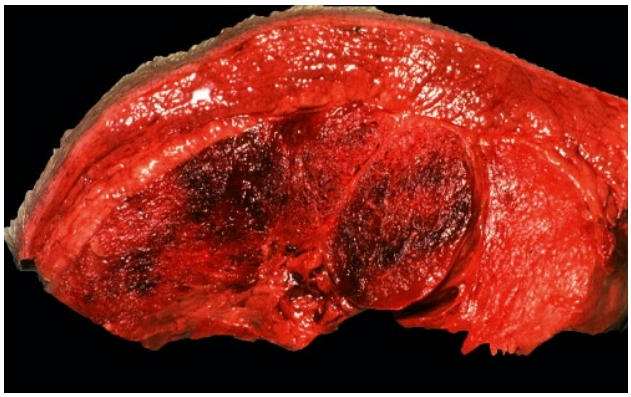


Figure 51.9 *Clostridium septicum* gas gangrene in a pig. Diffuse necrotizing and emphysematous myositis. *Source:* Courtesy of Dr. Greg Stevenson.

acute infection may show loss of skin and subcutaneous tissue with exposure of underlying muscle.

Microscopically, the edematous subcutis contains large numbers of degenerating acute inflammatory cells and few to many bacteria. Septic thrombi in subcutaneous veins and lymphatics are commonly found. Affected skeletal muscle fibers undergo coagulation necrosis with fragmentation and lysis, and bacteria are readily found between degenerating muscle fibers (Silva et al. 2016).

Clostridium perfringens type A-associated uterine gangrene and decomposition of its contents may follow dystocia and attempts to assist with delivery. Foul-smelling, reddish watery vulvar discharge may be seen, and death ensues in 12–24 hours. The uterus is usually dark green or black, is malodorous, and contains gas bubbles. There may be foul-smelling reddish fluid in the peritoneal cavity. Decomposition of the remainder of the carcass is rapid, and lesions are rarely identified in other sites.

Diagnosis

A presumptive diagnosis is based upon typical history of rapid progression and gross lesions. Laboratory confirmation is achieved by detection of the microorganisms involved by any of the methods described below. Bacteria can be observed in direct smears of affected subcutis or muscle, and fluorescent-labeled antibody staining is a rapid and accurate method to positively identify the species of clostridia present (Batty and Walker 1963; Silva et al. 2016). Bacteriologic culture is an effective alternative confirmatory method if done by a qualified veterinary microbiologist. However, culture can be time consuming (Martig 1966). For culture, care must be exercised in collection of samples to prevent fecal contamination as clostridia originating from the intestine may overgrow primary pathogens and/or result in false-positive tests. Also, swarming of *C. septicum* may cause small numbers of the organism to appear predominant, resulting in a false-positive diagnosis. Confirmation can also be by immunohistochemistry for each of the *Clostridium* spp. that can be performed on formalin-fixed, paraffin-embedded tissues. Polymerase chain reaction is a very sensitive and specific diagnostic test that can be performed on fresh or formalin fixed tissues.

Treatment and prevention

Prevention is preferred to treatment, given the fulminant clinical course. Sanitary procedures should be followed when making injections or performing surgery, and housing and equipment should be maintained free of exposed sharp objects. Immunization against toxin antigens provides lifelong immunity (Green et al. 1987), and use of vaccines may be considered on premises where the disease recurs. Treatment with antimicrobials may be successful if applied early (Zeller 1956). Experimental prophylactic use of tetracyclines, penicillin, or chloramphenicol prevents disease in mice (Taylor and Novak 1952).

Blackleg

Blackleg is a disease typically described in young cattle and rarely in other animal species, with an endogenous pathogenesis and produced exclusively by *C. chauvoei* (Abreu and Uzal 2016). There have been very few substantiated reports of blackleg in swine (Eggleston 1950; Gualandi 1955; Sterne and Edwards 1955), and information about the condition in this species is minimal.

Etiology and pathogenesis

Blackleg is produced exclusively by *C. chauvoei*. See section on gas gangrene for a brief description of this microorganism and its virulence factors. Pathogenesis of *C. chauvoei* infection in pigs has been little explored. It is believed that infection may be by the oral route rather than beginning as a wound infection as in the case of gas gangrene. If this is the case, the organisms may lie dormant in various tissues until a microenvironment favorable for their growth is generated; this microenvironment is thought to be induced by localized trauma. The disease has occurred in swine kept under poor hygienic conditions on premises with previous losses of cattle from blackleg (Gualandi 1955; Sterne and Edwards 1955). Disease may follow consumption by swine of meat from blackleg-affected calves (Eggleston 1950), and in these cases swelling of the face and throat is prominent.

The roles of toxin A (TccA), which is necrotizing, hemolytic, and lethal, and beta toxin, a DNase (Ramachandran 1969), have not been fully defined. Flagellar expression is associated with virulence, and phase variation occurs in motility and flagellation (Tamura et al. 1995). Flagella are apparently immunogenic (Kojima et al. 2000; Verpoort et al. 1966).

Clinical signs and lesions

Signs include high fever, anorexia, depression, and lameness, with crepitant lesions and sudden death. Lesions are thought to be more common in legs (Mavenyengwa and Matope 1995). Gross lesions are often dry and emphysematous at the center, but edematous, hemorrhagic, and necrotic at the periphery. Microscopically, lesions are similar to those described in cattle and consist of myofiber necrosis with little leukocytic infiltration (Abreu and Uzal 2016).

Diagnosis, treatment, and prevention

A diagnosis of blackleg can be made only by bacterial identification, due to similarities in the clinical presentation and pathology of other histotoxic clostridial infections. The fluorescent antibody test, applied to impression smears of infected tissue, is a rapid and practical method of identification (Batty and Walker 1963; Uzal et al. 2016). Bacterial isolation may be difficult in

decomposing specimens, since *C. chauvoei* is easily overgrown by other bacteria, including *C. septicum*. Polymerase chain reaction is also available to detect this microorganism on fresh or formalin fixed tissues.

Prevention of *C. chauvoei* infection requires minimizing exposure. *C. chauvoei* is not known to be a common soil organism, but anecdotal evidence suggests that keeping swine on known contaminated premises or allowing them to eat carcasses of ruminants dead of blackleg are risk factors.

Neurotoxigenic clostridia

Neurotoxic clostridial diseases include tetanus caused by *C. tetani* and botulism caused by *T. botulinum*. Neither disease is zoonotic; hence there is no public health concern.

Tetanus

Clostridium tetani causes tetanus, characterized by toxin-mediated, uncontrollable spasms of voluntary muscles. Swine of all ages may be affected, but most cases involve young pigs and originate with castration wounds or umbilical infection (Popoff 2016b).

Etiology and epidemiology

Clostridium tetani is a slender, anaerobic, gram-positive rod. It forms terminal spores that are ubiquitous in the environment. Spores often enter via traumatic wounds, including, but not limited to, those from tail docking and castration (Popoff 2016b). Sows may be infected by contamination of uterine prolapses.

Pathogenesis

Development of tetanus depends upon the presence of *C. tetani* in a tissue environment that will support spore germination, vegetative cell growth, and toxin production. Spores usually gain entrance via a deep penetrating wound. Spore germination is facilitated by the presence of foreign bodies or facultative anaerobes that reduce oxygen tension in tissues. Bacterial multiplication and production of the primary toxin tetanospasmin (TeNT) is enhanced by tetanolysin, a cholesterol-binding toxin that inhibits neutrophil and macrophage chemotaxis and causes local tissue necrosis. Spores may remain latent in healed wounds for 10 years or more. *C. tetani* is not invasive and remains localized at the primary site of infection (Popoff 2016b).

Toxin-containing vesicles pass by retrograde axonal transport along the motor nerve fibers from neuromuscular junctions at the site of the infection, acting eventually on the inhibitory neurons in the ventral horn of the spinal cord. The TeNT cleaves synaptobrevin, a protein involved in the exocytosis of neurotransmitters by neurons, resulting in tetany.

Clinical signs and lesions

Tetanus is characterized by generalized skeletal muscle spasms. The incubation period ranges from several days to several weeks. In general, shorter incubation periods are associated with a more acute and fulminating course and a higher fatality rate.

The earliest sign is a stiffened gait, and disease progresses rapidly over 1–2 days. Ears become erect, the tail extends straight out, the head is slightly elevated, and the nictitating membrane may protrude. The pig becomes incapable of walking, and the skeletal muscles are very firm on palpation. Ultimately, the pig lies in lateral recumbency in opisthotonus, with front and rear legs extended posteriorly. Tetanic spasms proceed from periodic to continuous and are noticeably heightened by sudden sensory stimuli. Tachycardia and increased respiration rate are common terminal signs, and white froth may be present around the mouth and external nares (Popoff 2016b).

In acute cases, respiratory failure resulting from severe skeletal muscle spasms is likely to be the single most important cause of death. Prolonged recumbency and nutrient deprivation may be factors contributing to death in animals with a relatively long survival time. Animals dying of tetanus do not show specific gross or microscopic lesions. Deep wounds associated with the port of entry may be found; although these support the diagnosis, they are not diagnostic in themselves.

Diagnosis

Diagnosis is based upon typical clinical signs. An obvious area of infection (e.g. a castration wound or umbilical abscess) may be apparent. Identification of typical rods with terminal spores on Gram-stained smears of a wound lends credibility to a diagnosis of tetanus (Popoff 2016b). The organism may be isolated by bacteriologic culture or identified by immunofluorescence (Batty and Walker 1963) from suspect wounds, but this is usually not necessary if there is adequate antemortem clinical observation of the affected animals.

Treatment and prevention

There is no practical way to eliminate spores from soil, so control is directed toward prevention of wound contamination by soil or feces. Good sanitation in the farrowing house, treatment of umbilical cords with antiseptics soon after birth, sanitary practices for castration and other surgeries, and treatment of wounds with antiseptics and affected animals with prophylactic parenteral antibiotics are recommended preventive measures against tetanus. Sharp objects that may cause wounds should be removed from the environment.

Passive immunization with tetanus antitoxin, prophylactic use of antibiotics, and/or active immunization with tetanus toxoid may be indicated. Prophylactic use of

large doses of long-acting penicillin or tetracyclines may be superior to antitoxin in preventing experimental tetanus if treatment is instituted within a few hours after infection (Veronesi 1966). Active immunity may be obtained from a single injection of alum-precipitated tetanus toxoid, and excellent protection for a year or more can be expected if three doses are given several weeks apart.

With even moderate clinical tetanus, prognosis is poor, and there is little evidence that treatment is of real benefit. Various suggested treatments include reopening castration wounds and flushing them with hydrogen peroxide, administration of antitoxin to neutralize toxin not already fixed by nervous tissue, administration of antibiotics, and the use of tranquilizers or barbiturates as muscle relaxants.

Botulism

Botulism is mostly produced by *C. botulinum*. This microorganism may produce seven botulinum neurotoxins (BoNTs), which are different protein neurotoxins that are immunologically distinct and are identified by letters from A to G. A novel type, BoNT/H, which seems to be a hybrid between BoNT/F and BoNT/A, has been reported but awaits further characterization (Popoff 2016a). Nevertheless, all BoNTs cause the typical flaccid paralysis of botulism in experimental animals, similar to that observed in humans and domestic and wild animals suffering from botulism (Popoff 2016a). Each of the BoNTs has unique geographic distribution and species susceptibility patterns (CDC 1998; Hatheway 1990, 1995; Shapiro et al. 1998; Smith 1977; Smith and Milligan 1979). Swine are susceptible to botulism but are in general considered to be among the least susceptible domestic animals.

Etiology and epidemiology

C. botulinum is strictly anaerobic and gram positive (Smith and Holdeman 1968) and forms oval, usually subterminal, spores. Growth is optimal at about 86 °F (30 °C). Spores are ubiquitous in soil throughout the United States (Kelch et al. 2000; Smith 1979; Whitlock and Williams 1999).

Botulism in swine is rare, so there are few recorded organism or toxin sources, but disease in swine due to consumption of dead fish (Beiers and Simmons 1967) and decomposing brewers' waste (Doiurtre 1967) has been reported. Disease in other species is associated with contaminated forage (Franzen et al. 1992; Kelly et al. 1984; Kinde et al. 1991; Le Maréchal et al. 2016; Popoff 2016a; Ricketts et al. 1984; Whitlock 1997; Wichtel and Whitlock 1991), contamination of grain by decomposing animal carcasses (Divers et al. 1986; Enfors et al. 1975; Galey et al. 2000; Whitlock and Williams 1999), or transport of BoNT by ravens or crows feeding on a decomposing carcass (Schoenbaum et al. 2000). Type D botulism has been linked to pica, in which phosphorus-deficient animals consume bones of carcasses of animals dead of botulism (Dobereiner et al. 1992).

Eating habits of non-confined pigs should make them likely candidates for botulism, but there appears to be innate resistance to toxin administration by the oral route. The swine GI tract may have a low permeability for botulinum toxin (Dack and Gibbard 1926; Scheibner 1955; Smith et al. 1971).

Pathogenesis

Potency of botulinum toxin varies among toxin types, and amount of toxin produced varies with strain. Botulism occurs after ingestion of preformed BoNT or by dissemination of toxin from an infected wound or focus of clostridial multiplication in the GI tract or elsewhere (Bernard et al. 1987; Hatheway 1995; Swerczek 1980). Absorption varies among species and with different areas of the GI tract (May and Whaler 1958).

Botulinum toxin is composed of an enzymatic light chain (a zinc-dependent endopeptidase) and a heavy chain that binds to receptors and facilitates internalization. The light chain cleaves proteins involved in exocytosis of neurotransmitters by neurons. Toxin types B, D, and F cleave synaptobrevin, types A and E act on SNAP-25, and type C toxin acts on syntaxin at the myoneural junction, preventing muscular contraction. Death is generally ascribed to asphyxia resulting from paralysis of the muscles of respiration.

Clinical signs and lesions

The latent period between consumption of toxic material and onset of signs ranges from 8 hours to 3 days or more, largely determined by the amount of toxin consumed (Beiers and Simmons 1967; Smintzis and Dunn 1950). Initial signs are weakness, incoordination, and staggering, with weakness appearing first in the forelegs, followed by involvement of the hind legs and general motor paralysis and dilation of the pupils (Smith et al. 1971). The clinical effect is progressive flaccid paralysis of voluntary muscles, which manifests in the end as lateral recumbency with complete flaccidity. Other clinical signs include anorexia, reduced vision or complete blindness, aphonia, excessive salivation, involuntary urination, and deep labored breathing (Beiers and Simmons 1967; Smintzis and Dunn 1950). There are no specific gross or microscopic lesions.

Diagnosis

A diagnosis of botulism should be considered in afebrile, alert animals with progressive weakness and recumbency. Because the pig apparently is quite highly resistant to botulism, a diagnosis should be made only after

thorough investigation and exclusion of other possible diagnoses (Beiers and Simmons 1967). Confirmation of the diagnosis should be based on detection of BoNT in feed, GI contents, liver, or serum. Mouse bioassay is still the test most frequently used around the world, although the sensitivity of the test is usually low. The sensitivity of this test for mammals is highest for feed, intermediate for GI content and liver, and lowest for serum (Le Maréchal et al. 2016; Muller 1967; Narayan 1967; Yamakawa et al. 1992).

Treatment and prevention

If botulism is suspected, an effort should be made to find the toxin source and prevent further consumption of suspect material by the herd. Antitoxin is the only specific treatment for botulism, and it has been effective in reducing mortality in humans after consumption of toxin-containing food (Lamanna and Carr 1967; Le Maréchal et al. 2016). Polyvalent antitoxins incorporating the types most commonly present in a geographic area are required for therapy in animals. Therapy aimed at reducing continued absorption of toxin from the intestine (administration of magnesium sulfate) might be helpful.

Prevention requires eliminating opportunities to consume potentially toxic material such as spoiled garbage and decomposing animal tissue. Prophylactic immunization with toxoids is not practical in swine because of the infrequent occurrence of the disease.

References

Abreu CC, Uzal FA. 2016. Blackleg. In Uzal FA, Songer JG, Prescott JF, et al. eds. *Clostridial Diseases of Animals.* Ames, IA: Wiley Blackwell.

Aikat BK, Dible JH. 1960. *J Pathol* 79:277.

Allo M, Silva J Jr, Fekety R, et al. 1979. *Gastroenterology* 76:351.

Arbuckle JBR. 1972. *Pathology* 106:65.

Awad MM, Ellemor DM, Bryant AE, et al. 2000. *Microb Pathog* 28:107.

Azuma R, Hamacka T, Shioi H, et al. 1983. *Nippon Juigaku Zameszshi* 45:135.

Bakker D, Corver J, Harmanus C, et al. 2010. *J Clin Microbiol* 48:3744–3749.

Barnes DM, Moon HW. 1964. *J Am Vet Med Assoc* 144:1391.

Batty I, Walker PD. 1963. *J Pathol* 85:517.

Beiers PR, Simmons GC. 1967. *Aust Vet J* 43:270.

Bergeland ME, Dermody TA, Sorensen DK. 1966. Porcine enteritis due to *Clostridium perfringens* type C. I. Epizootiology and diagnosis. *Proc US Livest Sanit Assoc* 70:601.

Bernard W, Divers TJ, Whitlock RH, et al. 1987. *J Am Vet Med Assoc* 191:73.

Bueschel DM, Jost BH, Billington SJ, et al. 2003. *Vet Microbiol* 94:121.

Buogo C, Capaul S, Haeni H, et al. 1995. *Vet Med Ser B* 42:51–58.

Burke MP, Opeskin K. 1999. Nontraumatic clostridial myonecrosis. *Am J Forensic Med Pathol* 20:158.

Centers for Disease Control and Prevention. 1998. Centers for Disease Control and Prevention: Botulism in the United States, 1899–1996. Handbook for Epidemiologists, Clinicians, and Laboratory Workers, Atlanta, GA.

Chan G, Farzan A, Soltes G, et al. 2012. *BMC Vet Res* 8:156

Chan G, Farzan A, Prescott JF, et al. 2013. *Can Vet J* 54:504–506.

Cho SK, Kim JY, Park JM. 1991. *Res Rep Rural Dev Adm Vet* 33:25–31.

Collins JE, Bergeland ME, Bouley D, et al. 1989. *J Vet Diagn Invest* 1:351–353.

Dack GM, Gibbard J. 1926. *J Infect Dis* 39:181.

Damme-Jongsten M, van Haagsma J, Notermans S. 1990. *Vet Rec* 126:191–192.

Diab SS. 2016. Diseases produced by *Clostridium perfringens* type C. In Uzal FA, Songer JG, Prescott JF, et al. eds. *Clostridial Diseases of Animals.* Ames, IA: Wiley Blackwell.

Diab SS, Uzal FA, Songer JG. 2016. Diseases produced by *Clostridium difficile.* In Uzal FA, Songer JG, Prescott JF, et al., eds. *Clostridial Diseases of Animals.* Ames, IA: Wiley Blackwell.

Divers TJ, Bartholomew RC, Messick JB, et al. 1986. *J Am Vet Med Assoc* 188:382.

Dobereiner J, Tokarnia CH, Langenegger J, et al. 1992. *Dtsch Tierarztl Wschr* 99:188.

Doiurtre MP. 1967. *Bull Off Int Epizoot* 67:1497.

Eggleston EL. 1950. *Vet Med* 45:253.

Enfors E, Gunnarsson A, Hurvell B, et al. 1975. *Svensk Vet* 27:333–339.

Estrada Correa AE, Taylor DJ. 1989. *Vet Rec* 124:606–611.

Farzan A, Kircanski J, DeLay J, et al. 2013. *Can J Vet Res* 77:45–53.

Field HL, Gibson EA. 1955. *Vet Rec* 67:31.

Finegold SM, Sutter VL, Mathisen GL. 1983. Normal indigenous intestinal flora. In Hentges D, ed. *Human Intestinal Microflora in Health and Disease.* New York: Academic Press.

Fisher DJ, Fernandez-Miyakawa ME, Sayeed S, et al. 2006. *Infect Immun* 74:5200–5210.

Franzen P, Gustafsson A, Gunnardsson A. 1992. *Svensk Veterinar tidning* 44:555–559.

Freeman J, Bauer MP, Baines SD, et al. 2010. *Clin Microbiol Rev* 23:529–549.

Galey FD, Terra R, Walker R, et al. 2000. *J Vet Diagn Invest* 12:204–209.

Garcia JP, Beingesser J, Fisher DJ, et al. 2012. *Vet Microbiol* 157:412–419.

Green DS, Green MJ, Hillyer MH, et al. 1987. *Vet Rec* 120:435–439.

Gualandi GL. 1955. *Arch Vet Ital* 6:57.

Hatheway CL. 1990. *Clin Microbiol Rev* 3:66–98.
Hatheway CL. 1995. *Curr Topics Microbio Immun* 195:55–75.
Hogh P. 1965. *Nord Vet Med* 17:1.
Hogh P. 1967. *Acta Vet Scand* 8:301.
Hunter PA, Dawson S, French GL, et al. 2010. *J Antimicrob Chemother* 65 Suppl 1:13–17.
Jaartsveld FHJ, Janssens FTM, Jobse CJ. 1962. *Tijdschr Diergeneeskd* 87:768.
Johannsen U, Menger S, Arnold P, et al. 1993. *Monatsh fur Veterinaermed* 48:267–273.
Kahn CM. 1924. *J Infect Dis* 35:423–478.
Kanakaraj R, Harris DL, Songer JG, et al. 1998. *Vet Microbiol* 63:29–38.
Kelch WJ, Kerr LA, Pringle JK, et al. 2000. *J Vet Diagn Invest* 12:453–455.
Kelly AR, Jones RJ, Gillick JC, et al. 1984. *Eq Vet J* 16:519–521.
Kennedy KK, Norris SJ, Beckenhauer WH, et al. 1977. *Am J Vet Res* 38:1515–1518.
Kinde H, Betty RL, Ardans A, et al. 1991. *J Am Vet Med Assoc* 199:742–746.
Kink JA, Williams JA. 1998. *Infect Immun* 66:2018–2025.
Kojima A, Uchida I, Sekizaki T, et al. 2000. *Vet Microbiol* 76:359–372.
Kuhnert P, Capaul SE, Nicolet J, et al. 1996. *Int J Syst Bacteriol* 46:1174–1176.
Lamanna C, Carr CJ. 1967. *Clin Pharmacol Ther* 8:286–332.
Le Maréchal C, Woudstra C, Fach P. 2016. Botulism. In Uzal FA, Songer JG, Prescott JF, et al., eds. *Clostridial Diseases of Animals*. Ames, IA: Wiley Blackwell.
Lindström M, Heikinheimo A, Lahti P, et al. 2011. *Food Microbiol* 28:192–198.
Macias Rioseco M, Beingesser J, Uzal FA. 2012. *Anaerobe* 18:357–360.
MacLennan JD. 1962. *Bacteriol Rev* 26:177.
Mansson I, Smith LDS. 1962. *Acta Pathol Microbiol Scand* 55:342–348.
Martig J. 1966. *Schweiz Arch Tierheilkd* 108:303.
Matishek PH, McGrinley M. 1986. *Am J Vet Res* 46:2147–2148.
Matthias D, Illner F, Bauman G. 1968. *Arch Exp Veterinarmed* 22:417.
Mavenyengwa M, Matope G. 1995. *Zimbabwe Vet J* 26:135–138.
May AJ, Whaler BC. 1958. *Br J Exp Pathol* 39:307.
Meer RR, Songer JG. 1997. *Am J Vet Res* 58:702–705.
Mehdizadeh Gohari I, Parreira VR, Nowell VJ, et al. 2015. *PLoS One* 10:e012268.
Meszaros J, Pesti L. 1965. *Acta Vet Acad Sd Hung* 15:465.
Miwa N, Nishima T, Kubo S, et al. 1996. *J Vet Med Sci* 58:197–203.
Miwa N, Nishima T, Kubo S, et al. 1997. *J Vet Med Sci* 59:89–92.
Morin M, Turgeon D, Jolette J, et al. 1983. *Can J Comp Med* 47:11.
Muller J. 1967. *Bull Off Int Epizoot* 67:1473–1478.
Narayan KG. 1967. *Zentralbl Bakteriol [Orig]* 202:212–220.
Niilo L. 1988. *Can Vet J* 29:658–664.
Ohnuna Y, Kondo H, Saino H, et al. 1992. *J Jpn Vet Med Assoc* 45:738–741.
Plaisier AJ. 1971. *Tijdschr Diergeneeskd* 96:324.
Popoff M. 2016a. *Clostridium botulinum* and *Clostridium tetani* neurotoxins. In Uzal FA, Songer JG, Prescott JF, et al., eds. *Clostridial Diseases of Animals*. Ames, IA: Wiley Blackwell.
Popoff M. 2016b. *Tetanus*. In Uzal FA, Songer JG, Prescott JF, et al. eds. *Clostridial Diseases of Animals*. Ames, IA: Wiley Blackwell.
Popoff M. 2016c. Toxins of histotoxic clostridia: *Clostridium chauvoei, Clostridium septicum, Clostridium novyi*, and *Clostridium sordellii*. In Uzal FA, Songer JG, Prescott JF, et al. eds. *Clostridial Diseases of Animals*. Ames, IA: Wiley Blackwell.
Post KW, Jost BH, Songer JG. 2002. *J Vet Diagn Invest* 14:258–259.
Princewill TJT. 1985. *Bull Anim Health Prod Afr* 33:323–326.
Princewill TJ, Oakley CL. 1976. *Med Lab Sci* 33:110–118.
Ramachandran S. 1969. *Indian Vet J* 46:754–768.
Ricketts SW, Greet TRC, Glyn PJ, et al. 1984. *Eq Vet J* 16:515–518.
Ripley PH, Gush AF. 1983. *Vet Rec* 112:201.
Rood JI. 2016. General physiological and virulence properties of the pathogenic clostridia. In Uzal FA, Songer JG, Prescott JF, et al., eds. *Clostridial Diseases of Animals*. Ames, IA: Wiley Blackwell.
Rood JI, Buddle JR, Wales AJ, et al. 1985. *Aust Vet J* 62:276–279.
Rood JI, Keyburn AL, Moore RJ. 2016. *Avian Pathol* 45:295–301.
Rood JI, Adams V, Lacey J. et al. Anaerobe. 2018 doi: 10.1016/j.anaerobe.2018.04.011. [Epub ahead of print].
Sayeed S, Uzal FA, Fisher DJ, et al. 2008. *Mol Microbiol* 67:15–30.
Scheibner G von. 1955. *Dtsch Tierarztl Wochenschr* 62:355.
Schoenbaum MA, Hall SM, Glock RD, et al. 2000. *J Am Vet Med Assoc* 217:365–368.
Schumacher VL, Martel A, Pasmans F, et al. 2013. *Vet Pathol* 50:626–629.
Shapiro RL, Hatheway C, Swerdlow DL. 1998. *Ann Intern Med* 129:221–228.
Silva ROS, Uzal FA, Oliveira Jr CA, et al. 2016. Gas gangrene (Malignant edema). In Uzal FA, Songer JG, Prescott JF, et al., eds. *Clostridial Diseases of Animals*. Ames, IA: Wiley Blackwell.
Smintzis G, Dunn D. 1950. *Bull Soc Sci Vet (Lyon)*:71.
Smith LDS. 1977. Botulism in animals. In Thomas CC, ed. *Botulism: The Organism, its Toxins, the Disease*. Springfield, IL: Charles C Thomas.
Smith LDS. 1979. *Rev Infect Dis* 1:637–641.
Smith LDS, Holdeman LV. 1968. *The Pathogenic Anaerobic Bacteria*. Springfield, IL: Charles C. Thomas.
Smith GR, Milligan RA. 1979. *J Hyg Camb* 83:237–241.
Smith LDS, Davis JW, Libke KG. 1971. *Am J Vet Res* 32:1327.
Songer JG, Meer RR. 1996. *Anaerobe* 2:197–203.
Songer JG, Uzal FA. 2005. *J Vet Diagn Invest* 17:528–536.

Songer JG, Post KW, Larson DJ, et al. 2000. *JSHAP* 8:185–189.
Songer JG, Harmon AH, Keel MK. 2016. Toxins of *Clostridium difficile*. In Uzal FA, Songer JG, Prescott JF, et al., eds. *Clostridial Diseases of Animals*. Ames, IA: Wiley Blackwell.
Sterne M, Edwards JB. 1955. *Vet Rec* 67:314.
Swerczek TW. 1980. *J Am Vet Med Assoc* 176:348–350.
Szabo S, Szent-Ivanyi T. 1957. *Acta Vet Acad Sci Hung* 7:413.
Szent-Ivanyi T, Szabo S. 1955. *Magy Allatorv Lapja* 10:403.
Tamura Y, Kijima-Tanaka M, Aoki A, et al. 1995. *Microbiology* 141:605–610.
Taylor DJ, Bergeland ME. 1992. Clostridial infections. In Leman AD, Straw BE, Mengeling WL, et al., eds. *Diseases of Swine*, 7th ed. Ames, IA: Iowa State University Press.
Taylor WI, Novak M. 1952. *Antibiot Chemother* 2:639.
Theoret JR, McClane BA. 2016. Toxins of *Clostridium perfringens*. In Uzal FA, Songer JG, Prescott JF, et al., eds. *Clostridial Diseases of Animals*. Ames, IA: Wiley Blackwell.
Uzal FA. 2016. Diseases produced by *Clostridium perfringens* type A in mammalian species. In Uzal FA, Songer JG, Prescott JF, et al., eds. *Clostridial Diseases of Animals*. Ames, IA: Wiley Blackwell.
Uzal FA, Saputo J, Sayeed S, et al. 2009. *Infect Immun* 77:5291–5299.
Uzal FA, Plattner BL, Hostetter JM. 2016. Alimentary system. In Maxie G, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. St. Louis, MO: Elsevier, pp. 1–257.
Veronesi R. 1966. Antibiotics versus antitetanic serum in the prevention of human tetanus. Principles on Tetanus. In: Proceedings, 2nd International Conference on Tetanus, p. 417.
Verpoort JA, Joubert FJ, Jansen BC. 1966. *S Afr J Agric Sci* 9:153–172.
Viscidi R, Laughon BE, Yolken R. 1983. *J Infect Dis* 148:93–100.
Walker PD, Murrell TGC, Nagy LK. 1980. *J Med Microbial* 13:445.
Warriner K, Xu C, Habash M, et al. 2016. *J Appl Microbiol* 122:542–553.
Waters EH, Orr JP, Clark EG, et al. 1998. *J Vet Diagn Invest* 10:104–108.
Waters M, Savoie A, Garmory HS, et al. 2003. *J Clin Microbiol* 41:3584–3591.
Weese JS, Wakeford T, Reid-Smith R, et al. 2010. *Anaerobe* 16:501–504
Whitlock RH. 1997. Botulism. *Vet Clin North Am Equine Pract* 13:107–128.
Whitlock RH, Williams JM. 1999. Botulism toxicosis of cattle. *Proc Bovine Practitioner* 32:45–53.
Wichtel JJ, Whitlock RH. 1991. *J Am Vet Med Assoc* 199:471–472.
Yaeger M, Funk N, Hoffman L. 2002. *J Vet Diagn Invest* 14:281–287.
Yamakawa K, Kamiya S, Yoshimura K, et al. 1992. *Microbiol Immunol* 36:29–34.
Zeller M. 1956. *Tierarztl Umsch* 11:406.

52

Colibacillosis

John M. Fairbrother and Éric Nadeau

Relevance

Diseases caused by *Escherichia coli* have been a recognized problem for as long as pigs have been raised. Early work in the 1960s and 1970s elucidated the pathogenic mechanisms of *E. coli*-induced neonatal diarrhea and led to the development of sow vaccines that effectively control this form of the disease by passive immunity. However, maternal vaccination does not protect pigs against the diarrhea and edema disease (ED) that occur in the post weaning period.

More recent advances in our understanding of how *E. coli* cause disease have led to a better classification of pathogenic strains based on the presence of virulence factors, permitting much more accurate diagnostic strategies, as opposed to traditional serotyping identifying the *E. coli* O and H types. Antimicrobial resistance is at a crisis point for pig producers because of limited treatment options and an increased public health concern due to the potential transfer of drug resistance genes directly by contact and indirectly into the food chain, water, manure, etc. This has provided the impetus to find alternative control strategies such as novel vaccines for weaned pigs.

All over the world, *E. coli* is an important cause of a wide range of diseases in pigs, including neonatal diarrhea, post weaning diarrhea (PWD), ED, septicemia, polyserositis, coliform mastitis (CM), and urinary tract infection (UTI). In particular, diarrhea and ED due to *E. coli* may result in significant economic losses due to morbidity, mortality, decreased weight gain, and cost of treatment, vaccinations, and feed supplements. *E. coli* PWD is also called post weaning enteric colibacillosis. Edema disease is also known as "bowel edema" or "gut edema" because edema of the submucosa of the stomach and the mesocolon is often a prominent feature of the disease. *E. coli* PWD and ED may occur independently, but they may also occur together in a single outbreak or in the same pig. PWD is endemic in many farms and its prevalence fluctuates over time and across regions. A more severe form of enteric *E. coli* infection may be observed at 2–3 weeks following weaning and is manifested as sudden death or severe diarrhea.

Postpartum dysgalactia syndrome (PPDS) or mastitis–metritis–agalactia (MMA) is an economically important disease complex characterized by reduced milk production postpartum, leading to pig starvation (see Chapter 18 for a more detailed discussion of this syndrome). Mastitis is the most important component, and coliforms, predominantly *E. coli*, are the most frequently isolated bacteria in affected sows. The term "coliform mastitis" is used to refer to puerperal mastitis in the pig.

UTI is present whenever microbes colonize any of the typically sterile sections of the urinary tract. It may or may not be accompanied by clinical or subclinical disease. In the pig, specific UTI caused by *Actinobaculum suis* (Chapter 64) is distinguished from nonspecific UTI caused by a variety of microbes, the subject of this chapter. According to Liebhold et al. (1995), a nonspecific UTI often predisposes for *A. suis* infection.

Certain *E. coli*, particularly those of serotype O157:H7, serogroup O26, and other non-O157 *E. coli*, may be present sporadically in the intestines and feces of normal pigs and are considered as zoonotic.

Etiology

Taxonomy, morphology, and laboratory cultivation

The genus *Escherichia* is named after the German pediatrician Theodor Escherich (1857–1911). It is classified with the family *Enterobacteriaceae*, which consists of gram-negative facultatively anaerobic rods. The species *E. coli* includes normal inhabitants of the gastrointestinal tract and strains causing a broad variety of intestinal and extraintestinal diseases in swine.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Escherichia coli are gram-negative, peritrichously flagellated rods of variable length and with a diameter of about 1 µm. Colonies on solid media reach their full size within 1 day of incubation and vary from smooth to rough or mucoid. A wide range of selective media is available for growth of *E. coli*. Hemolysis, although not itself a significant virulence factor in enterotoxigenic *E. coli* (ETEC) and edema disease *E. coli* (EDEC), is frequently used as a marker for pathogenicity in isolates of these pathotypes producing the F4 (K88) or F18 adhesin and certain isolates producing F6 (987P). All other ETEC and an increasing number of ETEC isolates producing F4 from pigs are nonhemolytic.

Species identification may be on biochemical characteristics, although there is no differentiating biochemical test for which 100% of strains give a positive reaction. Commercially available identification kits therefore make use of up to 50 tests to achieve a high level of accuracy. Many larger diagnostic laboratories now use MALDI-TOF mass spectrometry that generates a spectral signature that is compared with a database for the identification of the microorganism. The determination of DNA relatedness, the scientific base of discrimination between species, is restricted to research laboratories.

Classification

Over the decades, several classification systems have been developed to subdivide the species into types. Serotyping is an established typing system to identify pathogenic *E. coli* strains, and serotypes have shown good association with certain virulence traits. Complete serotyping includes determination of the surface O (somatic; polysaccharide), K (capsular or microcapsular), H (flagellar), and F (fimbrial) antigens. Unlike salmonellae, only a small percentage of *E. coli* isolates are typable with available antisera, since serotyping has been limited to isolates of proven or suspected pathogenicity. Presently, at least 188 O, 103 K, 56 H, and over 20 F antigens are officially recognized. A serogroup refers to strains that share only one of these antigens (e.g. serogroup O149), whereas a serotype refers to strains sharing a combination of these antigens (e.g. serotype O149:H10). Although certain porcine pathogenic *E. coli* belong to a limited number of serotypes, serotyping is less used today for diagnostic purposes having been replaced by direct detection of genes coding for bacterial traits involved in pathogenesis of disease, called virulence factors. The term pathotype is used to identify types of *E. coli* on the basis of their virulence mechanism as indicated by the presence of particular virulence factor combinations, which characterize the way in which disease is caused. This system identifies broad classes or categories of pathogenic *E. coli*, such as ETEC; Shiga toxin-producing *E. coli* (STEC), which includes the EDEC and enterohemorrhagic *E. coli* (EHEC); enteropathogenic *E. coli* (EPEC); and extraintestinal pathogenic *E. coli* (ExPEC) (Gyles and Fairbrother 2010). Detection of virulence factors is important for the identification of pathogenic *E. coli*, and the term virotype is now used to refer to the combination of these factors for a particular isolate. Table 52.1 summarizes important pathotypes of *E. coli*

Table 52.1 Important pathotypes, adhesins, toxins, and serogroups of pathogenic *E. coli* in pigs.

Pathotype	Adhesins	Toxins	O serogroups	Diseases
ETEC	F5(K99), F6(987P), F41	STa	O8, O9, O20, O64, O101	Neonatal diarrhea
	F4(K88)	STa, STb, LT, EAST1, α-hemolysin	O8, O138, O141, O145, O147, O149, O157	Neonatal diarrhea Diarrhea in young pigs preweaning
	F4(K88), AIDA, unknown	STa, STb, LT, EAST1, α-hemolysin	O8, O138, O139, O141, O147, O149, O157, O?:K48	Post weaning diarrhea
	F18, AIDA	STa, STb, LT, Stx(VT), EAST1, α-hemolysin	O8, O138, O139, O141, O147, O149, O157	
EPEC	Eae (intimin)		O45, O103	
STEC (VTEC)	F18, AIDA	Stx2e(VT2e), EAST1, α-hemolysin	O138, O139, O141, O147	Edema disease
	Eae (intimin)	Stx1 and/or Stx2	O157	None in pigs, bloody diarrhea and hemolytic uremic syndrome in humans
ExPEC	P, S	CNF	O6, O8, O9, O11, O15, O17, O18, O20, O45, 060, O78, O83, O93, O101, O112, O115, O116	Colisepticemia/polyserositis
	P, S	CNF	O1, O4, O6, O18	Urogenital infection

and their common virulence factors and serogroups as well as diseases they cause.

Enterotoxigenic *E. coli* (ETEC)

ETEC is the most important pathotype in pigs and includes strains that elaborate one or several enterotoxins that induce secretory diarrhea (Fairbrother et al. 2005; Gyles and Fairbrother 2010). Two major classes of enterotoxin are produced by porcine ETEC: heat-stable toxin (ST) and heat-labile toxin (LT). Heat-stable toxin has been further divided into STa (also called STI, ST1, or STaP) and STb (also called STII or ST2) based on solubility in methanol and biological activity. Likewise, two subgroups of LT, LTI and LTII, have been described. The enteroaggregative heat-stable toxin 1 (EAST1) is widespread among porcine ETEC. The role of this toxin in porcine diarrhea remains controversial as, alone, EAST1 does not seem capable to produce disease, but together with LT it is efficient in producing diarrhea. The *E. coli* enterotoxins and their activity have been reviewed in detail elsewhere (Dubreuil et al. 2016; Gyles and Fairbrother 2010).

ETEC must attach to the apical surface of small intestinal mucosal epithelial cells or the adjacent mucus layer in order to colonize and produce a combination of enterotoxins in the microenvironment of the glycocalyx. The level of intestinal colonization determines whether or not disease will result from infection. Bacterial cells attach by fimbrial adhesins that are hairlike appendages composed of protein subunits, extending from the bacterial cell (Figure 52.1). Fimbriae are classified by serologic reactivity and over 20 types have been identified. Of these, F5 (K99), F6, and F41 are generally isolated from neonatal diarrhea, F4 from both neonatal diarrhea and PWD, and F18 from PWD and ED.

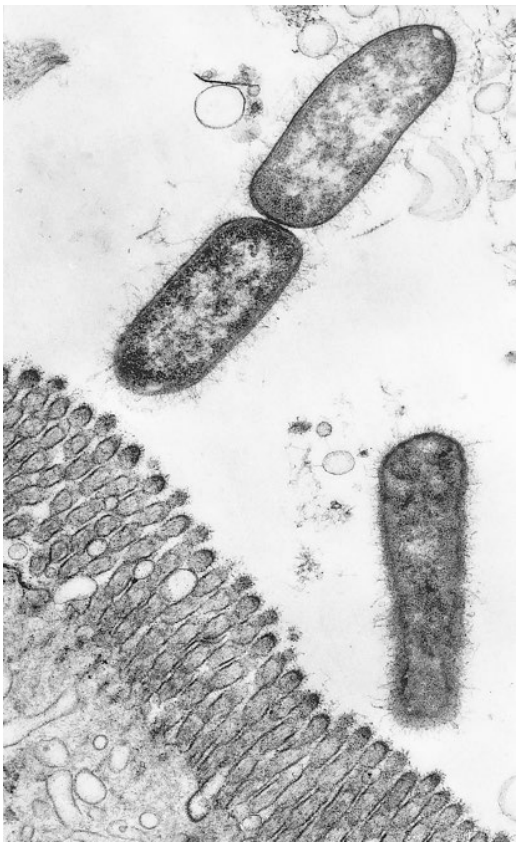


Figure 52.1 Electron micrograph of fimbrial attachment typical of enterotoxigenic *E. coli* (ETEC) in the intestine. Hairlike fimbriae extend from the surface of bacterial cells that are located approximately half a bacterial width away from the microvilli of intestinal epithelial cells.

Neonatal ETEC

ETEC that cause neonatal diarrhea usually produce only STa and may have one or more of the fimbriae F4, F5, F6, and F41. The prevalence of F4-, F5-, and F6-fimbriated ETEC from neonatal diarrhea cases from various studies was recently reviewed, showing both temporal and geographic variations (Dubreuil et al. 2016). Of these, the F4-ETEC most often belong to the serogroups O149, O8, O147, and O157, and the F5-ETEC, F6-ETEC, and F41-ETEC to serogroups O8, O9, O64, and O101 (Gyles and Fairbrother 2010).

Post weaning ETEC

ETEC that cause diarrhea in post weaning or older suckling pigs typically produce one or more of the enterotoxins including STa, STb, LT, and EAST-1 (Zhang et al. 2007). These PWD strains usually have either F4 or F18 as fimbrial adhesin (Fairbrother et al. 2005; Francis 2002; Frydendahl 2002; Luppi et al. 2016; Mainil et al. 2002; Zhang et al. 2007). However, some F4- and F18-negative PWD virotypes have been identified, the most important of which are shown in Table 52.2 (Do et al. 2006; Frydendahl 2002; Jahanbakhsh et al. 2016; Liu et al. 2014; Wang et al. 2011). The role of these F4- and F18-negative virotypes in the development of diarrhea has not yet been established. ETEC isolates of the STb or STb:EAST-1 virotypes from weaned pigs may also produce a non-fimbrial adhesin involved in diffuse adherence (AIDA-I) (Mainil et al. 2002; Ngeleka et al. 2003), originally detected in *E. coli* isolates from humans with diarrhea.

The fimbriae F4 and F18 have several variant subtypes based on antigenic differences. For F4 variants ab, ac, and ad have been described. However, almost all belong to F4ac and are often referred to simply as F4. For F18 there are two known variants, ab and ac. Though early studies associated the F18ac variant with ETEC causing PWD and F18ab with F18-ETEC causing ED, with some exceptions, a more recent report showed that both variants may be involved in causing PWD or ED in pigs (DebRoy et al. 2009). At present in PWD, most F4-positive isolates

Table 52.2 Common serovirotypes of pathogenic *E. coli* from pigs with post weaning diarrhea or edema disease.

Fimbrial adhesin	Serovirotypes
F4	O149:LT:STb:EAST-1, O149:LT:STa:STb:EAST-1, O149:LT:STb, O?:STa:EAST-1:Stx2e
F18	O149:LT:STb:EAST-1, O138:STa:STb, O138:LT:STb:EAST1:Stx2e, O139: Stx2e:(AIDA), O147:STa:STb:AIDA, O?:STa:STb, O?:STa:STb:Stx2e
Not known	O?:STa:STb, O?:STb:EAST-1:AIDA, O?:LT:STb:EAST-1:AIDA, O45/O?:Eae:(EAST-1)

Note: Virulence factors in parentheses are not always present when tested and are not always tested for in all laboratories.

are O149, whereas F18 isolates are more heterogeneous and include serogroups O139, O138, O141, O147, and O157. Common serovirotypes of pathogenic ETEC from pigs with PWD are listed in Table 52.2.

Certain strains produce enterotoxins and Stx2e (see STEC below), as well as either of the fimbrial variants F18ab and F18ac. These strains are classified as ETEC rather than STEC, since they produce clinical PWD more than ED. Mixed infections of F18-STEC and F4-ETEC may be observed. In these cases, the predominant clinical sign is often diarrhea caused by the F4-ETEC, although histopathological evidence of ED may be present. Mixed infections of F18-ETEC and F4-ETEC are also observed.

ETEC may also be associated with secondary septicemia, particularly in older piglets. These isolates most frequently belong to the serovirotypes listed in Table 52.2.

Enteropathogenic *E. coli* (EPEC)

Another pathotype of *E. coli* found in pigs with PWD is known as EPEC. EPEC were initially associated with diarrhea in children, especially in developing countries. These bacteria do not attach by fimbria; rather they have a complex secretion system that injects over 20 effector proteins into the host enterocyte, leading to intimate adherence of the bacteria to the host intestinal epithelium and to development of a characteristic "attaching and effacing" (AE) lesion (Figure 52.2). EPEC, which induce formation of AE lesions, are called attaching and effacing *E. coli* (AEEC). EPEC from different animal species may have different virulence factors, but all possess a variant of the AE factor Eae or intimin, a bacterial outer membrane protein adhesin that is responsible for intimate attachment. Hence, the presence of Eae (intimin) is indicative of an EPEC. EPEC do not possess any virulence factors of classic ETEC PWD or ED strains (Zhu et al. 1994).

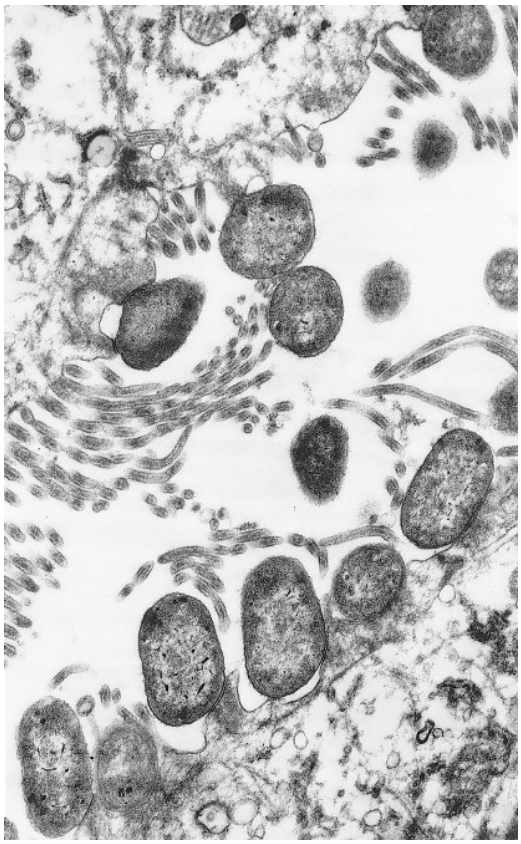


Figure 52.2 Electron micrograph of intimate attachment by enteropathogenic *E. coli* (EPEC) and typical attaching and effacing (AE) lesions. Bacteria attach intimately to the apical intestinal epithelial cell membrane, induce dense regions in the apical cell cytoplasm, and efface cell microvilli.

Shiga toxin-producing *E. coli* (STEC), edema disease *E. coli* (EDEC), and enterohemorrhagic *E. coli* (EHEC)

STEC produces one or more of a family of cytotoxins that are known as Shiga toxins (Stx) or verotoxins (VT) (Mainil 1999). The two names are used interchangeably in the literature. Many STEC without fimbriae are probably not pathogenic, but are present in the normal intestinal microbiota in pigs. In pigs the most important STEC are those that cause ED; these are known as EDEC. These strains produce the Shiga toxin variant Stx2e (VT2e) and may possess the fimbrial variant F18ab or F18ac (DebRoy et al. 2009). Common serovirotypes of EDEC are listed in Table 52.2. Another subgroup of STEC, also known as EHEC, is highly pathogenic in humans (e.g. O157:H7). Most EHEC are AE, also possessing Eae and the same secretion system as EPEC.

Some zoonotic EHEC are occasionally recovered, at a low rate, from pigs (see section "Public Health").

E. coli causing fatal shock

Enteric colibacillosis complicated with shock also occurs in young pigs before and after weaning. *E. coli* associated with this disease are usually F4-ETEC (serogroup O149, O157, or O8) (Faubert and Drolet 1992) or Stx2e-producing *E. coli* that are associated with ED.

Extraintestinal pathogenic *E. coli* (ExPEC)

ExPEC are a heterogeneous group of *E. coli* so named because their normal habitat is in the intestinal tract yet they are able to invade, cause bacteremia, and induce septicemia or localized extraintestinal infections such as meningitis or arthritis (Fairbrother and Ngeleka 1994). In contrast to ETEC, EPEC, and STEC, they are not characterized by a constant group of virulence factors. Rather they possess a large number of virulence factors that vary greatly between strains. They often possess fimbrial antigens of the P, S, and F1C families that contribute to bacterial colonization (Dozois et al. 1997) as well as cytotoxins such as hemolysin and cytotoxic necrotizing factor (CNF). They usually contain one and sometimes several iron capturing systems, such as aerobactin, which permit them to survive in the blood and other tissues outside the intestines (Gyles and Fairbrother 2010). ExPEC possess lipopolysaccharides (LPS) (O-antigen) and capsules (K-antigens), which protect the bacteria against killing by serum complement and phagocytes.

Serogroups O6, O8, O9, O11, O15, O17, O18, O20, O45, O60, O78, O83, O93, O101, O112, O115, and O116 have most commonly been identified in isolates associated with septicemia (Gyles and Fairbrother 2010).

Coliform mastitis (CM) *E. coli*

CM in sows appears to be from fecal contaminants and is noncontagious. Multiple serological types of *E. coli* isolates from cases of mastitis may be found within a herd, between distinct glands of one sow, or even between subcomplexes within one gland (Awad-Masalmeh et al. 1990; Morner et al. 1998). Heterogeneity of mastitis isolates has also been demonstrated by random amplified polymorphic DNA genotyping (Ramasoota et al. 2000). This great variety of coliform bacteria associated with CM indicates an abundant reservoir of potentially pathogenic bacteria. One study demonstrated the presence of ExPEC virulence genes in *E. coli* isolates from both CM cases and healthy sows, suggesting a role for other factors in CM (Gerjets et al. 2011).

Nonspecific urinary tract infection (UTI) *E. coli*

One or more of a number of bacterial species may cause nonspecific UTI, including *E. coli*. *E. coli* isolates from UTI in pigs have not been well characterized. Isolates from pyelonephritis in pigs differ from those of human uropathogenic isolates with respect to virulence profile, being rarely hemolytic and less frequently possessing P and F1C fimbriae (Krag et al. 2009).

Genetics of virulence

In most *E. coli* diseases, virulence genes determine pathogenicity and are encoded by plasmids, bacteriophages, or pathogenicity islands (PAIs) (Gyles and Fairbrother 2010). These genes are plasmid-encoded for enterotoxins and fimbriae or pili, phage-encoded for Stx, and PAI-encoded for the AE lesion. In strains from most extraintestinal infections, however, the genes encoding for fimbriae, cytotoxins, and hemolysin are chromosomally located. In the laboratory plasmids can easily be transmitted from donor to recipient strains. However, such exchanges of genetic material do not appear to play a major role in the field as the genetic makeup of pathogenic *E. coli* strains is remarkably stable. This may be because a whole set of virulence factors is involved in the virulence of a particular strain, and certain recipient strains may not express transmitted plasmid-determined functions. The clinically important development of antimicrobial resistance is an exception to this observation.

Public health

Certain EHEC, particularly EHEC O157:H7, O26, and other non-O157 serogroups, may be present sporadically in the intestines and feces of normal pigs and are zoonotic, as they may cause bloody diarrhea, hemorrhagic colitis, and/or hemolytic uremic syndrome in humans infected through food or water contaminated by animal feces (Fairbrother and Nadeau 2006). Pigs are not considered a major source of O157 EHEC, the prevalence rate being usually very low. Cattle and other ruminants are the main reservoir of zoonotic EHEC. ED bears some similarity to the human diseases caused by EHEC, which produce closely related Shiga toxins. However, the human EHEC strains colonize the intestine by a mechanism distinct from EDEC, and the Shiga toxins target different organs (Gyles and Fairbrother 2010). Serotypes associated with ED are also different from those of EHEC that cause disease in humans.

Escherichia coli of the intestinal ecosystem, both commensal and pathogenic, may acquire antimicrobial resistance following administration of antimicrobials, whether in the feed, water, or by injection, for the purpose of promoting growth, improving feed efficiency, preventing diseases (prophylaxis), or as treatment, including metaphylaxis, for bacterial infections. These strains may be resistant to antimicrobials that are preferred treatments for serious infections in humans and

for which limited alternatives are available, referred to as critically important or very high importance antimicrobials, such as third- and fourth-generation cephalosporins (e.g. ceftiofur) and fluoroquinolones (e.g. enrofloxacin). Such strains constitute a public health threat when they enter the food chain.

Epidemiology

Escherichia coli infections are widespread, occurring in both industrialized and developing countries and in temperate, subtropical, and tropical climates. Diarrhea (neonatal ETEC, PWD ETEC, and EPEC), ED due to EDEC, systemic infections due to ExPEC, CM, and UTI occur in pigs in all countries where pigs are raised commercially.

The primary habitat of *E. coli* in the pig is the gastrointestinal tract, and *E. coli* is the dominant aerobe in this habitat. *E. coli* populations in the pig fecal microbiota and in the farm environment are highly dynamic and show high levels of diversity (Marchant and Moreno 2013). Dominant strains change at intervals from 1 day to several weeks, leading to successive waves of dominant strains (Katouli et al. 1995). It has been reported that the *E. coli* microbiota is highly structured, and that different compartments of the gut are preferentially colonized by distinct phylogenetic *E. coli* clones (Dixit et al. 2004), though another study suggested that porcine *E. coli* populations might not be as structured (Schierack et al. 2007). Numbers in the large intestine average around 10^7 colony-forming units (CFU)/g of contents; however, *E. coli* contribute less than 1% to the total bacterial count.

Escherichia coli are found in fecal-contaminated feed, water, soil, and the environment of the pig barn. Long survival times in the environment are promoted by low temperature and sufficient moisture, among other factors. In slurry samples, a porcine *E. coli* O139:K82 strain remained viable for more than 11 weeks (Burrows and Rankin 1970). The spread of pathogenic *E. coli* is presumed to mainly occur via other pigs and contaminated barn environment, but also via aerosols, contaminated feed and water, contaminated trucks that transport pigs, other vehicles, and possibly other animal species. Airborne transmission between pigs in wire cages 1.5 m apart was repeatedly observed in transmission experiments with an F4-ETEC strain (Wathes et al. 1989).

Intestinal infections caused by ETEC, EDEC, and EPEC are considered contagious. The same strain is usually found in many sick pigs and often in consecutive batches of pigs. A more recent phenomenon is the emergence in sick pigs of a more pathogenic Stx-producing F18-ETEC lineage demonstrating a high level of multidrug resistance, thus representing a high risk to swine production (Kusumoto et al. 2016). In contrast, infections caused by ExPEC and *E. coli* causing CM and UTI do not behave as communicable diseases. Mixed infections by more than one strain are frequent and are acquired by invasion of preexisting intestinal bacteria in the case of ExPEC and from fecal and/or environmental contamination of teats and the urethra in the case of CM and UTI.

Routine cleaning and disinfection are usually insufficient to break the cycle of infection by *E. coli* (Hampson et al. 1987). Under experimental conditions, however, transmission can be prevented by strict hygienic measures (Kausche et al. 1992).

There are only limited data on the susceptibility of *E. coli* isolates to commonly used disinfectants. According to a Danish study, *E. coli* fecal isolates from livestock did not appear to have developed resistance to benzalkonium chloride, H_2O_2, chlorhexidine, formaldehyde, or zinc chloride (Aarestrup and Hasman 2004). However, Beier et al. (2005) demonstrated that reduced chlorhexidine susceptibility in virulent *E. coli* isolates from newborn pigs with diarrhea correlated with resistance to gentamicin and streptomycin. These findings underline the potential for transfer of this resistance and the possible impact of environmental contamination.

Neonatal *E. coli* diarrhea

Neonatal *E. coli* diarrhea is observed most commonly in pigs aged from 0 to 4 days and is caused by ETEC. The newborn pig, on leaving the uterus and before reaching the teats of the sow, encounters the heavily contaminated environment of the farrowing crate and the skin of the dam, resulting in ingestion of microbes from the intestinal microbiota of the sow. In conditions of poor hygiene or in a continuous-farrowing system, buildup of pathogenic strains increases risk for an outbreak.

Pathogenesis

In the presence of the appropriate predisposing environmental conditions and host factors, ETEC proliferate in the intestine and cause disease by means of specific virulence factors (Table 52.1; see also etiology above). Risk factors for the development of various *E. coli* diseases in pigs are summarized in Table 52.3. ETEC must be able to adhere to and colonize the intestinal mucosa to permit the release of sufficient enterotoxin to cause diarrhea. Adherence to specific receptors on mucosal epithelial cells and in the adjacent mucus layer is mediated by fimbria on the bacterial surface (Figure 52.1).

ETEC affecting neonates mostly colonize the posterior jejunum and ileum. Piglets are most susceptible to infection with F5-ETEC and F6-ETEC during the first several days of life and subsequently become more resistant. This susceptibility could be related to a reduction of the

Table 52.3 Risk factors for development of *E. coli* diseases.

	Etiology	Risk factors		
Diseases	***E. coli* pathotype + others**	**Host**		**Environment**
Neonatal diarrhea	F4(K88)-, F5(K99)-, F6(K987P)-, F41-ETEC	• Susceptibility to F5(K99) and F6(987P) high; decreases with age • Susceptibility to F18 low; increases with age • No effect of age on susceptibility to F4(K88) • First gestation: low levels of colostral antibodies		Ambient temperature less than 25 °C (77 °F)
Septicemia	ExPEC	• Low transfer of colostrum		
Young pig diarrhea	F4(K88)-ETEC, rotavirus, coccidia, TGE virus			
Enteric colibacillosis complicated by shock	F4(K88)-ETEC	• Some pigs resistant to F18 due to lack of receptor		
Edema disease	F18-STEC	• Up to 50% of pigs may be resistant to F4 due to lack of receptor • Earlier weaning age • Stress • Loss of specific antibodies from milk	Rapidly growing pigs	• High protein diet • Transportation • Mixing of pigs • Diet changes • Diet elements – Low level of milk and other products of animal source – Certain ingredients such as soybeans – Presence of other infections, such as PRRSV or PRV
Post weaning diarrhea	F4(K88)-, F18-ETEC AIDA-ETEC, EPEC, mixed *E. coli* pathotypes			
Urogenital infection	ExPEC	• Last period of pregnancy • Parturition • Trauma at coitus		

number of receptors present on intestinal epithelial cells with age or inhibition of colonization by preferential binding of bacteria to F6 receptors present in the mucus rather than to those on the intestinal epithelium. Not all pigs have epithelial cell receptors for F4 and are thus resistant to infection by F4-ETEC (van den Broeck et al. 1999). This genetic resistance is inherited in a simple Mendelian way with the allele for the receptor being dominant. A similar genetic resistance has not been observed for the other fimbriae (F5, F6, F41) of neonatal porcine ETEC.

ETEC adhering to the intestinal mucosa produce enterotoxins that change the water and electrolyte flux of the small intestine and may lead to diarrhea if the excess fluid from the small intestine is not absorbed in the large intestine. Excessive net secretion leads to dehydration, metabolic acidosis, and eventually death. Most strains of neonatal ETEC produce STa, which is active in the intestine of infant mice and young piglets of less than 2 weeks of age, but is less active in older pigs. This could be due to differences in the concentration of intestinal receptors with age. Based on the concentration and affinity of the STa receptors, the posterior jejunum appears to be the major site of hypersecretion in response to STa.

In suckling pigs, the severity of ETEC-induced disease depends on antibody titers in the milk of the sow (Sarmiento et al. 1988b). The colostrum and the milk may contain nonspecific bactericidal factors and specific antibody (mainly IgG and IgA, but also IgM) that inhibit the adherence of pathogenic *E. coli* to the intestine. If the dam has not been vaccinated or exposed to the pathogenic *E. coli* present in the environment of the piglets, specific antibodies are not present in the colostrum and the milk, and suckling piglets are susceptible to intestinal infection. Similarly when individual piglets do not have access to colostrum, due to injury or inability to compete or due to agalactia or insufficient teats of the sow, they are more susceptible to infection.

Low ambient temperatures in the farrowing house also impact severity of disease. In pigs kept at temperatures of

less than 25 °C (77 °F), intestinal peristaltic activity is greatly reduced, and passage of bacteria and protective antibodies through the intestine is delayed (Sarmiento 1983). Increased numbers of pathogenic *E. coli* in the intestinal tract of these pigs result in a more severe diarrhea than in pigs kept at 30 °C (86 °F).

Clinical signs

Clinical signs and age most commonly affected are summarized for the various diseases caused by *E. coli* in Table 52.4. Neonatal diarrhea may first be observed 2–3 hours after birth and may affect one or a few pigs or whole litters. Gilt litters are more often affected than sow litters. Morbidity in an affected herd is extremely variable. The average is 30–40%, but may be as high as 80% in some herds. Mortality can reach 70% in affected litters. In a low proportion of pigs, the infection may be so rapid that death occurs before the development of diarrhea.

Diarrhea may be very mild with no evidence of dehydration or may be clear, watery, and profuse. The feces vary in color from clear to whitish or various shades of brown. In very severe outbreaks, a small proportion of affected animals may vomit. In severe cases, 30–40% of total body weight may be lost and result in signs of dehydration. Abdominal musculature may be flaccid, pigs may be sluggish with sunken eyes, and skin may be bluish gray in color and parchment-like in texture. The loss of fluid and weight results in the exaggeration of bony prominences. These animals usually die. In more chronic or less severely affected cases, the anus and perineum may be inflamed from contact with the alkaline diarrheic fecal material. Pigs with less severe dehydration may continue to drink and, if treated appropriately, recover with only minimal long-term effects.

Lesions

Few specific lesions are observed. Gross lesions may include dehydration, dilation of the stomach (which may contain undigested milk curd), venous infarcts on the greater curvature of the stomach, and dilation of the small intestine with some congestion of the small intestinal wall.

Microscopically, layers of *E. coli* may be observed adhering in patches to the mucosal epithelial cells of the jejunum and ileum. Adhering bacteria may be found only in the crypts of Lieberkühn, or more often covering the crypts and the tips of the villi. Other lesions sometimes observed include vascular congestion in the lamina propria with hemorrhages into the intestinal lumen, increased numbers of neutrophils and macrophages in the lamina propria, and mild villous atrophy.

Table 52.4 Association of clinical signs, prevalence, and age with various clinical diseases due to *E. coli*.

	Age period[a]				
Clinical disease	**Newborn[b]**	**Suckling**	**Post weaned**	**Grow–finish**	**Adult**
Neonatal diarrhea[c] Severe watery diarrhea, dehydration, mortality up to 70% in affected litters	■				
Septicemia Shock, depression, death, polyarthritis	■				
Young pig diarrhea Low mortality, diarrhea of moderate severity, decreased weight gain		■			
Enteric colibacillosis complicated by shock Rapid death, cyanosis of extremities, diarrhea		■			
Edema disease[c] Sudden death, possibly paralysis, and eyelid edema, sporadic mortality up to 65%			■	■	
Post weaning diarrhea[c] Initially deaths, with severe to moderate diarrhea, decreased weight gain, mortality up to 25% in untreated animals			■	■	
Urogenital infection Sporadic cystitis often after mating, pyelonephritis 2 weeks postpartum					■
Coliform mastitis First several days following farrowing, generally short duration. Clinically similar to lactational failure					■

[a] Most commonly affected age periods shaded.
[b] First several days.
[c] Most important clinical diseases.

Diagnosis

Neonatal diarrhea caused by ETEC must be differentiated from other common infectious causes of diarrhea including *Clostridium difficile* and *Clostridium perfringens* types A and C (Chapter 51); transmissible gastroenteritis virus (TGEV) (Chapter 31); porcine epidemic diarrhea virus (PEDV) (Chapter 31); rotavirus groups A, B, and C (Chapter 43); and PRRSV (Chapter 41). In suckling pigs 5 days and older, *Isospora suis* (Chapter 66) must also be considered. It may be helpful to determine fecal pH as ETEC produces alkaline feces, whereas malabsorptive diarrheas produced by TGEV, PEDV, and rotaviruses produce acidic feces.

Diagnosis of neonatal ETEC is based on clinical signs, microscopic lesions, and the presence of small gram-negative rod-shaped bacteria colonizing the small intestinal mucosa. Colonization can be visualized in formalin-fixed, paraffin-embedded tissues by routine histopathology, or *E. coli* organisms can be definitively identified and visualized using immunohistochemistry or in frozen sections using indirect immunofluorescence. This diagnosis is strengthened by the isolation from rectal swabs or intestines of *E. coli* possessing appropriate virulence factors. Criteria used to identify causative *E. coli* in diarrhea in pigs are summarized in Table 52.5.

Swabs or samples of intestinal contents should be inoculated onto blood and MacConkey agar or other media, which are selective for *Enterobacteriaceae* and allow differentiation of lactose fermenting from lactose-non-fermenting gram-negative enteric bacilli. Use of transport medium such as alginate swabs or Stuart's medium should be considered if isolation could not be done within 24 hours.

Morphology and lactose fermentation on MacConkey agar are a first indication of potential *E. coli*. To identify the species as *E. coli*, it is essential to determine the capacity of colonies to transform indole, since 99% of *E. coli* strains are indole positive. Identification can be completed by the citrate assay (*E. coli* are not able to use citrate as the only carbon source) and by the methyl red assay.

A diagnostic laboratory can use OK typing sera (serotyping) against serogroups most prevalent in its geographical region to obtain a rapid presumptive diagnosis. Common O serogroups associated with disease in pigs are shown in Table 52.1. Complete O and H serotyping can only be carried out in a few reference laboratories.

The use of serotyping for primary identification of porcine pathogenic *E. coli* is less relevant today because of the presence of non-typable (NT) pathogenic strains, not all strains of a given serotype are pathogenic, the need of large number of reference antisera, and the growing availability of efficient genetic methods to identify virulence factors. Virotyping, or determination of the virulence factors, is thus a more definitive way of identifying pathogenic *E. coli* (Table 52.1).

The presence of enterotoxins and cytotoxins may be detected by tests for biological activity, which are no longer commonly used in routine diagnosis. Antigen ELISA tests have been developed for the detection of Stx-producing cultures or for direct detection of the Stx-producing bacteria in the feces and may be used more widely in diagnostic laboratories.

Slide agglutination, with or without latex particles, is a simple and easy method to determine the presence of fimbrial adhesins of ETEC, which are expressed in culture media, except F18, which is rarely expressed *in vitro*. This method is commonly used for the identification of F4-ETEC. Antigen ELISA is also used to determine the presence of adhesins. However, bacteria must be grown on the appropriate media for detection of F5 and F41, which are only well expressed in culture media low in glucose or alanine. Other fimbriae, such as F1 and F6, undergo phase variation and may be very poorly expressed after several passages in the laboratory.

Currently, genotypic analysis is more commonly used to define the virotypes involved in an infection. Techniques include colony or DNA hybridization and polymerase chain reaction (PCR) for the detection of genes encoding for virulence factors such as toxins and

Table 52.5 Criteria used to identify causative *E. coli* in diarrhea.

	Pathogenic *E. coli* identified						
	ETEC						
Criterion	F4(K88)	F18	F5(K99), F6(987P), F41	AIDA	F18-STEC	EPEC	ExPEC
Hemolytic colonies	All (not discriminatory)		None		All	None	Some
OK serotyping	Most (some non-ETEC identified)			Not known	Most	Few	Some
F adhesin serotyping	All	Not reliable	Most (not reliable)	Not done	Not reliable	Not done	
Virotyping	All						Some

adhesins (Francis 2002; Frydendahl 2002; Harel et al. 1991; Wray and Woodward 1994). PCR may also be used to detect pathogenic *E. coli in situ* in formalin-fixed, paraffin-embedded tissues.

Immunity

Immunity to enteric *E. coli* infections is humoral and is initially provided through passive immunity via the maternal colostrum and lactogenic antibodies in the milk of the sow and subsequently by active local intestinal immune responses. The concentration of IgG in colostrum is severalfold higher than in sow plasma and declines rapidly during the first 24 hours of secretion, with IgA becoming the dominant immunoglobulin class in milk (Rooke and Bland 2002). The latter protects the gut against *E. coli* infection. Protective immunity is based on the presence of antibodies to surface antigens, especially the fimbrial adhesins F4, F5, F6, and F41. Antibodies to the polysaccharide capsule of ETEC may also be protective. Anti-fimbriae and anti-capsular antibodies function by preventing attachment of the ETEC to enterocytes. Infection with ETEC positive for fimbriae, for example, F5, is unlikely to result in cross-protection against infection with ETEC positive for another fimbria such as F4 or F41 unless some protection is conferred by antibodies against shared capsular antigens.

Prevention and control

Treatment with antimicrobials at the earliest sign of diarrhea may be on an individual or litter basis, by mouth or parenteral injection. It is important to confirm the diagnosis of *E. coli* infection by culture and to perform antimicrobial sensitivity tests because antimicrobial sensitivity varies greatly among *E. coli* isolates. *In vitro* resistance of *E. coli* isolates to a wide range of antimicrobial agents has dramatically increased over the last several years. Commonly used antimicrobials are ampicillin, apramycin, ceftiofur, gentamicin, neomycin, spectinomycin, furazolidone, and potentiated sulfa drugs. An alternative approach to the antibiotic treatment of enteric *E. coli* infection is the use of bacteriophages, an approach that has been successful experimentally (Jamalludeen et al. 2009) but has not been extensively applied in the field.

Fluid therapy, consisting of electrolyte replacement solutions containing glucose given orally, is useful for the treatment of dehydration and acidosis. Drugs that inhibit the secretory effects of enterotoxin, such as chlorpromazine and berberine sulfate, may be useful for the treatment of diarrhea, although many of these drugs have undesirable side effects. The use of such antisecretory drugs as bencetimide and loperamide, alone or in combination with antibacterial agents, has also been suggested (Solis et al. 1993).

The principles of control of *E. coli* neonatal diarrhea revolve around hygiene and management strategies to reduce the buildup of the causative *E. coli* and increasing immunity in sows and piglets. A program for prevention of enteric *E. coli* infection should be aimed at reduction of numbers of pathogenic *E. coli* in the environment by good hygiene, maintenance of suitable environmental conditions, and provision of a plentiful supply of colostrum at birth and a high level of immunity. Strategies commonly used for the control of enteric *E. coli* infections are summarized in Table 52.6.

One of the most important factors in prevention of enteric *E. coli* infection and septicemia is the maintenance of piglets at an adequate environmental temperature, free of drafts, and on a low-heat-conducting floor. This is particularly true for piglets of below average weight, which lose heat more rapidly because they have a greater skin surface area per unit body weight.

Stringent biosecurity should be used to control the introduction of different *E. coli* virotypes or other infectious agents into the herd. Animals in the herd will have little immunity to *E. coli* fimbrial antigens with which they have not had contact.

Good hygiene in the farrowing area leads to a reduction in the numbers of *E. coli* presented to the piglet to a level that it is able to control through its own defense mechanisms. Farrowing crates should be thoroughly cleaned and disinfected between litters, and farrowing rooms should be managed as all-in/all-out with thorough disinfection between farrowing groups. Sow fecal material should be minimized in farrowing crates by sizing crates to sow size that minimize area of fecal contamination and by utilizing perforated flooring that allows fecal material to drop through. Litters farrowed onto such floors have a noticeably lower incidence of diarrhea than those on solid concrete floors.

A dry, warm environment reduces the moisture available for survival and growth of *E. coli*. This is largely affected by ventilation rates, although if room temperature is too high, sows tend to try and spread water over their lying area to cool themselves, thereby defeating other hygienic procedures. The sow should be at a temperature of approximately 22 °C (72 °F), necessitating a warmer creep area for the piglets. It is important to ensure that younger piglets are maintained at a constant temperature of 30–34 °C (86–93 °F) to prevent chilling, which predisposes to neonatal colibacillosis.

Maternal vaccination has been one of the most effective ways of preventing neonatal ETEC diarrhea in piglets. One of the earliest vaccination techniques consisted of taking the small intestinal contents (or scour) from a piglet with diarrhea, culturing it in milk, and feeding the culture to pregnant sows, usually about a month before parturition (Kohler 1974). This technique is effective, conferring an immunity lasting

Table 52.6 Strategies commonly used for the control of enteric *E. coli* infections.

	Strategies that result in:	
	Reduced number of pathogenic *E. coli*	**Increased resistance of animals to infection**
Preweaning diarrhea	• Warmth • Hygiene • Gate and floor design • Quarantine • All-in/all-out • Farrowing	• Maternal vaccination – F4(K88), F5(K99), – F6(987P), F41 • Pig hyperimmune – γ-globulin
Post weaning diarrhea and edema disease	• Increase weaning age • Warmth • Diet – Highly digestible – Milk-based protein – Restricted feed intake • Hygiene • Water additive – Organic acids • Feed supplements – Organic acids – ZnO – Spray-dried plasma – Probiotics • Antimicrobial peptides	• Live oral nontoxigenic F4(K88) and F18 *E. coli* vaccines • Oral powdered egg yolk from F4(K88)- and F18-immunized hens • Stx2e toxoid vaccine (edema disease) • Selection of F4(K88)- and F18-resistant animals

throughout the suckling period, and is still used, particularly in the United States.

Commonly used commercially available vaccines are given parenterally and may be killed whole cell bacterins or purified fimbrial vaccines, with or without LT toxoid. Both types of vaccines appear to work equally well. Bacterins usually contain strains representing the most important serogroups and producing the fimbrial antigens F4, F5, F6, and F41 (Gyles and Fairbrother 2010). Two doses are usually given parenterally at 3 or 4 weeks apart, the second generally administered 2–3 weeks prior to parturition. Most vaccines comprise antigens for other pathogens, such as for *C. perfringens*, and rotavirus. Some vaccines may include antigens for the different F4ab, F4ac, and F4ad variants. However, it is not clear that these vaccines would be more efficient than others including only F4ac since most cases are due to F4ac-ETEC and immunologic cross-reactivity between the three variants is reported. One sow vaccine including also bacterins of F18ab and F18ac *E. coli* has been recently approved in several European countries. In cases where vaccination is ineffective, it is important to identify the serotype of the isolated causative strain for possible inclusion in an autogenous bacterin. Further characterization of these isolates may identify new or variant fimbrial adhesins important in the pathogenesis of ETEC diarrhea.

Post weaning *E. coli* diarrhea and edema disease

Escherichia coli PWD and ED are treated in one section because they often both occur in the same age group of pigs, the causative bacteria share certain virulence factors, and some strains of *E. coli* can cause both diseases. There are also important differences in the two diseases.

Lactogenic passive protection decreases with aging of suckling piglets, and older unweaned piglets can be affected by *E. coli* diarrhea and ED as levels of protective antibody wane in maternal milk; however, loss of milk antibodies at weaning contributes significantly to susceptibility of pigs to PWD and ED (Deprez et al. 1986; Sarmiento et al. 1988b). As a result, most disease is observed post weaning.

PWD and ED are caused by strains of *E. coli* that possess adhesion factors enabling the bacteria to colonize the small intestine and that elaborate one or several protein exotoxins. They may occur independently, but PWD and ED may also occur in a single outbreak or in the same pig.

PWD is most commonly caused by ETEC and mediated by enterotoxins but can also be caused by EPEC that do not possess any virulence factors of classic PWD or ED strains. Common PWD and ED serovirotypes are summarized in Table 52.2, and virulence factors are listed in Table 52.1 and described in the etiology section above. PWD ETEC strains usually have either F4 or F18 variants F18ac or F18ab as fimbrial adhesins, although some F4- and F18-negative virotypes exist. Most PWD F4-positive isolates are serogroup O149, and F18 isolates are O139, O138, O141, O147, or O157. PWD ETEC are observed in pigs worldwide, and in a given geographical area, certain serovirotypes predominate (Do et al. 2010; Fairbrother et al. 2000; Francis 2002; Frydendahl 2002; Liu et al. 2014; Wang et al. 2011). Outbreaks of F4-positive *E. coli* disease tend to involve only one strain at any one time. Occasionally, two potential pathogens are isolated, but one usually predominates in any given outbreak. Exceptionally, in one case, multiple outbreaks in herds involving different serogroups were detected in 47% of 84 herds (Awad-Masalmeh et al. 1988). Mortality is often 1.5–2% of pigs and can reach 25% if no treatment is given.

ED is caused by EDEC that colonize the small intestine and elaborate a Shiga toxin, Stx2e, which enters the bloodstream and damages vessel walls, resulting in edema in targeted tissues. Most notably, cerebral edema leads to the predominant nervous signs characteristic of the disease. Both F18ab and F18ac variants may be involved (DebRoy et al. 2009). Most ED strains belong to serogroups O138, O139, O141, and O147. ED most often manifests as sporadic cases or small outbreaks limited to a specific age group. The case mortality rate ranges from 50 to over 90%, and the course of the disease in the herd varies from 4 to 14 days. The disease typically disappears as abruptly as it appears. Recurrence on the same site is common.

Some F18ab- and F18ac-positive strains of *E. coli* produce both enterotoxins and Stx2e. Pigs infected by one of these strains generally exhibit clinical PWD more than ED. Outbreaks of disease may also be caused by mixed infections of both ETEC and EDEC strains. When this occurs, clinical diarrhea usually predominates even though microscopic lesions of ED may be present.

The age group primarily affected by PWD and/or ED varies based on absolute pig age and diet. There are some differences between *E. coli* with F4 and those with F18. Receptors for F4 are fully expressed on enterocytes of pigs from birth to adult age, rendering pigs of all ages potentially susceptible to infection. F4-positive strains most often cause outbreaks of PWD in the very first days after weaning.

In contrast F18 receptors important in ED and some PWD are expressed age dependently. It was first reported that the F18 receptors are not yet fully expressed by piglets under about 20 days of age (Nagy et al. 1992). However, a more recent study reported that the expression of the F18 receptors in the intestines was found from 10 days of age, increasing gradually with age in the suckling period, and then maintained highly expressed from about 3 weeks of age (Coddens et al. 2007). Hence, *E. coli* with F18 fimbriae do not cause diarrhea or ED in neonatal pigs and usually cause ED or PWD between 5 and 14 days after weaning or after introduction to fattening herds. Fimbrial receptors are subject to modulation by feed lectins such as constituents of leguminous plants (Kelly et al. 1994). It is speculated that feed-induced changes of the receptor are involved in the observed reduction in colonization by F18-positive *E. coli* in the first days after weaning (Bertschinger et al. 1993).

Older suckling pigs may acquire F18 *E. coli* infection from previously contaminated farrowing crates or their dam and develop disease and/or carry it into the weaner unit. The contaminated environment of the weaner unit appears to be also an important source of pathogenic *E. coli* strains. Intestinal infections caused by *E. coli* are contagious and are transmitted to other pigs by the oral route via fecal–oral transmission, contaminated immediate environment, contaminated feed and drinking water, handlers, or aerosol. The same strain is usually found in many sick pigs and often in consecutive batches of pigs (Jorsal et al. 1996). These strains are usually only shed for a few days after infection, probably due to the development of immunity. Not all infected pigs develop disease; the degree of colonization determines whether disease results from infection.

Pathogenesis

Colonization

When ingested in sufficient numbers, *E. coli* causing PWD and/or ED colonize and then proliferate rapidly to attain massive numbers to the order of 10^9/g of contents. For ETEC and EDEC, colonization requires attachment of fimbrial adhesins (mainly F4 and F18) to complementary receptors on the small intestinal epithelium or in the mucus coating in the mid-jejunum to ileum. On the other hand, for EPEC, the eae adhesin (intimin) binds to its complementary receptor on the apical surface of host epithelial cells in the small and large intestines, with heaviest colonization in the duodenum and cecum.

Epithelial cell receptors for pathogenic ETEC and EDEC are not present in every pig. Certain pigs do not have receptors for F4 and are thus resistant to infection by F4-ETEC. Receptors are prerequisite for both the development of clinical signs and immune responses with F4-positive *E. coli* (van den Broeck et al. 1999). This genetic resistance to infection is inherited in a simple Mendelian way, and the allele for the receptor is dominant. Subsequent studies have demonstrated up to eight

pig phenotypes (A to H) based on susceptibility of brush borders of different pigs to adherence of isolates, producing the variants F4ab, F4ac, and F4ad (Baker et al. 1997; Hu et al. 1993; Li et al. 2007). The F4 receptors per se are still unknown, and different receptor types have been proposed to play a role in the binding of F4 to enterocytes (Nguyen et al. 2013). The loci encoding porcine intestinal receptors for F4ab and F4ac are closely linked on chromosome 13 (Edfors-Lilja et al. 1995). Susceptibility of pigs to F4ab/ac -ETEC diarrhea has been linked to a polymorphism on MUC4 gene (Goetstouwers et al. 2014). However, the correlation between MUC4 polymorphism and F4 susceptibility is not completely consistent, particularly for resistance, a proportion of pigs with a MUC4-resistant genotype showing intestinal adhesion of F4ac *E. coli* and/or becoming seropositive after immunization with F4 (Goetstouwers et al. 2014; Nguyen et al. 2013; Rasschaert et al. 2007). This strongly suggests the presence of other receptor(s) for F4ac. Other genes on pig chromosome 13 have attracted scientific interest as F4ab/ac receptor, including MUC13, MUC20, and TFRC (reviewed by Xia et al. 2015). It has been shown that pigs carrying F4ab/ac receptors had greater average daily weight gain than pigs exempt of these receptors (Yan et al. 2009).

Specific intestinal receptors for F18 have not yet been fully identified. However, the importance of F18 receptor in colonization of F18 *E. coli* and development of disease was demonstrated; the susceptibility to colonization by F18 *E. coli* is controlled by a dominant allele and resisted by a recessive allele (Bertschinger et al. 1993; Frydendahl et al. 2003). The receptor for F18 fimbriae is controlled in a single locus on chromosome 6 close to the locus for stress susceptibility. Pigs with at least one copy of the dominant allele for the receptor are susceptible to intestinal colonization. Susceptibility to F18 *E. coli* infections was reported to be dependent on the activity of the FUT1 gene, encoding the alpha(1,2)-fucosyltransferase. The association between FUT1 M307 genotypes and adhesion phenotypes was reported to be very strong for both F18ab and F18ac (Frydendahl et al. 2003). The complexity of the F18 receptor expression was recently demonstrated by differential gene expression analysis (Dong et al. 2016).

Other factors may also effect bacterial colonization and severity of disease. Low room temperature in the weaner rooms appears to be responsible for a more severe course of PWD (Wathes et al. 1989). This is likely because chilling reduces intestinal peristaltic activity and enhances bacterial colonization. In contrast, under experimental conditions, ED is not aggravated by cold stress (Kausche et al. 1992). Endogenous as well as orally administered proteases may reduce the receptor activity for F4 fimbriae (Mynott et al. 1996), thus reducing the severity of F4-mediated diarrhea. Several predisposing factors, such as a weaner diets containing soya and field peas or PRRSV infection, may enhance bacterial colonization and development of the AE lesions of EPEC (Neef et al. 1994).

Mechanisms of ETEC post weaning diarrhea

Like neonatal ETEC, post weaning ETEC adhere to the intestinal mucosa and produce enterotoxins, which change the water and electrolyte flux of the small intestine. Excessive secretion leads to dehydration, metabolic acidosis, and possibly death.

Post weaning ETEC strains produce one or more of the enterotoxins STa, STb, LT, and EAST-1. STb is antigenically and genetically unrelated to STa and is poorly immunogenic. LT is a highly immunogenic molecule, and recent studies have shown that LT also promotes adherence of ETEC *in vitro* and *in vivo* (Fekete et al. 2013).

EAST-1 is commonly found in F4-ETEC strains from pigs with diarrhea and in F18:Stx2e strains from pigs with ED (Choi et al. 2001). The mechanism of action of EAST-1 is proposed to be identical to that of STa. Overall, most studies suggest that EAST1 toxin alone does not induce diarrhea in the animals tested (Dubreuil et al. 2016).

Mechanisms of EPEC post weaning diarrhea

EPEC use a type III secretion system (T3SS) to translocate bacterial effectors directly into the intestinal epithelial cells. Porcine EPEC attach to the intestinal mucosa and cause lesions similar to those observed for EPEC isolated from human infantile diarrhea (Hélie et al. 1991). They attach intimately to the intestinal epithelial cell membrane by means of a bacterial outer membrane protein termed "EPEC attaching and effacing factor" (Eae) or "intimin." Eae is the product of a chromosomal PAI in EPEC composed of over 40 genes that code for proteins involved in intimate adherence to and signal transduction within the host cell (Dean and Kenny 2009; Nataro and Kaper 1998). One of these proteins is the "translocated intimin receptor" (Tir) that is injected into the host cell cytoplasm where it reappears on the host cell surface and acts as receptor for the bacterial intimin (Gyles and Fairbrother 2010). Attached EPEC efface the microvilli and sometimes invade the epithelial cells (Figure 52.2) (Zhu et al. 1994). The mechanisms by which EPEC induce diarrhea are poorly understood. Effacement of microvilli and the consequent loss of absorptive surface area may lead to malabsorptive diarrhea. The rapid onset of diarrhea suggests a more active secretory mechanism that may be due to EPEC signaling activity on intracellular mediators (Gyles and Fairbrother 2010). Other possible mechanisms include an increased permeability of tight junctions between epithelial cells, a localized inflammatory response at the lesion site, or chloride

secretion following polymorphonuclear (PMN) leukocyte transmigration. The clinical outcome of the infection is difficult to evaluate, as mixed infection, such as with F4-ETEC, often occurs.

Mechanisms of edema formation by edema disease *E. coli* (EDEC)

ED is a Stx2e toxemia that results in severe edema in specific sites in pigs that have absorbed Stx2e from the intestine following colonization by an EDEC. Highly purified Stx2e induces a dose-dependent disease indistinguishable from ED when administered intravenously to pigs (MacLeod and Gyles 1990). Colonization by EDEC develops over 3–6 days on the tips and sides of villi in the distal jejunum and ileum and is mediated by plasmid-encoded F18ab or F18ac fimbriae. It has been reported that F18-STEC infection results in a slower colonization of the gut than F4-ETEC, the latter showing a peak of fecal excretion at 2 days postchallenge compared with 3–5 days for F18-STEC (Verdonck et al. 2002).

The Stx2e toxin is part of the AB_5 toxin family and binds to globotriaosylceramide (Gb3) or preferably to globotetraosylceramide (Gb4) receptors. The Stx2e produced by EDEC in the intestine is absorbed into the circulation and causes vascular damage in target organs. The toxin also binds to Gb4 on red blood cells. Thus, vessels are subjected to prolonged toxin exposure (Boyd et al. 1993). The toxin can be detected in endothelial cells of small blood vessels of the intestine and in microvillous membranes of enterocytes at the base of the villi by immunological methods (Waddell et al. 1996). The Stx2e toxin does not appear to be absorbed from the intestine under normal conditions, but the addition of deoxycholate to the intestine allows absorption of Stx2e to occur (Waddell and Gyles 1995), and it is possible that bile could influence absorption. Strains of EDEC may pass from the intestine to the mesenteric lymph nodes and produce Stx2e toxin there, providing another mechanism for absorption of toxin into the blood.

The most consistent injury observed in field cases, after injection of partially purified toxin (Gannon et al. 1989) and in pigs inoculated orally with live EDEC (Kausche et al. 1992), is a degenerative angiopathy of small arteries and arterioles. The edema fluid found in various tissues is low in protein and is the result of a mild increase in vascular permeability.

A distinct type of ED is characterized by terminal bloody diarrhea and hemorrhagic lesions in the cardiac region of the stomach, the ileum, and the large intestine (Bertschinger and Pohlenz 1983). Gannon et al. (1989) observed acute hemorrhagic gastroenteritis in some of the pigs to which a high dose of Stx2e was administered. Epithelial necrosis secondary to necrosis of small arteries and arterioles may be responsible for luminal hemorrhage.

Clinical Signs

Post weaning diarrhea

Diarrhea in pigs from late suckling through the post weaning period is similar to that observed in neonatal piglets but tends to be less severe. Diarrhea is seen as yellowish or grey fluid, lasting up to a week and causing dehydration and emaciation. Over several days, most of the pigs in a group may be affected, and mortality of up to 25% may be observed. Peaks of diarrhea vary between farms but are generally seen in the first 3 weeks after weaning. Severe cases have been observed at 6–8 weeks after weaning when the pigs enter the growing barns.

Edema disease

ED mostly occurs during the first few weeks after weaning, although cases may be observed through to the grower barns. The disease may be sporadic or may affect an entire herd and may be first recognized as sudden death without signs of sickness. Some affected pigs become inappetent, develop swelling of the eyelids and forehead (Figure 52.3), emit a peculiar squeal, and show incoordination and respiratory distress. Some of these pigs will soon become recumbent and die. There is usually no diarrhea or fever. In mild cases, subcutaneous edema is accompanied by pruritus, which disappears after recovery. In some pigs with or without dyspnea, respiration is accompanied by a snoring sound. Watery diarrhea with clots of fresh blood may appear in a few pigs at the terminal stage.

Subclinical ED may occur where pigs are clinically normal but develop vascular lesions and may have a decreased growth rate. Chronic ED occurs in a low proportion of pigs recovering from acute attacks of ED or *E. coli* PWD caused by strains that also produce Stx2e. This condition was called cerebrospinal angiopathy before its association with ED became apparent. For periods varying from days to several weeks after intestinal infection, growth stops, and sick pigs often show unilateral

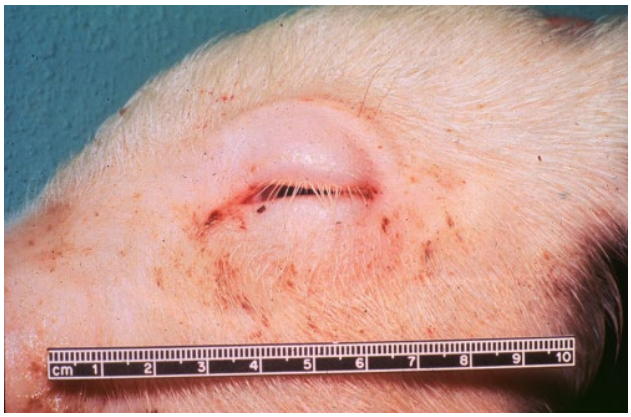


Figure 52.3 Edematous swelling of eyelids in a pig with edema disease. *Source:* Courtesy of Dr. Greg Stevenson.

nervous disturbances such as circling movements, twisting of the head, or atrophy of limb muscles with progressive weakness. Subcutaneous edema is rare.

Lesions

Post weaning diarrhea

Pigs dead from *E. coli* PWD are generally in good condition but severely dehydrated with sunken eyes and some cyanosis. The stomach is often distended with dry feed. The gastric fundus is variably hyperemic. The small intestine is dilated, slightly edematous, and hyperemic. The contents vary from watery to mucoid, with a characteristic smell. The mesentery is heavily congested. Contents of the large intestine most often are light greenish or yellowish and are mucoid to watery. Pigs dying late in an outbreak look emaciated and exhibit a strong smell of ammonia. There are irregularly shaped superficial ulcerations in the gastric fundus and large intestine. The feces may look yellow and pasty. If the causative strain of ETEC also produces Stx2e, lesions typical of ED (see below) are mild or absent.

Microscopic lesions in pigs with ETEC PWD are few. Bacterial layers (gram-negative rods) may be observed in patches on the apical surface of villous epithelial cells in the ileum and, less consistently, jejunum (Sarmiento et al. 1988a), although absence of this observation does not exclude ETEC infection. The mucosa and the epithelium remain normal in appearance; however, increased numbers of neutrophils may be observed in the superficial lamina propria.

Microscopically in pigs infected with EPEC, a multifocal colonization of the brush border of mature enterocytes by *E. coli* arranged in palisades with enterocyte degeneration and light to moderate inflammation of the lamina propria is observed, the latter being mostly in the ileum (Hélie et al. 1991). Colonization is most intense in the duodenum and cecum, bacteria are sometimes observed in intracytoplasmic vacuoles in enterocytes, and colonized enterocytes swell and slough, resulting in mild to moderate villous atrophy in the small intestine. On transmission electron microscopy, bacteria are intimately attached to the cytoplasmic membrane of mature enterocytes and arranged in regular palisades, parallel to the microvilli, with effacement of adjacent microvilli. The bacterial cell wall and the apical cell membrane of the enterocyte are separated by a narrow regular gap of 10 nm at the cupping pedestal, and apical dense regions are seen at attachment sites (Figure 52.2).

Edema disease

Pigs dying of ED are mostly in good condition. Edema is variable in specific sites of predilection and may be absent in some animals. Subcutaneous edema may occur and is most often in the eyelids and face (Figure 52.3). Gelatinous edema that is barely detectable up to 2 cm in thickness in the submucosa of the gastric cardia and occasionally fundus is characteristic. The mesocolon is commonly edematous, and edema of the small intestinal mesentery and gallbladder is sometimes observed. The mesenteric and colic nodes may be swollen, edematous, and congested. Pericardial, pleural, and peritoneal cavities sometimes contain a slight increase of serous fluid that contains strands of fibrin.

The stomach is typically full of dry, fresh-looking feed, and the small intestine is relatively empty. Some think that this is a manifestation of delayed gastric emptying, since some animals have a period of anorexia before death. Colonic contents may be diminished in amount and some pigs may be constipated. There may be varying degrees of pulmonary edema and a characteristic patchy, sublobular congestion. In some cases, this may be the only observable lesion. Cases with laryngeal edema have also been observed. A few epicardial and endocardial petechiae may occur. This lesion must not be confused with mulberry heart disease (Chapter 14) where hemorrhages are also throughout the myocardium.

In some pigs with ED, a form of hemorrhagic gastroenteritis occurs, which is quite different from that described with *E. coli* PWD. In addition to marked edema, the edematous submucosa of the cardiac region of the stomach and the mucosa of the lower small and upper large intestine show extensive hemorrhage. Watery diarrhea with clots of coagulated blood occurs shortly before death in some of these pigs.

Microscopically, patchy layers of bacteria are adherent to the distal jejuna and ileal mucosa early in the course of ED (Bertschinger and Pohlenz 1983). Contrasting with *E. coli* PWD, the colonization has often disappeared when pigs with ED become moribund (Bertschinger and Pohlenz 1983).

The hallmark microscopic lesion of ED is a degenerative angiopathy affecting small arteries and arterioles with associated edema of surrounding tissues (Clugston et al. 1974). Sites of predilection are those mentioned above where edema is observed as well as in the brain. The dense arterial network in the mesocolon adjacent to the colic lymph nodes is frequently affected. Vascular lesions may be subtle, affecting only some segments of arterioles, and difficult to detect in acute cases, but are more readily apparent in surviving pigs or those affected subclinically (Kausche et al. 1992).

Acute changes include necrosis of smooth muscle cells in the tunica media evidenced by pyknotic and karyorrhectic nuclear debris and hyaline change in cytoplasm (Figure 52.4). In the walls of some affected vessels, fibrinoid material is deposited. Endothelial cells may also be swollen. In acute experimental cases, edema of the leptomeninges and perivascular spaces has been demonstrated. Affected vessels in the brain may be surrounded

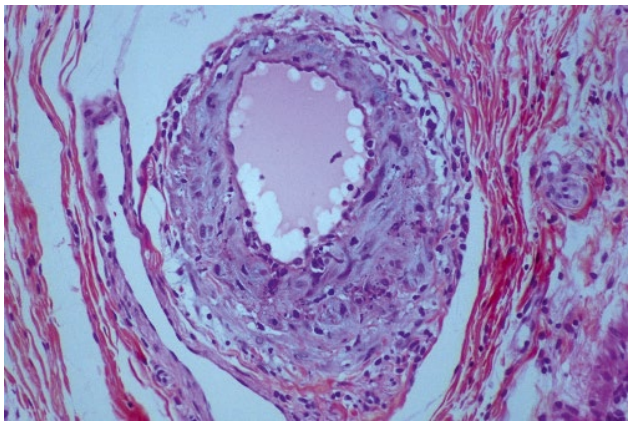


Figure 52.4 Necrosis of smooth muscle cells of the tunica media of a small arterial blood vessel in a pig with edema disease. Note pyknosis and karyorrhexis of smooth muscle nuclei. *Source:* Courtesy of Dr. Richard Drolet.

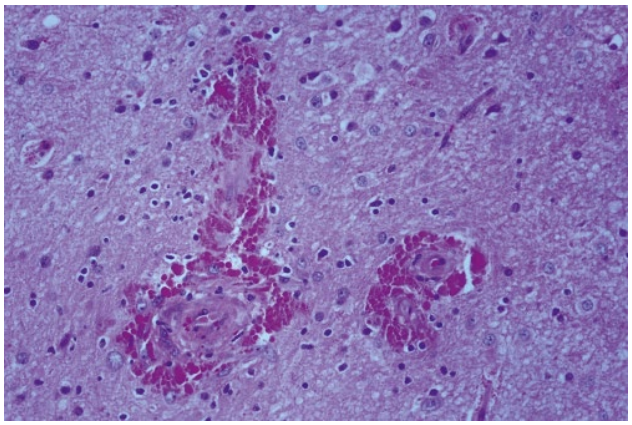


Figure 52.5 Cerebrospinal angiopathy in a pig with edema disease. Note hyaline degeneration of arteriolar walls with perivascular eosinophilic droplets. *Source:* Courtesy of Dr. Richard Drolet.

by eosinophilic, periodic acid–Schiff (PAS)-positive droplets (Bertschinger and Pohlenz 1974) (Figure 52.5). In older lesions, there may be proliferation of adventitial and medial cells. Thrombosis is not usually a feature of uncomplicated naturally occurring ED.

In cases of ED where hemorrhagic gastroenteritis is observed, vessels in affected areas of the gastric, lower small intestinal, and upper colonic wall have changes strikingly similar to those observed in human hemorrhagic colitis caused by EHEC. These include swelling, vacuolation and proliferation of endothelial cells, subendothelial fibrin deposition, medial necrosis, perivascular edema, and microthrombus formation.

In pigs that have recovered from natural outbreaks or survived for several days following acute signs, there may be multifocal encephalomalacia in the brain stem together with typical lesions in the small arteries and arterioles (Kausche et al. 1992). Malacia is thought to be due to ischemia secondary to vascular injury.

Diagnosis

In enteric post weaning *E. coli* infections, a presumptive diagnosis is made based on clinical presentation of diarrhea early after weaning (or sometimes later as previously described), marked dehydration, and low to moderate mortality. Mild diarrhea may be the only clinical presentation in less severe cases. The gross lesions, including the characteristic smell, are also helpful. Differentials should include other common infectious causes of non-bloody diarrhea in weaned pigs including rotaviruses (Chapter 43), TGEV (Chapter 31), salmonellosis (Chapter 59), PEDV (Chapter 31), and, in older weaned pigs, proliferative enteropathy (Chapter 58).

Likewise in ED, a presumptive diagnosis is made based on the sudden appearance of neurologic disease in thriving pigs in the first weeks after weaning. Partial ataxia or a staggering gait is the most consistent sign, and subcutaneous edema in the palpebrae and over the frontal bones is also a cardinal sign when present. Sudden death without clinical signs has been observed in some cases. Characteristic lesions of edema in gastric mucosa and mesentery are helpful when present, but may be absent in a significant number of cases, especially when severe diarrhea has preceded ED. Differentials should include other common causes of nervous signs in weaned pigs including pseudorabies (Chapter 35), teschoviral encephalitis (Chapter 40), *Streptococcus suis* (Chapter 61)- or *Haemophilus parasuis* (Chapter 54)-induced meningitis, and water deprivation/salt intoxication (Chapter 68). ED should be included in the differential diagnostic when sudden death is observed in the first weeks after the weaning.

Definitive diagnosis of ETEC or EPEC PWD and ED is based on the presence of typical lesions and culture of the causative *E. coli* along with confirmation of appropriate virulence factors (Tables 52.1 and 52.2). Criteria used to identify causative *E. coli* in diarrhea in pigs are summarized in Table 52.5. Serotyping continues to be used today in some laboratories, but virotyping (identification of virulence factors) should be performed to confirm the case. Colonization can be visualized in formalin-fixed, paraffin-embedded tissues by routine histopathology, or *E. coli* organisms can be definitively identified and visualized using immunohistochemistry or in frozen sections using indirect immunofluorescence. However, absence of observed colonization does not exclude ETEC or EDEC. Methods for culture and serovirotyping are discussed previously in the section on diagnosis of neonatal ETEC.

Culture of the small intestine in ETEC and small intestine and colon in EPEC and ED usually yields pure or

nearly pure cultures of hemolytic (ED and ETEC) or nonhemolytic (EPEC) *E. coli*. However in ED bacterial numbers may have declined in more protracted cases (Bertschinger and Pohlenz 1983); therefore, a negative bacteriologic result does not exclude the diagnosis of ED. Because all F18 and most F4 *E. coli* causing ED and ETEC PWD are hemolytic, the presence of hemolytic colonies is often used as a rapid means for confirming a presumptive diagnosis of these conditions. However, this method would exclude EPEC and an increasing number of F4-ETEC that produce nonhemolytic colonies on blood agar. This may be an important consideration when putting into place prevention strategies, such as vaccination. In addition, in cases of diarrhea with mixed infections of hemolytic and nonhemolytic ETEC, the assumption that the presence of hemolytic colonies indicated that F4- or F18-ETEC was the only causative agent may result in the nonhemolytic ETEC remaining undetected. Likewise, in cases of diarrhea mixed with subclinical ED, this assumption would result in the ED remaining undetected. These are important considerations in light of the increasing prevalence of mixed pathogenic *E. coli* infections associated with cases of diarrhea, particularly later in infection or in groups with a more endemic presentation.

In cases of subacute or chronic ED or of ED in adults, culture is of little value since causative EDEC are typically no longer a dominant strain in the intestines. Subacute or chronic ED is diagnosed by lesions, especially by the demonstration of subacute to chronic arteriopathy and possibly lesions of focal encephalomalacia. Diagnosis of ED in adult pigs may require additional effort, often requiring postmortem examination and histopathology of more than one pig. Death of adults is occasionally caused by cerebral hemorrhage (stroke) from rupture of severely damaged arterioles, with an apparent affinity for the basal ganglia, especially the corpus striatum.

Immunity

Protective acquired immunity is based on the presence of mucosal secretory antibodies, first IgM and then IgA, to fimbrial adhesins, particularly F4 and F18, in weaned pigs. There are differences in kinetics of infection and immunity in weaned pigs infected with F4- and F18-ETEC (Verdonck et al. 2002). Infection with F4-ETEC results in a more rapid intestinal colonization, induction of anti-fimbrial antibodies, and switch from IgM to IgA and IgG than for F18-ETEC. There are conflicting results concerning cross-protection between strains with fimbrial variants F18ab and F18ac, a first study showing evidence of crosswise anti-colonization immunity between F18ab and F18ac *E. coli* (Sarrazin and Bertschinger 1997), and another one concluding heterologous protection may not be very effective (Bertschinger et al. 2000). However, effective cross-protection has been demonstrated with an oral commercial live vaccine recently approved in Europe. This vaccine comprising a live nonpathogenic F18ac *E. coli* showed protection against a challenge with an F18ab-ETEC.

Protective acquired antibodies are produced against Stx2e in pigs that survive ED. Weiler et al. (1995) showed that pigs that had survived an outbreak of ED had antibodies reacting in an antigen ELISA to the B subunit of Stx2e. Vaccine studies using various forms of Stx2e toxoid have demonstrated protection in ED models (see section on active and passive immunization below).

Prevention and control

Treatment

In post weaning colibacillosis, treatment with antimicrobials and electrolytes should be administered. Sick pigs must be treated parenterally as they eat and drink very little. Subsequently the antimicrobial may be given in the water or feed or as metaphylaxis treatment. Attractive rehydration fluid should be offered to counteract dehydration and acidosis or injected intraperitoneally if the pig is anorectic. Such fluids may contain glucose, glycine, citric acid, and potassium dihydrogen phosphate in an isotonic solution (Bywater and Woode 1980). Uptake should be equal to the loss (i.e. up to 25% of the body weight).

Control of bacterial proliferation therapeutically is much more effective in *E. coli* PWD than in ED, because in ED Stx2e has already been absorbed into the circulation and been bound to receptors when clinical signs become visible. In general, pigs showing neurological signs have a poor prognosis. The development of bacterial resistance against a wide range of antimicrobial drugs makes the efficacy of antimicrobial therapeutics uncertain. Antimicrobial susceptibility testing is indispensable for selection of effective drugs. Antimicrobials that reach therapeutic concentrations in the intestinal lumen, such as amoxicillin/clavulanic acid, fluoroquinolones, cephalosporins, apramycin, ceftiofur, neomycin, or trimethoprim, must be selected. However, antimicrobials identified as "Critically important in human medicine," such as fluoroquinolones and cephalosporins of third and fourth generations, should be used only as a last resort.

For groups of pigs during outbreaks of ED, withholding the feed is thought to impair colonization and is a valuable measure still recommended to prevent new clinical cases. In-feed or water medication, selected by sensitivity testing of isolated EDEC, administered a few days before the onset of ED can reduce the outcome of the outbreak.

Preventive husbandry

Nurseries should be managed as all-in/all-out facilities and should be thoroughly cleaned of organic matter and disinfected prior to use. Water lines and water systems should likewise be disinfected using shock chlorination or a similar chemical process. Management of the weanling pig should minimize environmental and other forms of stress such as unnecessary mixing of litters, chilling, transportation, and assignment to new pens. Recently weaned pigs should be housed in a draft-free environment at a constant temperature of about 29.5 °C (85 °F).

Passive and active immunoprophylaxis

Various strategies involving passive immunity for the prevention of PWD and ED have been used with varying success. An improved weight gain and lower frequency of diarrhea were observed in early weaned (10 days of age) pigs fed a spray-dried porcine plasma-based diet, partly due to the presence of specific anti-ETEC antibodies (Owusu-Asiedu et al. 2002). Similarly, spray-dried porcine plasma had an inhibitory effect on *E. coli* enterotoxemia that lasted only as long as the plasma was fed (Deprez et al. 1990). However, the potential link between spray-dried porcine plasma products and PEDV transmission in North America led to a ban, or voluntary removal, of these products in several North American and European areas (Opreissnig et al. 2014). Passive immune protection against colonization with F4- and F18-positive *E. coli* may be attained by feeding eggs from vaccinated hens or IgY from egg yolk, although effectiveness is not always observed in challenge studies (Li et al. 2015). Antiserum produced by injecting a horse with Stx2e toxoid was effective in protecting pigs from ED in two Danish herds with this disease (Johansen et al. 2000).

Several commercial vaccines are now available for the prevention of *E. coli* PWD and ED. Vaccination against ETEC in pigs has been recently reviewed (Melkebeek et al. 2013). Injectable vaccines, such as those administered to sows for the prevention of neonatal diarrhea, stimulate mostly systemic rather than mucosal immunity, giving rise to circulating antibodies, which do not reach intestinal bacteria in high enough levels to be very effective (Van den Broeck et al. 1999). Such vaccines may even suppress the mucosal immune response upon subsequent oral infection with a pathogenic *E. coli* (Bianchi et al. 1996). Though systemic induction of serum IgA by parenteral vaccines may result in the presence of secretory IgA in the intestinal tract, the latter would be present only as long as the systemic response persists, as no antigen-specific IgA antibody-secreting cells are present in the gut-associated lymphoid tissue (GALT) (Melkebeek et al. 2013). A new vaccine comprising different *E. coli* bacterins was recently approved in some European countries for vaccination of sows and gilts for the passive immunization of piglets in order to prevent neonatal diarrhea and to reduce mortality and clinical signs due to PWD (up to 21 days of age) and to ED (up to 28 days of age).

Live attenuated and wild-type avirulent *E. coli* vaccines have been shown to be an effective approach to prevent and control F4 and F18 pathogenic *E. coli*. Live nonpathogenic *E. coli* vaccine strains carrying fimbrial adhesins may be administered to weaned piglets in the drinking water or to unweaned piglets by oral drenching at least one week prior to the expected onset of diarrhea. Oral administration of a live nonpathogenic F4 *E. coli* vaccine to 17- to 18-day-old pigs immediately following weaning induced a significant protection after challenge with a F4-ETEC strain administered at 7 and 21 days after vaccination and resulted in reduction of clinical signs of PWD, ileal colonization and fecal shedding of F4-ETEC, and normal growth rates after challenge (Fairbrother et al. 2017). Live nonpathogenic F4 *E. coli* vaccines are commercially available (Europe and North America), and a bivalent live vaccine comprising a F4 nonpathogenic *E. coli* strain and a F18 nonpathogenic *E. coli* strain has been recently approved in Europe and Canada for immunization of pigs against F4- and F18-positive *E. coli* PWD.

Another approach is the oral administration of purified F4 and/or F18 fimbriae as a vaccine for the control of outbreaks of *E. coli*-associated diarrhea in weaned pigs (reviewed by Melkebeek et al. 2013). The use of such experimental subunit vaccines, with or without the mucosal adjuvant CT, results in a specific intestinal mucosal immune response and a significant reduction in fecal excretion of the pathogenic F4 *E. coli*. Contrary to F4, oral delivery of purified F18 could not induce a protective response against pathogenic F18 *E. coli* challenge infection (Verdonck et al. 2007).

Several approaches for the control of ED have been investigated. A genetically modified Stx2e toxoid was found to prevent overt and subclinical ED when vaccinated pigs were challenged with EDEC (Bosworth et al. 1996). In two Danish herds with persistent ED problems, vaccination with a Stx2e toxoid almost totally eliminated mortality due to ED (Johansen et al. 1997). A commercial injectable vaccine based on genetically modified recombinant Stx2e that showed reduction of mortality due to ED (Fricke et al. 2015) is now licensed in Europe and Canada.

As with ETEC PWD, live avirulent F18-positive *E. coli* strains have been orally administered to pigs at least 1 week prior to the expected onset of ED. Commercial oral live vaccines comprising an avirulent F18 *E. coli* are available in North America for immunization of recently weaned pigs against ED, but no efficacy studies are yet published. The abovementioned bivalent F4/F18 live vaccine comprising a F4 and a F18 nonpathogenic strain has been recently licensed in Canada for immunization of pigs against F4-PWD, F18-PWD, and F18-ED.

Antimicrobial prophylaxis

At present preventive feed medication with antibiotics is widely practiced in most countries despite serious drawbacks such as nonacceptance by the consumer, impaired buildup of immunity, and selection of resistant bacteria. Resistance is often induced within days or a few weeks. Isolates from *E. coli* PWD and ED show the highest rate of resistance within porcine *E. coli*. In addition to the classes of antimicrobials mentioned above for parenteral therapy, aminoglycosides and colistin are widely used for chemoprophylaxis. However, colistin resistance in pigs has been increasingly reported worldwide in the last several years (Rhouma et al. 2016). In addition, since the first description of the plasmid-mediated colistin resistance *mcr-1* gene in food animals, food, and humans in China in November 2015 (Liu et al. 2016), the number of reports in animal sources rapidly increased. Recently, the European Antimicrobial Advice Ad Hoc Expert Group of the European Medicines Agency recommended that colistin should be classified as "Critically important antimicrobial" (category 2), reserved for situations when there is no affective alternative.

Dietary preventive measures

A number of nutritional strategies appear promising as alternative means of maintaining performance and controlling PWD in weaned pigs without using in-feed antimicrobials and have been reviewed elsewhere (Heo et al. 2013; Kil and Stein 2010; Thacker 2013).

Restriction of feed intake, high fiber diets, and ad libitum feeding of fiber have been reported as effective deterrents to the development of ED and PWD (Bertschinger et al. 1978). The nutritive value of the feed may be reduced by increasing fiber content to 15–20% and reducing crude protein and digestible energy to one-half of the normal values. The addition of fiber to normal diets or feeding high-quality alfalfa coupled with restriction of daily feed intake may be beneficial. A low protein diet with amino acid supplementation may decrease production of toxic protein metabolites and reduce PWD (Heo et al. 2013). Animal source proteins seem to provide protection against PWD. Addition of dairy products to feed delayed the occurrence of PWD and reduced mortality (Tzipori et al. 1980), possibly due to greater digestibility or stimulation of higher feed intake (Lalles et al. 2007).

A lower mortality due to *E. coli* enterotoxemia and improved weight gains were reported after introduction of rations with a reduced acid-binding capacity. A similar effect is ascribed to organic acids. Organic acids contribute to the maintenance of an acidic gastrointestinal tract, which may control potentially pathogenic bacteria. However, mortality due to ED was not reduced by the inclusion of a mixture of organic and inorganic acids in the feed (Johansen et al. 1996). This result is not surprising in view of the highly regulated pH close to the mucosal surface (McEwan et al. 1990).

Zinc oxide may offer an alternative to antimicrobials. Feeds with contents between 2400 and 3000 ppm of zinc reduce diarrhea and mortality and improve growth. The prevention of disease in piglets improves growth and feed conversion efficiency. Positive response at 1500 ppm has been reported using growth rate as an indicator. The protective effects of zinc oxide may be due not to antibacterial activity but to a protection of intestinal cells from ETEC infection by inhibition of bacterial adhesion and internalization and modulation of cytokine gene expression (Roselli et al. 2003). However, care must be taken to avoid overdose or feed pigs for a too long period since toxicity has been reported. Environmental considerations should be included in discussions of zinc oxide at such high levels. Recently, different studies suggest that therapeutic doses of zinc co-select for antibiotic resistance development in weaned pigs, though further research is needed to confirm the link between the use of zinc and antimicrobial resistance (Vahjen et al. 2015).

Several successful dietary supplements have been reported. Oral administration of immunostimulatory beta-glucans from the cell wall of yeasts reduces the susceptibility of weaned piglets to F4-ETEC infection (Stuyven et al. 2009). Exogenous as well as endogenous proteases lower the activity of intestinal F4 receptors. Bromelain, a protease from pineapple stems, administered orally to pigs reduced the binding of F4-ETEC to brush borders in a dose-dependent manner (Mynott et al. 1996). Wide ranges of antimicrobial peptides, which are components of the host defense system, have been identified, which may potentially replace antibiotics (Thacker 2013). Other alternatives include clay minerals, essential oils, and recombinant enzymes, although most of these compounds produce inconsistent results and they are rarely as effective as antibiotics.

Addition of colicin E1, a member of a class of bacteriocins produced by and effective against *E. coli*, in the diet of young pigs decreased the incidence and severity of PWD caused by F18-ETEC and improved the growth performance of the piglets (Cutler et al. 2007).

Prebiotics selectively stimulate the proliferation of potentially beneficial microorganisms in the gastrointestinal tract. For example, administration of a heat-killed and dried *Enterococcus faecalis* strain significantly reduced the incidence of clinical signs due to STEC (Tsukahara et al. 2007).

Some promising results have been obtained using probiotics, potentially beneficial microorganisms. Feeding of a diet supplemented with a *Lactobacillus sobrius* strain isolated from the pig intestine resulted in a significant decrease in ETEC numbers and increased daily weight gain in weaned pigs challenged with an F4-ETEC (Konstantinov et al. 2008). *Lactobacillus plantarum*,

given to piglets in early life, improved performance and effectively prevented the diarrhea in young piglets induced by F4-ETEC challenge by improving function of the intestinal barrier by protecting intestinal morphology and intestinal permeability (Yang et al. 2014). The administration of *Pediococcus acidilactici* or *Saccharomyces cerevisiae boulardii* was effective in reducing F4-ETEC attachment to the ileal mucosa, and at least the presence of *P. acidilactici* resulted in the modulation of the expression of intestinal inflammatory cytokines, in pigs challenged with F4-ETEC (Daudelin et al. 2011). On the other hand, others reported no efficacy of feeding of *Lactobacillus* spp., *Enterococcus faecium*, and *Bacillus cereus* strain "toyoi" to experimentally and/or naturally infected pigs (De Cupere et al. 1992).

Feeding of a diet supplemented with fermented soybeans (especially *Rhizopus*-fermented soybean, but also *Bacillus*-fermented soybean) reduced the excretion of ETEC and the incidence, severity, and duration of diarrhea in weaned pigs (Kiers et al. 2003).

Breeding of resistant pigs

Augmentation of the presence of both the F18 and F4 resistance loci in the pig population through breeding is an attractive approach to prevent PWD and ED. However, it will be important to avoid co-selection of unwanted traits closely linked with loci coding for the F18 and F4 receptors. It cannot be predicted if additional types of adhesive fimbriae or new variants of known types will emerge, which could bind to yet unidentified receptors. Availability of techniques for large-scale selection of resistant animals remains an important challenge.

A few genetic markers are used by some genetic and breeding companies to increase the proportion of F4- and F18-resistant pigs. A PCR-RFLP test detecting FUT1 M307 polymorphism, correlated with the gene controlling expression of the *E. coli* F18 receptor, could be a simple and inexpensive method for large-scale selection of animals (Frydendahl et al. 2003).

The transmembrane mucins MUC13 and MUC4 provide potential markers for selection of ETEC F4ab/ac-resistant animals, although it is probable that other receptors are involved (Rasschaert et al. 2007; Zhang et al. 2008). However Rasschaert et al. (2007) calculated the sensitivity and specificity for the *in vitro* villous adhesion test, with MUC4 genotyping as the gold standard, as 100 and 24%, respectively. Low specificity was evidenced by a high proportion of pigs identified as genetically resistant showing adhesion of F4ac *E. coli* on the intestine. On the other hand, the immunity aspect should be considered if breeding for resistance becomes available for F4-ETEC, as resistant sows (F4 receptor-negative sows) do not develop and transfer F4-specific antibodies in their colostrum. Thus, heterozygous piglets are not passively protected from development of neonatal diarrhea due to these strains.

E. coli causing fatal shock

Enteric colibacillosis complicated with shock occurs in young pigs before and after weaning. *E. coli* associated with this disease are F4-ETEC (serogroup O149, O157, or O8) or Stx2e-producing *E. coli* that are associated with ED.

In these cases of ETEC or ED, infection progresses so rapidly that death occurs due to shock before diarrhea is observed or before diarrhea proves fatal in the case of ETEC or before cerebral edema proves fatal in ED. This phenomenon is probably due to the rapid release of large amounts of LPS by the colonizing ETEC. The lipid A portion of LPS stimulates the overproduction of mediators of inflammation including tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6. Aggregation and subsequent degranulation of neutrophils activated by these mediators causes damage to the vascular endothelium, loss of fluid, and hypovolemic shock. Modulation of the coagulation pathway also leads to fibrin deposition and clot formation.

Clinical signs are minimal. Apparently healthy young pigs die suddenly or decline rapidly with cyanosis of the extremities. A yellowish to brownish diarrhea is sometimes observed.

Characteristic gross lesions include marked congestion of the small intestinal and stomach walls and blood-tinged intestinal contents (Figure 52.6). Microscopically, severe congestion of the gastric and small intestinal mucosae is commonly associated with microvascular fibrinous thrombi. Necrosis of villi with marked infiltration of neutrophils occurs in severe cases. There is only occasional hemorrhage in the lamina propria of the jejunum and the ileum (Faubert and Drolet 1992).

Immunity, diagnosis, prevention, and control are as described for EPEC and ED.

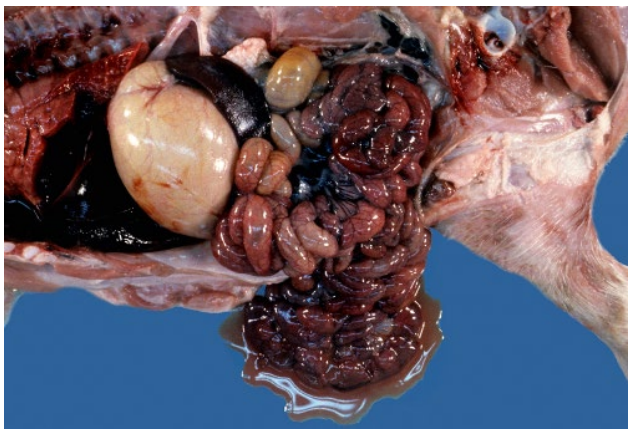


Figure 52.6 Marked congestion of the small intestine with blood-tinged contents in a weaned pig with enteric colibacillosis complicated with shock. *Source:* Courtesy of Dr. Richard Drolet.

Systemic *E. coli* infections

E. coli may induce systemic infections, such as septicemia, or localized extraintestinal infections, such as meningitis or arthritis, resulting from bacteremia (Gyles and Fairbrother 2010). Primary septicemia due to *E. coli* occurs sporadically or rarely as small outbreaks predominantly in newborn to 4-day-old pigs; septicemia can be secondary to diarrhea or other compromising diseases in young and even weanling pigs (Fasina et al. 2015).

Piglets lacking immunity due to an absence of ingested colostrum or to ingestion of colostrum lacking specific antibody are most at risk for primary septicemia. Agalactia in sows, low birth weights, an inadequate number of functional nipples, or any other factor that reduces consumption of colostrum may result in susceptible piglets. Although these piglets usually develop septicemia within the first few days of life, cases may occur throughout the suckling period, or exceptionally in pigs up to 80 days of age. Poor environmental temperature control and inadequate hygiene increase the risk of systemic infection. The intestine is considered as the major route of *E. coli* invasion. The disease can be experimentally induced by oral or intragastric inoculation (Ngeleka et al. 1993). Bacterial invasion may also be via the respiratory tract or contamination of the umbilicus. Strains involved are known as ExPEC and are a heterogeneous group that possesses a large number of virulence factors (discussed in the section on etiology above). Nevertheless, only a limited number of serogroups are represented (Table 52.1).

Secondary septicemia may develop after invasion by ETEC. The ETEC serovirotypes most often involved are listed in Table 52.2. Older suckling piglets suffering from secretory diarrhea caused by ETEC are most often affected. Sows may also be affected soon after farrowing. Other risk factors may include enteric viruses that damage the intestine and alter the bacterial environment or infection by PRRSV that results in immunosuppression, permitting invasion by ETEC leading to death (Nakamine et al. 1998).

Pathogenesis

Bacteria pass through the mucosa of the alimentary tract, probably by endocytic uptake into intestinal epithelial cells or through the intercellular spaces formed by lateral plasma membranes of adjacent epithelial cells, to locate in the mesenteric lymph nodes before entering the bloodstream. Bacterial invasion may result in a generalized infection (septicemia, polyserositis) with bacteria disseminated in various extraintestinal organs such as the lung, liver, spleen, kidney, and brain or in a localized infection (meningitis or arthritis).

The exact role of each virulence factor in the pathogenicity of ExPEC is not always clear, although it appears that the greater number of virulence factors present in an ExPEC isolate, the greater its pathogenic potential. LPS, K-antigen of the capsule and O-antigen, and production of siderophores such as aerobactin are thought to allow the bacteria to invade the host and escape its defense mechanisms. These determinants increase bacterial resistance to the bactericidal effect of complement and to phagocytosis and allow bacterial growth in body fluids with low concentrations of free iron (Ngeleka et al. 1992). Fimbriae appear to be important for the survival and spread of bacteria within the host and subsequent bacterial pathogenicity, in part by promoting bacterial resistance to the bactericidal effects of phagocytosis (Ngeleka et al. 1994).

Clinical signs

Clinical signs of septicemia include depression, lameness, reluctance to move, anorexia, rough hair coat, and labored respiration, due in part to the effect of bacterial endotoxin or cytotoxins or to the effects of inflammatory cytokines induced by these bacterial products (Jesmok et al. 1992; Nakajima et al. 1991). The affected piglets may show sternal recumbency and the abdomen may be somewhat distended. Sometimes piglets become unconscious, with convulsions and paddling movements; they may be in good bodily condition, but cyanosis of the extremities may be observed. Some piglets are found dead, whereas others are comatose without any sign of diarrhea. These clinical signs may develop within 12 hours after birth, and piglets can die within 48 hours. In older piglets, the clinical signs may include periodic diarrhea or other clinical signs, which precede the onset of acute septicemia with clinical signs resembling those in the newborn pigs.

Lesions

In acute primary septicemia, there may be no gross lesions other than congestion of the intestine, the mesenteric lymph nodes, and the extraintestinal organs. In subacute cases, subserous or submucosal hemorrhages and fibrinous polyserositis (Figure 52.7) with gross signs of pneumonia are usually observed, often accompanied by fibrinopurulent arthritis and meningitis. Histological examination of the lung reveals expansion of alveolar septa with edema and neutrophilic infiltration, but alveoli are mostly free of exudates.

In secondary septicemia resulting from enteric colibacillosis, icterus, petechial hemorrhages in the serosal membranes, and splenomegaly accompanied by severe diarrhea and dehydration may be observed. In many cases of secondary systemic *E. coli* infection, presumably occurring in the terminal stages of the underlying disease, lesions attributable to systemic ETEC are slight or no lesions at all are recorded.

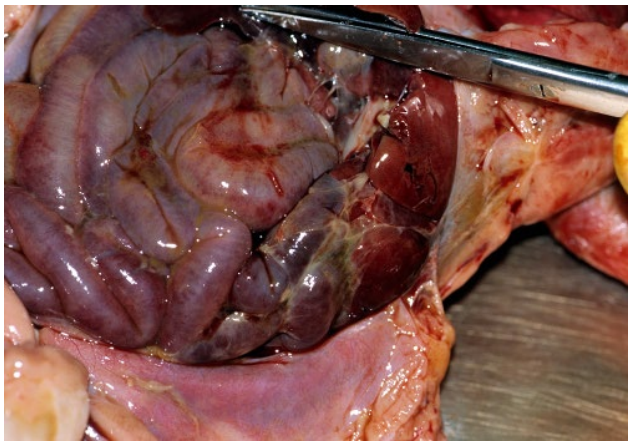


Figure 52.7 Fibrinous peritonitis in a 2-day-old piglet with polyserositis due to *E. coli* (ExPEC). *Source:* Courtesy of Dr. Richard Drolet.

Diagnosis

Systemic colibacillosis should be suspected with the appearance of the clinical signs and lesions described above, especially when observed in pigs under 4 days of age. Differentials should include other causes of systemic bacterial infections. In older suckling and especially weanling pigs with polyserositis, differentials should include *H. parasuis* (Chapter 54), *Mycoplasma hyorhinis* (Chapter 56), and *S. suis* (Chapter 61).

Positive blood culture results are essential to make the diagnosis of bacteremia. However, treatment cannot be delayed until the results of blood cultures are obtained. Blood must be withdrawn from the vein aseptically and deposited into both aerobic and anaerobic blood culture bottles. Diagnosis is confirmed by the isolation in pure culture or by the predominance in extraintestinal organs such as the spleen, liver, brain, lungs, and pericardial, pleural, and peritoneal fluids of one of the typical *E. coli* serovirotypes associated with primary septicemia (Table 52.1) or, for secondary septicemia, one of the ETEC serovirotypes listed in Table 52.2. Methods used to isolate, identify, and serovirotype are as described in the diagnostic section for neonatal *E. coli* diarrhea.

Prevention and control

In septicemia, treatment may be useful in subacute cases of infection but is mostly ineffective after the appearance of clinical signs. However, the remaining unaffected littermates and adjacent litters should be treated prophylactically with antibiotics. Vaccination is rarely considered for the control of septicemia due to *E. coli*. However, in the case of small outbreaks of septicemia, careful monitoring of the causative serogroup(s) and autovaccination of the pregnant sows might be beneficial. The most effective prevention is in ensuring that piglets consume adequate colostrum by focusing on management practices that enable optimal lactation and suckling. Additionally, facility design and husbandry practices to minimize environmental contamination by feces will reduce exposure and transmission of offending strains of *E. coli* to suckling pigs.

Coliform mastitis

The term "coliform mastitis" is used to refer to mastitis in swine, underlining the parallel of this disease with CM in the cow. Up to 80% of dysgalactic sows may have gross lesions of mastitis (Ross et al. 1981). Wegmann et al. (1986) reported that *E. coli* or *Klebsiella pneumoniae* were isolated from 79% of mammary complexes in sows with mastitis. Mortality among affected sows is low, but in piglets nursing multiparous sows with PDS, it has been reported to be as high as 55.8%, compared with 17.2% in piglets nursing healthy sows (Bäckström et al. 1984).

The fecal microbiota is a reservoir for extraintestinal coliform infections such as mastitis and UTI. Various coliform strains are found in the intestine of each individual pig, and mixed infections of more than one strain are often found in any one pig. In about one-third of the sows with mastitis, identical isolates were found in mastitic glands, the uterine contents, and the urinary bladder (Bertschinger et al. 1977a). The intestinal microbiota of the sow, the oral microbiota of the neonatal piglet, and environmental bacteria may significantly contribute to contamination of the nipples. Awad-Masalmeh et al. (1990) found identical O serogroups of *E. coli* in mammary secretion and in feces of about one-fourth of sows with CM. The bedding of the sow is very important, permitting feces and urine to contaminate the udder (Muirhead 1976). *Klebsiella* spp. may also originate from wood shavings used for bedding. Counts of coliform bacteria on teat ends and incidence of intramammary *E. coli* infections were considerably greater in sows farrowing in conventional farrowing crates as compared with those observed in sows farrowing in pens where they could lie down in a clean resting area (Bertschinger et al. 1990).

A great heterogeneity of isolates, evidenced by multiple serological types and by random amplified polymorphic DNA genotyping, may be cultured in cases of mastitis within a herd, between distinct glands of one sow, or even between subcomplexes within one gland (Awad-Masalmeh et al. 1990; Morner et al. 1998; Ramasoota et al. 2000).

Pathogenesis

Mastitis has been reproduced in the sow by intramammary instillation of as few as 120 organisms of a strain of *K. pneumoniae*. External contamination of the nipples

was as successful on gestation day 111 as at 2 hours after completion of farrowing (Bertschinger et al. 1977b). However, it is not clear when spontaneous invasion of the cistern takes place. Significant numbers of coliform bacteria have been observed in about one-fourth of mammary glands cultured immediately prior to parturition (McDonald and McDonald 1975). New infections seem to most commonly occur from before the birth of the piglets to 2 days postpartum (Bertschinger et al. 1990).

The bacteria are located in the ductular and alveolar lumina, either free or within phagocytic cells. Little adhesion to surfaces is observed. At postmortem examination the causative bacteria are frequently isolated from regional lymph nodes, whereas isolations from the liver, spleen, or kidney are rare (Ross et al. 1981).

Multiplication of bacteria in the mammary secretion is controlled by antimicrobial mechanisms, including a variety of inhibitors acting in concert and conferring on the dry udder a nearly total resistance to coliform proliferation. A lower opsonic activity observed in mammary secretions of sows at parturition (Osterlundh et al. 1998) as well as a lower phagocytic capacity of PMN cells in colostrum as compared with milk (Osterlundh et al. 2001) may explain the increased susceptibility to the development of CM at parturition. CM is a self-limiting disease. The bacteria generally disappear between 1 and 6 days after parturition (Bertschinger et al. 1990), but in severe cases they may persist in necrotic foci throughout lactation (Löpfe 1993).

CM in the sow is associated with massive accumulation of neutrophils in the lumina of affected glands and may be accompanied by a severe leukopenia (Bertschinger et al. 1977b). Severe reaction is the consequence of massive and persistent multiplication of inoculated bacteria, susceptibility to infection being associated with impaired function of circulating neutrophils (Löfstedt et al. 1983). The cause of the impaired neutrophil function is not well understood. Magnusson et al. (2001) showed that sows were more susceptible to experimental infection immediately prior to parturition than at 4 days before parturition, the number of neutrophils in the blood being greater in the former. These findings suggest a role for the number of circulating neutrophils at the time of infection in the development of clinical CM in the sow. Nevertheless, Osterlundh et al. (2002) have shown in experimental inoculation studies that impaired chemotactic or phagocytic capacity of blood granulocytes does not appear to be involved in susceptibility of sows to develop clinical CM at parturition.

The systemic signs of CM are induced by bacterial endotoxin (Bertschinger et al. 1990). The development of clinical signs of CM, like high fever, is associated with the degree of local expression of regulatory cytokines such as IL-1 and IL-6 (Zhu et al. 2008).

Clinical signs

In CM, the initial signs are most often detected on the first or second day and more rarely on the third day after farrowing, although they may be observed as early as during parturition. The first clinical signs are pyrexia, listlessness, weakness, and loss of interest in the piglets. Affected sows prefer sternal recumbency. In severe cases they become stiff, do not stand, and may even become comatose. Consumption of feed and water is either reduced or absent. Body temperature is moderately elevated and only rarely exceeds 42 °C (107 °F). Afebrile cases have been reported. On the other hand, many normal sows will have rectal temperatures that exceed the 39.7 °C (103 °F) limit on the day of parturition and for 2 days thereafter. In affected sows, respiratory and heart rates are increased. In general, clinical signs do not last for more than 2–3 days.

The clinical findings in sows with proven CM may be quite similar to those in sows with lactational failure. Interpretation of clinical parameters is also rendered difficult by the presence of subclinical CM in apparently healthy sows. The behavior of the piglets is very helpful in the early detection of lactational failure. Undernourished piglets look gaunt. They frequently try to suck, move from nipple to nipple, nibble at litter, and lick urine from the floor. If the sow gives access to the nipples, the periods of suckling are shortened. After suckling, the piglets wander instead of resting in close contact with their littermates.

Precise localization of mammary lesions is often not possible because reddening and heat of the skin extend over several subcomplexes. The reliable clinical assessment of the state of the mammary tissue is difficult because of subcutaneous fat and considerable subcutaneous edema. If palpable, the mastitic tissue is firmer and palpation may cause pain. The red color of the skin is blanched by finger pressure, which causes a depression of the tissue lasting for some time due to edema. Mere clinical examination will at best detect only a portion of the affected subcomplexes (Persson et al. 1996). The inguinal lymph nodes may be swollen.

Lesions

Lesions of CM are confined to the mammary glands and regional lymph nodes. Grossly, the exudate from inflamed subcomplexes resembles serous to creamy pus. It may contain clots of fibrin or blood. The subcutaneous tissue may be edematous over affected parts of the udder. Irregularly scattered foci of mastitis may be detected in different subcomplexes. The appearance of affected mammary tissue varies from slightly increased firmness and grayish discoloration to sharply demarcated, red-mottled, hard, and dry areas.

Microscopically, an acute purulent exudative mastitis with congestion is observed. The severity varies greatly, both between and within subcomplexes, ranging from a small number of neutrophils in the alveolar lumina to severe purulent infiltration with necrosis. Acute purulent lymphadenitis may be present in the inguinal and iliac lymph nodes. Large necrotic foci surrounded by fibrous tissue may persist through the lactation in severe cases (Löpfe 1993).

Diagnosis

Any hypogalactia at the beginning of lactation arouses suspicion of CM. The diagnosis may be supported by fever, anorexia, reluctance to stand, lying on the gland, and disinterest in the piglets. In severe cases some affected glands may be reddened, swollen, and firm, and the secretion may look abnormal.

A reliable rapid test for use on the farm is not available. Due to the normal higher cell content of sow milk compared with cow milk, tests developed for use with the cow cannot be recommended. Bacteriological and cytological examinations of the secretion are only valuable if all glands are sampled or if affected glands are known. The pH is of limited diagnostic value (Persson et al. 1996), but cytological examination allows differentiation between healthy and mastitic complexes at least during the first 48 hours after parturition (Wegmann and Bertschinger 1984). Because mastitis is a local process, samples must be taken from individual complexes and not pooled. A suggested threshold value for the total cell count is 5×10^6 cells/mL of milk and fewer than 70% PMN. Culture of the secretion may be necessary in unclear cases. Methods for culture are as described for neonatal *E. coli* diarrhea.

Immunity

An episode of CM apparently does not result in protection against homologous reinfection (Bertschinger and Bühlmann 1990). Ringarp (1960) reported a higher incidence in sows than in gilts, as well as repeated occurrence, up to 10 times, in individual sows.

Prevention and control

In CM, therapeutic measures are usually not undertaken before the sow shows signs of dysgalactia. Thus treatment may at best shorten the period of underfeeding of the piglets. Antimicrobial therapy is complicated by the heterogeneous pattern of antimicrobial susceptibility of individual isolates not only within a herd but also within a sow. Therefore sensitivity testing is of little value in individual cases.

Much attention should be given to the piglets. They may either be fostered by other sows or remain with their mother and receive a milk substitute. A sterile 5% glucose solution can be repeatedly injected intraperitoneally every several hours, or a more concentrated solution may be administered intragastrically. When the piglet obtains insufficient amounts of milk, protection against chilling is particularly important.

Strategies for prevention of CM include protection of the teats from bacterial contamination (Muirhead 1976). Optimal prophylaxis is achieved by designing farrowing accommodation in which the sow is prevented from lying down in her own feces. When this is not possible, periodic washing and disinfection of contaminated pens and of the newly housed sows are not highly effective prophylactic measures. If cases of CM accumulate, the bedding materials should be checked. Drastic reduction of the sow's ration shortly before parturition is a widespread practice. Reduction of the daily feed allowance may substantially lower the incidence of agalactia (Persson et al. 1989).

Chemoprophylaxis appears to be the most promising method of control for CM where accommodation cannot be improved. The prevalence of drug resistance and the wide variety of bacteria associated with the disease in a herd must be considered when the drug is selected. Feed medication should be replaced by individual application of the drug in a small amount of feed because the feed consumption of the sow in the periparturient period is quite variable. Minimizing the period of treatment helps to postpone the emergence of drug resistance. Antimicrobials demonstrating some effectiveness include a combination of trimethoprim, sulfadimidine, and sulfathiazole; apramycin; and enrofloxacin.

Nonspecific urinary tract infection

Like CM, nonspecific UTI behaves like a noncontagious infectious disease of endogenous origin. The fecal microbiota is a reservoir for extraintestinal infections such as mastitis and UTI. Contamination and ascent of the urethra by fecal microbiota is more likely in females than males. Under intensive confinement conditions, vulvas of sows are often placed in direct contact with feces (Smith 1983). The dog-sitting position helps to force fecal material into the vagina. Sows resting for long periods of time void urine at longer intervals, and urinary stasis favors bacterial ascent in the urethra and proliferation in the bladder. However, housing conditions have not been studied with respect to UTI. The age distribution of UTI favors the concept of continuous exposure to fecal contamination, since the prevalence of UTI increases with sow parity and increasing parity is associated with greater vulvar and urethral trauma and laxity (Becker et al. 1985). Low water consumption may also have a role in predisposing to UTI.

Pathogenesis

In humans and in dogs, colonization of the lower genital tract and of the urinary tract by uropathogenic *E. coli* is greatly facilitated by fimbrial adhesins, particularly type 1 and P fimbriae (Gyles and Fairbrother 2010). Similarly, P fimbriae and mannose-sensitive hemagglutination indicative of type 1 pili were found in *E. coli* isolates from the urine of pigs with bacteriuria (de Brito et al. 1999). Serovirotypes of *E. coli* commonly isolated from UTI are listed in Table 52.1. Bacteria most likely ascend through the urethra. Invasion is favored by the short wide urethra of the female pig, the relaxation of the sphincter muscle in late pregnancy and puerperium, trauma to the urethra and bladder at coitus and parturition, abnormal bacterial colonization of the sinus urogenitalis and the genital organs, incomplete closure of the vulva, and catheterization of the bladder. Asymptomatic bacteriuria may result in cystitis with spontaneous remission. Nonspecific infection may promote colonization of the bladder by *A. suis* (Liebhold et al. 1995). Bacterial colonization may lead to shortening and deformation of the ureteric valve, promoting vesicoureteric reflux (Carr et al. 1990).

UTI may predispose to MMA due to ascending invasion of the uterus at parturition and of the mammary glands from contamination of the lying area. Identical OK serotypes of *E. coli* have been found in the urinary bladder and uterus or bladder and mammary gland of sows with MMA (Bertschinger et al. 1977a).

Clinical signs

In the vast majority of nonspecific UTI cases, there are no clinical signs. Sows with a significant bacteriuria tend to wean small litters, have increased intervals between litters, show a lower fertility rate, and exhibit an inferior body condition (Akkermans and Pomper 1980). Sows with cystitis may void urine in small quantities with straining or be observed in a dog-sitting position (Becker et al. 1988).

Vulvar discharge may appear as dried deposits around the vulva, on the underside of the tail, or more often as a pool on the floor underneath the sow (Dial and MacLachlan 1988a). The discharge may be mucoid, mucohemorrhagic, or purulent and is observed most often during the final phase of urination. However, discharge may result from inflammation of any part of the urogenital tract. Significant discharge is more often the consequence of endometritis than of UTI.

Severe pyelonephritis becomes clinically manifest during the first 2 weeks postpartum in 40% of the cases (Stirnimann 1984). Typical cases exhibit a rectal temperature below 38 °C (100 °F), a heart rate over 120, polypnea, cyanosis, ataxia, and more rarely generalized tremor. The blood concentrations of urea and creatinine are higher than normal.

Lesions

The gross lesions of cystitis begin as focal or diffuse mucosal hyperemia (Dial and MacLachlan 1988b). Subsequently, there may be mucosal ulceration with fibrinopurulent exudate over affected areas. The bladder wall becomes thickened. Similar lesions occur in the ureters and the renal pelvis if infection ascends the urinary tract. In pyelonephritis the inflammatory process extends into the renal parenchyma. Multiple unevenly distributed foci of inflammation mostly affecting the renal poles are observed (Isling et al. 2010). Wedge-shaped foci extend from the distorted pelvis to the cortex. Fibrosis of the kidneys may occur with time.

Microscopic bladder lesions can be found even in sows with nonspecific UTI and no proteinuria. They consist of a prominent goblet cell proliferation and of intraepithelial cysts containing a few granulocytes. The epithelial layer is infiltrated with neutrophils, whereas mononuclear cells dominate in the lamina propria (Liebhold et al. 1995). In kidneys, tubulointerstitial infiltration with neutrophils and mononuclear cells and tubular destruction are the main findings (Isling et al. 2010).

Diagnosis

Clinical examination of the animal is of little value in the diagnosis of UTI. Bacterial culture of the urinary tract is complicated by the presence of the normal microbiota colonizing the vagina and the distal part of the urethra. Therefore, distinction between contamination and infection is based on the number of bacteria in the urine. A viable count of 10^5 CFU/mL is interpreted as indicative of infection and 10^4 CFU/mL as suspicious. Catheterization of the sow is possible but does not circumvent contamination and involves the risk of setting up a new UTI.

Immunity

Serum antibody against the infecting *E. coli* strain can regularly be detected in sows with pyelonephritis, less often in sows with cystitis, and rarely in sows with asymptomatic bacteriuria (Wagner 1990). *E. coli* strains may persist in the urinary tract despite high antibody concentrations in the urine.

Prevention and control

Treatment of urogenital infections of swine is frustrating (Dial and MacLachlan 1988b). For UTI, therapy with broad-spectrum or combined antimicrobials, such as trimethoprim/sulfonamides, is recommended due to the variable susceptibilities of the diverse bacteria involved and the frequent acquisition of resistance (Berner 1990). Prolonged parenteral treatment may be recommended, although

subclinical UTI often persists after treatment (Becker et al. 1988). Treatment of affected sows with specific antimicrobial drugs before parturition may be helpful.

Strategies for control of UTI include decreasing environmental exposure by improving fecal drainage and housing conditions. Frequency of urination may be increased by giving access to an exercise yard and by increasing water intake, being achieved by a salt content of 1% in the diet (Smith 1983). Water accessibility and flow rates in water lines and nipples should also be evaluated. Decreased water consumption can also be caused by poor palatability.

References

Aarestrup FM, Hasman H. 2004. *Vet Microbiol* 100:83–89.

Akkermans JPWM, Pomper W. 1980. The significance of a bacteriuria with reference to disturbances in fertility. *Proc Congr Int Pig Vet Soc* 6:44.

Awad-Masalmeh M, Reitinger H, Quakyi E, et al. 1988. Observations on the isolation and characterization of *E. coli* derived from edema disease cases. *Proc Int Congr Pig Vet Soc* 10:114.

Awad-Masalmeh M, Baumgartner W, Passernig A, et al. 1990. *Tierarztl Umsch* 45:526–535.

Bäckström L, Morkoc AC, Connor J, et al. 1984. *J Am Vet Med Assoc* 185:70–73.

Baker DR, Billey LO, Francis DH. 1997. *Vet Microbiol* 54:123–132.

Becker HA, Kurtz R, Von Mickwitz G. 1985. *Prakt Tierarztl* 66:1006–1011.

Becker W, Kurtz R, Von Mickwitz G. 1988. *Prakt Tierarztl* 69:41–45.

Beier RC, Bischoff KM, Ziprin RL, et al. 2005. *Bull Environ Contam Toxicol* 75:835–844.

Berner H. 1990. *Dtsch Tierarztl Wochenschr* 97:20–24.

Bertschinger HU, Bühlmann A. 1990. Absence of protective immunity in mammary glands after experimentally induced coliform mastitis. *Proc Congr Int Pig Vet Soc* 11:175.

Bertschinger HU, Pohlenz J. 1974. *Schweiz Arch Tierheilkd* 116:543–554.

Bertschinger HU, Pohlenz J. 1983. *Vet Pathol* 20:99–110.

Bertschinger HU, Pohlenz J, Hemlep I. 1977a. *Schweiz Arch Tierheilkd* 119:223–233.

Bertschinger HU, Pohlenz J, Middleton-Williams DM. 1977b. *Schweiz Arch Tierheilkd* 119:265–275.

Bertschinger HU, Eggenberger U, Jucker H, et al. 1978. *Vet Microbiol* 3:281–290.

Bertschinger HU, Bürgi E, Eng V, et al. 1990. *Schweiz Arch Tierheilkd* 132:557–566.

Bertschinger HU, Stamm M, Vögeli P. 1993. *Vet Microbiol* 35:79–89.

Bertschinger HU, Nief V, Tschape H. 2000. *Vet Microbiol* 71:255–267.

Bianchi AT, Scholten JW, van Zijderveld AM, et al. 1996. *Vaccine* 14:199–206.

Bosworth BT, Samuel JE, Moon HW, et al. 1996. *Infect Immun* 64:55–60.

Boyd B, Tyrrell G, Maloney M, et al. 1993. *J Exp Med* 177:1745–1753.

de Brito BG, Leite DS, Linhares RE, et al. 1999. *Vet Microbiol* 65:123–132.

Burrows MR, Rankin JD. 1970. *Br Vet J* 126:32–34.

Bywater RJ, Woode GN. 1980. *Vet Rec* 106:75–78.

Carr J, Walton JR, Done SH. 1990. Observations on the intravesicular portion of the ureter from healthy pigs and those with urinary tract disease. *Proc Congr Int Pig Vet Soc* 11:286.

Choi C, Cho W, Chung H, et al. 2001. *Vet Microbiol* 81:65–71.

Clugston RE, Nielsen NO, Smith DLT. 1974. *Can J Comp Med* 38:34–43.

Coddens A, Verdonck F, Tiels P, et al. 2007. *TVet Microbiol* 122:332–341.

Cutler SA, Lonergan SM, Cornick N, et al. 2007. *Antimicrob Agents Chemother* 51:3830–3835.

Daudelin JF, Lessard M, Beaudoin F, et al. 2011. *Vet Res* 42: doi: https://doi.org/10.1186/1297-9716-42-69.

De Cupere F, Deprez P, Demeulenaere D, et al. 1992. *J Vet Med B Infect Dis Vet Public Health* 39:277–284.

Dean P, Kenny B. 2009. *Curr Opin Microbiol* 12:101–109.

DebRoy C, Roberts E, Cheuchenzuber W, et al. 2009. *J Vet Diagn Invest* 21:359–364.

Deprez P, Van den Hende C, Muylle E, et al. 1986. *Vet Res Commun* 10:469–478.

Deprez P, De Cupere F, Muylle E. 1990. The effect of feeding dried plasma on experimental *Escherichia coli* enterotoxemia in piglets. *Proc Congr Int Pig Vet Soc* 11:149.

Dial G, MacLachlan NJ. 1988a. *Compend Contin Educ Vet* 10:63–71.

Dial G, MacLachlan NJ. 1988b. *Compend Contin Educ Vet* 10:529–540.

Dixit SM, Gordon DM, Wu XY, et al. 2004. *Microbiology* 150:1735–1740.

Do TN, Cu PH, Nguyen HX, et al. 2006. *J Med Microbiol* 55:93–99.

Do TN, Trott DJ, Nadeau E, et al. 2010. Comparison of the pathotypes and virotypes of pathogenic *Escherichia coli* in diseased pigs in Vietnam and Quebec, Canada. In Proceedings of the International Pig Veterinary Society Congress, p. 766.

Dong WH, Dai CH, Sun L, et al. 2016. *Anim Genet* 47:448–435.

Dozois CM, Clement S, Desautels C, et al. 1997. *FEMS Microbiol Lett* 152:307–312.

Dubreuil JD, Isaacson RE, Schifferli DM. 2016. *EcoSal Plus* 7:1–80.

Edfors-Lilja I, Gustafsson U, Duval-Iflah Y, et al. 1995. *Anim Genet* 26:237–242.

Fairbrother JM, Nadeau E. 2006. *Escherichia coli*: On-farm contamination of animals. *Rev Sci Tech* 25:555–569.
Fairbrother JM, Ngeleka M. 1994. Extraintestinal *Escherichia coli* infections in pigs. In Gyles CL, ed. *Escherichia coli in Domestic Animals and Humans*. Wallingford, UK: CAB International.
Fairbrother JM, Higgins R, Desautels C. 2000.Trends in pathotypes and antimicrobial resistance of *E. coli* isolates from weaned pigs. *Proc Congr Int Pig Vet Soc* 16:17.
Fairbrother JM, Nadeau E, Gyles CL. 2005. *Anim Health Res Rev* 6:17–39.
Fairbrother JM, Nadeau É, Bélanger L, et al. 2017. *Vaccine* 35:353–360.
Fasina FO, Bwala DG, Madoroba E. 2015. *Onderstepoort J Vet Res* 82: doi: https://doi.org/10.4102/ojvr.v82i1.986.
Faubert C, Drolet R. 1992. *Can Vet J* 33:251–256.
Fekete PZ, Mateo KS, Zhang WP, et al. 2013. *Vet Microbiol* 164:330–335.
Francis DH. 2002. *J Swine Health Prod* 10:171–175.
Fricke R, Bastert O, Gotter V, et al. 2015. *Porcine Health Manag* 1:6: doi: https://doi.org/10.1186/2055-5660-1-6.
Frydendahl K. 2002. *Vet Microbiol* 85:169–182.
Frydendahl K, Kare Jensen T, Strodl Andersen J, et al. 2003. *Vet Microbiol* 93:39–51.
Gannon VPJ, Gyles CL, Wilcock BP. 1989. *Can J Vet Res* 53:306–312.
Gerjets I, Traulsen I, Reiners K, et al. 2011. *Vet Microbiol* 152:361–367.
Goetstouwers T, Van Poucke M, Coppieters W, et al. 2014. *PLoS One* 9(8): doi:https://doi.org/10.1371/journal.pone.0105013
Gyles CL, Fairbrother JM. 2010. *Escherichia coli*. In Gyles CL, Prescott JF, Songer JG, et al., eds. *Pathogenesis of Bacterial Infections in Animals*, 4th ed. Ames, IA: Wiley-Blackwell. pp. 267–308.
Hampson DJ, Fu ZF, Robertson ID. 1987. *Epidemiol Infect* 99:149–153.
Harel J, Lapointe H, Fallara A, et al. 1991. *J Clin Microbiol* 29:745–752.
Helie P, Morin M, Jacques M, et al. 1991. *Infect Immun* 59:814–821.
Heo JM, Opapeju FO, Pluske JR, et al. 2013. *J Anim Physiol Anim Nutr* 97:207–237.
Hu ZL, Hasler-Rapacz J, Huang SC, et al. 1993. *J Hered* 84:157–165.
Isling LK, Aalbaek B, Schroder M, et al. 2010. *Acta Vet Scand* 52:48.
Jahanbakhsh S, Smith MG, Kohan-Ghadr HR, et al. 2016. *Int J Antimicrob Agents* 48:194–202.
Jamalludeen N, Johnson RP, Shewen PE, et al. 2009. *Vet Microbiol* 136:135–141
Jesmok G, Lindsey C, Duerr M, et al. 1992. *Am J Pathol* 141:1197–1207.
Johansen M, Baekbo P, Thomsen LK. 1996. Control of edema disease in Danish pig herds. *Proc Congr Int Pig Vet Soc* 14:256.
Johansen M, Andresen LO, Jorsal SE, et al. 1997. *Can J Vet Res* 61:280–285.
Johansen M, Andresen LO, Thomsen LK, et al. 2000. *Can J Vet Res* 64:9–14.
Jorsal SE, Aarestrup FM, Ahrens P, et al. 1996. Oedema disease in Danish pig herds: Transmission by trade of breeding animals. *Proc Congr Int Pig Vet Soc* 14:265.
Katouli M, Lund A, Wallgren P, et al. 1995. *Appl Environ Microbiol* 61:778–783.
Kausche M, Dean EA, Arp LH, et al. 1992. *Am J Vet Res* 53:281–287.
Kelly D, Begbie R, King TP. 1994. *Nutr Res Rev* 7:233–257.
Kiers JL, Meijer JC, Nout MJ, et al. 2003. *J Appl Microbiol* 95:545–552.
Kil DY, Stein HH. 2010. *Can J Anim Sci* 90:447–460.
Kohler EM. 1974. *Am Vet J* 35:331–338.
Konstantinov SR, Smidt H, Akkermans ADL, et al. 2008. *FEMS Microbiol Ecol* 66:599–607.
Krag L, Hancock V, Aalbaek B, et al. 2009. *Vet Microbiol* 134:318–326.
Kusumoto M, Hikoda Y, Fujii Y, et al. 2016. *J Clin Microbiol* 54:1074–1081.
Lalles JP, Bosi P, Smidt H, et al. 2007. Nutritional management of gut health in pigs around weaning. *Proc Nutr Soc* 66:260–268.
Li Y, Qiu X, Li H, et al. 2007. *J Genet Genomics* 34:591–599.
Li XY, Wang LL, Zhen YH, et al. 2015. *J Anim Sci Biotechnol* 6: doi: https://doi.org/10.1186/s40104-015-0038-8.
Liebhold M, Wendt M, Kaup F-J, et al. 1995. *Vet Rec* 137:141–144.
Liu WX, Yuan CW, Meng XQ, et al. 2014. *Vet J* 199:286–289.
Liu YY, Wang Y, Walsh TR, et al. 2016. *Lancet Infect Dis* 16:161–168.
Löfstedt J, Roth JA, Ross RF, et al. 1983. *Am J Vet Res* 44:1224–1228.
Löpfe PJ. 1993. Experimentelle Mastitis bei der Sau: Korrelation der pathologisch-anatomischen und histologischen Befunde mit den klinischen Befunden 4–30 Tage nach der Ansteckung mit *E. coli* und Klebsiella pneumoniae. DVM thesis, Zurich.
Luppi A, Gibellini M, Gin T, et al. 2016. *Porcine Health Manag* 2: doi: 10.1186/s40813-016-0039-9.
MacLeod DL, Gyles CL. 1990. *Infect Immun* 58:1232–1239.
Magnusson U, Pedersen Morner A, Persson A, et al. 2001. *J Vet Med B Infect Dis Vet Public Health* 48:501–512.
Mainil J. 1999. Shiga/verocytotoxins and Shiga/verotoxigenic *Escherichia coli* in animals. *Vet Res* 30:235–257.
Mainil JG, Jacquemin E, Pohl P, et al. 2002. *Vet Microbiol* 86:303–311.
Marchant M, Moreno MA. 2013. *Appl Environ Microbiol* 79:853–859.
McDonald TJ, McDonald JS. 1975. *Cornell Vet* 65:73–83.
McEwan GTA, Schousboe B, Skadhauge E. 1990. *J Vet Med A Physiol Pathol Clin Med* 37:439–444.
Melkebeek V, Goddeeris BM, Cox E. 2013. *Vet Immunol Immunopathol* 152:37–42.
Morner AP, Faris A, Krovacek K. 1998. *Zentralbl Veterinarmed B* 5:287–295.

Muirhead MR. 1976. *Vet Rec* 99:288–292.
Mynott TL, Luke RK, Chandler DS. 1996. *Gut* 38:28–32.
Nagy B, Casey TA, Whipp SC, et al. 1992. *Infect Immun* 60:1285–1294.
Nakajima Y, Ishikawa Y, Momotani E, et al. 1991. *J Comp Pathol* 104:57–64.
Nakamine M, Kono Y, Abe S, et al. 1998. *J Vet Med Sci* 60:555–561.
Nataro JP, Kaper JB. 1998. *Clin Microbiol Rev* 11:142–201.
Neef NA, McOrist S, Lysons RJ, et al. 1994. *Infect Immun* 62:4325–4332.
Ngeleka M, Harel J, Jacques M, et al. 1992. *Infect Immun* 60:5048–5056.
Ngeleka M, Jacques M, Martineau-Doizé B, et al. 1993. *Infect Immun* 61:836–843.
Ngeleka M, Martineau-Doizé B, Fairbrother JM. 1994. *Infect Immun* 62:398–404.
Ngeleka M, Pritchard J, Appleyard G, et al. 2003. *J Vet Diagn Invest* 15:242–252.
Nguyen VU, Goetstouwers T, Coddens A, et al. 2013. *Vet Immunol Immunopathol* 152:93–100.
Opreissnig T, Ziao CT, Gerber PF, et al. 2014. *PLoS One* 9:e104766.
Osterlundh I, Holst H, Magnusson U. 1998. *Theriogenology* 50:465–477.
Osterlundh I, Holst H, Magnusson U. 2001. *Am J Vet Res* 62:1250–1254.
Osterlundh I, Hulten F, Johannisson A, et al. 2002. *Vet Immunol Immunopathol* 90:35–44.
Owusu-Asiedu A, Baidoo SK, Nyachoti CM, et al. 2002. *J Anim Sci* 80:2895–2903.
Persson A, Pedersen AE, Göransson L, et al. 1989. *Acta Vet Scand* 30:9–17.
Persson A, Pedersen Mörner A, Kuhl W. 1996. *Acta Vet Scand* 37:293–313.
Ramasoota P, Krovacek K, Chansiripornchai N, et al. 2000. *Acta Vet Scand* 41:249–259.
Rasschaert K, Verdonck F, Goddeeris BM, et al. 2007. *Vet Microbiol* 123:249–253.
Rhouma M, Beaudry F, Letellier A. 2016. *Int J Antimicrob Agents* 48:119–126.
Ringarp N. 1960. *Acta Agric Scand* Suppl 7.
Rooke JA, Bland IM. 2002. *Livest Prod Sci* 78:13–23.
Roselli M, Finamore A, Garaguso I, et al. 2003. *J Nutr* 133:4077–4082.
Ross RF, Orning AP, Woods RD, et al. 1981. *Am J Vet Res* 42:949–955.
Sarmiento JI. 1983. Environmental temperature: A predisposing factor in the enterotoxigenic *Escherichia coli*–induced diarrhea of the newborn pig. MS thesis, University of Guelph, Ontario.
Sarmiento JI, Casey TA, Moon HW. 1988a. *Am J Vet Res* 49:1154–1159.
Sarmiento JI, Dean EA, Moon HW. 1988b. *Am J Vet Res* 49:2030–2033.
Sarrazin E, Bertschinger HU. 1997. *Vet Microbiol* 54:133–144.
Schierack P, Walk N, Reiter K, et al. 2007. *Microbiology* 153:3830–3837.
Smith WJ. 1983. *Pig News Info* 4:279–281.
Solis CA, Sumano LH, Marin HJA. 1993. *Pig Vet J* 30:83–88.
Stirnimann J. 1984. *Schweiz Arch Tierheilkd* 126:597–605.
Stuyven E, Cox E, Vancaeneghem S, et al. 2009. *Vet Immunol Immunopathol* 128:60–66.
Thacker PA. 2013. *J Anim Sci Biotechnol* 4: doi: 10.1186/2049-1891-4-35.
Tsukahara T, Inoue R, Nakanishi N, et al. 2007. *J Vet Med Sci* 69:103–109.
Tzipori S, Chandler D, Smith M, et al. 1980. *Aust Vet J* 56:274–278.
Vahjen W, Pietruszynska D, Starke IC, et al. 2015. *Gut Pathog* 7:doi: https://doi.org/10.1186/s13099-015-0071-3.
Van den Broeck W, Cox E, Goddeeris BM. 1999. *Infect Immun* 67:520–526.
Verdonck F, Cox E, van Gog K, et al. 2002. *Vaccine* 20:2995–3004.
Verdonck F, Tiels P, van Gog K, et al. 2007. *Vet Immunol Immunopathol* 120:69–79.
Waddell TE, Gyles CL. 1995. *Infect Immun* 63:4953–4956.
Waddell TE, Lingwood CA, Gyles CL. 1996. *Infect Immun* 64:1714–1719.
Wagner S. 1990. Die Immunreaktion bei der durch *Escherichia coli* bedingten chronischen Harnwegsinfektion des weiblichen Schweines. DVM thesis, University of München.
Wang XM, Liao XP, Liu SG, et al. 2011. *Foodborne Pathog Dis* 8:687–692.
Wathes CM, Miller BG, Bourne FJ. 1989. *Anim Prod* 49:483–496.
Wegmann P, Bertschinger HU. 1984. Sequential cytological and bacteriological examination of the secretions from sucked and unsucked mammary glands with and without mastitis. *Proc Congr Int Pig Vet Soc* 8:287.
Wegmann P, Bertschinger HU, Jecklin H. 1986. A field study on the prevalence of coliform mastitis (MMA) in Switzerland and the antimicrobial susceptibility of the coliform bacteria isolated from the milk. *Proc Congr Int Pig Vet Soc* 9:92.
Weiler LH, Franke S, Menge C, et al. 1995. *Dtsch Tierarztl Wochenschr* 102:40–43.
Wray C, Woodward MJ. 1994. Laboratory diagnosis of *Escherichia coli* infections. In Gyles CL, ed. *Escherichia coli in Domestic Animals and Humans*. Wallingford: CAB International. pp. 595–628.
Xia PP, Zou YJ, Wang YT, et al. 2015. *Appl Microbiol Biotechnol* 99:4953–4959.
Yan XM, Ren J, Huang X, et al. 2009. *J Anim Sci* 87:334–339.
Yang KM, Jiang ZY, Zheng CT, et al. 2014. *J Anim Sci* 92:1496–1503.
Zhang W, Zhao M, Ruesch L, et al. 2007. *Vet Microbiol* 123:145–152.
Zhang B, Ren J, Yan X, et al. 2008. *Anim Genet* 39:258–266.
Zhu C, Harel J, Jacques M, et al. 1994. *Infect Immun* 62:4153–4159.
Zhu Y, Magnusson U, Fossum C, et al. 2008. *Vet Immunol Immunopathol* 125:182–189.

53

Erysipelas

Tanja Opriessnig and Tania A. Coutinho

Relevance

The causative agent of erysipelas, *Erysipelothrix rhusiopathiae*, was first isolated from a pig in 1882 by Louis Pasteur and was first characterized in experimentally infected pigs in 1886 by Friedrich Löffler. Erysipelas occurs sporadically in the pig population, but there is evidence that more severe and prevalent outbreaks occur in recurring intervals of approximately 10 years. Several recent reports of swine erysipelas outbreaks in countries with relevant pig production raise concerns on reemergence of this disease (Bender et al. 2010; Coutinho et al. 2011; Ding et al. 2015; To et al. 2012).

Swine erysipelas, when uncontrolled, is economically significant and capable of affecting all stages of pork production. The greatest losses can be attributed to cases of sudden death and acute septicemia in grow–finish pigs. A frequent sequel of surviving an acute infection is chronic lameness and arthritis, which results in poor growth and abattoir condemnations. *E. rhusiopathiae* is also relevant from a public health perspective, albeit infections in humans are rare (Reboli and Farrar 1989).

Etiology

Erysipelothrix rhusiopathiae belongs to a group of bacteria that represent a new class, *Erysipelotrichia*, in the phylum *Firmicutes* (Ogawa et al. 2011). The genus *Erysipelothrix* comprises seven species to date: *E. rhusiopathiae* (Migula 1900; Skerman et al. 1980), *Erysipelothrix tonsillarum* (Takahashi et al. 1987), *Erysipelothrix* sp. strain 1, *Erysipelothrix* sp. strain 2, *Erysipelothrix* sp. strain 3 (Takahashi et al. 2008), *Erysipelothrix inopinata* (Verbarg et al. 2004), and *Erysipelothrix larvae* sp. nov. (Bang et al. 2015). The most relevant species for pigs is *E. rhusiopathiae*, though strains of *E. tonsillarum* have been isolated from cases of chronic arthritis and vegetative valvular endocarditis (Bender et al. 2011; Takahashi et al. 1996).

Erysipelothrix spp. are nonmotile, non-sporulating, non-acid-fast, facultatively intracellular gram-positive rods that are facultative anaerobes and grow between 41 °F (5 °C) and 111 °F (44 °C), with optimal growth occurring between 86 °F (30 °C) and 98.6 °F (37 °C) (Carter 1990). On agar media, colonies of *Erysipelothrix* spp. are clear, circular, and small (0.1–0.5 mm in diameter) after 24 hours of incubation at 95 °F (35 °C) or 81 °F (27 °C), with increased size (0.5–1.5 mm in diameter) after 48 hours, and they are either smooth or rough, being slightly larger with an irregular edge. Most strains induce a narrow zone of partial hemolysis on blood agar media, usually with a greenish color. Rough colonies are not typically associated with hemolysis. *E. rhusiopathiae* is relatively inactive in commonly used biochemical tests, producing acid but no gas from certain fermentable carbon compounds and hydrogen sulfide in triple-sugar iron agar (Carter 1990).

Based on heat-stable cell wall antigens, *Erysipelothrix* spp. strains can be differentiated by precipitation reactions using hyperimmune rabbit antiserum into at least 28 serotypes, 1a, 1b, 2–26, and N, the latter referring to strains lacking these antigens (Kucsera 1973; Wood and Harrington Jr 1978) (Table 53.1). Cases of clinical erysipelas are predominantly caused by serotype 1a, 1b, or 2 (Wood and Harrington Jr 1978).

Public health

Though human infections by *E. rhusiopathiae* are rarely reported, this bacterium has long been recognized as zoonotic (Rosenbach 1909). Most human cases are a consequence of occupational exposure (butchers, abattoir workers, veterinarians, farmers, fishermen, fish handlers, and housewives) to infected animals or their tissues (Reboli and Farrar 1989) and occur via scratches

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 53.1 Characteristics of strains associated with different *Erysipelothrix* spp.

Species	Isolated from	Pathogenic to pigs	Serotypes	Surface protective antigen type
E. rhusiopathiae	Terrestrial and marine mammals, birds, reptiles, fish, and arthropods	Yes	1a, 1b, 2, 4, 5, 6, 8, 9[a], 11, 12, 15, 16, 17, 19, 21, N	A, B, C, or combinations
E. tonsillarum	Terrestrial mammals and fish	Variable	3, 7[a], 10[a], 14, 20, 22, 23, 25, 26	None
Erysipelothrix sp. strain 1	Pigs	Yes	13	None
Erysipelothrix sp. strain 2	Pigs	Yes	9[b], 10[b], 18	C
Erysipelothrix sp. strain 3	Pigs	Yes	7[a]	None
E. inopinata	Sterile-filtered vegetable broth[c]	No	Not determined	Not determined
E. larvae sp. nov.	Beetle	No	Not determined	Not determined

[a] Few strains.
[b] Majority of the strains.
[c] Never isolated from an animal source.

or traumatic penetration of the skin. Not accidentally the most common clinical manifestation in humans is known by names such as "whale finger," "seal finger," "speck finger," "blubber finger," "fish poisoning," "fish handler's disease," and "pork finger" (Reboli and Farrar 1989) since cutaneous infections are usually confined to the extremities. The clinical form in humans is called erysipeloid and manifests as acute localized cellulitis with skin reddening. Additional clinical presentations include a generalized cutaneous form and a septicemic form, with endocarditis present in about one-third of the patients (Principe et al. 2016). These conditions should not be confused with "human erysipelas," which refers to a skin infection caused by mainly group A and G streptococci (Bläckberg et al. 2015). Human-to-human transmission of *E. rhusiopathiae* has not been established to date (Reboli and Farrar 1989).

Epidemiology

Erysipelothrix rhusiopathiae is worldwide in distribution and is ubiquitous. The domestic pig is considered the most important reservoir. Besides pigs, a variety of vertebrate and invertebrate species are known to harbor the organism providing a broad reservoir (Wang et al. 2010).

Approximately 30–50% of apparently healthy pigs harbor *E. rhusiopathiae* in their tonsils and other lymphoid tissues (Stephenson and Berman 1978). Carriers and pigs with acute erysipelas can shed the organism in their excretions (urine, feces) and secretions (saliva, nasal mucus) for extended periods of time. The bacterium can be isolated from oral fluid of an acutely infected population for 1–3 days and demonstrated by real-time PCR for 1–9 days (Giménez-Lirola et al. 2013). Transmission of *E. rhusiopathiae* is believed to occur directly via oronasal secretions and feces and indirectly via environmental contamination (Wood and Harrington Jr 1978). While there is no evidence of *E. rhusiopathiae* growth in the environment, its presence in facilities and on equipment is well documented (Bender et al. 2010; Reboli and Farrar 1992). Pigs can be infected by ingestion of contaminated feed or water and through contamination of skin wounds. Pigs less than 3 months of age (due to a protective effect of passively acquired immunity) or pigs older than 3 years of age (due to repeated subclinical disease) are generally least predisposed to erysipelas.

Survival of *E. rhusiopathiae* in soil is less than 35 days, with no evidence of establishment of stable populations (Wood 1973). *E. rhusiopathiae* is inactivated by moist heat at 131 °F (55 °C) but is resistant to salting and many other food preservation methods (Conklin and Steele 1979). It may remain viable for 12 days under direct exposure to sunlight and for months in unburied carcasses or in carcasses buried to a depth of 7 ft (2.13 m) (Reboli and Farrar 1989). *Erysipelothrix* spp. are inactivated by commonly available disinfectants (Conklin and Steele 1979).

Pathogenesis

The route of exposure to *E. rhusiopathiae* in pigs is primarily oral with initial infection of the tonsils or gastrointestinal mucosa. Cytokeratin 18 positive cells of tonsillar crypt epithelium are likely the main invasion sites of *E. rhusiopathiae* (Harada et al. 2013). The bacterium is able to survive and replicate within macrophages (Ogawa et al. 2011; Shimoji 2000) and is consistently

detected within peripheral tissues (Harada et al. 2013). Bacteria may also enter through skin abrasions or via mechanical vectors such as arthropod bites (Chirico et al. 2003). Usually, bacteremia develops within 24 hours in the absence of an effective immune response. Subsequent septicemia results in distribution of the organisms throughout the body. In the early septicemic stage, damage occurs to capillaries and venules of most body organs and synovial membranes (Schulz et al. 1975a). At 36 hours after subcutaneous inoculation, endothelial swelling, monocyte adherence to vascular walls, and hyaline thrombosis occur (Schulz et al. 1975b). This process is referred to as a shock-like generalized coagulopathy that leads to fibrinous thrombosis, diapedesis, invasion of vascular endothelium by bacteria, and deposition of fibrin in perivascular tissues (Schulz et al. 1975a, 1976a). Eventually, there is connective tissue activation in predisposed sites of infection including the joints, heart valves, and skin (Schulz et al. 1976b). Hemolysis and ischemic necrosis can occur in severe cases. Sequestration of *E. rhusiopathiae* in the cytoplasm of chondrocytes of articular cartilage has been reported (Franz et al. 1995) and likely provides protection from host immunity, contributing to chronic arthritis.

Marked differences in virulence among strains of *E. rhusiopathiae* exist and may be modulated by virulence factors (Wang et al. 2010). Important virulence factors include neuraminidase, capsular polysaccharides, and surface proteins. Neuraminidase is an enzyme that cleaves sialic acids from glycoproteins, glycolipids, and polysaccharides on host cell walls, providing bacterial nutrients and aiding in bacterial adhesion and tissue invasion (Schauer 1985). The amount of secreted neuraminidase is proportional to the degree of virulence in *E. rhusiopathiae* strains (Krasemann and Müller 1975); none is secreted by nonpathogenic *E. tonsillarum* (Wang et al. 2005). The polysaccharide capsule of *E. rhusiopathiae* provides resistance to phagocytosis by leukocytes and intracellular killing by macrophages (Shimoji 2000). The *E. rhusiopathiae* capsule plays a major role in bacterial adherence and immune evasion. Its phosphorylcholine component, along with surface protective antigen A (SpaA), has been demonstrated to be involved in bacterial adherence to endothelial cells in the absence of platelet-activating factor receptor mediation *in vitro* (Harada et al. 2014). Although SpaA, a choline-binding protein, is expressed in higher amounts in highly virulent strains (Galán and Timoney 1990) and has long been known to be a major protective antigen of *E. rhusiopathiae* (Makino et al. 1998), the extent of its contribution to bacterial virulence is unknown. Other bacterial surface proteins that may contribute to virulence include novel adhesins RspA and RspB (Shimoji et al. 2003), which are important in early biofilm formation.

Experimental evidence linking susceptibility to swine erysipelas with host genetics is lacking. Sudden changes in weather, especially hot summer weather, or other stressors have been implicated in increased incidence of the disease.

Clinical signs

Three clinical forms of swine erysipelas – acute, subacute, and chronic – have been described (Conklin and Steele 1979; Grieco and Sheldon 1970). The acute form is septicemic disease that manifests as sudden onset of acute death, abortions, depression, lethargy, pyrexia (104–108 °F [40–42 °C] or greater), withdrawal, lying down, painful joints evidenced by stiff stilted gait, reluctance to move and/or vocalization during movement, partial or complete inappetence, and characteristic pink, red, or purple raised firm rhomboid or squared "diamond skin" lesions. In dark-skinned animals, the skin lesions are best appreciated by palpation or by observing areas with raised hairs. In nonfatal cases, the skin lesions will gradually disappear within 4–7 days.

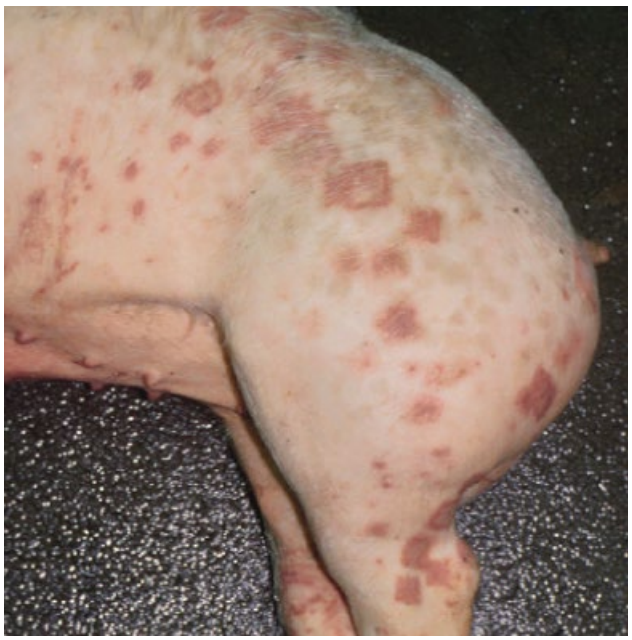
The subacute form is clinically less severe than the acute form. Animals do not appear as sick, temperatures are not as high or persistent, appetite may remain unaffected, skin lesions may be few in number or absent, mortality will be lower, and animals will recover more rapidly. There may be infertility, litters with increased numbers of mummies or of small size, and pre- or postparturient vulvar discharges. Some cases may be so mild that they remain unnoticed (subclinical).

Chronic erysipelas follows acute, subacute, or sometimes subclinical erysipelas in a proportion of surviving animals. The most economically significant form is chronic arthritis that may appear as soon as 3 weeks after the initial outbreak. Affected animals are mildly to markedly lame with associated reduction in feed intake. Firm enlargement of hock, stifle, or carpal joints may be observed. Chronic erysipelas can also manifest as respiratory distress, lethargy, cyanosis, or sudden death as consequences of vegetative valvular endocarditis that may lead to cardiac insufficiency and pulmonary edema.

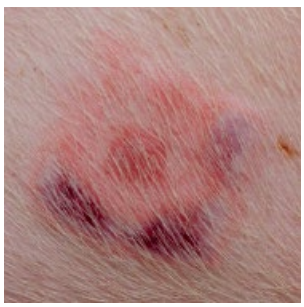
Morbidity and mortality vary depending on the immune status of a herd. In outbreaks of acute swine erysipelas in naïve herds, mortality can quickly rise to 20–40%. In subclinically or chronically affected herds, the morbidity and mortality associated with *Erysipelothrix* spp. vary and are dependent on herd management, environment, and other concurrent infections.

Lesions

The nearly pathognomonic gross lesions of acute swine erysipelas consist of multifocal pink to purple rhomboid (diamond-shaped) slightly raised skin lesions (Figure 53.1) predominately around the snout, ears, jowls, throat, abdomen, and thighs. The skin of extremities can also be purple. In addition to skin lesions, other typical lesions of septicemia include enlarged and congested lymph nodes, enlarged spleen, and edematous and congested lungs. Petechiae and ecchymosis may be found in the renal cortex (Figure 53.2), the heart (epicardium and atrial myocardium), and occasionally elsewhere. Joints may be slightly enlarged, and the synovium and periarticular tissues are typically distended by serofibrinous exudates that may also fill the joint cavity. In some outbreaks, fattening hogs may be found dead without any gross lesions.



(a)



(b)

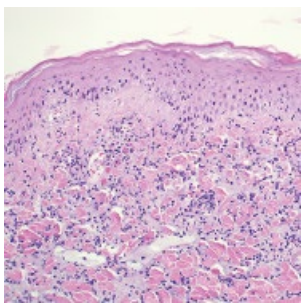


Figure 53.1 Rhomboid skin lesions in a pig infected with *Erysipelothrix rhusiopathiae*. *Source:* Courtesy of Dr. Patrick Halbur. Inset (a): larger magnification. Inset (b): microscopic lesions. Note diffuse moderate suppurative and mild necrotizing dermatitis.

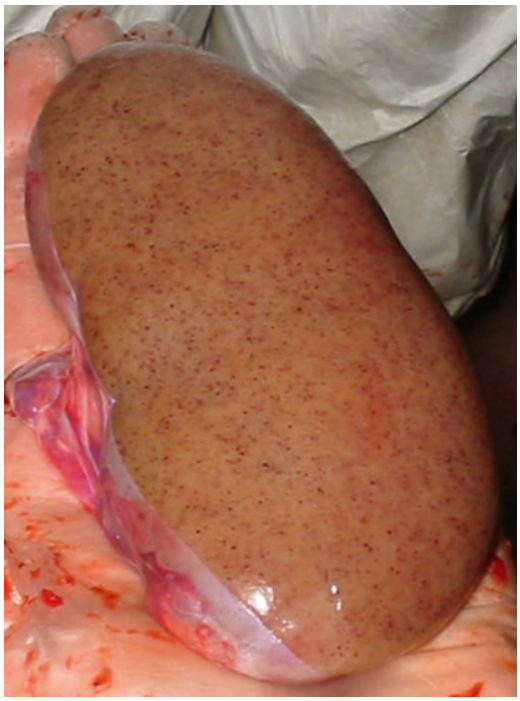


Figure 53.2 Pinpoint petechial hemorrhages on the cortex of a kidney from a pig suffering from swine erysipelas. *Source:* Courtesy of Dr. João Gomes-Neto.

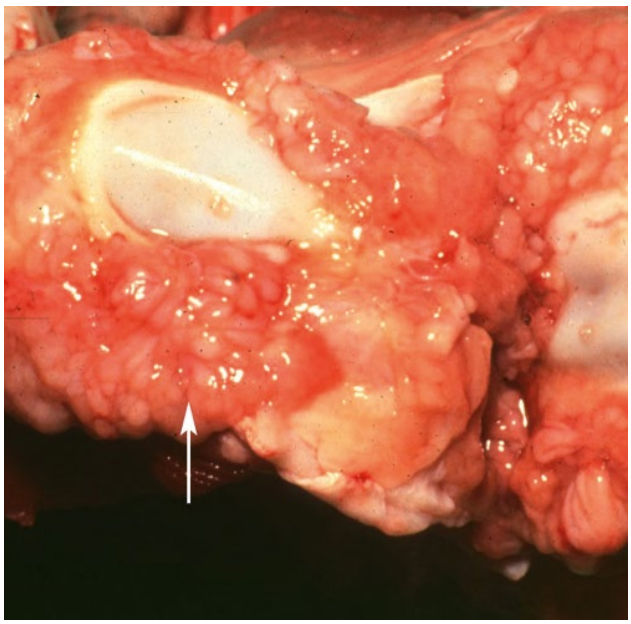


Figure 53.3 Proliferative synovitis in the stifle from a pig with swine erysipelas. *Source:* Courtesy of Dr. Greg Stevenson.

Chronic lesions may include chronic arthritis often involving joints of one or more legs or the intervertebral articulations. Proliferative synovial membranes (Figure 53.3) and serosanguinous effusion in the joint cavity are also observed. The joint capsule is often

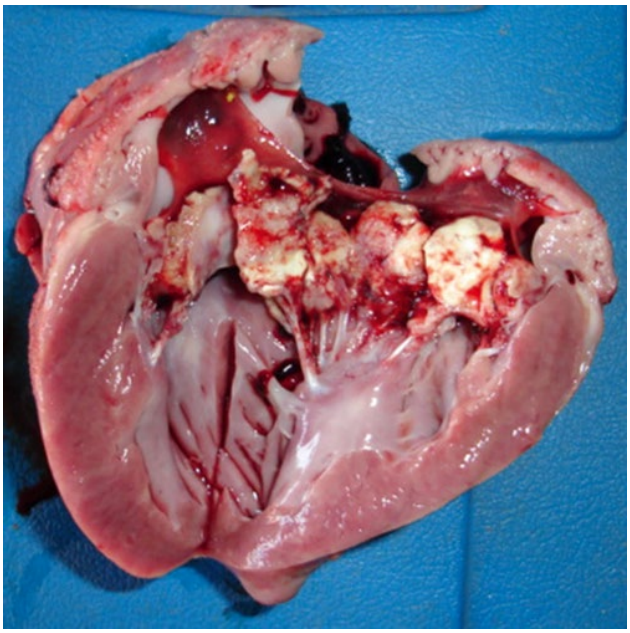


Figure 53.4 Valvular endocarditis in a pig with naturally occurring swine erysipelas. Note the proliferative granular growth on the heart valve. *Source:* Courtesy of Dr. João Gomes-Neto.

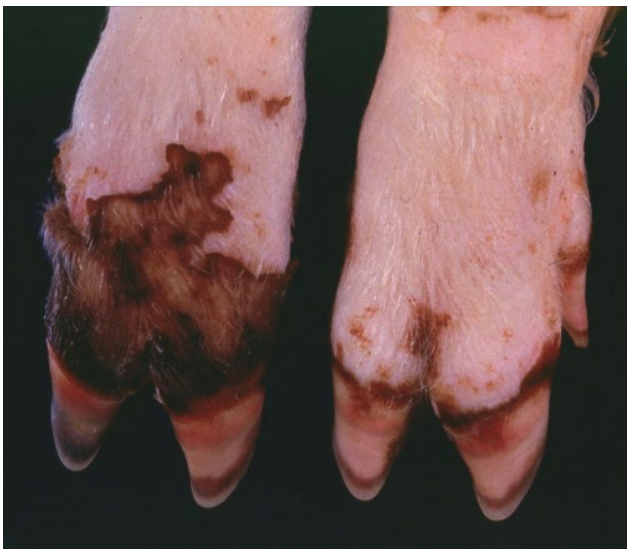


Figure 53.5 Ischemic necrosis of the skin on extremities in an 8-week-old pig with swine erysipelas. Note the dark dry partially detached skin. *Source:* Courtesy of Dr. Greg Stevenson.

hyperemic. There may be proliferation and erosion of the articular cartilage that lead to fibrosis, ankylosis, and spondylitis. Valvular endocarditis (Figure 53.4) can be seen as proliferative granular growth on the heart valves (mitral valve most common). Ischemic necrosis of the rhomboid skin lesions and of skin on the extremities is also observed as dry, dark, and sometimes partially detached skin (Figure 53.5).

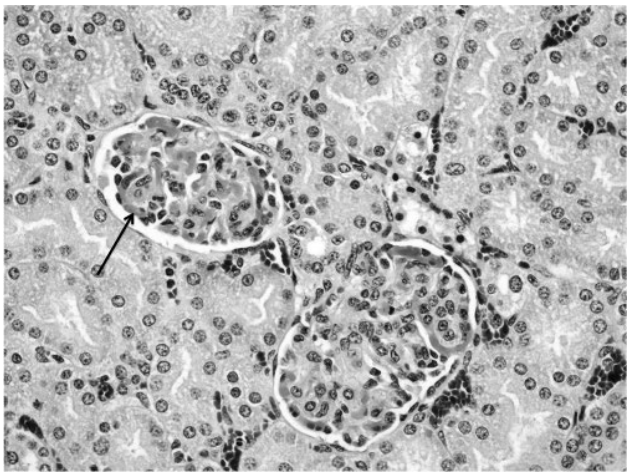


Figure 53.6 Changes in glomeruli in a kidney from a pig 2 days after experimental infection with *Erysipelothrix rhusiopathiae*. Note the inflammatory changes and congestion.

Microscopic lesions in acute erysipelas are predominantly in blood vessels, resulting in associated ischemia and necrosis. Capillaries and venules in the dermis are often dilated and congested. Microthrombi and bacterial emboli may occlude vessels, leading to circulatory stasis and focal necrosis (Figure 53.6). Neutrophils infiltrate the affected dermis. Similar hyperemia, vasculitis, neutrophilic infiltrates, and focal necrosis can also be observed in the brain, heart, kidneys, lungs, liver, spleen, and synovial membranes. Consequences of alveolar septal vessel damage include acute interstitial and exudative pneumonia characterized by serous exudates expanding alveolar septa and flood alveoli that also often contain aggregates of macrophages. Damage to glomerular vessels may result in hemorrhages that are grossly visible on the renal cortex. Affected lymph nodes are hyperemic, hemorrhagic, and infiltrated with neutrophils. Segmental hyaline and granular necrosis of muscle fibers followed by fibrosis, calcification, and regeneration also occurs. As lesions become subacute, infiltrates of monocytes, lymphocytes, and macrophages accumulate in sites of inflammation.

Chronic arthritis is characterized by marked hyperplasia of synoviocytes, resulting in thickened villous proliferations on synovial membranes that also have stromal thickening due to infiltrates of lymphocytes, plasma cells, and macrophages as well as neovascularization. In later stages, marked fibrosis may be observed in synovial membranes and periarticular tissues. Articular cartilage may be focally to extensively necrotic with associated fibrinous to fibrinopurulent exudates. Vegetative valvular endocardial lesions are composed of irregular fibrin lamina comprising fibrin, necrotic cellular debris, mixed inflammatory cells, bacterial colonies, and granulation tissue.

Diagnosis

Timely and accurate diagnosis of erysipelas is important since effective treatments are available and there are a wide variety of conditions that may be confused with swine erysipelas. Differentials for acute swine erysipelas include septicemia and sudden death in grow–finish pigs due to *Salmonella* Choleraesuis (Chapter 59), *Actinobacillus suis* or *Actinobacillus pleuropneumoniae* (Chapter 48), *Haemophilus parasuis* (Chapter 54), *Streptococcus suis* (Chapter 61), and other bacteria. Skin lesions resembling swine erysipelas can also be observed with classical swine fever virus (Chapter 39), porcine dermatitis and nephropathy syndrome (Chapter 30), or *A. suis* (Chapter 48) septicemia. For diagnosis of *Erysipelothrix* spp., a variety of tests are available (Table 53.2). Selection of diagnostic assays should be based on cost, required turnaround time, and availability in different geographic regions.

Isolation of *Erysipelothrix* spp. from tissues with morphological lesions provides a definitive laboratory diagnosis. Direct culture from non-contaminated specimens is usually fast and easy and can be conducted using basic laboratory equipment. Chronic cases and contaminated specimens often require prior selective enrichment methods (Bender et al. 2009; Harrington Jr and Hulse 1971; Wood 1965). Other gram-positive, non-sporulating rod-shaped bacteria that can be confused with *Erysipelothrix* spp. include members of the genera *Brochothrix*, *Corynebacterium*, *Lactobacillus*, *Listeria*, *Kurthia*, and *Vagococcus* (Bender et al. 2009).

To overcome the difficulties of bacterial isolation from specimens of antimicrobial-treated pigs or chronically affected pigs, an immunohistochemical assay has been developed and found to be highly sensitive and specific when compared with direct culture techniques (Opriessnig et al. 2010). In contrast the use of fluorescent antibodies in frozen tissue sections was found less sensitive compared with culture methods (Harrington Jr et al. 1974).

Several polymerase chain reaction (PCR) methods have been developed for the rapid detection of *Erysipelothrix* spp. Among PCR assays, genus-specific methods (Makino et al. 1994; Shimoji et al. 1998a), a multiplex PCR assay capable of differentiating between *E. rhusiopathiae* and *E. tonsillarum* (Yamazaki 2006), a species-specific PCR assay capable of distinguishing four *Erysipelothrix* species (Takeshi et al. 1999), a single nucleotide polymorphism-based PCR assay able to differentiate a Japanese live vaccine strain from field isolates (Shiraiwa et al. 2015), and a quantitative real-time multiplex assay capable of detecting and differentiating *E. rhusiopathiae*, *E. tonsillarum*, and *Erysipelothrix* spp. strain 2 (Pal et al. 2009) have been described.

Serological assays including plate, tube, and microtitration agglutination, passive hemagglutination, hemagglutination inhibition, complement fixation, enzyme-linked immunosorbent assays (ELISA), and indirect immunofluorescence assays have been used to detect antibodies against *E. rhusiopathiae* in pigs (Imada et al. 2003; Wang et al. 2010). However, since 30–50% healthy pigs are carriers and serum antibody responses to *E. rhusiopathiae* infection can persist for days, weeks, or even throughout the life of the pig, serology assays are most useful to evaluate vaccination success in breeding herds.

Genetic or antigenic properties of *Erysipelothrix* spp. isolates can be utilized to gain insight into the origin and relatedness of individual isolates. Methods include serotyping, genomic fingerprinting, and pulsed-field gel electrophoresis (PFGE). The standard serotyping method

Table 53.2 Application of different diagnostic assays for identification of *Erysipelothrix* spp.

	Preferred sample			
Assay	**Fresh tissues[a]**	**Formalin-fixed, paraffin-embedded tissues[a]**	**Fluids or blood**	**Estimated time required (h)**
Direct isolation	X[b]		X[b]	24–48
Enrichment	X		X	48–72
Fluorescent antibody assay	X			24–48
Immunohistochemistry	X			27
		X		3
Conventional PCR	X		X	5
Real-time PCR	X		X	3
Serology			X	5

[a] Tissues include the spleen, lungs, liver, affected sections of the skin, kidney, and lymph nodes.
[b] Not treated with antibiotics.

utilizes a double agar-gel precipitation test with type-specific rabbit antisera and antigen (Imada et al. 2004; Kucsera 1973); its execution depends on availability of antiserum and requires about 3 days for completion. Genomic fingerprints can be created for species for which minimal information is available by random amplified polymorphic DNA (RAPD) analysis (Okatani et al. 2000, 2004; Imada et al. 2004). Among the current DNA-based typing methods, PFGE has been considered the gold standard by some (Olive and Bean 1999), and using restriction enzyme *Sma*I resulted in distinct PFGE patterns, allowing differentiation among *Erysipelothrix* isolates (Coutinho et al. 2011; Okatani et al. 2001; Opriessnig et al. 2004); however, the turnaround time of this assay is 3–4 working days.

Immunity

Both humoral immunity and cell-mediated immunity play a role in host defense against *E. rhusiopathiae* infection. Therapy with antiserum has been widely used as treatment for acute septicemia. *E. rhusiopathiae* bacteria opsonized with immune serum are readily eliminated by neutrophils, peripheral mononuclear cells, or macrophages, suggesting a type I phagocytosis mediated by IgG antibodies (Shimoji 2000) and participating antigens, notably Spa proteins (Imada et al. 2003; Makino et al. 1998). The presence of cellular immunity against *E. rhusiopathiae* was confirmed in mice experimentally immunized with acapsular *E. rhusiopathiae* (Shimoji et al. 1998b); however, its relative contribution to protection and involved bacterial antigens are presently unknown.

A significant level of cross-protection is observed among different *E. rhusiopathiae* strains. Pigs vaccinated with a live serotype 2 vaccine were protected against clinical erysipelas when challenged with serotypes 1a, 1b, 2, 5, 8, 11, 12, 18, 19, and 21 but developed local dermal lesions after serotype 9 and 10 challenge (Takahashi et al. 1984). Cross-protection is best understood when focusing on the Spa proteins, which are known to elicit highly protective antibodies (Galán and Timoney 1990). Three Spa proteins have been identified to date, namely, SpaA, SpaB, and SpaC (Makino et al. 1998; To and Nagai 2007) (Table 53.1), and appear not restricted to certain serotypes (Ingebritson et al. 2010). In the mouse model, homologous cross-protection of recombinant SpaA, SpaB, or SpaC proteins was confirmed, but heterologous cross-protection varied (To and Nagai 2007). Furthermore, mice immunized with an *E. rhusiopathiae* serotype 2 SpaA strain were protected when challenged with homologous Spa-type strains; however, protection varied against heterologous Spa strains (Ingebritson et al. 2010). Since Spa classifications are predictive of cross-protection, a new classification scheme for *E. rhusiopathiae* isolates based on Spa genes has been suggested (Ingebritson et al. 2010).

The degree and duration of passively acquired immunity are thought to be related to the immune status of the dam and colostrum uptake of the offspring. Sows are routinely vaccinated against *E. rhusiopathiae*, and maternally derived antibodies in their offspring are generally considered protective for up to approximately 8 weeks of age (Pomorska-Mól et al. 2012).

Prevention and control

Antimicrobial therapy early in the course of *E. rhusiopathiae* infection usually results in a good response in affected pigs within 24–36 hours. The bacterium is highly susceptible to penicillin, which remains the treatment of choice (Chuma et al. 2010; Coutinho et al. 2011; Ding et al. 2015; Zhang et al. 2015). Most *E. rhusiopathiae* strains are also susceptible to cephalosporins, lincosamides, macrolides, quinolones, and tetracyclines; however, antimicrobial susceptibility should be carefully evaluated on a case-by-case basis due to the emergence of resistant isolates (Chuma et al. 2010; Coutinho et al. 2011). The mechanisms by which *E. rhusiopathiae* strains develop resistance to antibiotics remain unclear, though resistance genes located in the chromosome (Zhang et al. 2015) and a small plasmid (Xu et al. 2015) have been described.

Prevention of swine erysipelas is best accomplished by immunization programs. Current vaccines are based on *E. rhusiopathiae* serotypes 1 or 2 and are either inactivated bacterins for intramuscular injection or attenuated (avirulent live) vaccines designed for whole herd mass treatment via the drinking water. Most bacterins are serotype 2 (Eamens et al. 2006), while most attenuated live vaccines contain serotype 1a isolates (Opriessnig et al. 2004). Vaccination is generally effective in preventing swine erysipelas, and the duration of immunity varies between 6 and 12 months for both correctly administered bacterins and avirulent vaccines (Swan and Lindsey 1998). Vaccination may not be as effective in preventing chronic arthritis since sequestration of *E. rhusiopathiae* in the cytoplasm of chondrocytes of articular cartilage may provide protection from host immunity. Vaccination of breeding animals reportedly reduces the incidence of periparturient vulvar discharge, decreases farrowing intervals, and increases the numbers of live-born pigs in clinically affected herds (Gertenbach and Bilkei 2002). Immunization programs can target growing pigs, breeding herds, or both.

Negative herds can be established by Cesarean derivation or medicated early weaning. However, given the ubiquity of *E. rhusiopathiae*, it is unlikely that negative herds can be maintained for extended periods of time.

References

Bang BH, Rhee MS, Chang DH, et al. 2015. *J Microb* 107:443–451.

Bender JS, Kinyon JM, Kariyawasam S, et al. 2009. *J Vet Diagn Invest* 21:863–868.

Bender JS, Shen HG, Irwin CK, et al. 2010. *Clin Vaccine Immunol* 17:1605–1611.

Bender JS, Irwin CK, Shen HG, et al. 2011. *J Vet Diagn Invest* 23:139–142.

Bläckberg A, Trell K, Rasmussen M. 2015. *BCM Infect Dis* 15:402.

Carter GR. 1990. *Erysipelothrix rhusiopathiae*. In Cole JR, ed. *Diagnostic Procedures in Veterinary Microbiology and Mycology*, 5th ed. Springfield, IL: Academic Press, pp. 195–196.

Chirico J, Eriksson H, Fossum O, et al. 2003. *Med Vet Entomol* 17:232–234.

Chuma T, Kawamoto T, Shahada F, et al. 2010. *J Vet Med Sci* 72:643–645.

Conklin RH, Steele JH. 1979. *Erysipelothrix* infections. In Steele JH, ed. *CRC Handbook Series in Zoonoses*, Vol 1. Boca Raton, FL: CRC Press, pp. 327–337.

Coutinho TA, Imada Y, Barcellos DESN, et al. 2011. *Diagn Microbiol Infect Dis* 69:123–129.

Ding Y, Zhu D, Zhang J, et al. 2015. *Emerg Microbes Infect* 4:e69.

Eamens GJ, Chin JC, Turner B, et al. 2006. *Vet Microbiol* 116:138–148.

Franz B, Davies ME, Horner A. 1995. *FEMS Immunol Med Microbiol* 12:137–142.

Galán JE, Timoney JF. 1990. *Infect Immun* 58:3116–3121.

Gertenbach W, Bilkei G. 2002. *J Swine Health Prod* 10:205–207.

Giménez-Lirola LG, Xiao CT, Zavala M, et al. 2013. *J Microbiol Methods* 92:113–121.

Grieco MH, Sheldon C. 1970. *Ann N Y Acad Sci* 174:523–532.

Harada T, Ogawa Y, Eguchi M, et al. 2013. *Vet Immunol Immunopathol* 153:260–266.

Harada T, Ogawa Y, Eguchi M, et al. 2014. *Vet Microbiol* 172:216–222.

Harrington R Jr, Hulse DC. 1971. *Appl Microbiol* 22:141–142.

Harrington R Jr, Wood RL, Hulse DC. 1974. *Am J Vet Res* 35:461–462.

Imada Y, Mori Y, Daizoh M, et al. 2003. *J Clin Microbiol* 41:5015–5021.

Imada Y, Takase A, Kikuma R, et al. 2004. *J Clin Microbiol* 42:2121–2126.

Ingebritson AL, Roth JA, Hauer PJ. 2010. *Vaccine* 28:2490–2496.

Krasemann C, Müller HE. 1975. *Zentralbl Bakteriol Orig A* 231:206–213.

Kucsera G. 1973. *Int J Syst Bacteriol* 23:184–188.

Löffler F. 1886. *Arb Kais Gesundheitsamte* 1:47–55.

Makino S, Okada Y, Maruyama T, et al. 1994. *J Clin Microbiol* 32:1526–1531.

Makino S, Yamamoto K, Murakami S, et al. 1998. *Microb Pathog* 25:101–109.

Migula W. 1900. *System der Bakterien*, vol 2. Jena: Gustav Fischer.

Ogawa Y, Ooka T, Shi F, et al. 2011. *J Bacteriol* 193:2059–2971.

Okatani AT, Hayashidani H, Takahashi T, et al. 2000. *J Clin Microbiol* 38:4332–4336.

Okatani AT, Uto T, Taniguchi T, et al. 2001. *J Clin Microbiol* 39:4032–4036.

Okatani AT, Ishikawa M, Yoshida S, et al. 2004. *J Vet Med Sci* 66:729–733.

Olive DM, Bean P. 1999. *J Clin Microbiol* 37:1661–1669.

Opriessnig T, Hoffman LJ, Harris DL, et al. 2004. *J Vet Diagn Invest* 16:101–107.

Opriessnig T, Bender JS, Halbur PG. 2010. *J Vet Diagn Invest* 22:86–90.

Pal N, Bender JS, Opriessnig T. 2009. *J Appl Microbiol* 108:1083–1093.

Pasteur L. 1882. *C R Acad Sci* 95:1120–1121.

Pomorska-Mól M, Markowska-Daniel I, Pejsak Z. 2012. *Vet J* 194:128–130.

Principe L, Bracco S, Mauri C, et al. 2016. *Infect Dis Rep* 8:6368.

Reboli AC, Farrar WE. 1989. *Clin Microbiol Rev* 2:354–359.

Reboli AC, Farrar WE. 1992. The genus Erysipelothrix. In Balows A, Truper HG, Dworkin M, et al., eds. *The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 2nd ed. New York: Springer, pp. 1629–1642.

Rosenbach PJ. 1909. *Z Hyg Infektionskr* 63:343–369.

Schauer R. 1985. *Trends Biochem Sci* 10:357–360.

Schulz LC, Drommer W, Seidler D, et al. 1975a. *Beitr Pathol* 154:27–51.

Schulz LC, Drommer W, Seidler D, et al. 1975b. *Beitr Pathol* 154:1–26.

Schulz LC, Ehard H, Drommer W, et al. 1976a. *Zentralbl Veterinarmed B* 23:617–637.

Schulz LC, Hertrampf B, Ehard H, et al. 1976b. *Z Rheumatol* 35:315–323.

Shimoji Y. 2000. *Microbes Infect* 2:965–972.

Shimoji Y, Mori Y, Hyakutake K, et al. 1998a. *J Clin Microbiol* 36:86–89.

Shimoji Y, Mori Y, Sekizaki T, et al. 1998b. *Infect Immun* 66:3250–3254.

Shimoji Y, Ogawa Y, Osaki M, et al. 2003. *J Bacteriol* 185:2739–2748.

Shiraiwa K, Ogawa Y, Egushi M, et al. 2015. *J Microbiol Meth* 117:11–13.

Skerman VBD, McGowan V, Sneath PHA. 1980. *Int J Syst Bacteriol* 30:225–420.

Stephenson EH, Berman DT. 1978. *Am J Vet Res* 39:187–188.

Swan RA, Lindsey MJ. 1998. *Aust Vet J* 76:325–327.

Takahashi T, Takagi M, Sawada T, et al. 1984. *Am J Vet Res* 45:2115–2118.

Takahashi T, Fujisawa T, Benno Y, et al. 1987. *Int J Syst Bacteriol* 37:166–168.
Takahashi T, Nagamine N, Kijima M, et al. 1996. *J Vet Med Sci* 58: 587–589.
Takahashi T, Fujisawa T, Umeno A, et al. 2008. *Microbiol Immunol* 52: 469–478.
Takeshi K, Makino S, Ikeda T, et al. 1999. *J Clin Microbiol* 37:4093–4098.
To H, Nagai S. 2007. *Clin Vaccine Immunol* 14:813–820.
To H, Sato H, Tazumi A, et al. 2012. *J Vet Med Sci* 74:949–953.
Verbarg S, Rheims H, Emus S, et al. 2004. *Int J Syst Evol Microbiol* 54:221–225.
Wang Q, Chang BJ, Mee BJ, et al. 2005. *Vet Microbiol* 107:265–272.
Wang Q, Chang BJ, Riley TV. 2010. *Vet Microbiol* 140:405–417.
Wood RL. 1965. *Vet Res* 26:1303–1308
Wood RL. 1973. *Cornell Vet* 63:390–410.
Wood RL, Harrington R Jr. 1978. *Am J Vet Res* 39:1833–1840.
Xu C, Zhang A, Yang C, et al. 2015. *Antimicrob Agents Chemother* 59:2462–2465.
Yamazaki Y. 2006. *J Vet Diagn Invest* 18:384–387.
Zhang A, Xu C, Wang H, et al. 2015. *Vet Microbiol* 177:162–167.

54

Glässer's Disease

Virginia Aragon, Joaquim Segalés, and A.W. (Dan) Tucker

Relevance

In 1910 K. Glässer discovered a bacillus in exudates from pigs with fibrinous polyserositis. The isolation and characterization of this fastidious bacillus required several years, and, after some changes, it was named *Haemophilus parasuis*. The disease caused by *H. parasuis* is characterized by fibrinous polyserositis and arthritis and is known as Glässer's disease.

Glässer's disease occurs in swine populations around the world irrespective of health status, and outbreaks of this economically significant disease may be linked to stress-associated events including coinfections and moving and commingling animals (Brockmeier et al. 2014). In the United States it is considered one of the main infectious problems in the nursery, also affecting growing pigs and sows (Holtkamp et al. 2007).

Etiology

The etiological agent of Glässer's disease was formally identified in 1931 by Lewis and Shope as *Haemophilus influenzae* (variety *suis*), due to the similarity to the human pathogen *H. influenzae*. Later, Biberstein and White (1969) determined that contrary to *H. influenzae*, the swine bacterium did not require the X factor (hemin) to grow. Following the standard nomenclature in this group of microorganisms, *H. influenzae* (variety *suis*) was renamed *H. parasuis*.

Haemophilus parasuis is a gram-negative bacterium and member of the family *Pasteurellaceae*. Revision of the classification of some members of this family is ongoing, and reclassification of *H. parasuis* as a different genus has been proposed (Inzana et al. 2016). In addition, high diversity in 16S rRNA gene sequence within the *H. parasuis* species and segregation into two distinct clusters have been reported (Angen et al. 2007).

Classification of *H. parasuis* strains

Haemophilus parasuis strains are heterogeneous in phenotypic and genotypic traits, including virulence. Strains are classified into 15 serovars (Kielstein and Rapp-Gabrielson 1992), although non-typable isolates represent a high percentage. Serotyping can be performed by agar-gel precipitation test (AGPT) (Kielstein and Rapp-Gabrielson 1992) or by indirect hemagglutination (del Rio et al. 2003b; Tadjine et al. 2004). *H. parasuis* serovars 4 and 5, along with non-typable isolates, are reported as the most prevalent in different countries (Angen et al. 2004; Cai et al. 2005; Oliveira et al. 2003a; del Rio et al. 2003b; Rubies et al. 1999; Tadjine et al. 2004; Turni and Blackall 2005). Discrepancies in serotyping results are reported (Turni and Blackall 2005) and are probably due to differences in methods, antisera, and reacting antigens. Recently, the analysis of loci encoding capsular polysaccharides from the 15 serovar reference strains has allowed design of polymerase chain reactions (PCRs) for molecular serotyping (Howell et al. 2013). These PCRs represent an invaluable tool for implementation of serotyping in many laboratories while reducing the number of non-typable isolates and the inconsistency of results.

Several studies have attempted to correlate serovar and virulence, but a strict relationship between these two traits has not been demonstrated (Table 54.1). As an example, serovar 7 strains were thought to all be non-virulent, but some serovar 7 strains have been isolated from systemic lesions of Glässer's disease, and disease has been reproduced with one of them (Aragon et al. 2010).

In order to increase the discrimination capacity of the typing methods, several genotyping methods have been used to classify *H. parasuis* strains. *H. parasuis* was first genotyped using fingerprinting methods, such as restriction endonuclease pattern, also known as restriction fragment length polymorphism (RFLP) (Smart et al. 1988) and enterobacterial repetitive intergenic consensus (ERIC)-PCR (Oliveira et al. 2003a; Rafiee et al. 2000).

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 54.1 Clinical and pathological outcome from experimental inoculation with strains from different serovars of *Haemophilus parasuis*.

Serovar	Clinical or pathological outcome	Route of inoculation	References
1,5,10,12,13,14	Death/moribundity	IP	Kielstein and Rapp-Gabrielson (1992)
2,4,8,15	Polyserositis		
3,6,7,9,11	Healthy		
1,5	Polyserositis	IN	Nielsen (1993)
2,3,4,6,7	Healthy		
1,5	Death/polyserositis	IN	Amano et al. (1994)
4	Polyserositis[a]		
10,12,4	Healthy	IN	Aragon et al. (2010)
4,15,10,5	Polyserositis		
7	Polyserositis[a]		

IP, intraperitoneal; IN, intranasal.
[a] In 1 out of 6 pigs.

Fingerprinting results are useful for local studies but are difficult to share and compare among laboratories. To facilitate the exchange of genotyping information, multilocus sequence typing was later developed (Olvera et al. 2006; Mullins et al. 2013). Genotyping methods confirmed the high heterogeneity of *H. parasuis*, allowed the identification of several *H. parasuis* strains in a single animal or farm, and established that strains isolated from systemic and respiratory sites represented two distinct groups.

There is no direct association between genotype and serovar (Oliveira et al. 2003a; Turni and Blackall 2010a). Lack of association between genotype and serovar has also been confirmed by three different RFLP-PCR protocols using *tbpA* (de la Puente Redondo et al. 2003) and *aroA* genes (del Rio et al. 2006).

Since the first report of the genome sequence of *H. parasuis* (Yue et al. 2008), the number of strains whose genome has been sequenced has increased quickly. Comparison of the genome of more than 200 clinical and nonclinical isolates allowed the detection of 48 putative virulence factors, including some factors identified by independent methods, such as the virulence-associated trimeric autotransporters (VtaAs), the cytolethal distending toxin (CDT), a *tonB*-dependent protein, and a serine protease (Howell et al. 2014).

Laboratory cultivation

Haemophilus parasuis is a small, nonmotile, pleomorphic (from single coccobacilli to filamentous chains) gram-negative bacterium in the family *Pasteurellaceae*, which requires V factor, nicotinamide adenine dinucleotide (NAD), but not X factor, hemin, for growth. In the laboratory *H. parasuis* grows on enriched chocolate agar, but not on blood agar. However it can also be cultured on blood agar with a *Staphylococcus* nurse streak as a source of V factor, showing the characteristic satellitic growth. *H. parasuis* requires 1–3 days to produce small brown-to-gray colonies on chocolate agar plates or small translucent nonhemolytic colonies on blood agar.

Epidemiology

Haemophilus parasuis is a member of the normal respiratory microbiota and is ubiquitous in swine herds worldwide. Colonization of the upper respiratory tract of piglets by *H. parasuis* occurs soon after birth through contact with the sow. However *H. parasuis* is not always detected in the nasal cavity of dams (Cerdà-Cuéllar et al. 2010), probably due to low quantity of bacteria in these animals. Attempts to isolate *H. parasuis* from the vagina of the sows have failed. This is in agreement with the production of *H. parasuis*-free piglets by snatch farrowing and subsequent artificial feeding. Snatch-farrowed colostrum-deprived piglets have been useful to experimentally reproduce disease with *H. parasuis* (Blanco et al. 2004; Oliveira et al. 2003b).

Haemophilus parasuis has been detected in nasal swabs of piglets with a maximum prevalence of colonization occurring at 60 days of age (Angen et al. 2007; Cerdà-Cuéllar et al. 2010). Different strains of *H. parasuis* can be isolated from the nasal cavities of piglets, and a single animal can carry more than one strain. Four to five strains can be isolated from a herd at a given time, and up to 16 different strains were isolated in a single farm during one production cycle (Cerdà-Cuéllar et al. 2010; Oliveira et al. 2003a; Olvera et al. 2006, 2007b). In spite of the variety of strains within a herd, usually one prevalent strain is associated with an outbreak (Rafiee et al. 2000).

Colonization of nasal mucous membranes by *H. parasuis* usually develops when piglets are still protected from Glässer's disease by circulating maternal IgM and IgG antibodies that are protective only for the strains to which each dam has been exposed. Disease may occur in pigs at different ages based on a number of factors including decay of maternal immunity, exposure to other strains by contact with other litters, mixing at weaning, or introduction of new stock. Additionally, immunity may be suppressed by porcine reproductive and respiratory syndrome virus (PRRSV) destruction and/or induced dysfunction of macrophages or by less defined mechanisms involving other pathogens, chilling, crowding, or other stressful events.

Domestic pig and wild boar are the only known hosts for this bacterium. *H. parasuis* has been isolated (Olvera et al. 2007a), and specific antibodies have been detected (Vengust et al. 2006) in wild boar, but only one case of Glässer's disease has been reported in this animal (Cuesta Gerveno et al. 2013).

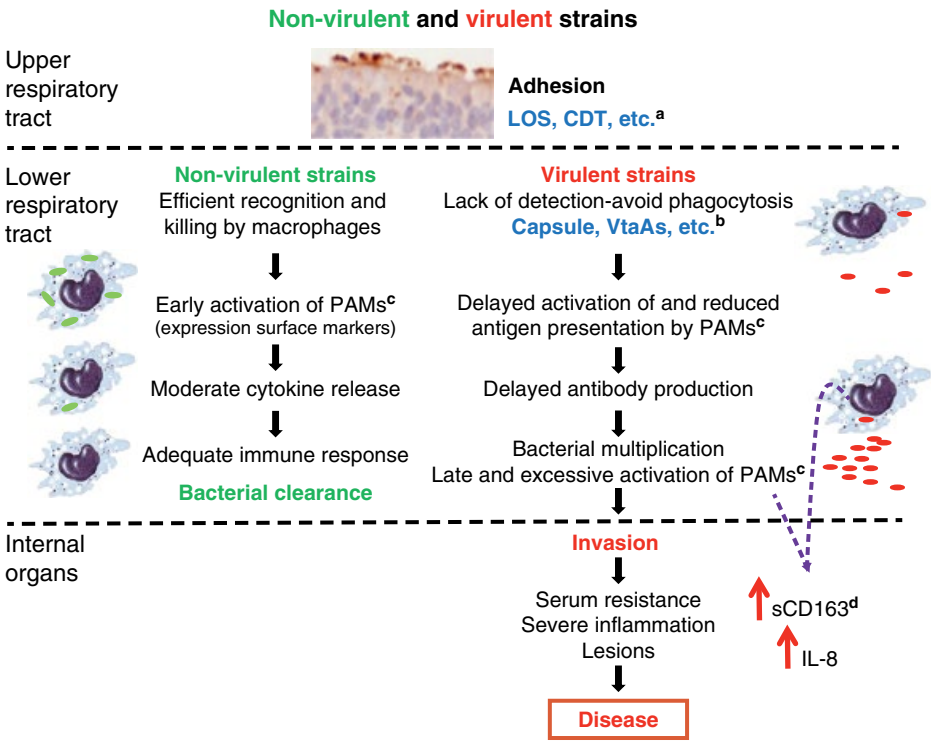
Transmission of *H. parasuis* occurs through contact of carrier or diseased pigs with susceptible animals. Thus, mixing pigs from different origins and ages is a risk factor for transmission. *H. parasuis* is very labile in the environment. Although there are not many studies on its resistance to disinfectants, efficacy of several formulations, including chloramine-T and quaternary ammonium compounds, has been reported (Rodríguez-Ferri et al. 2010).

Pathogenesis

Haemophilus parasuis pathogenesis requires entry into the host, evasion of host defenses, bacterial multiplication, and damage to tissues (Figure 54.1). Disease caused by *H. parasuis* has been reproduced with virulent strains by different inoculation routes (Amano et al. 1994; Blanco et al. 2004; Palzer et al. 2015a). After intranasal inoculation *H. parasuis* was detected in the nasal mucosa where suppurative rhinitis and epithelial cell degeneration were observed (Vahle et al. 1997). Induction of adhesins and biofilm formation may play an important role at this stage, especially in the normal colonization by non-virulent strains (Bello-Ortí et al. 2014b; Jin et al. 2006). Bacterial adhesion to and invasion of epithelial cells, induction of apoptosis, and cytokine release can also be important events for *H. parasuis* colonization (Bouchet et al. 2009; Frandoloso et al. 2012a). CDT and sugar residues of the lipooligosaccharide (LOS) are involved in the interaction with epithelial cells (Xu et al. 2013; Zhang et al. 2012b, 2013, 2014; Zhou et al. 2016). After nasal colonization, the bacterium is isolated from lung and later only virulent strains from internal organs after a short passage through blood (Vahle et al. 1995). In the lung, virulent strains of *H. parasuis* survive the phagocytic activity of porcine alveolar macrophages (PAMs) and can be detected in the lung tissue by immunohistochemistry (IHC). In contrast non-virulent strains are efficiently cleared from the lung by PAMs and are retained in the upper respiratory tract (Bello-Orti et al. 2014a; Olvera et al. 2009). Macrophage activation is delayed by virulent strains, and lung infection is established (Costa-Hurtado et al. 2013). Phagocytosis resistance is likely associated with the expression of capsule (Olvera et al. 2009), as well as with other genes, including two virulence-associated trimeric autotransporters (*vtaA*), VtaA8 and VtaA9 (Costa-Hurtado et al. 2012). In agreement, several genes with homology to putative virulence factors, such as several *vtaAs* and *siaB* (involved in sialic acid utilization), or a protease, were expressed *in vivo* in the infected lung (Bello-Ortí et al. 2015; Jin et al. 2008). Survival in the lung by virulent strains is accompanied by changes in bacterial metabolism, including upregulation of iron uptake genes (Bello-Ortí et al. 2015). Besides, *H. parasuis* possess a neuraminidase that operates as scavenger of sialic acid for nourishment or modification of the bacterial surface to evade the immune system (Martínez-Moliner et al. 2012; Perry et al. 2013).

Haemophilus parasuis invades endothelial cells and induces apoptosis and production of proinflammatory interleukin-6 (IL-6) and interleukin-8 (IL-8) (Bouchet et al. 2008; Frandoloso et al. 2013). These phenomena may play a role in the passage to the blood and across the blood–brain barrier. LOS has a partial role in endothelial adhesion and induction of inflammation (Bouchet et al. 2008; Zhang et al. 2013, 2014). Virulent strains are able to survive the bactericidal effect of the blood complement (Cerdà-Cuéllar and Aragon 2008) and can reach systemic sites. Several bacterial factors are involved in serum resistance, including the polysaccharide biosynthesis protein CapD (Wang et al. 2013) and the outer membrane protein P2 (Zhang et al. 2012a). Systemic spread of *H. parasuis* induces a severe increase in proinflammatory IL-8 and soluble CD163 in blood (Costa-Hurtado et al. 2013), with the subsequent inflammation and production of the characteristic lesions of Glässer's disease in serosae. In the spleen of infected pigs, genes involved in immune response were differentially expressed and included inflammatory molecules, acute-phase proteins, adhesion molecules, complement, and genes with functions in antigen processing and presentation (Chen et al. 2009).

Severity of disease depends on the virulence of the *H. parasuis* strain, the immunity of the piglets, the concomitant presence of other pathogens in the herd, and the genetic resistance of the host. *H. parasuis* can act as primary or secondary pathogen. Immunosuppressive events, such as viral infections that alter the immune system, allow *H. parasuis* strains that are usually restricted



[a] Sugar residues of the capsular lipooligosaccharide (LOS) and bacterial cytolethal distending toxin (CDT) are important in adhesion to host epithelial cells
[b] Bacterial capsule and two virulence-associated trimeric autotransporters (VtaA8 and VtaA9) interfere with phagocytosis by host leukocytes
[c] Pulmonary alveolar macrophages (PAMs)
[d] During tissue invasion, the number of activated macrophages increases with an associated increase in proinflammatory cytokines sCD163 and interleukin-8

Figure 54.1 Main events in *Haemophilus parasuis* pathogenesis. Virulent strains delay activation of alveolar macrophages, survive in the lung, and invade systemic organs, while non-virulent strains are cleared from the lung by alveolar macrophages and remain in the upper respiratory tract. Invasion of virulent strains induces severe inflammation and the characteristic lesions of Glässer's disease. Blue represents some virulence factors.

to the respiratory tract to invade and be isolated from systemic sites (Olvera et al. 2009). An epidemiological association between *H. parasuis* infection and PRRSV, porcine circovirus type 2 (PCV2), and influenza virus has been reported (Kim et al. 2002; Li et al. 2009; Palzer et al. 2015b; Unterweger et al. 2016). Exacerbation of disease by *H. parasuis* with PRRSV was experimentally confirmed by concurrent infection with both agents (Yu et al. 2012), although earlier studies failed to find such association (Solano et al. 1997). A work by Brockmeier (2004) linked higher nasal colonization by *H. parasuis* to prior colonization by *Bordetella bronchiseptica*. The influence of genetic background on resistance to *H. parasuis* systemic infection has been evaluated. Blanco et al. (2008) reported that piglets obtained from six different sire boars significantly varied regarding their resistance, which was associated with higher levels of antibacterial activity in the lung (Wilkinson et al. 2010). Some genetic markers (BAT2, Mx1, and EHMT2 variations) have been also associated with the risk of infection by *H. parasuis* (Wang et al. 2012).

Even though domestic pig and wild boar are the only natural hosts for *H. parasuis*, the guinea pig model is suitable to reproduce lesions (Morozumi et al. 1982; Rapp-Gabrielson et al. 1992), and the mouse model has been proven useful to study the protective capacity of experimental vaccine candidates (Zhou et al. 2009).

Clinical signs

Clinical signs are mainly observed in 4- to 8-week-old pigs, although the age of affected animals may vary, depending on the level of acquired maternal immunity and colonization. Also, Glässer's disease cases can be sporadically observed in adult pigs. Based on experimental

infections using *H. parasuis*-free piglets (Aragon et al. 2010), the incubation period varies depending on the infecting strain and ranges from less than 24 hours to 4–5 days post inoculation.

Peracute disease has a short course (<48 hours) and may result in sudden death without characteristic gross lesions (Peet et al. 1983). The typical clinical signs of acute Glässer's disease include high fever (41.5 °C), coughing, abdominal breathing, swollen joints with lameness, and central nervous signs such as lateral decubitus, paddling, and trembling (Vahle et al. 1995). These signs may be seen jointly or independently. Animals with mild to moderate clinical signs usually survive the acute phase of disease and may develop a chronic stage characterized by rough hair, reduced growth rate, and lameness. Dyspnea and coughing have also been described together with *H. parasuis* isolation from lungs with cranioventral consolidation (Little 1970). Other very sporadic clinical outcomes have been associated with *H. parasuis* infection, such as acute myositis of the masseter muscles in gilts (Hoefling 1991).

Morbidity and mortality rates associated with Glässer's disease in affected farms have been traditionally very variable, but they usually vary from 5 to 10% in conventional farms. Concomitant stressors, mainly viral infections that alter the immune system, also modulate disease prevalence. As stated above an increase of Glässer's disease prevalence has been described in farms affected by other pathogens, such as PRRSV, PCV2, and influenza.

Lesions

Peracutely diseased pigs usually die without characteristic gross lesions, but may show petechial hemorrhages in some tissues (Peet et al. 1983). Histologically, those pigs show septicemia-like microscopic lesions such as disseminated intravascular coagulation and micro-hemorrhages (Amano et al. 1994). Increased serosanguinous fluid in the thoracic and abdominal cavities, without fibrin, can also be seen in peracute cases of *H. parasuis* infection.

Acute systemic infection is characterized by the development of fibrinous or fibrinopurulent polyserositis, polyarthritis, and meningitis (Figure 54.2). The fibrinous exudate can be observed on the pleura, pericardium, peritoneum, synovia, and meninges and is usually accompanied by an increased amount of fluid (Oliveira et al. 2003b; Vahle et al. 1995). Fibrinous

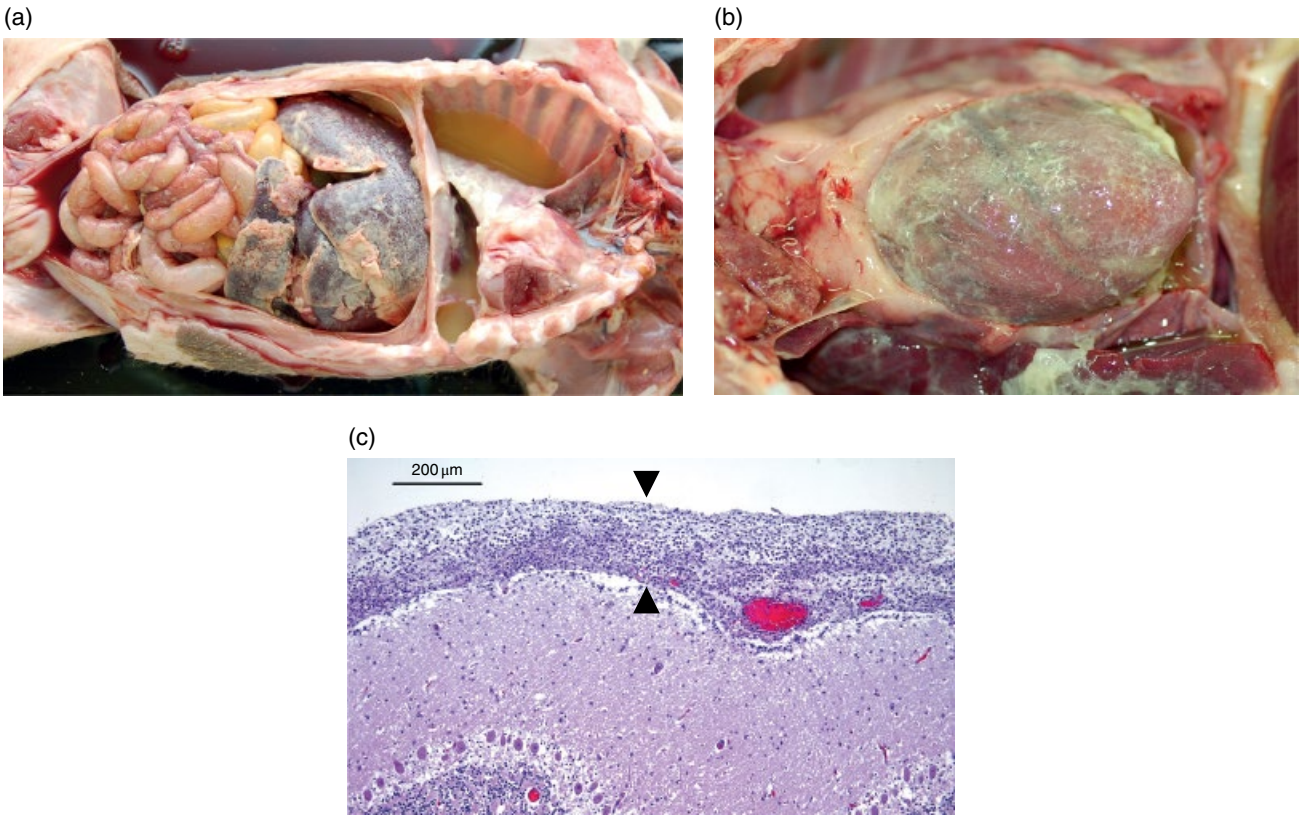


Figure 54.2 Gross and microscopic lesions found in *Haemophilus parasuis* systemic infection. (a) Polyserositis characterized by fibrinopurulent exudate on serosal membranes in peritoneal and thoracic cavities. Note the significant amount of fluid in the thorax. (b) Fibrinopurulent exudate on pericardial surface. (c) Brain, fibrinopurulent meningitis.

pleuritis may be found with or without cranioventral consolidation due to catarrhal purulent bronchopneumonia (Little 1970). Lack of characteristic gross lesions is also common in a number of pigs showing neurological clinical signs. Histopathological examination of typical Glässer's disease reveals fibrinous to fibrinopurulent serositis and usually does not contribute additional useful information to the diagnosis except the detection of potential fibrinopurulent meningitis (Figure 54.2c). Recently, pyelitis has been described as a consistent histopathological finding in experimentally infected conventional pigs with *H. parasuis* serovar 5 (Palzer et al. 2015a).

Chronically affected animals usually show severe fibrosis of the pericardium, pleura, and/or peritoneum, as well as chronic arthritis.

Diagnosis

The clinical signs and lesions described for *H. parasuis* systemic infection are not pathognomonic of this agent, and other agents must be included in a differential diagnosis. Fibrinous polyserositis may be caused by other gram-negative bacteria such as nonhemolytic *Escherichia coli* (Chapter 52). Polyserositis caused by nonhemolytic *E. coli* is sporadic and usually affects suckling piglets (Nielsen et al. 1975). Beta-hemolytic Shiga toxin 2e (Stx2e)-positive *E. coli* can cause central nervous system signs in recently weaned pigs (edema disease) similar to those observed with *H. parasuis* systemic infection. However, these pigs do not develop fibrinopurulent meningitis typical of *H. parasuis* (Moxley 2000). *Mycoplasma hyorhinis* is another important cause of fibrinous polyserositis in nursery pigs, and it is frequently found coinfecting pigs with *H. parasuis* (Chapter 56; Palzer et al. 2015b). *Streptococcus suis* usually affects pigs at the same age as *H. parasuis* and may cause similar lesions (Chapter 61; Reams et al. 1994). Other agents involved in development of lameness and arthritis include *Erysipelothrix rhusiopathiae* (Chapter 53) and *Mycoplasma hyosynoviae* (Chapter 56). However, both agents are more likely to cause chronic non-purulent arthritis in finishing pigs (Hariharan et al. 1992).

Considering that *H. parasuis* is a colonizer of the upper respiratory tract of healthy pigs, detection of this microorganism in the nasal cavity and trachea does not imply disease development. Systemic isolates are more likely to carry important virulence factors, and, therefore, those isolates should be pursued for laboratory confirmation of morbidity and mortality caused by this agent.

Haemophilus parasuis can be isolated from the fibrinous exudate and parenchyma of affected internal organs and from lung lesions in pneumonia cases. It is recommended to take several samples from the same animal. *H. parasuis* is a fastidious microorganism with limited survival at room temperature and special requirements for *in vitro* growth. The chances of isolating *H. parasuis* can be considerably improved by utilizing swabs with Amies transport media and submitting samples under refrigeration to the laboratory (del Rio et al. 2003a). Although *H. parasuis* isolation can be a difficult task, it is important to obtain an isolate for antibiotic sensitivity assessment and typing.

H. parasuis can also be detected directly in the lesions of affected tissues by IHC (Amano et al. 1994; Bello-Orti et al. 2014a; Vahle et al. 1995) or *in situ* hybridization (ISH) (Jung et al. 2004). However the specificity of these techniques is not fully clarified, and their uses have been restricted to research activities.

PCR is a sensitive and specific method to detect *H. parasuis*. Several methods are described in the literature, varying from conventional gel-based tests to real-time detection (Angen et al. 2007; Jung et al. 2004; Oliveira et al. 2001; Turni et al. 2010b). PCR detects *H. parasuis* even when the organism is no longer viable, and it represents a great advantage in sensitivity compared with bacterial isolation. A real-time PCR showed a detection limit of 0.83–9.5 CFU per reaction and was more sensitive and specific than previously reported gel-based methods (Turni et al. 2010b). PCR can be used also for differentiation between virulent and non-virulent isolates. A multiplex test based on the detection of a fragment of *vtaA*s can be used to further characterize isolates regarding invasiveness potential (Olvera et al. 2012). This information is relevant, especially when selecting isolates for antibiotic sensitivity or autogenous vaccine production.

ELISA can detect antibodies against *H. parasuis*, although this technique has mostly been used for research purposes. Whole cell indirect ELISAs have been used to characterize the transfer of maternal immunity from sows to piglets and to demonstrate seroconversion post-vaccination (Baumann and Bilkei 2002; Cerdà-Cuéllar et al. 2010; Solano-Aguilar et al. 1999). Blanco et al. (2004) utilized a commercial ELISA to characterize the decay of maternal antibodies and identify a window of susceptibility in conventional pigs for experimental infection. The specificity of experimental and commercially available *H. parasuis* ELISAs has not been extensively evaluated, and their ability to detect antibodies against different *H. parasuis* serovars and strains has been poorly characterized.

Immunity

Both innate and acquired immune responses are important in *H. parasuis* infection. Although phagocytosis, antibody opsonization, and complement attack were

previously recognized as key defenses, new information on the interplay between pathogen and host humoral and cellular immune responses is emerging.

Immune responses

IHC and ISH methods have demonstrated that following infection *H. parasuis* is phagocytosed by neutrophils and macrophages and can be found as degenerated bacteria in dilated phagosomes (Amano et al. 1994; Segales et al. 1999). PAMs promptly phagocytose non-virulent strains, whereas virulent strains show resistance to phagocytosis. Once opsonized by antibodies, these virulent strains can be successfully internalized and killed by PAMs (Olvera et al. 2009). Virulent strains appear to be resistant to complement-mediated killing, while non-virulent strains are susceptible to complement activation and killing via the non-antibody-mediated pathway (Cerdà-Cuéllar and Aragon 2008).

H. parasuis stimulates the production of proinflammatory cytokines IL-8 and IL-6 by porcine airway epithelial cells upon adhesion (Bouchet et al. 2009; Chen et al. 2015). IL-8 is a potent chemoattractant for leukocytes, whereas IL-6 is an important mediator in the development of acute-phase response. Increased levels of IL-1α have been reported in pigs undergoing severe disease following experimental infection, whereas IL-4, IL-10, tumor necrosis factor-alpha (TNF-α), and interferon gamma (IFN-γ) were expressed in significantly higher levels by survivors (Martín de la Fuente et al. 2009a). Swift-onset global changes in circulating immune cell populations have been described following *H. parasuis* challenge including a rapid increase in CD163+ monocyte lineages alongside a depletion of gamma/delta TCR+ T cells (Frandoloso et al. 2012b).

Development of antibodies against *H. parasuis* following exposure or vaccination has been demonstrated in colonized, convalescent, and vaccinated pigs. Pigs exposed to *H. parasuis* live cultures or vaccinated with killed bacterins generate a transient IgM response followed by a solid and progressively increasing IgG antibody response. Pigs with high IgG titers are protected against challenge (Martín de la Fuente et al. 2009b). Pigs exposed to live *H. parasuis* cells develop antibodies against specific surface proteins, including VtaAs and oligopeptide permease A (OppA), which are not always elicited by killed vaccines (Macedo et al. 2016; Olvera et al. 2010). The role of CD8+ cytotoxic T cells, which rapidly increase in numbers in challenged pigs, is not fully understood but is proposed to be linked to MHC class I presentation based on the ability of *H. parasuis* to penetrate epithelial and endothelial cells (Frandoloso et al. 2012b).

Protective immunity and cross-protection

Protective immunity against *H. parasuis* infection has been linked mainly to the development of specific antibodies, which are known to successfully opsonize *H. parasuis* and facilitate phagocytosis by PAMs (Olvera et al. 2009). Complete protection against challenge is usually obtained with homologous vaccination, whereas partial protection is generally observed with heterologous challenges (Martín de la Fuente et al. 2009b; Miniats et al. 1991; Nielsen 1993; Takahashi et al. 2001).

Exposure and colonization with live *H. parasuis* cells are also known to generate protective immunity. Pigs exposed by aerosol to different live *H. parasuis* strains developed circulating antibodies and resisted challenge with virulent heterologous strains (Brockmeier et al. 2013; Nielsen 1993). However, pigs colonized with virulent strains serve as reservoirs for transmission to naive pigs (Brockmeier et al. 2013) and constitute a risk for disease.

Cross-protection between different serovars and even within the same serovar is variable and difficult to predict (Rapp-Gabrielson et al. 1997). The design of new universal vaccines has exploited immunoproteomic approaches to identify secreted and surface proteins with protective potential (Li et al. 2015; Olvera et al. 2011; Zhou et al. 2009). Most promising among these approaches is the demonstration of protection from *H. parasuis* challenge after immunization using a mutated transferrin-binding protein (TbpB) cloned from a virulent strain of *H. parasuis* – with evidence of a robust TH2 response (Martínez-Martínez et al. 2016).

Maternal immunity

Maternal antibodies are an important source of protective immunity for susceptible piglets. Pigs lacking maternal immunity are susceptible to systemic infection using low doses of *H. parasuis*, whereas pigs with maternal antibodies are protected against challenge (Blanco et al. 2004; Oliveira et al. 2003b).

Levels of maternal antibodies directly influence the susceptibility of the offspring to *H. parasuis* systemic infection. Solano-Aguilar et al. (1999) demonstrated that pigs from vaccinated gilts had significantly higher maternal antibody titers compared with those from non-vaccinated gilts. Pigs from vaccinated gilts were protected against challenge at 28 days of age, whereas pigs from non-vaccinated gilts were fully susceptible to systemic infection at 21 days of age. More recently Cerdà-Cuéllar et al. (2010) found that maternal immunity decayed by 20 days in pigs from non-vaccinated sows but not until 60 days in those from vaccinated sows. These timings coincided with high levels of isolation and detection of *H. parasuis* in the nasal cavities of these pigs. Pigs from

vaccinated sows tended to have a lower amount and reduced variety of *H. parasuis* strains compared with pigs from non-vaccinated sows. Maternal immunity can interfere with piglet vaccination, measured as antibody induction (Pomorska-Mól et al. 2011), although this interference was not observed when vaccination was performed during an acute outbreak of Glässer's disease (Oh et al. 2013).

Prevention and control

Antibiotics are widely used to prevent and control *H. parasuis* disease, but increasing pressure to reduce reliance on antibiotics, particularly for population-level prophylaxis, puts more emphasis on vaccination strategies to prevent systemic infection and mortality.

Antibiotic treatment remains an essential control measure in the face of severe outbreaks of *H. parasuis* systemic infection. Injectable treatments tend to be more effective than water or feed medication, since pigs clinically affected by *H. parasuis* systemic infection are less likely to ingest the required antibiotic dose via food and water intake. Nonsteroidal anti-inflammatory treatment may assist recovery (Viehmann et al. 2013).

Antibiotic susceptibility profiles are variable in different countries and reflect the selection of drugs used in each region. For example, Danish, British, and Australian isolates are reported as broadly susceptible to most antibiotics, whereas high rates of resistance to commonly used antibiotics are reported for Chinese and Spanish isolates (Aarestrup et al. 2004; Dayao et al. 2014; El Garch et al. 2016; Martín de la Fuente et al. 2007; Zhou et al. 2010). Resistance to tetracycline and beta-lactam antibiotics has been linked to the presence of resistance genes in plasmids carried by *H. parasuis* (Lancashire et al. 2005; Moleres et al. 2015). Recent data indicate that low variability in the composition of the nasal microbiota at weaning, a condition that may be associated with antibiotic use, is a predisposing factor for disease development by *H. parasuis* (Correa-Fiz et al. 2016).

Haemophilus parasuis is a commensal of the upper respiratory tract and colonizes virtually every pig. The early colonization of pigs with virulent strains in the presence of maternal immunity actually prevents disease and mortality post weaning (Oliveira et al. 2004). However, natural colonization during the suckling period usually is not protective against the wide variety of strains circulating in different swine populations owing to poor heterologous protection (Oliveira et al. 2004). Therefore, commingling of pigs from different sources and ages is an important cause of increased mortality due to Glässer's disease and should be avoided when possible. Although elimination of *H. parasuis* from a herd is difficult due to early maternal colonization of neonatal piglets, it may be achieved through Cesarean derivation into pig-free premises. However, except in circumstances where standards of biosecurity are exceptional, elimination is not recommended because such herds are highly susceptible to severe outbreaks if exposed to virulent *H. parasuis* due to a lack of immunity. Strict biosecurity that forbids introduction of *H. parasuis*-positive swine or fomites is essential to prevent high mortality in such cases.

Vaccination is an effective measure to prevent mortality. Commercially available bacterin vaccines are based on serovar 5, combined serovars 4 and 5, or combined serovars 1 and 6 for use in sows and pigs. All these products show limited cross-protection to heterologous strains. Studies have failed to show interference from maternal antibody where sow and piglet vaccination are practiced concurrently in acute disease contexts (Oh et al. 2013). More recently, a live attenuated serovar 5 strain has become available in North America with cross-protection claimed for serovars 4 and 13 (Oliveira et al. 2013). Autogenous vaccines are highly effective in protecting susceptible pigs (McOrist et al. 2009), but it is essential to use typing methods to ensure that the appropriate systemic isolate is incorporated into the vaccine preparation by sampling from systemic rather than respiratory locations.

References

Aarestrup FM, Seyfarth AM, Angen Ø. 2004. *Vet Microbiol* 101:143–146.

Amano H, Shibata M, Kajio N, et al. 1994. *J Vet Med Sci* 56:639–644.

Angen Ø, Svensmark B, Mittal KR. 2004. *Vet Microbiol* 103:255–258.

Angen Ø, Oliveira S, Ahrens P, et al. 2007. *Vet Microbiol* 119:266–276.

Aragon V, Cerda-Cuellar M, Fraile L, et al. 2010. *Vet Microbiol* 142:387–393.

Baumann G, Bilkei G. 2002. *Vet Rec* 151:18–21.

Bello-Orti B, Costa-Hurtado M, Martinez-Moliner V, et al. 2014a. *Vet Microbiol* 170:430–437.

Bello-Ortí B, Deslandes V, Tremblay YD, 2014b. *Vet Res* 45:104.

Bello-Ortí B, Howell KJ, Tucker AW, et al. 2015. *Vet Res* 46:102.

Biberstein EL, White DC. 1969. *J Med Microbiol* 2:75–78.

Blanco I, Galina-Pantoja L, Oliveira S, et al. 2004. *Vet Microbiol* 103:21–27.

Blanco I, Canals A, Evans G, et al. 2008. *Can J Vet Res* 72:228–235.
Bouchet B, Vanier G, Jacques M, et al. 2008. *Vet Res* 39:42.
Bouchet B, Vanier G, Jacques M, et al. 2009. *Microb Pathog* 46:108–113.
Brockmeier SL. 2004. *Vet Microbiol* 99:75–78.
Brockmeier SL, Loving CL, Mullins MA, et al. 2013. *Clin Vaccine Immunol* 20:1466–1472.
Brockmeier SL, Register KB, Kuehn JS, et al. 2014. *PLoS One* 9:e103787.
Cai X, Chen H, Blackall PJ, et al. 2005. *Vet Microbiol* 111:231–236.
Cerdà-Cuéllar M, Aragon V. 2008. *Vet J* 175:384–389.
Cerdá-Cuéllar M, Naranjo JF, Verge A, et al. 2010. *Vet Microbiol* 145:315–320.
Chen H, Li C, Fang M, et al. 2009. *BMC Genomics* 10:64.
Chen Y, Liu T, Langford P, et al. 2015. *Mol Immunol* 65:360–366.
Correa-Fiz F, Fraile L, Aragon V. 2016. *BMC Genomics* 17:404.
Costa-Hurtado M, Ballester M, Galofré-Milà N, et al. 2012. *Vet Res* 43:57.
Costa-Hurtado M, Olvera A, Martinez-Moliner V, et al. 2013. *Infect Immun* 81:2327–2333.
Cuesta Gerveno JM, Risco Pérez D, Gonçalves Blanco P, et al. 2013. *J Vet Diagn Invest* 25:297–300.
Dayao DA, Kienzle M, Gibson JS, et al. 2014. *Vet Microbiol* 172:586–589.
El Garch F, de Jong A, Simjee S, et al. 2016. *Vet Microbiol* 194:11–22.
Frandoloso R, Martínez-Martínez S, Gutiérrez-Martín CB, et al. 2012a. *Vet Microbiol* 154:347–52.
Frandoloso R, Martínez-Martínez S, Yubero S, et al. 2012b. *Cell Immunol* 277:74–82.
Frandoloso R, Pivato M, Martínez-Martínez S, et al. 2013. *BMC Vet Res* 9:207.
Hariharan H, MacDonald J, Carnat B, et al. 1992. *J Vet Diagn Invest* 4:28–30.
Hoefling DC. 1991. *J Vet Diagn Invest* 3:354–355.
Holtkamp D, Rotto H, Garcia R. 2007. *Swine News Newsletter* 30:4.
Howell KJ, Weinert LA, Luan S-L, et al. 2013. *J Bacteriol* 195:4264–4273.
Howell KJ, Weinert LA, Chaudhuri RR, et al. 2014. *BMC Genomics* 15:1179.
Inzana TJ, Dickerman AW, Bandara AB. 2016. Taxonomic reclassification of "Haemophilus parasuis" to Glaesserella parasuis gen. nov., comb. nov. In Proceedings of 4th Prato Conference Pathogens of Bacterial Infections Animal, p. 13.
Jin H, Zhou R, Kang M, et al. 2006. *Vet Microbiol* 118:117–123.
Jin H, Wan Y, Zhou R, et al. 2008. *Environ Microbiol* 10:3326–3336.
Jung K, Ha Y, Kim SH, et al. 2004. *J Vet Med Sci* 66:841–845.
Kielstein P, Rapp-Gabrielson VJ. 1992. *J Clin Microbiol* 30:862–865.
Kim J, Chung HK, Jung T, et al. 2002. *J Vet Med Sci* 64:57–62.
de la Puente Redondo VA, Navas Mendez J, Garcia del Blanco N, et al. 2003. *Vet Microbiol* 92:253–262.
del Rio ML, Gutierrez B, Gutierrez CB, et al. 2003a. *Am J Vet Res* 64:1176–1180.
del Rio ML, Gutierrez CB, Rodriguez Ferri EF. 2003b. *J Clin Microbiol* 41:880–882.
del Rio ML, Martin CB, Navas J, et al. 2006. *Res Vet Sci* 80:55–61.
Lancashire JF, Terry TD, Blackall PJ, et al. 2005. *Antimicrob Agents Chemother* 49:1927–1931.
Lewis PA, Shope RE. 1931. *J Exp Med* 54:361–371.
Li JX, Jiang P, Wang Y, et al. 2009. *Prev Vet Med* 91:274–279.
Li M, Song S, Yang D, et al. 2015. *Vaccine* 33:1695–1701.
Little TW. 1970. *Vet Rec* 87:399–402.
Macedo N, Oliveira S, Torremorell M, et al. 2016. *Res Vet Sci* 107:62–67.
Martín de la Fuente AJ, Tucker AW, Navas J, et al. 2007. *Vet Microbiol* 120:184–191.
Martín de la Fuente AJ, Ferri EF, Tejerina F, et al. 2009a. *Res Vet Sci* 87:47–52.
Martin de la Fuente AJ, Rodriguez-Ferri EF, Frandoloso R, et al. 2009b. *Res Vet Sci* 86:248–253.
Martínez-Martínez S, Frandoloso R, Rodríguez-Ferri EF, et al. 2016. *Vet Immunol Immunopathol* 179:18–25.
Martínez-Moliner V, Soler-Llorens P, Moleres J, et al. 2012. *Microbiology* 158:2117–2124.
McOrist S, Bowles R, Blackall P. 2009. *J Swine Health Prod* 17:90–96.
Miniats OP, Smart NL, Rosendal S. 1991. *Can J Vet Res* 55:37–41.
Moleres J, Santos-López A, Lázaro I, et al. 2015. *Appl Environ Microbiol* 81:3255–3267.
Morozumi T, Hiramune T, Kobayashi K. 1982. *Natl Inst Anim Health Q (Tokyo)* 22:23–31.
Moxley RA. 2000. Edema disease. *Vet Clin North Am Food Anim Pract* 16:175–185
Mullins MA, Register KB, Brunelle BW, et al. 2013. *Vet Microbiol* 162:899–906.
Nielsen R. 1993. *Acta Vet Scand* 34:193–198.
Nielsen NC, Bille N, Riising HJ, et al. 1975. *Can J Comp Med* 39:421–426.
Oh Y, Han K, Seo HW, et al. 2013. *Can J Vet Res* 77:183–190.
Oliveira S, Galina L, Pijoan C. 2001. *J Vet Diagn Invest* 13:495–501.
Oliveira S, Blackall PJ, Pijoan C. 2003a. *Am J Vet Res* 64:435–442.
Oliveira S, Galina L, Blanco I, et al. 2003b. *Can J Vet Res* 67:146–150.
Oliveira S, Pijoan C, Morrison R. 2004. *J Swine Health Prod* 12:123–128.
Oliveira SR, Simonson RR, Mahlberg JD, et al., inventors. 2013. Modified live *Haemophilus parasuis* vaccine. US patent 8,404,253.
Olvera A, Cerda-Cuellar M, Aragon V. 2006. *Microbiology* 152:3683–3690.
Olvera A, Cerda-Cuellar M, Mentaberre G, et al. 2007a. *Vet Microbiol* 125:182–186.

Olvera A, Cerda-Cuellar M, Nofrarias M, et al. 2007b. *Vet Microbiol* 123:230–237.
Olvera A, Ballester M, Nofrarias M, et al. 2009. *Vet Res* 40:24.
Olvera A, Pina S, Perez-Simo M, et al. 2010. *Vet Res* 41:26.
Olvera A, Pina S, Pérez-Simó M, et al. 2011. *Vaccine* 29:2797–2802.
Olvera A, Pina S, Macedo N, et al. 2012. *Vet J* 191:213–218.
Palzer A, Austin-Busse RL, Ladinig A, et al. 2015a. *Tierarztl Prax Ausg G Grosstiere Nutztiere* 43:91–96.
Palzer A, Haedke K, Heinritzi K, et al. 2015b. *Can Vet J* 56:285–287.
Peet RL, Fry J, Lloyd J, et al. 1983. *Aust Vet J* 60:187.
Perry MB, MacLean LL, Gottschalk M, et al. 2013. *Carbohydr Res* 378:91–97.
Pomorska-Mól M, Markowska-Daniel I, Rachubik J, et al. 2011. *Vet Res Commun* 35:337–343.
Rafiee M, Bara M, Stephens CP, et al. 2000. *Aust Vet J* 78:846–849.
Rapp-Gabrielson VJ, Gabrielson DA, Schamber GJ. 1992. *Am J Vet Res* 53:987–994.
Rapp-Gabrielson V, Kocur GJ, Clark JT, et al. 1997. *Vet Med* 92:83–90.
Reams RY, Glickman LT, Harrington DD, et al. 1994. *J Vet Diagn Invest* 6:326–334.
Rodríguez Ferri EF, Martínez S, Frandoloso R, et al. 2010. Comparative efficacy of several disinfectants in suspension and carrier tests against *Haemophilus parasuis* serovars 1 and 5. *Res Vet Sci* 88:385–389.
Rubies X, Kielstein P, Costa L, et al. 1999. *Vet Microbiol* 66:245–248.
Segales J, Domingo M, Solano GI, et al. 1999. *Vet Microbiol* 64:287–297.
Smart NL, Miniats OP, MacInnes JI. 1988. *Can J Vet Res* 52:319–324.
Solano GI, Segalés J, Collins JE, et al. 1997. *Vet Microbiol* 55:247–257.
Solano-Aguilar GI, Pijoan C, Rapp-Gabrielson V, et al. 1999. *Am J Vet Res* 60:81–87.
Tadjine M, Mittal KR, Bourdon S, et al. 2004. *J Clin Microbiol* 42:839–840.
Takahashi K, Naga S, Yagihashi T, et al. 2001. *J Vet Med Sci* 63:487–491.
Turni C, Blackall PJ. 2005. *Vet Microbiol* 106:145–151.
Turni C, Blackall PJ. 2010a. *Aust Vet J* 88:255–259.
Turni C, Pyke M, Blackall PJ. 2010b. *J Appl Microbiol* 108:1323–1331.
Unterweger C, Wöchtl B, Spergser J, et al. 2016. *Tierarztl Prax Ausg G Grosstiere Nutztiere* 44:259–265.
Vahle JL, Haynes JS, Andrews JJ. 1995. *J Vet Diagn Invest* 7:476–480.
Vahle JL, Haynes JS, Andrews JJ. 1997. *Can J Vet Res* 61:200–206.
Vengust G, Valencak Z, Bidovec A. 2006. *J Vet Med B Infect Dis Vet Public Health* 53:24–27.
Viehmann M, Postiasi S, Balka G, et al. 2013. *Tierarztl Prax Ausg G Grosstiere Nutztiere* 41:225–232.
Wang SJ, Liu WJ, Yang LG, et al. 2012. *Mol Biol Rep* 39:8169–8176.
Wang X, Xu X, Wu Y, et al. 2013. *Vet Microbiol* 164:184–189.
Wilkinson JM, Sargent CA, Galina-Pantoja L, et al. 2010. *BMC Genomics* 11:455.
Xu C, Zhang L, Zhang B, et al. 2013. *Vet J* 195:200–204.
Yu J, Wu J, Zhang Y, et al. 2012. *Vet Microbiol* 158:316–321.
Yue M, Yang F, Yang J, et al. 2008. *J Bacteriol* 191:1359–1360.
Zhang B, Feng S, Xu C, et al. 2012a. *FEMS Microbiol Lett* 326:109–115.
Zhang B, He Y, Xu C, et al. 2012b. *Vet Microbiol* 157:237–242.
Zhang B, Xu C, Zhang L, et al. 2013. *Vet Microbiol* 162:713–723.
Zhang B, Yu Y, Zeng Z, et al. 2014. *Microb Pathog* 74:33–37.
Zhou M, Guo Y, Zhao J, et al. 2009. *Vaccine* 27:5271–5277.
Zhou X, Xu X, Zhao Y, et al. 2010. *Vet Microbiol* 141:168–173.
Zhou Q, Feng S, Zhang J, et al. 2016. *Front Cell Infect Microbiol* 6:100.

55

Leptospirosis

Zbigniew J. Arent and William A. Ellis

Relevance

Leptospirosis is a cause of reproductive loss in breeding herds and has been reported in swine from all parts of the world; however, knowledge of the incidence and economic impact of the disease is largely confined to Argentina and Brazil and to the intensive pig industries of the Northern Hemisphere, Australia, and New Zealand, where its importance has apparently declined.

Endemic infection in a herd of swine may produce little evidence of clinical disease, but when it is first introduced into a susceptible breeding herd, or during periods of waning herd immunity, it can cause very appreciable losses through infertility, abortion, and the full-term birth of dead pigs or weak pigs of reduced viability.

Leptospires persist in the kidneys and female and male genital tracts of carrier swine and are excreted in urine and genital fluids. Survival outside the host is favored by warm moist conditions. Transmission is by direct or indirect contact with a carrier pig or other animal. Interruption of transmission from infected pigs or other hosts to susceptible pigs is the critical factor in control.

Etiology

Leptospirosis of swine is a disease caused by a variety of morphologically similar but antigenically and genetically distinct, small, motile, aerobic spirochetes belonging to the genus *Leptospira*. They are thin helical motile, gram-negative organisms that are often hooked at one or both ends. They range in length from about 6 to 20 µm, with amplitude of approximately 0.1–0.15 µm and a wavelength of about 0.5 µm. Under adverse nutritional conditions, leptospires may be greatly elongated, while under conditions such as high salt concentrations, aging culture or in tissues, leptospires may form coccoid cells of about 1.5–2 µm diameter. They divide by binary fission and stain poorly with aniline dyes. Unstained cells are visible only by dark-field microscopy. In a suitable liquid environment, motility is accomplished by rotating along the long axis, but an undulating action is observed in semisolid media. Culture requires special media containing mammalian serum or albumin for cultivation (Faine et al. 1999).

The major structural components are an outer membrane that surrounds a double-membrane structure in which the cytoplasmic membrane and peptidoglycan cell wall are closely associated. Two flagella with polar insertions are located in the periplasmic space and are responsible for motility (Adler and de la Peña-Moctezuma 2010). Within the outer membrane, lipopolysaccharide (LPS) constitutes the main antigen for *Leptospira*. In addition to LPS, structural and functional proteins form part of the leptospiral outer membrane. A large proportion of such proteins are lipoproteins with LipL32, LipL41, LipL21, and Loa22 (probable lipoproteins) being the most abundant on the cell surface (Cullen et al. 2005; Haake and Zückert 2015). Integral membrane proteins such as the porin OmpL1 are also located in the outer membrane (Shang et al. 1995).

The genus *Leptospira* includes both saprophytic and pathogenic species. The pathogenic family consists of 14 pathogenic species: *Leptospira alexanderi*, *Leptospira alstonii*, *Leptospira borgpetersenii*, *Leptospira broomii*, *Leptospira inadai*, *Leptospira interrogans*, *Leptospira fainei*, *Leptospira kirschneri*, *Leptospira kmetyi*, *Leptospira licerasiae*, *Leptospira noguchii*, *Leptospira santarosai*, *Leptospira weilii*, and *Leptospira wolffii*, with more than 260 serovars. There are seven saprophytic species (Levett 2015). There are differences in the global distribution of some of the *Leptospira* species: *L. interrogans*, *L. borgpetersenii*, and *L. kirschneri* have a worldwide distribution, whereas *L. noguchii* and *L. santarosai* are found mainly in North and South America, while *L. weilii* is found mainly in China and Eastern Asia. Strains that cause disease in pigs are found mainly in the *L. interrogans* and *L. borgpetersenii* species.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

At the subspecies level, serovar classification of *Leptospira* is still widely used as it facilitates serodiagnostic, epidemiology, and prevalence studies. It is based on the expression of the surface-exposed epitopes in a mosaic of the LPS antigens, while the specificity of these epitopes depends on their sugar composition and orientation (Adler and de la Peña-Moctezuma 2010). In addition, the concept of serogroups that clusters antigenically related strains is still used as the basis of selecting cross-reactive strains for serological tests.

Public health

Leptospirosis is a potential occupational zoonosis of those who work with pigs in regions in which infection in swine is common, especially farmers, veterinarians, and abattoir workers (Faine et al. 1999). Infection is by direct or indirect contact of mucous membranes or skin wounds with the urine of an infected pig. A majority of cases are asymptomatic or present as sudden onset of an approximately weeklong febrile sickness with any combination of chills, conjunctival suffusion, severe headache, myalgia of lower back and legs, abdominal pain, vomiting, and diarrhea. In approximately 5–10% of cases, the clinical course progresses rapidly to include icterus, renal failure, cough, dyspnea, and hemoptysis that may be fatal.

Epidemiology

The epidemiology of swine leptospirosis is potentially very complicated, since swine can be infected by any of the pathogenic serovars. Fortunately, only a small number of serovars are endemic in any particular region or country. Furthermore, leptospirosis is a disease that shows a natural nidality, and each serovar tends to be maintained in specific maintenance hosts. Therefore, in any region, pigs are infected by serovars maintained by pigs or by serovars maintained by other animal species present in the area. The relative importance of these incidental infections is determined by the opportunity that prevailing social, management, and environmental factors provide for contact and transmission of leptospires from other species to pigs.

Pigs act as maintenance hosts for serovars belonging to the Pomona and Australis serogroups, while Icterohemorrhagiae, Grippotyphosa, and Tarassovi serogroups are among the more commonly identified incidental infections in swine.

Pomona infection

Leptospira serovars belonging to serogroup Pomona are one of the most commonly identified types of *Leptospira* in livestock. The classical serological identification of Pomona serogroup members has been very difficult because of very close antigenic relationships between strains. Taking into account genotypic classification, the members of serogroup Pomona are found in four Leptospira species, namely, *L. interrogans*, *L. kirschneri*, *L. noguchii* and *L. santarosai*, but only two of them, namely, *L. interrogans* with serovar Pomona and *L. kirschneri* with serovar Mozdok, have been the most commonly isolated from pigs worldwide. Introduction of genetic methods demonstrated strain differences at the subserovar level and some types within the serovar Pomona such as type Pomona (sensu stricto), Kennewicki and Monjakov.

Infections with these serovars have been extensively studied and provide a suitable model with which to illustrate general concepts of swine leptospirosis. Many strains of serovars Pomona types Pomona and Kennewicki, especially those found in the United States and Canada, are adapted to swine. They have been the cause of widespread clinical disease in swine in North and South America, Australia, New Zealand, parts of Asia, and Eastern and Central Europe and are endemic in many of these regions. There is now evidence of high levels of infection in parts of Africa (Agunloye 2001) and Southeast Asia (Al-Khleif et al. 2009). Such strains are apparently absent from the more westerly parts of Europe where the rodent-maintained serovar Mozdok may cause occasional outbreaks of clinical disease (Barlow 2004; Rocha 1990; Zieris 1991).

In parts of North America, the prevalence of Pomona infection in pigs has declined markedly from the high levels observed in the 1950s and early 1960s. No carriers were detected in a 1989 meat plant survey carried out in Iowa (Bolin and Cassells 1992). In contrast, Baker et al. (1989) recovered serovar Pomona type Kennewicki from almost 10% of pigs in a small survey in Canada.

Leptospires have a particular affinity for the kidneys of infected pigs, where they persist, multiply, and are voided in urine. This characteristic is very important in the transmission of infection.

Infection is introduced into a susceptible herd by three possible routes: the introduction of infected stock, exposure to a contaminated environment, or contact with an alternative infected animal vector. Carrier pigs are probably the most common route of introduction. Replacement gilts or infected boars have been identified as important means of introducing infection (Ellis 2006).

The importance of free-living species as possible sources of Pomona infection of pigs depends on geographical location. In North America, the skunk has been incriminated as a source of Pomona outbreaks in pigs (Mitchell et al. 1966), and the move to indoor housing has reduced the opportunity for skunk-to-pig transmission.

Once Pomona has been introduced into a pig population, a high prevalence of infection is established. Only low infective doses are required to transmit infection (Chaudhary et al. 1966a,b). If direct contact is prevented, indirect contact through contaminated effluent, water, or soil ensures transmission. The presence of moisture is critical for indirect transmission. The organisms cannot withstand desiccation, but when infected urine is deposited in damp soil or water with a neutral or slightly alkaline pH, the organisms may survive for extended periods (Ellis 2006).

During the initial herd infection, clinical disease may occur in all ages of sows. Following the initial establishment of infection, an endemic cycle typical of that in a maintenance host population is set up (Hathaway 1981). Piglets are passively protected in the first weeks of life by colostrum-derived immunoglobulins from infected dams (Bolt and Marshall 1995a; Fish et al. 1963). The duration of this passive protection depends primarily on the quantity of immunoglobulins received in colostrum (Chaudhary et al. 1966b). A study of grower pigs in New Zealand has shown that *Leptospira* infection becomes apparent in piglets from 12 weeks of age and by slaughter up to 90% may be infected. The intensity of shedding in urine is greatest in the first 3–4 weeks of infection after which it declines and becomes intermittent (Bolt and Marshall 1995a,b). Infection between groups of fattening pigs is often by urine-contaminated effluent from a common drainage system (Buddle and Hodges 1977). In herds with endemic infection, clinical disease is usually restricted to gilts that have either been reared in isolation since weaning and reintroduced into the herd or more commonly brought in from an uninfected herd.

Australis infection

Serovar Bratislava and to a lesser extent the closely related serovar Muenchen have emerged as major swine-maintained leptospiral infections but remain poorly understood due to difficulties in culturing these strains. Serologic data has indicated widespread infection in Europe, the United States, Canada, Australia, Brazil, South Africa (Ellis 2006), Nigeria (Agunloye 2001), Korea (Choi et al. 2001), and Japan, (Kikuchi et al. 2009) among others.

In contrast to the high seroprevalences reported worldwide, serovar Bratislava has only been recovered from pigs in a few countries, namely, the Netherlands (Hartman et al. 1975), the United Kingdom (Ellis et al. 1991), the United States (Bolin and Cassells 1990, 1992; Ellis and Thiermann 1986), Germany (Schonberg et al. 1992), and Vietnam (Boqvist et al. 2003).

The epidemiology of these strains is poorly understood. There are specific pig-adapted strains; other strains that are maintained by pigs, dogs, horses, and hedgehogs; and other strains that are found only in wildlife. Among the pig strains there are genotypes that are more likely to be associated with disease (Ellis et al. 1991; Ellis unpublished data).

Two very distinct serological profiles may be seen in endemically infected herds. In indoor sow units infected with pig-adapted strains of Bratislava, the prevalence of sows with antibody titers of greater than 1:100 in the microscopic agglutination test (MAT) is usually very low, although many sows will have titers of less than 1:100. This is thought to result from infection primarily due to venereal transmission. In contrast in units where the sows are kept outside, the seroprevalences (≥1:100) may be greater than 50%. This is thought due to systemic infection as a result of exposure to infected rodent urine.

Although the renal carrier state does become established, urinary excretion is poor compared with Pomona, and transmission within the fattening house is inefficient. Important additional carrier sites have been identified, namely, the upper genital tracts of sows and boars (Bolin and Cassells 1992; Ellis et al. 1986b,c; Power 1991). Venereal transmission is thought to play an important role in the spread of Bratislava infection. Boars are frequently the only new animals introduced into pig breeding units and are clearly a potential means of introducing *Leptospira* infections.

Tarassovi infection

There is much less information available on the epidemiology of Tarassovi infection in pigs. The pig was previously thought to act as a maintenance host for some strains of Tarassovi found in Eastern Europe and Australia, but declining seroprevalences would suggest that this is not so (Wasinski 2005). High seroprevalences associated with reproductive failure have recently been reported from Vietnam (Boqvist et al. 2007).

Many strains of Tarassovi have been recovered from free-living animals, further supporting the view that Tarassovi infections are incidental infections of pigs resulting from wildlife contact. Work in the United States supports this view. Tarassovi has not been recovered from swine, but there is serologic evidence of infection in pigs in the southeastern states (Cole et al. 1983) where it has been isolated from raccoons, skunks, and opossums (McKeever et al. 1958; Roth 1964).

Canicola infection

Although organisms belonging to this serogroup have been recovered from swine in a number of countries, little is known of the epidemiology of serovar Canicola infection in pigs. Conventional wisdom has been that infection is acquired from dogs, the recognized maintenance host for this serovar, although wildlife may also be

a source (Paz-Soldan et al. 1991). The long period of urine shedding observed in infected pigs (at least 90 days) and the ability of Canicola to survive for up to 6 days in undiluted pig urine (Michna 1962) suggest that intraspecies transmission could occur.

Icterohemorrhagiae infection

Serologic evidence of Icterohemorrhagiae serogroup infection has been reported in many countries, but few isolations have been made from pigs. Both serovars Copenhageni and Icterohemorrhagiae may be involved and are probably introduced to susceptible stock via an environment contaminated with infected urine from the brown rat (*Rattus norvegicus*), the maintenance host for these serovars. Urinary excretion is less than 35 days (Schnurrenberger et al. 1970), and intraswine transmission is inefficient (Hathaway 1985). It is believed, in the absence of supporting isolation data, that vaccine-induced antibodies are responsible for the seroprevalences to Icterohemorrhagiae observed in the United States. High seroprevalences to Icterohemorrhagiae associated with clinical disease have been detected in some pig populations in Brazil (Osava et al. 2010).

Grippotyphosa infection

Serovar Grippotyphosa infection is maintained by wildlife hosts, and incidental infection of pigs gives rise to low prevalences of antibodies in swine in various regions, particularly Eastern and Central Europe and the United States, and it has been recovered from pigs in Russia and the United States in the past (Ellis 2006). A high seroprevalence has been reported in Thailand (Puchadapirom et al. 2006).

Hardjo infection

Serovar Hardjo infection is maintained by cattle worldwide, and where cattle and pigs come in close contact, the opportunity arises for infection in pigs to occur. There have been reports of the isolation of Hardjo from pigs in the United Kingdom (Ellis et al. 1986a; Hathaway et al. 1983) and the United States (Bolin and Cassells 1992), but persistence in renal tissue was not a feature of experimental infection (Hathaway et al. 1983), suggesting that intraspecies transmission is unlikely.

Pathogenesis

The most important route of natural infection has not been determined. It is thought to be via the mucous membranes of the eye, mouth, or nose. Infection via the vaginal route is also possible, and transmission through milk from an infected dam has been demonstrated experimentally. A period of bacteremia, which may last for a week, begins 1 or 2 days after infection. During this period leptospires can be isolated from most organs of the body and also from cerebrospinal fluid (Ellis 2006). This primary bacteremic phase ends with the appearance of circulating antibodies, which are detectable usually after 5–10 days (Hanson and Tripathy 1986). A secondary bacteremic period (after 15–26 days) has been reported in experimental Hardjo infection (Hathaway et al. 1983).

Antileptospiral agglutinins appear at detectable levels in the blood at approximately 5–10 days after infection and reach maximum levels at around 3 weeks. Peak titers vary considerably (1:1,000 to 1:100,000 in the MAT), and these may be maintained for up to 3 weeks, after which a subsequent gradual decline occurs. Low titers may be detectable for several years in many animals.

Following the period of leptospiremia, leptospires localize in the proximal renal tubules where they multiply and are voided in the urine. The duration and intensity of urinary shedding varies from pig to pig and with the infecting serovar. In the case of Pomona infection, the intensity of excretion is highest during the first month of shedding, when more than a million leptospires may be present in each milliliter of urine; urine shedding is very constant during this period. A variable period of intermittent, low-intensity leptospiruria then ensues, and this may last for up to 2 years in some cases.

Leptospires also localize in the uterus of pregnant sows, and abortion, production of stillborn pigs, and neonatal disease frequently result from intrauterine infections occurring in the last half of the gestation period. Abortions and stillbirths usually occur 1–4 weeks following infection of the gilt or sow (Hanson and Tripathy 1986), by which time most sows have developed detectable antibody titers. Since pig fetuses are capable of producing antibodies during the latter stages of gestation, some stillborn piglets will have detectable titers.

The pathogenesis of reproductive disease is poorly understood, but some authors believe that transplacental infection, occurring during the very limited period of maternal leptospiremia, is the sole cause. While this may be true for systemic infections such as Pomona, the low antibody titers detected in sows aborting Bratislava-infected fetuses has led to the hypothesis that infection occurs as a result of waning uterine immunity being unable to prevent transplacental infection by leptospires present in the genital tract. Horizontal transmission to littermates not infected during the period of maternal leptospiremia may also occur. Once the placental barrier is breached, septicemia results in large numbers of leptospires in all fetal tissues (Ellis 2006).

An additional feature seen in Bratislava infection but not reported for the other swine leptospiral infections is the persistence of leptospires in the oviduct and uterus of nonpregnant sows and in the seminal vesicles, bulbourethral glands, prostate, and testes of boars (Ellis 2006; Oliveira et al. 2007).

Clinical signs

The vast majority of swine leptospiral infections are subclinical. Two groups of pigs are most likely to experience clinical infections: the young piglet and the pregnant sow.

Acute leptospirosis

This phase usually coincides with the period of bacteremia. In experimental infections, many pigs exhibit transient anorexia, pyrexia, and listlessness at this time (Hanson and Tripathy 1986). However, the mild nature of these signs means that in natural infections, especially in endemically infected herds where perhaps only one or two animals may be affected, this phase of infection usually goes unrecognized.

There have been a few reports of jaundice and hemoglobinuria in naturally occurring outbreaks (Ferguson et al. 1956), particularly in cases of infection in piglets under 3 months of age by Icterohemorrhagiae (Field and Sellers 1951; Modric et al. 2006). A high proportion of these undergo spontaneous recovery within a week of when clinical signs develop. The small number of such reports suggests that this more severe form of disease is rare.

Chronic leptospirosis

Abortions, stillbirths, the birth of weak piglets of reduced viability, and reduced litter size are primary signs of chronic leptospirosis, particularly Pomona infection. It is this aspect of leptospirosis that can cause considerable economic loss (Azevedo et al. 2008; Ellis 2006).

Information as to the importance of leptospirosis as a cause of abortion in national swine herds is not available. If it were, it would vary from country to country depending on prevalence as well as epidemiologic and management factors, including the implementation of control measures. From the limited and dated information available, it would appear that even in countries where vaccination has been widely practiced, leptospirosis is a common cause of swine abortion. In Ontario, for example, 6% of swine abortions were attributed to Pomona infection (Anon 1986). Endemic Tarassovi infection was considered to be the cause of a 3% abortion rate in herds in Poland (Wandurski 1982). Fearnley et al. (2008) found 4 of 24 fetal diagnostic submissions from the United Kingdom to be polymerase chain reaction (PCR) positive. Acute outbreaks can still give rise to severe losses; Saravi et al. (1989) described an outbreak in a herd in which 19% of pregnant sows aborted, while the proportion of stillborn per litter rose from 8% prior to the outbreak to 28% during the outbreak. Differences in strain pathogenicity also contribute to different prevalences of clinical abortion in infected herds (Nagy 1993).

A very high prevalence of serovars belonging to the Australis serogroup in aborted pig litters has been observed in part of the United Kingdom (Ellis et al. 1986a). Similar strains have also been recovered from aborted piglets in the United States (Bolin and Cassells 1990; Bolin et al. 1991). Rehmtulla et al. (1992) reported fetal Bratislava infection following abortions in 16% of sows in a herd in Ontario. Egan (1995) reported FAT positive prevalences ranging from 5 to 23% in diagnostic submissions in Ireland. Published experimental evaluations of the significance of such microbiological findings are not available. There has, however, been an absence of significant isolations of other abortifacient agents from these cases, and the farrowing rate and the number of live piglets born/sow improves significantly following either Bratislava vaccination (Frantz et al. 1989) or the use of an antibiotic medication program (Ellis et al. 1989).

Following abortions due to Pomona, there does not appear to be any subsequent limitation on reproductive performance, even in pigs that remain infected for long periods (Ferguson and Powers 1956; Kemenes and Suveges 1976; Mitchell et al. 1966).

Infertility is a feature of Bratislava infection (Hathaway and Little 1981; Jensen and Binder 1989; Van Til and Dohoo 1991). Split-herd trials, carried out using a Bratislava bacterin, have demonstrated significant improvements in sow fertility (Frantz et al. 1989).

Lesions

The main pathological changes are essentially the same for all infections, with the primary lesion being damage to the membranes of the endothelial cells of small blood vessels.

In acute leptospirosis there are no pathognomonic gross changes. Pathologic changes in acute Pomona infection are very limited, reflecting the mild nature of acute clinical disease. Hanson and Tripathy (1986) reported little gross or histopathologic change in swine killed during the acute phase of leptospirosis. Burnstein and Baker (1954) reported that petechial and ecchymotic hemorrhages could be seen in the lungs of some pigs, and histologic examinations have revealed minor renal tubular damage, focal liver necrosis, lymphocytic infiltration of the adrenal

glands, and meningoencephalitis with perivascular lymphocytic infiltration (Burnstein and Baker 1954; Chaudhary et al. 1966a; Sleight et al. 1960).

In chronic leptospirosis, gross lesions are confined to the kidneys and consist of scattered small gray foci, often surrounded by a ring of hyperemia. Microscopic examination reveals progressive multifocal interstitial nephritis (Burnstein and Baker 1954; Cheville et al. 1980; Langham et al. 1958). The renal interstitium contains focal-to-coalescing aggregates of lymphocytes, macrophages, and plasma cells. Some glomeruli may be swollen, atrophic, or fibrotic. Bowman's capsule may be thickened, containing eosinophilic granular material (Langham et al. 1958). Renal tubular epithelial cells may be degenerate and sloughing, resulting in necrotic cellular luminal debris, or they may be hyperplastic. Older lesions mainly consist of fibrosis and interstitial inflammatory cell infiltrations. Chronic lesions with accompanying acute inflammatory changes are still noticeable as long as 14 months' postinfection (Morter et al. 1960).

Experimental studies indicate that leptospires can invade the mammary gland of pigs and produce mild focal nonsuppurative mastitis (Tripathy et al. 1981).

The gross pathology of fetuses aborted as a sequela of Pomona infection is nonspecific and probably due to intrauterine autolysis, and includes edema of various tissues, serous or bloodstained fluid in body cavities, and sometimes petechial hemorrhages in renal cortices (Fennestad and Borg-Petersen 1966; Ryley and Simmons 1954a,b; Wrathall 1975). Jaundice may be seen in some aborted piglets (Hathaway et al. 1983). Multifocal hepatic necrosis, presenting as small grayish-white spots, may be a frequent finding (Fennestad and Borg-Petersen 1966; Fish et al. 1963; Ryley and Simmons 1954a,b). Microscopic examination may reveal small foci of interstitial nephritis and multifocal hepatic necrosis. Placentas from aborted fetuses are grossly normal (Fennestad and Borg-Petersen 1966; Fish et al. 1963).

Diagnosis

A diagnosis of clinical or subclinical leptospirosis in swine may be required for a variety of reasons, including (1) confirmation of clinical disease, (2) assessment of herd infection and/or immune status for control or eradication on a herd or national basis, or (3) determination of the status of individual animals for introduction into a closed herd or for trade.

The mild, often inapparent clinical signs of acute leptospirosis make clinical diagnosis difficult, and the lack of specificity of lesions makes pathological diagnosis difficult; therefore, diagnosis is usually based on the results of laboratory tests. Laboratory tests for leptospirosis fall into two groups: (1) indirect serologic tests that assess antibody levels and (2) direct tests that detect the organism. The selection of tests depends on the purpose for testing and availability of tests.

Serologic tests

Serologic testing is the most widely used method for diagnosing leptospirosis, and MAT (OIE 2008) is the standard serologic test. The minimum antigen requirements are that the test should employ representative strains of all the serogroups known to exist in the particular country, plus those known to be maintained by pigs elsewhere.

MAT is used primarily as a herd test. To obtain useful information, at least 10 animals or 10% of the herd, whichever is the greater, should be tested. A retrospective diagnosis of both acute leptospirosis and Pomona abortion may be made when the majority of affected animals have titers of 1 : 1000 or greater. Increasing the sample size and sampling a number of different cohorts markedly improves epidemiologic information, investigations of clinical disease, assessments of vaccination needs, and public health tracebacks.

As an individual animal test, MAT is very useful in diagnosing acute infection; rising antibody titers in paired acute and convalescent serum samples are diagnostic. The presence of antibody in fetal serum is diagnostic of leptospiral abortion.

MAT has severe limitations in the diagnosis of chronic infection in individual pigs, both in the diagnosis of abortion and in the identification of renal or genital carriers. Infected animals may have MAT titers below the widely accepted minimum significant titer of 1 : 100 (Ellis et al. 1986b,c). In these cases a competitive ELISA has proved useful (Frizzell et al. 2004).

Tests that detect leptospires

Confirmation of leptospires in the internal organs (such as the liver, lung, and brain) and body fluids (blood, cerebrospinal, thoracic, peritoneal) of clinically affected animals gives a definitive diagnosis of acute clinical disease or, in the case of a fetus, a diagnosis of leptospiral abortion and probable chronic infection of its mother.

Confirmation of leptospires in the male or female genital tract, the kidney, or urine, in the absence of evidence of generalized infection, is diagnostic of chronic infection. Failure to demonstrate leptospires in the urine of a pig does not rule out the possibility of the animal being a chronic renal carrier; it merely indicates that the pig was not excreting detectable numbers of leptospires at the time of testing.

Bacterial culture

Culture, especially from clinical material, is difficult and time consuming and is a job for laboratories specializing in the identification of isolates. Culture from renal carriers is very useful in epidemiologic studies to determine which serovars are present in an animal species or in a particular group of animals or geographic location.

Culture is the most sensitive direct method provided that antibiotic residues are absent, that tissue autolysis is not advanced, and that tissues for culture have been stored at a suitable temperature of 4 °C (39 °F) and, in the case of urine, at a suitable pH.

Culture should be carried out in a semisolid (0.1–0.2% agar) bovine serum albumin medium containing either Tween 80 (Johnson and Harris 1967) or a combination of Tween 80 and Tween 40 (Ellis 1986) and preferably with a small amount of fresh rabbit serum (0.4–2%). A dilution culture method should be used (Ellis 1986). Contamination may be controlled by a variety of selective agents including 5-fluorouracil, nalidixic acid, fosfomycin, and a cocktail of rifamycin, polymyxin, neomycin, 5-fluorouracil, bacitracin, and actidione. Culture media containing 5-fluorouracil at levels between 200 and 500 µg/mL should be used as transport media for the submission of samples (Ellis 1990). Cultures should be incubated at 29–30 °C (84–86 °F) for at least 12 weeks, preferably for 26 weeks (Ellis 1986). They should be examined by dark-field microscopy every 1–2 weeks.

Other methods to detect leptospires

Leptospires do not stain satisfactorily with the aniline dyes, and silver staining techniques lack sensitivity and specificity (Baskerville 1986). Dark-ground microscopy of fetal fluids or urine leads to false-positive diagnoses and should be avoided.

The demonstration of leptospires by immunochemical tests (immunofluorescence, immunoperoxidase, immunogold) is more suited to most laboratory situations; however, these tests are "number-of-organisms" dependent and lack the sensitivity of culture. They provide no information as to the infecting serovar (Ellis 1990) and require as reagent high IgG titer antileptospire sera. Immunofluorescence is the method of choice for the diagnosis of fetal leptospirosis.

Identification of leptospiral DNA by PCR should be the most sensitive method of detecting leptospires in tissues and fluids since PCR is not dependent on viable organisms. There have been few reported uses of PCR as a tool in the diagnosis of swine leptospirosis, and poor correlations with culture have been reported (Miraglia et al. 2008), but some studies have shown promise (Oliveira et al. 2007).

Prevention and control

Interruption of transmission from infected pig or other infected host to susceptible pig is the critical factor in control. Control of leptospirosis is dependent on the combined use of three strategies: antibiotic therapy, vaccination, and management. Unfortunately, not all these options are available in every country. Vaccines are not available in many Western European countries, while problems of antibiotic residues may make the use of antibiotic therapy difficult in other situations. In the United States, the most useful antibiotic for leptospiral control/treatment programs, streptomycin, is no longer available for veterinary use. Control programs must therefore be modified to meet local conditions.

Vaccination induces immunity of relatively short duration. Immunity to infection is probably never 100% and, at best, lasts little more than 3 months (Ellis et al. 1989; Kemenes and Suveges 1976). Immunity to clinical disease is believed to last somewhat longer, although exact duration is not known. Vaccination markedly reduces the prevalence of infection in swine herds (Kemenes and Suveges 1976; Wrathall 1975) but will not eliminate infection (Cargill and Davos 1981; Edwards and Daines 1979; Hodges et al. 1976). Improvements in bovine leptospirosis vaccines have resulted in protective immunity lasting approximately 12 months. There is a need for the development of similar vaccines for swine that should contain appropriate serovars of pig-maintained strains plus strains maintained by other regional hosts. Mixed genotype infection of serovar Bratislava indicates that natural infection by one genotype may not always confer immunity to infection by the heterologous genotype of the same serovar (Arent et al. 2016).

Antibiotics alone will not eliminate pig-maintained leptospiral infections from individual carriers or control infection in herds. Despite claims by some authors that either systemic streptomycin at 25 mg/kg body weight (Alt and Bolin 1996; Dobson 1974) or oral tetracyclines at levels of 800 g/ton of feed (Stalheim 1967) will eliminate carriers, others have reported that these regimes do not work (Doherty and Baynes 1967; Hodges et al. 1979). Work on the use of alternative antibiotic therapy regimes indicates that oxytetracycline (40 mg/kg for 3 or 5 days), tylosin (44 mg/kg for 5 days), or erythromycin (25 mg/kg for 5 days) may be effective in eliminating Pomona from the kidneys of experimentally infected pigs (Alt and Bolin 1996).

The main management factor in the control of leptospirosis is the prevention of direct or indirect contact with free-living vectors or other domestic stock. Strict biosecurity should be implemented, including stringent rodent control programs. When faced with an outbreak of clinical disease, the best option is to treat both affected and at-risk stock with streptomycin at

25 mg/kg body weight, to immediately vaccinate the at-risk stock, and then to introduce a regular vaccination program. If vaccination is not an available option, then a feed medication program, using either chlortetracycline or oxytetracycline at 600–800 g/ton of feed, should be introduced. This ration is fed either continuously or on a 1-month-on/1-month-off basis. Alternatively, it may be fed for two periods of 4 weeks in the year, preferably one in the spring and the other in the autumn.

The use of artificial insemination is an important tool in the control of Bratislava infection.

References

Adler B, de la Peña-Moctezuma A. 2010. *Vet Microbiol* 140:287–296.

Agunloye CA. 2001. *Trop Vet* 19:188–190.

Al-Khleif A, Damriyasa IM, Bauer C, et al. 2009. *Dtsch Tierarztl Wochenschr* 116:389–391.

Alt DP, Bolin CA. 1996. *Am J Vet Res* 57:59–62.

Anon. 1986. *Can Vet J* 27:290–294.

Arent Z, Frizzell C, Gilmore C, et al. 2016. *Vet Microbiol* 190:19–26.

Azevedo SSD, Soto FRM, Morais ZMD, et al. 2008. *Vet Arhiv* 78:13–21.

Baker TF, McEwen SA, Prescott JF, et al. 1989. *Can J Vet Res* 53:290–294.

Barlow AM. 2004. *Pig J* 54:123–131.

Baskerville A. 1986. Histological aspects of diagnosis of leptospirosis. In Nijhoff M, ed. *The Present State of Leptospirosis Diagnosis and Control*. Dordrecht, Netherlands: Martinus Nijhoff Publishers, pp. 33–43.

Bolin CA, Cassells JA. 1990. *J Am Vet Med Assoc* 196:1601–1604.

Bolin CA, Cassells JA. 1992. *J Vet Diag Invest* 4:87–89.

Bolin CA, Cassells JA, Hill HT, et al. 1991. *J Vet Diag Invest* 3: 152–154.

Bolt I, Marshall RB. 1995a. *NZ Vet J* 43:10–15.

Bolt I, Marshall RB. 1995b. *NZ Vet J* 43:204.

Boqvist S, Montgomery JM, Hurst M, et al. 2003. *Vet Microbiol* 93:361–368.

Boqvist S, Thi VTH, Magnusson U. 2007. Endemic leptospira infection in pigs in southern Vietnam: epidemiology and clinical affection. In Proceedings of the 12th International Conference of the Association of Institutions for Tropical Veterinary Medicine (AITVM), Montpellier, France, August 20–22, 2007, pp. 401–404.

Buddle JR, Hodges RT. 1977. *NZ Vet J* 25: 56 and 65–66.

Burnstein T, Baker A. 1954. *J Infect Dis* 94:53–54.

Cargill CF, Davos DE. 1981. *Aust Vet J* 57:236–238.

Chaudhary RK, Fish NA, Barnum DA. 1966a. *Can Vet J* 7:106–12.

Chaudhary RK, Fish NA, Barnum DA. 1966b. *Can Vet J* 7:121–127.

Cheville NF, Huhn R, Cutlip RC. 1980. *Vet Pathol* 17:338–351.

Choi C, Park YC, Paik MA, et al. 2001. *Vet Rec* 148:416.

Cole JR, Hall RF, Ellinghausen HC, et al. 1983. Prevalence of leptospiral antibodies in Georgia cattle and swine, with emphasis on Leptospira interrogans serovar tarassovi. *Proc US Anim Health Assoc* 87:199–210.

Cullen PA, Xu X, Matsunaga J, et al. 2005. *Infect Immun* 73:4853–4863.

Dobson KJ. 1974. *Aust Vet J* 50:471.

Doherty PC, Baynes ID. 1967. *Aust Vet J* 43:135–137.

Edwards JD, Daines D. 1979. *NZ Vet J* 27:247–248.

Egan J. 1995. *Irish Vet J* 48:399–402.

Ellis WA. 1986. The diagnosis of leptospirosis in farm animals. In Nijhoff M, ed. *The Present State of Leptospirosis Diagnosis and Control*. Dordrecht, Netherlands: Martinus Nijhoff Publishers, pp. 13–31.

Ellis WA. 1990. Leptospirosis. In OIE Manual of Recommended Diagnostic Techniques and Requirements for Biological Products for List A and B Diseases, Paris, Vol. 2, sec. 7, pp. 1–11.

Ellis WA. 2006. Leptospirosis. In Straw BE, Zimmermann JJ, D'allaire S, et al., eds. *Diseases of Swine*, 9th ed. Ames, IA, Blackwell Publishing, pp. 691–700.

Ellis WA, Thiermann AB. 1986. *Am J Vet Res* 47:1458–1460.

Ellis WA, McParland PJ, Bryson DG, et al. 1986a. *Vet Rec* 118:63–65.

Ellis WA, McParland PJ, Bryson DG, et al. 1986b. *Vet Rec* 118:294–295.

Ellis WA, McParland PJ, Bryson DG, et al. 1986c. *Vet Rec* 118:563.

Ellis WA, Montgomery JM, McParland PJ. 1989. *Vet Rec* 125:319–321.

Ellis WA, Montogomery JM, Thiermann AB. 1991. *J Clin Microbiol* 29:957–961.

Faine S, Adler B, Bolin C, et al. 1999. *Leptospira and Leptospirosis*, 2nd ed. Armadale, Australia: MedSci.

Fearnley C, Wakeley PR, Gallego-Beltran J, et al. 2008. *Res Vet Sci* 85:8–16.

Fennestad KL, Borg-Petersen C. 1966. *J Infect Dis* 116:57–66.

Ferguson LC, Powers TE. 1956. *Am J Vet Res* 17:471–477.

Ferguson LC, Lococo S, Smith HR, et al. 1956. *J Am Vet Med Assoc* 129:263–265.

Field HI, Sellers KC. 1951. *Vet Rec* 63:78–81.

Fish NA, Ryu E, Holland JJ. 1963. *Can Vet J* 4:317–327.

Frantz JC, Hanson LE, Brown AL. 1989. *Am Vet Res* 50:1044–1047.

Frizzell C, Mackie DP, Montgomery JM, et al. 2004. *Pig J* 53:195–199.

Haake DA, Zückert WR. 2015. *Curr Top Microbiol Immunol* 387:187–221.

Hanson LE, Tripathy DN. 1986. Leptospirosis. In Leman AD, Straw BE, Glock RB, et al., eds. *Diseases of Swine*, 6th ed. Ames, IA: Iowa State University Press, pp. 591–599.

Hartman EG, Brummelman B, Dikken H. 1975. *Tijdschr Diergeneeskd* 100:421–425.
Hathaway SC. 1981. *NZ Vet J* 29:109–112.
Hathaway SC. 1985. *Pig News Inf* 6:31–34.
Hathaway SC, Little TWA. 1981. *Vet Rec* 108:224–228.
Hathaway SC, Ellis WA, Little TWA, et al. 1983. *Vet Rec* 113:153–154.
Hodges RT, Stocker RP, Buddle JR. 1976. *NZ Vet J* 24:37–39.
Hodges RT, Thompson J, Townsend KG. 1979. *NZ Vet J* 27:124–126.
Jensen AM, Binder M. 1989. *Dansk Vet* 72:1181–1187.
Johnson RC, Harris VG. 1967. *J Bacteriol* 94:27–31.
Kemenes F, Suveges T. 1976. *Acta Vet Acad Sci Hung* 26:395–403.
Kikuchi N, Shikano M, Hatanaka M, et al. 2009. *J Vet Epidemiol* 13:95–99.
Langham RF, Morse EV, Morter RL. 1958. *Am J Vet Res* 19:395–400.
Levett PN. 2015 *Curr Top Microbiol Immunol* 387:11–20.
McKeever S, Gorman GW, Chapman JF, et al. 1958. *Am J Trop Med Hyg* 7:646–655.
Michna SW. 1962. *Vet Rec* 74:917–919.
Miraglia F, Moreno AM, Gomes CR, et al. 2008. *Braz J Microbiol* 39:501–507.
Mitchell D, Robertson A, Corner AH, et al. 1966. *Can J Comp Med Vet Sci* 30:211–217.
Modric Z, Turk N, Artukovic B, et al. 2006. *Hrvatski Veterinarski Vjesnik* 29:223–230.
Morter EV, Morse EV, Langham RF. 1960. *Am J Vet Res* 21:95.
Nagy G. 1993. *Acta Vet Hung* 41:315–324.
OIE. 2008. Leptospirosis. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Vol. 1, 6th ed. Paris: Office International Des Epizooties, pp. 251–264.
Oliveira SJD, Bortolanza F, Passos DT, et al. 2007. *Braz J Vet Res Anim Sci* 44:18–23.
Osava CF, Salaberry SRS, Nascimento CCN, et al. 2010. *Biosci J* 26:202–207.
Paz-Soldan SV, Dianderas MT, Windsor RS. 1991. *Trop Anim Health Prod* 23:233–240.
Power SB. 1991. *Vet Rec* 128:43.
Puchadapirom P, Niwetpathomwat A, Luengyosluechakul S. 2006. *Thai J Vet Med* 36:86.
Rehmtulla AJ, Prescott JF, Nicholson VM, et al. 1992. *Can Vet J* 33:344–345.
Rocha T. 1990. *Vet Rec* 126:602.
Roth EE. 1964. *Am J Vet Med Assoc* 101:211.
Ryley JW, Simmons GC. 1954a. *Queens J Agric Sci* 11:61–74.
Ryley JW, Simmons GC. 1954b. *Aus Vet J* 30:203–208.
Saravi MA, Molinari R, Soria EH, et al. 1989. *Rev Sci Tech Off Int Epizoot* 8:697–718.
Schnurrenberger PR, Hanson LE, Martin RJ. 1970. *Am J Epidemiol* 92:223–239.
Schonberg A, Hahnhey B, Kampe U, et al. 1992. *J Vet Med Series B* 39:362–368.
Shang ES, Exner MM, Summers TA, et al. 1995. *Infect Immun* 63:3174–3181.
Sleight SO, Langham RF, Morter RL. 1960. *J Infect Dis* 106:262–269.
Stalheim OHV. 1967. *Am J Vet Res* 28:161–166.
Tripathy DN, Hanson LE, Mansfield ME, et al. 1981. Pathogenesis of Leptospira pomona in lactating sows and transmission to piglets. *Proc US Anim Health Assoc* 85:188.
Van Til LD, Dohoo IR. 1991. A serological survey of leptospirosis in Prince Edward Island swine herds and its association with infertility. *Can J Vet Res* 55:352–355.
Wandurski A. 1982. *Med Weter* 38:218–220.
Wasinski B. 2005. *Med Weter* 61:46–49.
Wrathall AE. 1975. Reproductive disorders in pigs. In Animal Health Review Series no. 11. Commonwealth Agricultural Bureaux.
Zieris H. 1991. *Monatsh Veterinarmed* 46:355–358.

56

Mycoplasmosis

Maria G. Pieters and Dominiek Maes

Overview

Pathogenic and nonpathogenic *Mycoplasma* spp. have been identified in swine. Four species are responsible for nearly all diseases. *Mycoplasma hyopneumoniae* causes enzootic pneumonia (EP) and is an important primary pathogen in the porcine respiratory disease complex (PRDC). *Mycoplasma hyorhinis* most commonly causes polyserositis and arthritis in nursery-age pigs. *Mycoplasma hyosynoviae* causes arthritis in grow–finish pigs, and *Mycoplasma suis*, formerly *Eperythrozoon suis*, causes infectious anemia in pigs.

Mycoplasmas are classified within the phylum *Tenericutes*, class *Mollicutes*, order *Mycoplasmatales*, and family *Mycoplasmataceae* (Nowak 1929). They are the smallest and simplest known self-replicating organisms, phylogenetically related to gram-positive bacteria. They are distinguished from other eubacteria by lacking a cell wall (Neimark 1986) and by having cholesterol in the plasma membrane, a low genomic G+C content, and unique usage of the UGA codon for tryptophan. Mycoplasmas likely evolved by degenerative, reductive, and regressive evolution, ending with a very small genome of 580–1380 kbp (Razin 1992). This small genome results in a slow growth rate due to an inability to rapidly synthesize ribosomes and dependence on host-derived nutrients. Most *Mycoplasma* spp. attach to and replicate on epithelial cells that line the respiratory and urogenital tracts, as well as the mucous membranes of the conjunctiva, the digestive tract, mammary glands, and joints. In contrast, *M. suis* attaches to and invades and replicates on and in red blood cells (RBCs).

Mycoplasma hyopneumoniae

M. hyopneumoniae infects only pigs and is ubiquitous, found worldwide in nearly all countries and pig farms where the organism has not been intentionally eliminated. It is the essential causative agent of a chronic insidious bronchopneumonia known as EP. It produces long-term colonization of ciliated respiratory epithelium in the conducting airways where it suppresses innate and acquired pulmonary immunity. This allows upper respiratory commensal bacteria such as *Pasteurella multocida*, *Streptococcus suis*, *Haemophilus parasuis*, and others to proliferate in the lungs and contribute to disease. EP is characterized by its chronic nature, high morbidity, low mortality, and decreased performance. The economic impact of EP is significant and primarily related to decreased average daily gain, increased feed conversion ratio, and increased number of days to reach market weight. EP occurs primarily in grower–finisher pigs in age-segregated production systems or sometimes in younger nursery-age pigs in continuous-flow systems. In addition to EP, *M. hyopneumoniae* can also contribute to PRDC through its potentiation of respiratory disease caused by certain viral pathogens, including porcine reproductive and syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). Although most swine herds are endemically infected with *M. hyopneumoniae*, when it is introduced into negative naïve herds, it can produce epidemics affecting all ages of pigs simultaneously.

Etiology

M. hyopneumoniae was first isolated over half a century ago (Goodwin et al. 1965; Mare and Switzer 1965) and was established as the agent causing EP. It is difficult to grow *in vitro* due to demanding nutritional requirements, slow growth rate, and overgrowth with other bacteria, especially *M. hyorhinis*. The medium universally employed for *M. hyopneumoniae* culture was developed by Friis (1975) and contains a wide array of compounds including antibiotics, serum of swine and equine origin, yeast extract, and a pH indicator. Recently Cook et al. (2016) claim to have optimized the original medium by inclusion of kanamycin to inhibit the growth of *M. hyorhinis*. Careful sample selection and handling to ensure that a significant amount of

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

M. hyopneumoniae is inoculated into media improves isolation rates (Anderson et al. 2016a).

M. hyopneumoniae grows more slowly in culture than other mycoplasmas, and isolation is often unsuccessful. Culture is typically attempted when a certain isolate of *M. hyopneumoniae* is needed, but not as a routine diagnostic method. Optimum samples for culture include typical pneumonic lesions and the surrounding normal-appearing lung, and bronchial swabs. Following inoculation of Friis broth medium, the medium is serially diluted, and dilutions are monitored for 3–30 or more days for growth. Growth is typically evidenced by color change but can be accurately measured using an ATP luminometry method (Calus et al. 2010). Broth growth is plated to solid agar media to clone. Growth may require days to weeks and colonies are best visualized using a stereoscopic microscope. Individual colonies are identified as *M. hyopneumoniae* by PCR (Stakenborg et al. 2006a) or are grown back in Friis broth for identification by other methods or for harvest and storage.

Strains of *M. hyopneumoniae* are antigenically and genetically diverse. The lack of clonality of *M. hyopneumoniae* was suspected and initially demonstrated by serological methods (Ro and Ross 1983), fingerprinting (Kokotovic et al. 1999), clinical presentation (Vicca et al. 2003), and *in vitro* growth (Meyns et al. 2007). Efforts to characterize *M. hyopneumoniae* at the molecular level are based on sequencing of a specific VNTR in the gene encoding the major adhesion protein P146 (Mayor et al. 2007), the length of fragments after digestion with restriction enzymes (Kokotovic et al. 1999; Stakenborg et al. 2006b), or the analysis of VNTR patterns in various loci (Dos Santos et al. 2015b; Nathues et al. 2011; Stakenborg et al. 2005b; Vranckx et al. 2011). Strains of *M. hyopneumoniae* differ in virulence, with high virulence strains inducing more severe pneumonia in a larger proportion of infected pigs (Meyns et al. 2007; Vicca et al. 2003; Woolley et al. 2012). Higher pathogenicity in high virulence strains is attributed to a higher capacity to multiply in the lungs and induction of a more severe inflammatory process (Meyns et al. 2007).

Epidemiology

Infections caused by *M. hyopneumoniae* are virtually present in every country where pigs are raised. Prevalence levels are likely high, but there is little published prevalence data. Switzerland is an exception where the prevalence is under 1% after completing an eradication program at national level (Stärk et al. 2007). However *M. hyopneumoniae* infections in wild boar and domestic swine are sporadically detected in Switzerland (Kuhnert and Overesch 2014; Kuhnert et al. 2011). Cross-transmission between domestic and feral swine has not been documented.

Swine of all ages are susceptible to colonization by *M. hyopneumoniae* on the epithelium of their nasal cavity and conducting airways. *M. hyopneumoniae* is shed in nasal secretions of infected animals (Goodwin 1972), and the most common mode of transmission occurs via close, usually nose-to-nose, contact between infected and susceptible pigs (Calsamiglia and Pijoan 2000; Rautiainen and Wallgren 2001). However nose-to-nose transmission is inefficient and spread is slow. Studies have demonstrated that 1 infected nursery-age pig will transmit *M. hyopneumoniae* to 1–2 naïve pigs during 4–6 weeks of continuous contact (Meyns et al. 2004; Roos et al. 2016; Villarreal et al. 2011). Colonization of pigs persists for long periods, typically 7–8 months (Pieters et al. 2009). Colonized pigs may or may not develop clinical pneumonia during this period, depending on a variety of factors, including strain virulence, infectious dose, host immunity, concurrent bacterial, viral, or other infections, and environment, among others (see section "Pathogenesis"). Colonized pigs appear to be infectious to other animals during the entire duration of infection, although consistency of shedding and concentration of *M. hyopneumoniae* shed may vary over time. Naïve contact pigs develop a higher rate of colonization when exposed to *M. hyopneumoniae*-infected pigs with clinical EP compared with lower rates when exposed to subclinically *M. hyopneumoniae*-infected pigs in the later months of their infection (Pieters et al. 2009).

M. hyopneumoniae is most commonly introduced into naïve populations by direct transmission via close contact through the introduction of infected pigs. However, airborne transmission of *M. hyopneumoniae* has been suspected for decades (Goodwin 1985) and has been confirmed over short distances (Fano et al. 2005). Otake et al. (2010) showed that air samples containing *M. hyopneumoniae* recovered from as far as 9.2 km from an infected farm remained infectious to inoculated animals. A number of studies of the Danish specific-pathogen-free (SPF) systems found that herd reinfections with *M. hyopneumoniae* often occurred in the autumn and winter when atmospheric conditions favored aerosol transmission (Jorsal and Thomsen 1988). *In utero* transmission of *M. hyopneumoniae* is not known to occur, and the role of vectors in transmission is considered to be minimal.

Once *M. hyopneumoniae* is introduced, a swine herd typically remains endemically infected. Infection is maintained in breeding herds by the constant introduction of naïve replacement animals, which allows for continuous pathogen transmission among resident females and incoming purchased or self-reared replacement gilts. Dams transmit *M. hyopneumoniae* via nasal secretions to their progeny during the suckling period (Calsamiglia and Pijoan 2000; Fano et al. 2007; Rautiainen and Wallgren 2001). The proportion of sows that shed

M. hyopneumoniae decreases by parity. In one study, 73% of parity 1, 42% of parity 2–4, 50% of parity 6–7, and 6% of parity 8–11 were shedding the organism (Calsamiglia and Pijoan 2000). In another study, 33% of pigs from parity 1 to 2 sows were *M. hyopneumoniae* positive at weaning compared with 21% from 3+ parity sows (Fano et al. 2006). Management practices at the sow and the piglet level seem to be risk factors for piglet colonization. Increasing risk of infection at weaning is associated with a higher proportion of positive dams, higher numbers of replacement gilts in the breeding herd (presumably affecting the proportion of positive dams), larger farrowing rooms, lower ambient temperatures in the suckling piglet nesting area, and longer lactation periods (Nathues et al. 2013a, 2013b; Pieters et al. 2014; Vangroenweghe et al. 2015). Cross-fostering of piglets would also likely increase prevalence of *M. hyopneumoniae* at weaning. Prevalence of colonization at weaning varies widely but in most reports is 0–15%.

Following weaning, infected pigs are mixed with larger cohorts of naïve pigs, increasing the potential for transmission. The prevalence of piglet colonization at weaning age has been proposed as a predictor of disease severity in the finishing phase (Fano et al. 2007; Sibila et al. 2007b). For example, with a 1:1 transmission ratio every 4 weeks, a 2% prevalence of infection at a 1-month weaning age would result in 4, 8, 16, and 32% prevalence of infection in the weaned cohort at 2, 3, 4, and 5 months of age, compared with a 6% prevalence of infection at weaning that would result in 12, 24, 48, and 96% prevalence of infection at the same ages in the finishing barn. In a continuous-flow system, transmission is further enhanced by direct contact or indirect exposure via a shared airspace with older pigs having a higher prevalence of infection (Giacomini et al. 2016; Vranckx et al. 2012). In age-segregated 2- or 3-site systems, EP is usually manifest in the mid- to late finishing phase; however, in continuous-flow systems, EP may be observed as early as 8 weeks of age. Investigation of differences between farms and production systems has identified numerous factors important in the dynamics and severity of *M. hyopneumoniae* disease on a herd level, including housing styles, ventilation systems, stocking densities, climatic conditions, and the type of system, i.e. age-segregated or continuous-flow 1-, 2-, or 3-site production systems (Sibila et al. 2004b; Vicca et al. 2002). Additionally, the presence of other infectious or toxic agents impact expression of disease (see section "Pathogenesis").

The application of molecular methods has allowed the differentiation of *M. hyopneumoniae* strains and their epidemiologic study in the field. In areas with low swine density or low prevalence of *M. hyopneumoniae*-positive herds, a single strain of *M. hyopneumoniae* has typically been detected in infected herds (Mayor et al. 2007; Vranckx et al. 2012). In contrast in swine-dense areas, multiple strains of *M. hyopneumoniae* have been frequently detected in individual swine herds (Michiels et al. 2017; Nathues et al. 2011). One study suggests that herds infected with larger numbers of strains have more severe lesions of EP at slaughter (Michiels et al. 2017).

Pathogenesis

The pathogenesis of *M. hyopneumoniae* is complex and involves long-term colonization of airway epithelium, stimulation of a prolonged inflammatory reaction, suppression and modulation of the innate and adaptive immune responses, and interaction with other infectious agents.

M. hyopneumoniae replicates nearly exclusively on ciliated respiratory epithelial cells lining the nasal cavity and conducing airways of pigs. However, it has rarely been isolated from the liver, spleen, and kidneys of experimentally inoculated pigs where no lesions or alterations in organ function are reported (Le Carrou et al. 2006; Marchioro et al. 2013; Marois et al. 2007).

Upon inhalation, *M. hyopneumoniae* organisms penetrate the mucus layer of the respiratory mucosa (Jenkins et al. 2006; Wilton et al. 2009), and within one day they are adherent to cilia of epithelial cells of the trachea, bronchi, and bronchioles (Tajima and Yagihashi 1982). The process of adherence to the cilia is complex and mediated between adhesins of the mycoplasma cells and receptors on the membrane of the cilia. A number of adhesins have been identified including Mhp182 (P102), Mhp183 (P97), Mhp 684 (P146), Mhp 493 (P159), Mhp 494 (P216), Mhp 683 (P135), Mhp 271, Mhp 107, and Mhp 108 (P116). Adherence is largely mediated via interactions of members of the P97 and P102 adhesin families and P159 with glycosaminoglycans and fibronectin that decorate the surface of ciliated respiratory epithelial cells (Adams et al. 2005; Hsu and Minion 1998; Seymour et al. 2012; Wilton et al. 2009).

Members of the P97 and P102 adhesin families and P159 are large-mass (>100 kDa), modular, multifunctional molecules that are cleaved at multiple sites, generating a complex mixture of cleavage fragments that remain attached on the extracellular membrane surface of *M. hyopneumoniae*. In the P97/P102 adhesin families, posttranslational cleavage is extensive and unorthodox (Jarocki et al. 2015; Tacchi et al. 2016). The resulting diversity of surface proteins is thought to regulate adhesion to the host tissue, potentiate host cell invasion, and avoid immune clearance (Bogema et al. 2012). Cleavage fragments derived from these adhesins bind heparin (Burnett et al. 2006; Deutscher et al. 2010), fibronectin (Deutscher et al. 2010; Seymour et al. 2011, 2012), and plasminogen (Robinson et al. 2013; Seymour et al. 2010, 2011, 2012). Binding of plasminogen facilitates conversion to plasmin by mammalian plasminogen activators

(Seymour et al. 2012). Plasmin functions as a proinflammatory agonist and likely facilitates tissue invasion, inflammatory lesions, and systemic infection (Bogema et al. 2012).

The conducting airways, especially in the apical and cardiac lobes, are progressively colonized by *M. hyopneumoniae* with corresponding damage to the mucociliary apparatus (Bendjennat et al. 1999; Blanchard et al. 1992). Within hours after the adherence of *M. hyopneumoniae* to the cilia, damage to cilia gives way to cilia loss and ultimately disruption of the epithelial cells (Blanchard et al. 1992; Park et al. 2002). This results in a reduced clearing capacity by the mucociliary apparatus and increases susceptibility to secondary bacterial and viral respiratory infections (Ciprian et al. 1994; Park et al. 2016).

M. hyopneumoniae has a modulating effect on the inflammatory response and the immune system. *M. hyopneumoniae* organisms stimulate alveolar macrophages and lymphocytes to produce proinflammatory and immunoregulatory cytokines that play a role in inducing lung inflammation and lymphoid hyperplasia (Meyns et al. 2007; Rodriguez et al. 2004), the primary features of lesions observed in EP. On the other hand, there is also evidence for the induction of anti-inflammatory cytokines that suppress the function of neutrophils (Asai et al. 1996) and macrophages (Caruso and Ross 1990), this likely aiding persistent infection by *M. hyopneumoniae* and opportunistic infection by other bacterial and viral agents. See more on modulation of the inflammatory and immune response in section "Immunity."

Not all respiratory infections with *M. hyopneumoniae* result in clinical pneumonia. Development of clinical pneumonia is dependent on the number of organisms in the respiratory tract, the virulence of the infecting strain(s) of *M. hyopneumoniae*, and the involvement of other respiratory pathogens. The number of organisms colonizing a pig is likely dependent on cumulated infectious doses, capacity of the *M. hyopneumoniae* strain(s) to multiply in the lungs, and time. Infection with *M. hyopneumoniae* often has its greatest economic significance due to interaction with other respiratory pathogens.

Pulmonary colonization by *M. hyopneumoniae* predisposes pigs to respiratory infections with opportunistic bacteria, typically upper respiratory commensals. This is due to reduced pulmonary clearance induced by *M. hyopneumoniae* through damage to cilia, induction of excessive thick mucus, reduction in phagocytic efficiency of neutrophils and macrophages, and likely other mechanisms. Combined experimental infections with *M. hyopneumoniae* and either *P. multocida* (Amass et al. 1994) or *Actinobacillus pleuropneumoniae* (Marois et al. 2009) result in more severe lesions compared with the single infections. Coinfections with *P. multocida* and *A. pleuropneumoniae* and with other bacteria such as *Bordetella bronchiseptica*, *H. parasuis*, *Trueperella pyogenes*, or streptococci are commonly found in field outbreaks of EP.

Infection with *M. hyopneumoniae* also potentiates some viral infections. An initial study focusing on the interaction between *M. hyopneumoniae* and PRRSV did not demonstrate a potentiating effect of either pathogen (Van Alstine et al. 1996). Later studies confirmed that *M. hyopneumoniae* significantly prolongs and increases the severity of PRRSV-induced pneumonia (Thacker et al. 1999). Similar studies demonstrated only transitory and/or inconsequential potentiation of influenza viral pneumonias (Deblanc et al. 2012; Thacker et al. 2001; Yazawa et al. 2004). Studies using dual *M. hyopneumoniae* and PCV2 challenge models indicated that *M. hyopneumoniae* infection potentiates the severity of PCV2-associated lung and lymphoid lesions and increases the amount and prolongs the presence of PCV2 antigen (Opriessnig et al. 2004; Seo et al. 2014). Additionally, the incidence of post weaning multisystemic wasting syndrome in pigs was also found to be increased (Opriessnig et al. 2004). Sibila et al. (2012) however could not demonstrate an interaction between *M. hyopneumoniae* and PCV2 infection. In herds facing concurrent infections with *M. hyopneumoniae* and PCV2, studies have confirmed that vaccination against PCV2 alone or in combination with vaccination against *M. hyopneumoniae* is more effective in preventing potentiation of lung lesions than *M. hyopneumoniae* vaccination alone (Raith et al. 2015; Seo et al. 2014).

Finally, some cofactors potentiate lesions of EP in *M. hyopneumoniae*-infected pigs. Lesions typical for EP have higher prevalence and are more severe in pigs that are concurrently infected with migrating *Ascaris suum* larvae (Flesja and Ulvesaeter 1980) or in pigs receiving feed contaminated with fumonisin B (Pósa et al. 2013).

Immunity

Innate as well as adaptive humoral and cellular immune responses are elicited in *M. hyopneumoniae*-infected pigs. Although the immune response prevents significant systemic spread of *M. hyopneumoniae*, it is unable to rapidly clear pulmonary infection resulting in persistent colonization of airways for up to 7 months. This results in a prolonged localized inflammatory and cellular immune response that is responsible for the majority of gross and microscopic lesions.

M. hyopneumoniae both stimulates and inhibits the innate immune response. Colonization by *M. hyopneumoniae* induces localized tissue histiocytes to secrete inflammatory cytokines that, in turn, induce a localized inflammatory response. This results in an influx of neutrophils and macrophages that predominate early in infection. Clearance of *M. hyopneumoniae* and other bacterial and viral agents is impaired by *M. hyopneumoniae*-induced damage to cilia, induction of excessive

mucus, and reduction in phagocytic efficiency of neutrophils and macrophages. The secretion of inflammatory cytokines increases as colonization by *M. hyopneumoniae* progresses, due to increasing numbers of accumulating inflammatory cells. Toll-like receptors 2 and 6 recognize lipoproteins in *M. hyopneumoniae*, inducing cytokine production (Muneta et al. 2003; Régia Silva Sousa et al. 2011). The extent of the cytokine induction can vary between strains of infecting *M. hyopneumoniae* (Meyns et al. 2007). The cytokine profile during infection with *M. hyopneumoniae* is contradictory. Proinflammatory cytokines like IL-1, IL-2, IL-6, IL-8, IL-12, IL-18, INF-γ, and TNF-α (Asai et al. 1993, 1994; Muneta et al. 2006; Thacker et al. 2000a; Thanawongnuwech et al. 2004), as well as anti-inflammatory cytokines like IL-10, have been measured in *M. hyopneumoniae*-infected pigs (Thanawongnuwech and Thacker 2003).

Shortly after neutrophils and macrophages arrive at sites of *M. hyopneumoniae* colonization, peribronchiolar and adjacent perivascular connective tissues are infiltrated by both B and T lymphocytes, leading to the production of *M. hyopneumoniae*-specific antibodies and the development of a cell-mediated immune response. Helper (CD4+) T cells are more prevalent than cytotoxic (CD8+) T cells (Sarradell et al. 2003). Over time lymphoid nodules with germinal-like centers form. Cell-mediated immunity (CMI) may be important in limiting spread of *M. hyopneumoniae* to other organs, but it is also responsible for enhancing lesions. Tajima et al. (1984) demonstrated in thymectomized pigs treated with antithymocyte serum and inoculated with *M. hyopneumoniae* that lesions of EP were less severe than in controls but that *M. hyopneumoniae* was isolated from the spleen of one of the thymectomized pigs but not in control pigs.

Antibodies against *M. hyopneumoniae* are detected in the serum and in the lower respiratory tract of infected pigs. Immunoglobulins M, G, and A have been measured in the lung (Messier et al. 1990) and IgG and IgA in tracheobronchial secretions of naturally and experimentally infected animals (Messier et al. 1990; Redondo et al. 2009; Rodriguez et al. 2004; Suter et al. 1985). Studies confirm a lack of correlation between the level of serum antibodies and protection from infection (Djordjevic et al. 1997).

Antibodies and immune cells specific for *M. hyopneumoniae* can be transferred from the dam to the baby pig via colostrum. Bandrick et al. (2008) demonstrated the transfer of CMI in a newborn piglet model in a *M. hyopneumoniae* endemically infected farm. Later, Bandrick et al. (2011) showed that the transfer of the *M. hyopneumoniae*-specific immunity was affected by time after birth for antibodies and was time and source dependent in the case of CMI. The effect of maternally transferred immunity has been a cause of debate. Maternal interference with the pig immune response to infection, or vaccination, has been demonstrated in terms of antibody levels, but interference is not evidenced for maternal CMI (Bandrick et al. 2014).

Clinical signs

Two clinical forms of *M. hyopneumoniae*-induced disease occur in swine herds: epidemic and endemic. Epidemics are uncommon, occurring only when *M. hyopneumoniae* enters a noninfected, immunologically naive herd. The spread of the disease occurs rapidly, and all age groups may be affected. Coughing, acute respiratory distress, fever, and deaths may occur. Typically, infection transitions to an endemic pattern in the herd within 2–5 months.

The endemic form of disease known as EP is the common form of disease and is observed in continuously infected herds. The most obvious clinical sign is nonproductive coughing in nursery, grower, and/or finisher pigs. Onset is insidious, affecting at first a few animals and slowly spreading to involve a considerable percentage of the pigs. Coughing may disappear after 2–3 weeks but can also persist throughout the entire finishing period. Under experimental infections, clinical signs are mainly characterized by slight fever, followed by dry coughing. Coughing appears from 10 to 14 days post inoculation, reaches a maximum peak at about 4–5 weeks, after which it disappears gradually (Arsenakis et al. 2016; Garcia-Morante et al. 2016; Vicca et al. 2003). Under field conditions, most infections with *M. hyopneumoniae* are complicated by coinfection with other opportunistic bacteria or with respiratory viruses. In this case, clinical signs are more severe and may include severe respiratory distress, fever, labored breathing, prostration, reduced appetite, and eventually mortality.

Lesions

Macroscopic lesions in the lungs of pigs infected with *M. hyopneumoniae* are found bilaterally in the apical, cardiac, and intermediate lobes and sometimes in the anterior parts of the diaphragmatic lobes. In inoculation studies they can be detected 7–10 days post infection (Kobisch et al. 1993; Underdahl et al. 1980) and reach their maximal extension 2–4 weeks post infection. The lesions are fawn/pink or plum colored and demarcated from normal tissue (Rodríguez et al. 2016). When the affected lung is incised, the consistency is moderately firm, and the cut surface is moist. The bronchial and mediastinal lymph nodes are enlarged. During the chronic stages, the lesions are deeper red. Recovering lesions consist of fissures of collapsed alveoli (interlobular scar retractions) and remain 2–4 months after infec-

tion (Kobisch et al. 1993; Sorensen et al. 1997). They are located mainly in the cardiac lobes. Although gross lesions are suggestive of EP, they are not pathognomonic for *M. hyopneumoniae*, as other organisms like swine influenza virus or the combination of *P. multocida* and Aujeszky's disease virus may produce similar lesions (Done 1991; Fuentes and Pijoan 1987).

Microscopic lesions can be staged depending on the duration of the disease. Early microscopic lesions consist of limited accumulations of neutrophils in the lumens and around the airways as well as in alveoli. Few lymphocytes are in the adventitia of arterioles and venules and around the airways. Second-stage lesions occur between 7 and 28 days post infection and are characterized by marked accumulation of neutrophils, fluid, and macrophages in alveoli; proliferation of type II alveolar pneumocytes; increasing accumulation of lymphocytes, histiocytes, and plasma cells around blood vessels and conducting airways; and development of peribronchial lymphoid nodules. Bronchial epithelium may be partially denuded of cilia or partially desquamated (Blanchard et al. 1992). Established third-stage lesions occur 17–40 days post infection and are characterized by progressive peribronchial lymphoid hyperplasia, increased perivascular mononuclear cell accumulation, and progressive development of alveolar pneumonia. Late and recovering fourth-stage lesions occur 69–210 days post infection. Large numbers of lymphocytes and fewer plasma cells form extensive peribronchial cuffs that also contain many large lymphoid nodules that may compress bronchial lumens. Scoring systems have been used to quantify microscopic lesions (Morris et al. 1995; Villarreal et al. 2009).

Lesions are often complicated by additional features characteristic of concurrent bacterial or viral infectious agents.

Diagnosis

M. hyopneumoniae does not cause pathognomonic clinical signs or lesions. Diagnosis of EP should include typical clinical signs, epidemiology, typical lesions, and confirmation of *M. hyopneumoniae* infection.

Nonproductive cough and/or decreased growth rate is suggestive of *M. hyopneumoniae*. Traditional methods to quantify coughs have proven valuable to indicate disease onset (Nathues et al. 2012). The use of computerized cough monitoring systems coupled with microphones physically located in the barns have been proposed as means to detect increases in cough activity and to trigger disease investigations (Guarino et al. 2008).

Typical microscopic lesions should be confirmed in lungs having suggestive gross lesions of EP. Several samples should be collected from areas with gross lesions, focusing on areas toward the middle and base of lung lobes and avoiding lung margins. Samples should be placed in fixative as soon as possible after death. When possible, several lungs should be sampled.

The traditional gold standard method for confirmation of *M. hyopneumoniae* infection is culture using Friis media (Friis 1975); however, slow growth, frequent overgrowth by other bacteria, high cost, and low sensitivity of the method limit usefulness. Culture is typically employed only when a bacterial isolate is needed.

Direct detection of the antigen or nucleic acid can be performed in the airway epithelium and lung tissue. Fluorescent antibody (FA) or immunohistochemistry (IHC) tests use specific antibodies to detect antigen in thin frozen sections or fixed sections of tissue, and *in situ* hybridization (ISH) uses specific nucleic acid probes to detect specific nucleic acids in thin fixed tissue sections (Boye et al. 2001). FA and IHC tests have been widely used but require high-quality *M. hyopneumoniae*-specific antibodies that are sometimes unavailable. Among these, IHC and ISH have the advantage of allowing visualization of *M. hyopneumoniae* antigen in conjunction with microscopic lesions, conferring a high level of confidence in a diagnosis of EP. However, all three methods suffer from several disadvantages. They cannot be used on antemortem samples. Since they require intact *M. hyopneumoniae*-colonized bronchial epithelial cells in the sections of tested lungs, samples from carcasses with postmortem degeneration or that have passed through a scalding process at slaughter can result in false-negative tests. Because colonization of lungs is nonuniform, several sections from each lung should be tested, or false negatives may result. Finally, the technologies employed are less sensitive than PCR, which has become the most widely used confirmatory test.

Various PCR assays have been developed and validated, including gel based, nested, and real time (Dubosson et al. 2005; Strait et al. 2008). High accuracy, fast turnaround, high throughput, and utility for use with tissue samples collected postmortem or clinical samples collected from live pigs have made real-time PCR the most used confirmatory test. Sample selection for *M. hyopneumoniae* detection by PCR can be challenging. The most sensitive samples are of lung exhibiting typical gross lesions and that include bronchi, i.e. not along lung margins. Clinical samples can also be used, including oral fluids, nasal swabs, tonsillar swabs, laryngeal swabs, tracheobronchial swabs, and tracheobronchial lavages, among others (Anderson et al. 2016a,b; Calsamiglia et al. 1999; Fablet et al. 2010; Pieters et al. 2017; Sibila et al. 2004a). The sensitivity of the sample types varies, with samples collected toward the lower respiratory tract exhibiting higher sensitivity. However, care should be exercised when interpreting PCR results, as the potential

for cross-contamination exists, and viable and nonviable cells will be equally detected.

Evaluation of lungs at slaughter has been used to collect samples for confirmatory testing of *M. hyopneumoniae* infection and to quantify prevalence and severity of lesions suggestive of EP. Prevalence of EP can be overestimated since similar lesions of other respiratory diseases can be misclassified as EP. To prevent this, samples should be collected for histopathology and confirmatory testing. Prevalence can also be underestimated since lesions of EP can be resolved by slaughter age in some herds and/or animals, depending on the epidemiology of EP in the herd, as it is influenced by the interaction of various herd-specific housing, pig flow, gilt acclimation, vaccination, and other factors. Lack of lesions at slaughter should not be considered proof of absence of EP in the herd without further careful clinical examination.

The serological response to *M. hyopneumoniae* is frequently measured for diagnostic purposes. Various ELISA assays are commercially available for detection of *M. hyopneumoniae*-specific IgG in serum samples. Assays differ based on type (i.e. direct, indirect, or blocking) and by type of antigen used (i.e. whole cell or individual proteins). The overall accuracy of various commercial ELISA assays has been investigated, and significant differences have not been documented (Erlandson et al. 2005; Pieters et al. 2017). However occasionally the number of false-positive results in known negative populations exceeds that expected based on published test accuracy. Data from Petersen et al. (2016) supports the hypothesis that infections with other nonpathogenic mycoplasmas, like *Mycoplasma flocculare*, could be the cause of unexpected false-positive rates. Western blot analysis has been proposed as means to sort out apparently false-positive findings (Ameri et al. 2006), but results obtained using this method have not been consistent.

There are several considerations when interpreting *M. hyopneumoniae* serological data in EP investigations. Antibodies to *M. hyopneumoniae* are detectable in pigs generally after 3–8 weeks post exposure, and seroconversion does not typically occur in all infected animals. Moreover, antibodies are not detectable during the entire duration of infection (Pieters et al. 2009). Maternally derived antibodies, antibodies generated after infection, and antibodies generated after vaccination are indistinguishable. For these reasons, serology is a useful diagnostic method mainly in populations and when vaccination status is known.

Treatment, control, and eradication

Treatment

Tetracyclines and macrolides are frequently used to treat and control *M. hyopneumoniae* infections in pigs. Other potentially effective antimicrobials include lincosamides, pleuromutilins, fluoroquinolones, florfenicol, aminoglycosides, and aminocyclitols (Hannan et al. 1997a; Inamoto et al. 1994; Ter Laak et al. 1991). Since *M. hyopneumoniae* lacks a cell wall, it is insensitive to antibiotics that interfere with the polymerization of cell wall precursors, such as beta-lactam antibiotics (e.g. penicillin, cephalosporin), and to polymyxin, oleandomycin, erythromycin, rifampicin, streptomycin, neomycin, trimethoprim, and sulfonamides. Sulfonamides have little influence on *M. hyopneumoniae*, although they are widely used for control of secondary bacterial infections associated with EP.

Acquired antimicrobial resistance of *M. hyopneumoniae* has been documented but is not currently pervasive enough to constitute a major problem for treatment. Inconsistent results have been reported in susceptibility testing of tetracyclines (Etheridge et al. 1979; Williams 1978). Inamoto et al. (1994) reported acquired antimicrobial resistance to tetracyclines (chlortetracycline and oxytetracycline) occurring in *M. hyopneumoniae* field strains isolated in Japan between 1970 and 1990. However, high MIC values for chlortetracycline should be interpreted with care because of the instability of the molecule in the culture medium (Pommier 2006). Studies have also reported acquired antimicrobial resistance of field strains to macrolides (tylosin, tilmicosin), lincosamides (lincomycin), and fluoroquinolones (enrofloxacin, flumequine) (Le Carrou et al. 2006; Stakenborg et al. 2005a; Tavío et al. 2014; Thongkamkoon et al. 2013; Vicca et al. 2007). Resistance against other antimicrobials has not yet been detected. The susceptibility to valnemulin and tiamulin has decreased in 1997 (Hannan et al. 1997b) to 2013 (Tavío et al. 2014; Thongkamkoon et al. 2013).

Many studies have assessed the efficacy of various antimicrobials used for the treatment and control of *M. hyopneumoniae* infections (Mateusen et al. 2002; Pallarés et al. 2015; Vicca et al. 2005). An overview of studies performed under experimental or field conditions is available (Del Pozo Sacristán 2014). For most tested antimicrobials, treated pigs had improved performance and decreased lesions and clinical signs of EP. The success of therapy under field conditions is also determined by other concurrent respiratory bacterial pathogens. Antimicrobial medication of weaned pigs (Pallarés et al. 2015; Vicca et al. 2005) and sows (Thacker et al. 2006) reduced the number of *M. hyopneumoniae* organisms in the respiratory tract.

Diseased pigs and pen mates should be treated immediately with antibiotics active against *M. hyopneumoniae*. Pigs with severe clinical signs should receive medication by injection, followed by oral medication. The use of anti-inflammatory drugs can be helpful in severely affected pigs. Although treatment may lead to

clinical improvement, clinical signs of EP and shedding of *M. hyopneumoniae* typically reappear after cessation of therapy.

Control

Improvement of management practices is essential to the control of *M. hyopneumoniae* infections. Environmental and management factors impacting control of respiratory disease in pigs, including EP, have been reviewed by Maes et al. (2008). Management practices that reduce spread of *M. hyopneumoniae* or decrease lung damage by other pathogens lead to considerable improvement. These include all-in/all-out production, proper gilt acclimation, stabilization of herd immunity, optimal stocking densities, prevention of other respiratory diseases, and optimal housing and climatic conditions. Use of early weaning strategies where pigs are weaned at 7–10 days of age and removed to an isolated site significantly reduces but does not always completely eliminate vertical transmission from sows (Dritz et al. 1996).

Gilt acclimation prior to introduction into breeding herds is critical to control of EP. The goal is the introduction of immune gilts that are not shedding *M. hyopneumoniae*. This is challenging since vaccination does not prevent infection or shedding of *M. hyopneumoniae*. Pieters and Fano (2016) proposed a method of gilt development and acclimation using strategic exposure of gilts to *M. hyopneumoniae* at an early age, with the aim that gilts will clinically recover, develop immunity, and cease shedding prior to entry to the sow farm. Early exposure of gilts at approximately 50 days of age is key to success as *M. hyopneumoniae* persistence is extremely long.

Strategic and pulse medication with antibiotics in chronically infected herds has also been used to control EP. For strategic medication, antibiotic is administered at treatment levels for 1–2 weeks, commencing some days prior to the predicted onset of EP. Pulse medication implies that antibiotic is provided intermittently during critical production stages of the pigs to prevent EP. It has been beneficial, but the results were less effective than other control measures (Le Grand and Kobisch 1996). Strategic and pulse medication strategies are discouraged due to the increased risk of development of antimicrobial resistance and the risk for antimicrobial residues in the pig carcasses at slaughter.

Vaccination is widely applied worldwide to control *M. hyopneumoniae* infections. Commercial vaccines mostly consist of inactivated, adjuvanted whole cell preparations that are administered intramuscularly. Vaccination improves daily weight gain (2–8%), feed conversion ratio (2–5%), and sometimes mortality rate. Additionally, shorter time to reach slaughter weight, reduced clinical signs and lung lesions, and lower treatment costs are observed (Maes et al. 1998, 1999). The financial benefit of vaccination depends on herd factors and also market conditions (Maes et al. 2003). Although protection against clinical pneumonia is often incomplete and vaccination does not prevent colonization, currently used vaccines reduce the number of organisms in the respiratory tract (Meyns et al. 2006; Vranckx et al. 2012; Woolley et al. 2014) and decrease the prevalence of infection in a herd (Sibila et al. 2007b). However, vaccination does not significantly reduce the transmission of *M. hyopneumoniae* (Meyns et al. 2006; Pieters et al. 2010; Villarreal et al. 2011).

The exact mechanisms of protection from vaccination are not fully understood. Studies suggest that systemic CMI is important for protection (Marchioro et al. 2013; Thacker et al. 2000b). Results indicate a considerable variation among individual pigs. Both endogenous CMI and maternally derived CMI contribute to the generation of primary and secondary *M. hyopneumoniae*-specific CMI responses. Bandrick et al. (2014) showed that neonatal piglet vaccination against *M. hyopneumoniae* produces CMI responses that are not hindered by the presence of maternally derived *M. hyopneumoniae*-specific CMI.

The importance of local mucosal antibodies remains unclear. Djordjevic et al. (1997) and Thacker et al. (1998) found that antibody concentrations in respiratory tract washings did not correlate with protection, whereas opposite results were obtained by Thacker et al. (2000b) and Sarradell et al. (2003). Commercial vaccines also induce serum *M. hyopneumoniae*-specific antibodies. The percentage of animals seroconverting after vaccination ranges from 30 to 100% and serological responses differ among vaccines (Thacker et al. 1998). Antibody titers following vaccination may, in the absence of natural infections that boost the immune system, decrease below detection limits 1–3 months after vaccination (Maes et al. 1999). It is generally accepted that levels of serum antibodies do not predict protective immunity, since no direct correlation could be demonstrated between serum antibody concentration and protection against *M. hyopneumoniae* challenge (Djordjevic et al. 1997; Thacker et al. 1998).

Different vaccination strategies have been adopted, depending on type of herd, production system and management practices, infection pattern, and preferences of the pig producer. Since colonization with *M. hyopneumoniae* may already occur during the first weeks of life (Sibila et al. 2007a,b; Vicca et al. 2002; Villarreal et al. 2010), vaccination of piglets is most commonly used. The efficacy has been demonstrated in numerous studies under experimental as well as field conditions (Jensen et al. 2002). Vaccination of suckling piglets (early vaccination; <4 weeks of age) is more common in single-site herds, whereas vaccination of nursery/early fattening pigs (late vaccination; between 4 and 10 weeks) is more often practiced in two- or three-site systems where late

infections are more common. Originally, double vaccination was the most frequent practice. Currently, one-dose vaccination with products licensed for single-dose application is more frequently used, mainly because it requires less labor and it can be implemented more easily in routine management practices on the farm (Baccaro et al. 2006). Single vaccination at either 7 or 21 days of age was equally efficacious in a pig herd with clinical respiratory disease during the second half of the fattening period (Del Pozo Sacristán et al. 2014). Arsenakis et al. (2016) showed vaccinating piglets 3 days prior to weaning conferred slightly better results than vaccination at weaning.

Vaccination of sows at the end of gestation is less commonly practiced. It aims to both reduce transmission of *M. hyopneumoniae* from sow to offspring and potentially protect the piglets against infection via maternally derived immunity. Ruiz et al. (2003) and Sibila et al. (2008) showed that vaccination of sows 5 and 3, and 6 and 3 weeks before farrowing, respectively, resulted in a lower number of positive piglets at weaning.

Vaccination of replacement gilts is recommended in endemically infected herds to avoid destabilization of the breeding stock immunity (Bargen 2004). This is particularly important when gilts are purchased from herds that are free from *M. hyopneumoniae* or from herds with a low infection level.

Although vaccination confers beneficial effects in most infected herds, the effects are variable between them. This may be due, among other factors, to improper vaccine storage conditions and administration, vaccination compliance, infection level, diversity of *M. hyopneumoniae* strains, and coinfections. The influence of maternally derived antibodies on vaccine responses in piglets is not fully elucidated. Martelli et al. (2006) showed that maternally derived antibodies have little or no effect on vaccine-induced priming or on subsequent anamnestic response.

Preliminary studies have shown that genetic selection for disease resistance in pigs may be helpful in the control of *M. hyopneumoniae* infections (Borjigin et al. 2016a), although positive effects are not consistent (Borjigin et al. 2016b).

Eradication

The ultimate means to control *M. hyopneumoniae* infection is elimination. Originally pioneered in small herds in Europe in the 1980s, a program using partial depopulation consisting of a 2-week period during which no animals younger than 10 months were allowed on the farm has been successful in Switzerland, Denmark, Sweden, and Finland (Baekbo et al. 1996; Heinonen et al. 1999, 2011; Rautiainen et al. 2001; Zimmerman et al. 1989). Since then, *M. hyopneumoniae* elimination programs have evolved and have been adapted to current swine production systems around the globe. Besides depopulation and repopulation, which can be successfully applied for *M. hyopneumoniae*, given that a source of negative replacement gilts is secured, partial depopulation (Swiss method), herd closure and medication, and herd medication without herd closure are practiced nowadays (Holst et al. 2015). Herd closure and medication seem to provide high success rate in North America. Holst et al. (2015) have described protocol details and compared different medication options. Reservations on elimination decisions are mostly based on the perceived risk for reinfection. Nevertheless, diagnostic data from *M. hyopneumoniae*-negative flows raised in pig-dense areas have instilled trust in the likelihood for success and overtime perseverance of the elimination efforts (Yeske, personal communication).

Mycoplasma hyorhinis

Although *M. hyorhinis* is considered a commensal bacterium in swine, it is well established as a cause of polyserositis and polyarthritis primarily in nursery-age pigs. It has also infrequently been incriminated in pneumonia, otitis, conjunctivitis, and abortion.

Etiology

Initially described by Switzer (1955), *M. hyorhinis* is a bacterium that grows well in enriched media and exhibits its morphological features less than 1 week after culture in agar (Kobisch and Friis 1996). It has the ability for uptake and metabolism of a wider range of carbohydrates than other porcine mycoplasmas (Ferrarini et al. 2016). This may explain its ability to invade tissues beyond the respiratory tract in pigs, potentially infect other mammals, and thrive as a common contaminant of cell cultures.

The complex system of membrane lipoproteins of *M. hyorhinis* was described by Rosengarten and Wise (1990) and is the basis of remarkable membrane lipoprotein diversity. A combination of multiple coding sequences and mutations results in variable protein expression and size, thought to be important for pathogenesis and immune evasion (Citti et al. 2000; Rosengarten and Wise 1990, 1991; Yogev et al. 1995).

Various methods have been employed to demonstrate variability among *M. hyorhinis* strains. Typing methods based on multilocus sequence and multiple-locus variable number tandem repeat analysis (MLVA) have been recently developed (Dos Santos et al. 2015a; Tocqueville et al. 2014). To date, complete sequencing of various *M. hyorhinis* strains, obtained from several host tissues and cell culture, has been completed and is in the public domain (Calcutt et al. 2012; Dabrazhynetskaya et al. 2014; Goodison et al. 2013).

Public health

The ubiquity of *M. hyorhinis* is supported by common findings of contamination of a wide variety of cell cultures derived from multiple mammalian species (Robinson and Wichelhausen 1956). *M. hyorhinis* is one of the most important bacterial considerations when evaluating cell culture purity, yet contamination of animal and human biologics has been reported. Huang et al. (2001) found a high association between the presence of *M. hyorhinis* and a variety of human carcinomas, but causality of *M. hyorhinis* in human disease is not established.

Epidemiology

Data on the prevalence of *M. hyorhinis* in swine at the country level is generally not available. However, it is considered to have worldwide distribution and be prevalent even in herds where *M. hyopneumoniae* has been eradicated (Luehrs et al. 2017). In recent years, *M. hyorhinis* has been recognized as an emerging pathogen in the United States and other countries. Whether there is a true increase in disease prevalence or the perceived increase is the result of improved diagnostic assays is uncertain. There is poor understanding of the epidemiology of *M. hyorhinis* due to its relatively recent recognition as an economically significant swine pathogen.

The main expressions of disease caused by *M. hyorhinis* are polyserositis and polyarthritis in recently weaned pigs up to 8–10 weeks of age (Gois et al. 1977). Older pigs affected with *M. hyorhinis* typically exhibit mild arthritis (Potgieter and Ross 1972); thus age susceptibility is suspected (Gois and Kuksa 1974). Acute arthritis has been observed up to 2 months after experimental inoculation with *M. hyorhinis*, and the bacterium can persist in joints up to 8 months after inoculation (Barden and Decker 1971).

Eustachitis and otitis (Kazama et al. 1994; Morita et al. 1995, 1998, 1999), abortions (Shin et al. 2003), and pneumonia (Lin et al. 2006) have also been suggested to be the result of *M. hyorhinis* infections. The capability of *M. hyorhinis* to cause pneumonia is controversial. Colonization can induce subtle microscopic lesions in the respiratory epithelium (Lee et al. 2016). However, detection in the lungs of healthy pigs is common, and Luehrs et al. (2017) found no correlation between detection of *M. hyorhinis* in the lungs and coughing or pneumonic lesions.

M. hyorhinis is considered a commensal, colonizing the tonsils and respiratory epithelium of the nasal cavity and conducting airways (Friis and Feenstra 1994; Gois and Kuksa 1974). A majority of colonized pigs have no apparent clinical disease. Like most other *Mycoplasma* species affecting swine, *M. hyorhinis* is thought to be transmitted in nasal secretions by direct contact among pen mates and in the farrowing house from colonized dams to piglets (Friis and Feenstra 1994; Kobisch and Friis 1996). The speed of transmission among pigs has not been investigated. Ross and Spear (1973) isolated the organism from nasal secretions of 10% of sows and 30–40% of weanling pigs. Clavijo et al. (2017) detected *M. hyorhinis* in nasal swabs by PCR in slightly more than 10% of suckling pigs. The level of bacterial colonization increases rapidly in piglets after weaning, and almost 100% detection at the group level has been reported at the end of the nursery period (Clavijo et al. 2017; Roos and Pieters 2017). These high levels of colonization with *M. hyorhinis* appear to remain through the finishing phases. Anderson et al. (2016c) showed that the bacterium can be detected in herds even after undergoing elimination efforts including herd closure and antibiotic treatment.

Molecular characterization of *M. hyorhinis* has been performed with MLST (Tocqueville et al. 2014) and MLVA typing (Dos Santos et al. 2015a). However, applied epidemiological investigations at the molecular level are lacking for *M. hyorhinis*.

Pathogenesis

M. hyorhinis binds to the ciliated respiratory epithelium of the nasal cavities and conducting airways via a family of variable lipoproteins that are embedded in the bacterial cell membrane (Xiong et al. 2016a, b). Inoculation of pigs with *M. hyorhinis* resulted in colonization of the respiratory epithelium, damaged cilia, reduced epithelial thickness, and increased numbers of goblet cells; however, gross and microscopic lesions of pneumonia were not produced (Lee et al. 2016). Systemic invasion from sites of mucosal colonization and development of systemic disease are well documented, but the mechanism of invasion is unknown. Friis and Feenstra (1994) suggest that damage to the respiratory tract by other pathogens favors systemic spread. In experimental models in pigs of *M. hyorhinis*-induced polyserositis and/or arthritis, the incubation period is 3–10 days post inoculation (Kobisch and Friis 1996). The surface lipoproteins of *M. hyorhinis* have been suggested as the main cause of inflammatory reactions as they stimulate cytokine production by macrophages (Muhlradt et al. 1998).

M. hyorhinis is more common in lungs of pigs with PRDC than those without PRDC, suggesting a possible role in pathogenesis (Kawashima et al. 1996; Kobayashi et al. 1996b). Co-inoculation with *M. hyorhinis* and PRRSV or PCV2 resulted in more severe lesions of PRRSV-like or PCV2-like interstitial pneumonia, respectively, but did not result in enhanced *M. hyorhinis*

systemic disease (Chen et al. 2016; Lee et al. 2016). At least under the conditions of these studies, *M. hyorhinis* augmented lesions of PRRSV- or PCV2-induced interstitial pneumonia, but neither virus enhanced *M. hyorhinis* invasion, spread, and production of polyserositis and/or arthritis.

Immunity

A humoral immune response is elicited following *M. hyorhinis* infection. Circulating *M. hyorhinis*-specific antibodies are primarily IgG (Gois et al. 1972; Ross et al. 1973) and can be detected 2 weeks post inoculation by latex agglutination (Gois et al. 1972) or ELISA (Gomes Neto et al. 2014) tests. Antibodies peak at 12 weeks and decrease to non-detectable by approximately 26 weeks post infection (Ross et al. 1973). Antibodies can also be detected in synovial fluids in higher concentrations than serum for up to 1-year post inoculation (Ross et al. 1973).

Clinical signs

Pigs 3–10 weeks of age are typically affected with *M. hyorhinis*-induced polyserositis and/or arthritis, although they may not exhibit clinical signs (Friis and Feenstra 1994). A portion of affected animals may exhibit lethargy, fever, and anorexia. Respiratory distress characterized by labored breathing and dyspnea can be observed in pigs with pleuritis. Swelling of one or more joints and lameness are frequently observed in pigs with arthritis (Barden and Decker 1971).

Lesions

Macroscopically, fibrinous serositis involving the pleura, pericardium, and/or peritoneum is most commonly observed in *M. hyorhinis* infections (Gois et al. 1977). Microscopic lesions include infiltration of lymphocytes, plasma cells, and macrophages in serosal connective tissues and variable amounts of fibrin containing neutrophils on serosal surfaces (Roberts et al. 1963). In joints affected with *M. hyorhinis*, synovial membranes are edematous with fibrinosuppurative exudate adherent to synoviocytes (Ennis et al. 1971) and expansion of the synovial stroma by infiltrates of lymphocytes, plasma cells and macrophages, and fewer neutrophils (Duncan and Ross 1969; Ennis et al. 1971). Synovial fluid contains fibrin, neutrophils, erythrocytes, and platelets (Duncan and Ross 1969). Protein titers in the synovial fluid are higher than those in serum and seem to be the result of local immunoglobulin production (Barden and Decker 1971; Barthel et al. 1972), even if the microorganism cannot be isolated from joints (Barden and Decker 1971; Ennis et al. 1971).

Diagnosis

Fibrinous polyserositis and/or arthritis in nursery-age pigs can also be caused by *H. parasuis* (Chapter 54), *S. suis* (Chapter 61), *Actinobacillus suis* (Chapter 48), or *M. hyosynoviae* (arthritis only; see below). Confirmation of *M. hyorhinis* as a cause of polyserositis and/or arthritis requires demonstration of characteristic lesions, confirmation of infection in the systemic site of the observed lesions, and elimination of other potentially causal agents by negative tests. Because *M. hyorhinis* is a commensal in the tonsil, nasal cavity, and lung, confirmation of infection in any of these sites does not confirm it as cause of disease in systemic sites (Friis and Feenstra 1994; Gomes Neto et al. 2012; Rovira et al. 2010).

Confirmation of *M. hyorhinis* is typically performed by culture and/or PCR. Appropriate samples for confirmation of disease include fibrin or swabs collected from serosal surfaces or joints with lesions or fluid from the pleural, pericardial, peritoneal, or joint cavities where lesions are present. Useful samples for epidemiologic studies may also include nasal swabs, tonsillar swabs, and tracheobronchial swabs (Friis and Feenstra 1994; Gois et al. 1969; Gomes Neto et al. 2012; Makhanon et al. 2012).

Bacterial isolation can be performed using enriched media such as the one described by Hayflick and Chanock (1965), although *M. hyorhinis* can be cultured in media used to grow *M. hyopneumoniae* (Friis 1975), in which case *M. hyorhinis* will overgrow other *Mycoplasma* species. Initial culture in broth or agar needs to be further confirmed with PCR or bacterial sequencing. Frequently, several passages and single-colony cloning are needed before an isolate can be obtained.

DNA hybridization (Taylor et al. 1984, 1985) and conventional, multiplex, and real-time PCR assays have been developed for detection of *M. hyorhinis* nucleic acid (Caron et al. 2000; Clavijo et al. 2014; Gomes Neto et al. 2015; Kang et al. 2012; Lin et al. 2006; Stakenborg et al. 2006a; Stemke et al. 1994; Timenetsky et al. 2006). The overall accuracy of PCR-based tests is high.

M. hyorhinis detection can also be achieved by immunofluorescence assays, although test accuracy may be low (Binder et al. 1989; Jansson 1974; Potgieter and Ross 1972). ISH assays have been developed for *M. hyorhinis* detection in fixed tissue, but are not routinely used for diagnostic testing (Boye et al. 2001; Kim et al. 2010; Resende et al. 2017).

Serology is not generally used for diagnosis. Efforts have been made to adapt at least a small proportion of the various research-based ELISAs for manufacturing (Binder et al. 1989; Duncan and Ross 1973; Friis and Feenstra 1994; Gois et al. 1972; Gomes Neto et al. 2014;

Kawashima et al. 1996), but commercial serologic tests are generally unavailable. Serologic response would not likely differentiate localized commensal infection from systemic disease-inducing infection.

Prevention and control

Due to the high prevalence of infection and lack of understanding of epidemiology and strain virulence, measures to eliminate or control herd infection are generally not considered. Efforts are primarily focused on mitigation of clinical disease through antibiotic therapy and, sometimes, vaccination to reduce or prevent disease.

Due to the lack of a cell wall, *M. hyorhinis* is naturally resistant to β-lactams. *M. hyorhinis* is generally susceptible to macrolides, lincomycin, and tetracyclines (Aarestrup et al. 1998; Ogata et al. 1971; Williams 1978; Wu et al. 2000). Decreased susceptibility or resistance to certain antimicrobial drugs has been suggested (Kobayashi et al. 1996a, 2005).

Commercial vaccines for *M. hyorhinis* are unavailable. Autogenous vaccines for *M. hyorhinis* have gained popularity among certain production systems, but efficacy evaluations of such products have not been performed or are not in the public domain.

Published reports of successful elimination of *M. hyorhinis* from swine herds are not available. Methods used in Switzerland to eliminate *M. hyopneumoniae* from the national swine herd did not eliminate *M. hyorhinis*.

Mycoplasma hyosynoviae

M. hyosynoviae, also belonging to the class *Mollicutes*, infects only swine. It is a commensal of the tonsils, nasal cavity, and conducting airways and occasionally invades systemically to produce acute arthritis in growing- and finishing-age swine.

Etiology

Originally described by Ross and Karmon in 1970, *M. hyosynoviae* was characterized from cultures obtained from swine joints, nose, and tonsils. Comparable with other *Mycoplasma* species, *M. hyosynoviae* requires enriched media for growth. A medium containing PPLO broth, mucin, and turkey serum has been employed to increase growth and potentiate virulence of *M. hyosynoviae*. Overgrowth with other swine *Mycoplasma* species, like *M. hyorhinis*, is a common problem in culture. In solid media *M. hyosynoviae* exhibits the typical *Mycoplasma* "fried egg" colony morphology within 3–4 days of incubation (Ross and Karmon 1970).

Epidemiology

Information is lacking on the prevalence of *M. hyosynoviae* around the globe, but it is thought to occur worldwide. Infections due to *M. hyosynoviae* have been documented in the United States, England, Denmark, Australia, Japan, Thailand, and Canada, among others. Clinical and diagnostic investigations from many other countries also confirm arthritis typical of *M. hyosynoviae*. An increase in detection of *M. hyosynoviae* in submissions to diagnostic laboratories in the United States in recent years (Gomes Neto et al. 2012) is presumably due to improved diagnostic capabilities and not increased prevalence.

Like other respiratory mycoplasmas, *M. hyopneumoniae* and *M. hyorhinis*, *M. hyosynoviae* is shed in nasal secretions and transmitted by close contact or presumably short distances by aerosol. The bacterium seems to be introduced to herds by persistently colonized replacement gilts (Ross and Spear 1973). Transmission among pen mates is thought to occur, although information on the speed of transmission and risk factors for colonization and/or infection have not been clearly identified. It has been proposed that bacterial shedding occurs during the acute (Ross and Spear 1973) and the chronic phase of infection (Hagedorn-Olsen et al. 1999).

Transmission from infected dam to piglet during the lactation period occurs but does not seem to be as frequent as for *M. hyopneumoniae* or *M. hyorhinis*. Early reports suggested that transmission did not take place in piglets younger than 4–8 weeks of age (Ross and Spear 1973). Subsequent studies confirmed tonsillar colonization of younger suckling pigs (Hagedorn-Olsen et al. 1999; Schwartz et al. 2014). In general, prevalence of colonization is low at weaning and typically peaks sometime between 10 and 16 weeks of age (Roos et al. 2016; Ross and Spear 1973; Schwartz et al. 2014). Lameness and lesions of arthritis are produced in only a portion of colonized pigs.

Pathogenesis

The conditions that trigger systemic spread of *M. hyosynoviae* from its commensal habitat on the respiratory mucosa and in tonsils are unknown as are risk factors for disease development. An inoculation study in 6- and 13-week-old naïve pigs resulted in similar prevalence and severity of clinical lameness and arthritis, suggesting that naïve pigs younger than those typically observed with disease in the field are susceptible (Lauritsen et al. 2008). Infection and disease have been characterized in naïve pigs in multiple studies utilizing intravenous or intranasal inoculation or by direct contact with *M. hyosynoviae*-infected seeder pigs (Gomes Neto et al. 2016; Hagedorn-Olsen et al. 1999). Detection of *M. hyosynoviae*

in the blood has been as early as 2 days post inoculation. Incubation period to lameness and arthritic lesions has been consistently determined to be 4–10 days post inoculation/exposure, with 30–75% of inoculated/exposed pigs developing disease. *M. hyosynoviae* can be detected in blood for only a few days post inoculation, from joints typically up to 21 days, and from tonsils for longer in both diseased and non-diseased pigs.

Virulence factors are unknown for *M. hyosynoviae* as are mechanisms of tissue damage. Strains are thought to differ in virulence (Gomes Neto et al. 2016), but convincing documentation is lacking.

Immunity

The immune response against *M. hyosynoviae* is not well characterized. A recent study by Lauritsen et al. (2017) suggests that maternally transferred antibodies are detectable in piglets and confer partial protection. Gomes Neto et al. (2014) followed a cohort of experimentally infected pigs, and at least until the last day of sampling (66 days post inoculation), only one of four inoculated animals had seroconverted to *M. hyosynoviae*.

Clinical signs

The main clinical sign associated with *M. hyosynoviae* infection is acute lameness affecting one or more joints in pigs 3–5 months of age. Involvement of stifle and/or hock joints can result in a dog-sitting posture. Lameness may last for only a few days or for an extended time. It typically decreases with time until there is full recovery unless complicated by conditions like osteochondrosis that can aggravate and extend lameness. As a consequence of pain, pigs can exhibit lack of appetite or reluctance to move, which ends up in weight loss and poor body condition. Thus, infections with *M. hyosynoviae* can be a significant welfare issue in the swine industry and contribute to the general culling and mortality observed in finishing pigs (Kobisch and Friis 1996; Nielsen et al. 2001).

Lesions

M. hyosynoviae causes lesions in one or more joints, while no joint-specific tropism has been demonstrated (Gomes Neto et al. 2016). In acute infections, the synovial stroma is expanded by serofibrinous effusion and sparse aggregates of lymphocytes and plasma cells, resulting in grossly moist swollen synovial membranes. Fibrin and few neutrophils may be adherent to synoviocytes and, along with blood, may create turbidity and variable color from yellow to red to brown in joint fluid (Hagedorn-Olsen et al. 1999; Nielsen et al. 2001). As lesions become subacute to chronic, the fibrin and neutrophilic infiltrates resolve, large numbers of lymphocytes and plasma cells expand the synovial stroma, and there is proliferation of synoviocytes, resulting in grossly proliferative synovial villi. The turbidity and coloration of the synovial fluid may remain or resolve with a more normal synovial fluid observed.

Diagnosis

M. hyosynoviae should be suspected in cases of acute lameness in finishing pigs over 10 weeks of age, along with other causes of lameness including *M. hyorhinis*, *E. rhusiopathiae* (Chapter 53), *H. parasuis* (Chapter 54), and *S. suis* (Chapter 61). Noninfectious causes such as osteochondritis dessicans and nutritional deficiencies/excesses as well as genetics, trauma, management practices, and facilities design, among others, should be evaluated as part of an exhaustive investigation (Chapter 19). Diagnosis requires demonstration of characteristic lesions and confirmation of *M. hyosynoviae* infection in affected joints. Acutely lame non-medicated pigs should be selected for sampling, and joints on affected legs should be examined and sampled. Joint fluid and/or synovial membranes should be collected for confirmation of infection, and synovial membranes should be fixed for histopathologic examination (Gomes Neto et al. 2012).

Gross and microscopic lesions in synovial membranes are sufficiently specific to strongly suggest *M. hyosynoviae*, but not pathognomonic, and must be accompanied by confirmation of infection. Confirmation by culture is only practiced in selected diagnostic laboratories, due to the intrinsic lack of sensitivity, and has been rapidly replaced by detection with highly accurate PCR-based tests (Gomes Neto et al. 2015; Makhanon et al. 2012). DNA identification in tissue can also be performed via ISH (Boye et al. 2001), but is not used as a routine diagnostic test. ELISA-based antibody tests have been developed (Nielsen et al. 2005), but are not commercially available for evaluation of the humoral immune response.

Prevention and control

Preventative measures for *M. hyosynoviae* infection are not routinely employed. Vaccines for *M. hyosynoviae* are not commercially available. Autogenous vaccines are applied in the field, although public data evaluating the efficacy of such products is generally lacking.

Antimicrobial treatments are the most common strategy for disease control. A large number of studies have shown the *in vitro* and field efficacy of macrolides, quinolones, and to a lesser extent tetracyclines (Burch and Goodwin 1984; Cooper et al. 1993; Friis et al. 1992; Hannan et al. 1989, 1997a,b; Kobayashi et al. 1996b; Schultz et al. 2012). Occasionally, reduced susceptibility

to antibiotics has been reported. Treatment early in the course of infection is crucial for effectiveness.

Mycoplasma (Eperythrozoon) suis

M. suis, formerly *E. suis*, is classified as a member of the family *Mollicutes* based on the physical characteristic of the organism and the 16S ribosomal RNA (rRNA) gene sequences (Neimark et al. 2002; Peters et al. 2008). It is a hemotrophic mycoplasma and causes infectious anemia in pigs.

Etiology

Hemotrophic mycoplasmas were previously classified in the genera *Eperythrozoon* and *Haemobartonella*. Based on 16S rRNA gene phylogenetic analysis, they were reclassified to the genus *Mycoplasma*, family *Mycoplasmataceae*, order *Mycoplasmatales*, and class *Mollicutes* (Messick et al. 2002; Neimark et al. 2001, 2002; Rikihisa et al. 1997). Hemotrophic mycoplasmas are clustered in the "pneumoniae" group (Neimark et al. 2001; Peters et al. 2008; Rikihisa et al. 1997); however, their tropism for RBCs and only moderate sequence similarity (77–83%) to any other *Mycoplasma* spp. (Uilenberg et al. 2004) have made their taxonomic classification uncertain.

M. suis is round to oval and 0.2–2 µm in diameter, and it adheres to the surface of erythrocyte membranes (Liebich and Heinritzi 1992). It may also invade erythrocytes, residing in membrane-bound vacuoles or free in the cytoplasm (Groebel et al. 2009), or adhere to endothelial cells (Sokoli et al. 2013). The organism cannot be cultured in cell-free media to date.

Epidemiology

Infections with *M. suis* have been reported worldwide. The use of PCR and serology based on recombinant antigens has improved knowledge of the distribution and prevalence of *M. suis* infections. Using PCR, the prevalence in feeder pigs (20–30 kg) in Germany was 14%, with 40% of the farms having positive animals (Ritzmann et al. 2009). In Brazil 18% of sows and 40% of piglets (9–30 days of age) were positive (Guimaraes et al. 2007). Song et al. (2014) used serology to assess the prevalence and risk factors in 69 pig farms from different provinces in China. Overall, 96% of the farms tested positive. The prevalence was higher in breeding animals (44–48%) than in suckling (13%), weaned (11%), and growing–finishing pigs (25%). The prevalence was significantly higher in summer and autumn than in other seasons. Antimicrobial treatment (odds ratio of 0.24) and frequency of disinfection (odds ratio of 0.23) were associated with a lower prevalence. The presence of mosquitoes and flies (odds ratio of 5.99) was associated with a higher prevalence. Using PCR, the prevalence in wild boar in Germany was 10% (Hoelzle et al. 2010), and the prevalence in pigs raised under extensive conditions in Brazil was 76% (Toledo et al. 2016).

The natural transmission routes of *M. suis* are largely unknown. Experimental transmission via intraperitoneal, oral, subcutaneous, and intravenous inoculation of infected blood has been described (Hoelzle 2008). Therefore, it is likely that an iatrogenic blood transfer due to contaminated instruments or small skin lesions due to fighting play an important role. Other described potential transmission routes include transplacental transmission (Henderson et al. 1997), blood-contaminated semen, and transmission by bloodsucking arthropods (Neimark et al. 2001; Prullage et al. 1993). Dietz et al. (2014) found shedding in saliva, in nasal and vaginal secretions, and in urine upon experimental infection. They suggested that blood-independent transmission as well as indirect transmission via environmental contamination could also play a role in the epidemiology of *M. suis* infections.

Pathogenesis

The pathogenesis of *M. suis*-induced disease is complex, and the organism has a repertoire of mechanisms to induce clinical signs and establish persistent infections (Hoelzle et al. 2014). Adhesion of *M. suis* to RBCs results in mechanical and/or osmotic damage that triggers extravascular hemolysis in the spleen, liver, lungs, and bone marrow, resulting in anemia (Groebel et al. 2009; Guimaraes et al. 2011). At least two proteins of *M. suis*, MSG1 and α-enolase, are involved in adhesion, and both also serve as enzymes in carbohydrate metabolism (Oehlerking et al. 2011; Schreiner et al. 2012a,b).

M. suis may also invade RBCs using an endocytosis-like invasion mechanism. Membrane-adhered *M. suis* induces through unknown mechanisms invagination and pinching-off of the RBC membrane, resulting in *M. suis* in intracellular vacuoles (Groebel et al. 2009; Guimaraes et al. 2011). The life span of parasitized RBCs is reduced through several mechanisms. *M. suis* scavenging of glucose and amino acids (Guimaraes et al. 2011) in RBCs likely leads to reduced energy production and oxidative stress. *M. suis*-induced damage of the RBC membrane (Zachary and Basgall 1985) is thought to induce eryptosis, the process of programmed cell death of RBCs (Lang et al. 2006; Felder et al. 2011). Eryptosis results in conformational and biochemical changes in the RBC membrane, leading to subsequent recognition and phagocytosis by macrophages (Felder et al. 2011; Lang et al. 2006). The occurrence and frequency of eryptosis depends on the virulence of the *M. suis* strain, whether pigs are splenectomized, and the phase of infection.

Autoimmune mechanisms also contribute to the development of *M. suis*-induced anemia. Both cold (IgM) and warm (IgG) reactive autoimmune antibodies targeting RBC antigens lead to extravascular and intravascular hemolysis. Cold autoreactive antibodies (so-called cold agglutinins) are found mainly during the chronic phase of the disease (Felder et al. 2010), and warm autoreactive IgG occurs mainly during the acute phase at the time of peak *M. suis* bacteremia (Felder et al. 2010; Hoelzle et al. 2006).

M. suis is also able to attach to endothelial cells (Sokoli et al. 2013), either singly or as biofilm-like microcolonies. This may contribute to persistence of infection by preventing phagocytosis by macrophages in major organs operative in extravascular hemolysis. *M. suis*-induced endothelial damage may also compound clinical anemia by causing coagulopathy, vascular thrombosis, and hemorrhagic diathesis (Sokoli et al. 2013).

Clinical signs

The incubation period and clinical signs after experimental infection with a virulent and RBC invasive strain depend on whether pigs are splenectomized. Splenectomized pigs develop severe anemia and massive hypoglycemia 5–8 days post infection, and most pigs need to be euthanized. By contrast, non-splenectomized pigs develop mild anemia, massive skin alterations with petechiae, and hemorrhagic diathesis between days 6 and 21 and seroconvert within 35 days post infection (Stadler et al. 2014). When using less virulent strains, infection in non-splenectomized pigs leads to none or marginal clinical symptoms or alterations in blood parameters (Heinritzi et al. 1990a,b).

The incubation period in naturally infected animals is highly variable with some infected pigs never exhibiting clinical disease. Infected animals can remain normal for months prior to exhibiting clinical disease, which is often related to stress, or individual susceptibility. *M. suis* infection under field conditions can cause acute hemolytic disease and death in young pigs, prepartum sows, and stressed weaned and feeder pigs. Pallor, fever, occasional icterus, and cyanosis of the extremities, especially the ears, are observed during the acute phases of the disease. More commonly, mild anemia, increased mortality, and poor growth rates are observed in suckling, weaned, and feeder pigs. Infection of sows may result in fever, anorexia, lethargy, dysgalactia, and poor maternal behavior (Strait et al. 2012). Clinical disease in sows typically occurs within 3–4 days of introduction to the farrowing room or immediately after farrowing. Chronic infections in animals with low or undetectable numbers of parasites result in unthriftiness, pallor, and occasionally skin hypersensitivity characterized by urticaria. In sows, chronic *M. suis* infection has been associated with decreased reproductive efficiency, although Zinn et al. (1983) found no appreciable impact on sow reproductive performance.

In all cases, simultaneous infections with other pathogens, poor management such as overcrowding, poor environmental conditions, and the presence of parasites may contribute to the severity of disease associated with *M. suis* infection.

Diagnosis

Diagnosis is based on clinical signs, hematology results, and the demonstration of the organism. PCR assays are available, are more sensitive for detection of *M. suis* than blood smears (Hoelzle et al. 2007b), and are effective in detection of *M. suis* in pigs that are carriers or subclinically infected (Hoelzle et al. 2003; Messick et al. 1999). A sensitive and specific real-time PCR assay is currently the best assay for detecting the organism. Other more recently developed tests include a specific and highly sensitive multiplex microbead immunoassay (Guimaraes et al. 2014) and a colloidal gold-based immunochromatographic assay for rapid detection (<10 minutes) of *M. suis* in porcine plasma (Meng et al. 2014).

Detection of serum antibodies is also possible. Mostly, ELISAs based on recombinant antigens such as rMSG1, rHspA1, or rPPA are used (Hoelzle et al. 2007a; Liu et al. 2012). The production of antibodies occurs in waves, with each reinfection or recrudescent episode resulting in the production of new antibodies. However, the antibody titers may persist only 2–3 months, resulting in frequent false-negative results (Heinritzi 1999).

Treatment and prevention

Treatment

The treatment of choice for infection is oxytetracycline at a dose of 20–30 mg/kg administered parenterally (Heinritzi 1999). Acutely diseased pigs require parenteral treatment due to lack of adequate feed consumption. Administration of oxytetracycline at times of stress or treatment in infected herds may help to prevent acute disease. However, treatment does not necessarily eliminate the organism from the pig, as *M. suis* may reside intracellularly in RBCs. Chlortetracycline fed to the entire sow herd at 22 mg/kg/d for 2 weeks resulted in a near-complete disappearance of dysgalactia in sows farrowing within 5 weeks after the start of treatment (Strait et al. 2012).

Supportive therapy and iron injections (200 mg iron dextran/pig) will help recovery and minimize mortality. Feeding affected sows multiple times a day to maintain blood glucose can help prevent acute death and dysgalactia.

Strategies for elimination of *M. suis* from infected herds are not available.

Prevention

Stopping the spread of the organism and preventing reinfection are critical to controlling the herd infection status. Transmission by needles and surgical instruments must be minimized by changing needles between sows and litters. No vaccine is currently available, and vaccine development is complicated by the lack of ability to culture *M. suis* and knowledge of the virulence factors. An attempt to produce a vaccine from a recombinant protein produced in *E. coli*, while inducing a humoral and cellular immune response, failed to protect against challenge (Hoelzle et al. 2009). If a herd is *M. suis*-free, new additions should also be from herds negative for the organism.

Other *mycoplasma* spp. in swine

Other mycoplasmas in swine are generally nonpathogenic and without consequence. They include species typically found in other animals and acholeplasmas that are common in a wide variety of animals and plants.

M. flocculare (Friis 1972) is nonpathogenic but of importance because of its similarity to *M. hyopneumoniae* (Petersen et al. 2016). It is widely distributed in swine where it is a commensal in the nasal cavities and lungs. Similarity of *M. flocculare* and *M. hyopneumoniae* in culture, antigenically and genetically, has made their differentiation difficult until recently. They share approximately 78% of coding DNA sequences and greater than 90% of predicted surface proteins (Siqueira et al. 2013). In recent years PCRs have been developed that can differentiate the two species (Blank and Stemke 2001; Stakenborg et al. 2006a; Strait et al. 2008a).

Little information is available on infection of the genitourinary tract with mycoplasmas in swine. Shin et al. (2003) demonstrated a cytopathic species of *M. hyorhinis* that was thought to be a cause of abortions in sows. Other species of mycoplasmas that have been isolated from swine include *M. sualvi*, *M. hyopharyngis*, *M. arginini*, *M. bovigenitalium*, *M. buccale*, *M. gallinarum*, *M. iners*, *M. mycoides*, and *M. salivarium*. In addition to mycoplasmas, acholeplasmas have occasionally been isolated from the respiratory tract of swine. Acholeplasmas differ from mycoplasmas by having a larger genome and are capable of growing in media that lacks sterols. No importance in their presence in pigs has been demonstrated.

References

Aarestrup FM, Friis NF, Szancer J. 1998. *Acta Vet Scand* 39:145–147.

Adams C, Pitzer J, Minion FC 2005. *Infect Immun* 73:7784–7787.

Amass S, Clark L, van Alstine W, et al. 1994. *J Am Vet Med Assoc* 204:102–107.

Ameri M, Zhou EM, Hsu WH. 2006. *J Vet Diagn Invest* 18:198–201.

Anderson A, Dalquist L, Leuwerke B, et al. 2016a. Improvement in *Mycoplasma hyopneumoniae* culture and isolation focusing on sample selection and handling. In Proc AD Leman Swine Conf.

Anderson A, Gimenez-Lirola L, Zimmerman J, et al. 2016b. Effect of pretreatments on *Mycoplasma hyopneumoniae* detection in oral fluids. In Proc Congr Int Pig Vet Soc, p. 234.

Anderson A, Lyons W, Geiger J, et al. 2016c. Effect of herd closure on the pattern of *Mycoplasma hyosynoviae* and *Mycoplasma hyorhinis* infection. In Proc Annu Meet Am Assoc Swine Vet, pp. 92–93.

Arsenakis I, Panzavolta L, Michiels A, et al. 2016. *BMC Vet Res* 12:63.

Asai T, Okada M, Ono M, et al. 1993. *Vet Immunol Immunopathol* 38:253–260.

Asai T, Okada M, Ono M, et al. 1994. *Vet Immunol Immunopathol* 44:97–102.

Asai T, Okada M, Yokomizo Y, et al. 1996. *Vet Immunol Immunopathol* 51:325–331.

Baccaro M, Hirose F, Umehara O, et al. 2006. *Vet J* 172:526–531.

Baekbo P, Kooij D, Mortensen S, et al. 1996. *Acta Vet Scand Suppl* 90:63–65.

Bandrick M, Pieters M, Pijoan C, et al. 2008. *Clin Vaccine Immunol* 15:540–543.

Bandrick M, Pieters M, Pijoan C, et al. 2011. *Vet Rec* 168:100.

Bandrick M, Theis K, Molitor TW. 2014. *BMC Vet Res* 10:124.

Barden JA, Decker JL. 1971. *Arthritis Rheum* 14:193–201.

Bargen L. 2004. *Can Vet J* 45:856–859.

Barthel CH, Duncan JR, Ross RF. 1972. *Am J Vet Res* 33:967–974.

Bendjennat M, Blanchard A, Loutfi M, et al. 1999. *Infect Immun* 67:4456–4462.

Binder A, van Wees C, Likitdecharote B, et al. 1989. *Berl Munch Tierarztl Wochenschr* 102:11–13.

Blanchard B, Vena M, Cavalier A, et al. 1992. *Vet Microbiol* 30:329–341.

Blank WA, Stemke GW. 2001. *Int J Syst Evol Microbiol* 51:1395–1399.

Bogema D, Deutscher A, Woolley L, et al. 2012. *Mol Biol* 3:1–11.

Borjigin L, Shimazu T, Katayama Y, et al. 2016a. *Anim Sci J* 87:321–329.

Borjigin L, Shimazu T, Katayama Y, et al. 2016b. *Anim Sci J* 87:638–645.
Boye M, Jensen TK, Ahrens P, et al. 2001. *APMIS* 109:656–664.
Burch DG, Goodwin RF. 1984. *Vet Rec* 115:594–595.
Burnett TA, Dinkla K, Rohde M, et al. 2006. *Mol Microbiol* 60:669–686.
Calcutt MJ, Foecking MF, Rosales RS, et al. 2012. *J Bacteriol* 194:1848.
Calsamiglia M, Pijoan C. 2000. *Vet Rec* 146:530–532.
Calsamiglia M, Pijoan C, Trigo A. 1999. *J Vet Diagn Invest* 11:246–251.
Calus D, Maes D, Vranckx K, et al. 2010. *J Microbiol Methods* 83:335–340.
Caron J, Ouardani M, Dea S. 2000. *J Clin Microbiol* 38:1390–1396.
Caruso JP, Ross RF. 1990. *Am J Vet Res* 51:227–231.
Chen D, Wei Y, Huang L, et al. 2016. *Vet Microbiol* 182:123–130.
Ciprian A, Cruz TA, de la Garza M. 1994. *Arch Med Res* 25:235–239.
Citti C, Watson-McKown R, Droesse M, et al. 2000. *J Bacteriol* 182: 1356–1363.
Clavijo MJ, Oliveira S, Zimmerman J, et al. 2014. *J Vet Diagn Invest* 26:755–760.
Clavijo MJ, Murray D, Oliveira S, et al. 2017. *Vet Rec* 81:68.
Cook BS, Beddow JG, Manso-Silván L, et al. 2016. *Vet Microbiol* 195:158–164.
Cooper AC, Fuller JR, Fuller MK, et al. 1993. *Res Vet Sci* 54:329–334.
Dabrazhynetskaya A, Solka V, Volokhov D, et al. 2014. *Genome Announc* 2:e00127-14.
Deblanc C, Gorin S, Quéguiner S, et al. 2012. *Vet Microbiol* 157:96–105.
Del Pozo Sacristán R. 2014. Treatment and control of *Mycoplasma hyopneumoniae* infections. PhD thesis, Ghent University Belgium, p. 189.
Del Pozo Sacristán R, Sierens A, Marchioro S, et al. 2014. *Vet Rec* 174:197.
Deutscher A, Jenkins C, Minion FC, et al. 2010. *Mol Microbiol* 78:444–448.
Dietz S, Mack SL, Hoelzle K, et al. 2014. *Vet Microbiol* 172:581–585.
Djordjevic SP, Eamens GJ, Romalis LF, et al. 1997. *Aust Vet J* 75:504–511.
Done S. 1991. *Vet Rec* 128:582–586.
Dos Santos LF, Clavijo MJ, Sreevatsan S, et al. 2015a. *J Microbiol Methods* 111:87–92.
Dos Santos LF, Sreevatsan S, Torremorell M, et al. 2015b. *Vet Microbiol* 175:374–381.
Dritz SS, Chengappa MM, Nelssen JL, et al. 1996. *J Am Vet Med Assoc* 208:711–715.
Dubosson CR, Conzelmann C, Miserez R, et al. 2005. *Vet Microbiol* 102:55–65.
Duncan JR, Ross RF. 1969. *Am J Pathol* 57:171–186.
Duncan JR, Ross RF. 1973. *Am J Vet Res* 34:363–366.
Ennis RS, Dalgard D, Willerson JT, et al. 1971. *Arthritis Rheum* 14:202–211.
Erlandson KR, Evans RB, Thacker BJ, et al. 2005. *J Swine Health Prod* 13:198–203.
Etheridge JR, Cottew GS, Lloyd LC. 1979. *Aust Vet J* 55:356–359.
Fablet C, Marois C, Kobisch M, et al. 2010. *Vet Microbiol* 143:238–245.
Fano E, Pijoan C, Dee S. *Vet Rec* 2005;157:105–108.
Fano E, Pijoan C, Dee SA, et al. 2006. Assessment of the effect of sow parity on the prevalence of *Mycoplasma hyopneumoniae* in piglets at weaning. In Proc Intl Pig Vet Congr, Copenhagen, Denmark, 1:96.
Fano E, Pijoan C, Dee S, et al. 2007. *Can J Vet Res* 71:195–200.
Felder K, Hoelzle K, Heinritzi K, et al. 2010. *BMC Vet Res* 6:18.
Felder K, Hoelzle K, Ritzmann M, et al. 2011. *Cell Physiol Biochem* 27:557–564.
Ferrarini MG, Siqueira FM, Mucha SG, et al. 2016. *BMC Genomics* 17:353.
Flesja K, Ulvesaeter H. 1980. *Acta Vet Scand Suppl* 74:1–22.
Friis NF. 1972. *Acta Vet Scand* 13:284–286.
Friis NF. 1975. *Nord Vet Scand* 27:337–339.
Friis NF, Feenstra AA. 1994. *Acta Vet Scand* 35:93–98.
Friis NF, Hansen KK, Schirmer AL, et al. 1992. *Acta Vet Scand* 33:205–210.
Fuentes M, Pijoan C. 1987. *Am J Vet Res* 48:1446–1448.
Garcia-Morante B, Segalés J, López-Soria S, et al. 2016. *Vet Res* 47:54.
Giacomini E, Ferrari N, Pitozzi A, et al. 2016. *Vet Res Commun* 40:81–88.
Gois M, Kuksa F. 1974. *Zentralbl Veterinarmed B* 21:352–361.
Gois M, Cerny M, Rozkosny V, et al. 1969. *Zentralbl Veterinarmed B* 16:253–265.
Gois M, Franz K, Kuksa F, et al. 1972. *Zentralbl Veterinarmed B* 19:379–390.
Gois M, Kuksa F, Sisak F. 1977. *Zentralbl Veterinarmed B* 24:89–96.
Gomes Neto JC, Gauger PC, Strait EL, et al. 2012. *J Swine Health Prod* 20:82–86.
Gomes Neto JC, Strait EL, Raymond M, et al. 2014. *Vet Microbiol* 174:163–171.
Gomes Neto JC, Bower L, Erickson BZ, et al. 2015. *J Vet Sci* 16:195–201.
Gomes Neto JC, Raymond M, Bower L, et al. 2016. *J Vet Sci* 17:489–496.
Goodison S, Urquidi V, Kumar D, et al. 2013. *Genome Annouc* 1: e00101-12.
Goodwin RF. 1972. *Res Vet Sci* 13:262–267.
Goodwin RF. 1985. *Vet Rec* 116:690–694.
Goodwin RFW, Pomeroy AP, Whittlestone P. 1965. *Vet Rec* 77:1247–1249.
Groebel K, Hoelzle K, Wittenbrink MM, et al. 2009. *Infect Immun* 77:576–584.
Guarino M, Jans P, Costa A, et al. 2008. *Comput Electron Agric* 62:22–28.

Guimaraes A, Biondo A, Lara A, et al. 2007. *Vet Rec* 160:50–53.
Guimaraes A, Santos A, SanMiguel P, et al. 2011. *PLoS One* 6:e19574.
Guimaraes A, Santos A, Timenetsky J, et al. 2014. *J Vet Diagn Invest* 26:203–212.
Hagedorn-Olsen T, Nielsen NC, Friis NF. 1999. *Zentralbl Veterinarmed A* 46:317–325.
Hannan PC, O'Hanlon PJ, Rogers NH. 1989. *Res Vet Sci* 46:202–211.
Hannan PC, Windsor GD, de Jong A, et al. 1997a. *Antimicrob Agents Chemother* 41:2037–2040.
Hannan PC, Windsor HM, Ripley PH. 1997b. *Res Vet Sci* 63:157–160.
Hayflick L, Chanock RM. 1965. *Bacteriol Rev* 29:185–221.
Heinonen M, Autio T, Saloniemi H, et al. 1999. *Acta Vet Scand* 40:241–252.
Heinonen M, Laurila T, Vidgren G, et al. 2011. *Vet J* 188:110–114.
Heinritzi K. 1999. Eperythrozoonosis. In Straw B, D'Allaire S, Mengeling WL, et al., eds. *Diseases of Swine*, 8th ed. Ames, IA: Iowa State University Press, pp. 413–418.
Heinritzi K, Peteranderl W, Plank G. 1990a. *Dtsch Tierarztl Wochenschr* 97:31–34.
Heinritzi K, Plank G, Peteranderl W, et al. 1990b. *Zentralbl Veterinarmed B* 37:412–417.
Henderson JP, O'Hagan J, Hawe SM, et al. 1997. *Vet Rec* 140:144–146.
Hoelzle L, 2008. *Vet Microbiol* 130:215–226.
Hoelzle LE, Adelt D, Hoelzle K, et al. 2003. *Vet Microbiol* 93:185–196.
Hoelzle L, Hoelzle K, Ritzmann M, et al. 2006. *Clin Vaccine Immunol* 13:116–122.
Hoelzle K, Grimm J, Ritzmann M, et al. 2007a. *Clin Vaccine Immunol* 14:1616–1622.
Hoelzle LE, Helbling M, Hoelzle K, et al. 2007b. *J Microbiol Methods* 70:346–354.
Hoelzle K, Doser S, Ritzmann M, et al. 2009. *Vaccine* 27:5376–5382.
Hoelzle K, Engels M, Kramer M, et al. 2010. *Vet Microbiol* 143:405–409.
Hoelzle L, Zeder M, Felder K, et al. 2014. *Vet J* 202:20–25.
Holst S, Yeske P, Pieters M. 2015. *J Swine Health Prod* 23:321–330.
Hsu T, Minion FC. 1998. *Infect Immun* 66:4762–4766.
Huang S, Li JY, Wu J, et al. 2001. *World J Gastroenterol* 7:266–269.
Inamoto T, Takahashi H, Yamamoto K, et al. 1994. *J Vet Med Sci* 56:393–394.
Jansson R. 1974. *Acta Vet Scand* 15:274–282.
Jarocki V, Santos J, Tacchi J, et al. 2015. *Open Biol* 5:140175.
Jenkins C, Wilton J, Minion FC, et al. 2006. *Infect Immun* 74:481–487.
Jensen CS, Ersboll AK, Nielsen JP. 2002. *Prev Vet Med* 54:265–278.
Jorsal SE, Thomsen BL. 1988. *Acta Vet Scand* 29:436–438.
Kang I, Kim D, Han K, et al. 2012. *Can J Vet Res* 76:195–200.
Kawashima K, Yamada S, Kobayashi H, et al. 1996. *J Comp Pathol* 114:315–323.
Kazama S, Yagihashi T, Morita T, et al. 1994. *Res Vet Sci* 56:108–110.
Kim B, Lee K, Han K, et al. 2010. *J Vet Med Sci* 72:1225–1227.
Kobayashi H, Morozumi T, Munthali G, et al. 1996a. *Antimicrob Agents Chemother* 40:1030–1032.
Kobayashi H, Sonmez N, Morozumi T, et al. 1996b. *J Vet Med Sci* 58:1107–1111.
Kobayashi H, Nakajima H, Shimizu Y, et al. 2005. *J Vet Med Sci* 67:795–800.
Kobisch M, Friis NF. 1996. *Rev Sci Tech* 15:1569–1605.
Kobisch M, Blanchard B, Le Potier MF. 1993. *Vet Res* 24:67–77.
Kokotovic B, Friis NF, Jensen JS, et al. 1999. *J Clin Microbiol* 37:3300–3307.
Kuhnert P, Overesch G. 2014. *Vet Microbiol* 174:261–266.
Kuhnert P, Overesch G, Belloy L. 2011. *Vet Microbiol* 152:191–195.
Lang F, Lang K, Lang P, et al. 2006. *Antioxid Redox Signal* 8:1183–1192.
Lauritsen KT, Hagedorn-Olsen T, Friis NF, et al. 2008. *Vet Microbiol* 130:385–390.
Lauritsen KT, Hagedorn-Olsen T, Jungersen G, et al. 2017. *Vet Immunol Immunopathol* 183:22–30.
Le Carrou J, Laurentie M, Kobisch M, et al. 2006. *Antimicrob Agents Chemother* 50:1959–1966.
Le Grand A, Kobisch M. 1996. *Vet Res* 27:241–253.
Lee J-A, Oh U-R, Lee J-B, et al. 2016. *Vet Immunol Immunopathol* 177:48–51.
Liebich HG, Heinritzi K. 1992. *Tierarztl Prax* 20:270–274.
Lin JH, Chen SP, Yeh KS, et al. 2006. *Vet Microbiol* 115:111–116.
Liu J, Zhou D, Cheng Z, et al. 2012. *Res Vet Sci* 93:48–50.
Luehrs A, Siegenthaler S, Gruetzner N, et al. 2017. *Vet Microbiol* 203:1–5.
Maes D, Deluyker H, Verdonck M, et al. 1998. *J Vet Med B* 45:495–505.
Maes D, Deluyker H, Verdonck M, et al. 1999. *Vaccine* 17:1024–1034.
Maes D, Verbeke W, Vicca J. 2003. *Livest Prod Sci* 83:85–93.
Maes D, Segalés J, Meyns T, et al. 2008. *Vet Microbiol* 126:297–309.
Makhanon M, Tummaruk P, Thongkamkoon P, et al. 2012. *Tropl Anim Health Prod* 44:313–318.
Marchioro S, Maes D, Flahou B, et al. 2013. *Vaccine* 31:1305–1311.
Mare CJ, Switzer WP. 1965. *Vet Med* 60:841–846.
Marois C, Kobisch M, Gautier-Bouchardon A. 2007. *Vet Microbiol* 120:96–104.
Marois C, Gottschalk M, Morvan H, et al. 2009. *Vet Microbiol* 135:283–291.

Martelli P, Terreni M, Guazzetti S, et al. 2006. *J Vet Med B Infect Dis Vet Public Health* 53:229–233.
Mateusen B, Maes D, Van Goubergen M, et al. 2002. *Vet Rec* 151:135–140.
Mayor D, Zeeh F, Frey J, et al. 2007. *Vet Res* 38:391–398.
Meng K, Sun W, Zhao P, et al. 2014. *Biosens Bioelectron* 55:396–399.
Messick JB, Cooper S, Huntley M. 1999. *J Vet Diagn Invest* 11:229–236.
Messick J, Walker P, Raphael W, et al. 2002. *Int J Syst Evol Microbiol* 52:693–698.
Messier S, Ross RF, Paul PS. 1990. *Am J Vet Res* 51:52–58.
Meyns T, Maes D, Dewulf J, et al. 2004. *Prev Vet Med* 66:265–275.
Meyns T, Dewulf J, de Kruif A. 2006. *Vaccine* 24:7081–7086.
Meyns T, Maes D, Calus D, et al. 2007. *Vet Microbiol* 120:87–95.
Michiels A, Vranckx K, Piepers S, et al. 2017. *Vet Res* 48:2.
Morita T, Fukuda H, Awakura T, et al. 1995. *Vet Pathol* 32:107–111.
Morita T, Sasaki A, Kaji N, et al. 1998. *Am J Vet Res* 59:869–873.
Morita T, Ohiwa S, Shimada A, et al. 1999. *Vet Pathol* 36:174–178.
Morris C, Gardner I, Hietala S. 1995. *Prev Vet Med* 21:323–337.
Muhlradt PF, Kiess M, Meyer H, et al. 1998. *Infect Immun* 66:4804–4810.
Muneta Y, Uenishi H, Kikuma R, et al. 2003. *J Interferon Cytokine Res* 23:583–590.
Muneta Y, Minagawa Y, Shimoji Y, et al. 2006. *J Interferon Cytokine Res* 26:637–644.
Nathues H, grosse Beilage E, Kreienbrock L, et al. 2011. *Vet Microbiol* 152:338–345.
Nathues H, Spergser J, Rosengarten R, et al. 2012. *Vet J* 193:443–447.
Nathues H, Doehring S, Woeste H, et al. 2013a. *Acta Vet Scand* 55:44.
Nathues H, Woeste H, Doehring S, et al. 2013b. *Acta Vet Scand* 55:30.
Neimark HC. 1986. In Madoff S, ed. *The Bacterial L-Forms*. New York: Marcel Dekker Inc., pp. 21–42.
Neimark H, Johansson K, Rikihisa Y, et al. 2001. *Int J Syst Evol Microbiol* 51:891–899.
Neimark H, Johansson KE, Rikihisa Y, et al. 2002. *Int J Syst Evol Microbiol* 52:683.
Nielsen OE, Nielsen NC, Friis NF. 2001. *J Vet Med A* 48:475–486.
Nielsen OE, Lauritsen KT, Friis NF, et al. 2005. *Vet Microbiol* 111:41–50.
Nowak J. 1929. *Ann Inst Pasteur (Paris)* 43:1330–1352.
Oehlerking J, Kube M, Felder K, et al. 2011. *J Bacteriol* 193:2369–2370.
Ogata M, Atobe H, Kushida H, et al. 1971. *J Antibiot* 24:443–451.
Opriessnig T, Thacker E, Yu S, et al. 2004. *Vet Pathol* 41:624–640.
Otake S, Dee S, Corzo C, et al. 2010. *Vet Microbiol* 145:198–208.
Pallarés F, Lasa C, Roozen M, et al. 2015. *Vet Rec Open* 2:e000079.
Park S, Yibchok-Anun S, Cheng H, et al. 2002. *Infect Immun* 70:2502–2506.
Park C, Jeong J, Kang I, et al. 2016. *BMC Vet Res* 12:25.
Peters I, Helps R, McAuliff L, et al. 2008. *J Clin Microbiol* 46:1873–1877.
Petersen A, Oneal D, Seibel J, et al. 2016. *Vet Microbiol* 192:204–212.
Pieters M, Fano E. 2016. *Vet Rec* 178:122–123.
Pieters M, Pijoan C, Fano E, et al. 2009. *Vet Microbiol* 134:261–266.
Pieters M, Fano E, Pijoan C, et al. 2010. *Can J Vet Res* 74:157–160.
Pieters M, Cline GS, Payne BJ, et al. 2014. *Vet Microbiol* 172:575–580.
Pieters M, Daniels J, Rovira A. 2017. *Vet Microbiol* 203:103–109.
Pommier P. 2006. In vitro, are all the tetracyclines equivalent? In Proc 19th IPVS Congr, Copenhagen, p. 411.
Pósa R, Magyar T, Stoev S, et al. 2013. *Vet Pathol* 50: 971–979.
Potgieter LND, Ross RF. 1972. *Am J Vet Res* 33:99–105.
Prullage JB, Willams RE, Gaffar SM. 1993. *Vet Parasitol* 50:125–135.
Raith J, Kuchling S, Schleicher C, et al. 2015. *Vet Rec* 176:124.
Rautiainen E, Wallgren P. 2001. *J Vet Med B Infect Dis Vet Public Health* 48:55–65.
Rautiainen E, Oravainen J, Virolainen JV, et al. 2001. *Acta Vet Scand* 42:3555–3564.
Razin S. 1992. In Maniloff J, Editor in chief. McElhaney RN, Finch LR, Baseman JB, eds. *Mycoplasmas: Molecular Biology and Pathogenesis*. Washington, DC: American Society for Microbiology, pp. 3–22.
Redondo E, Masot AJ, Fernández A, et al. 2009. *J Comp Pathol* 140:260–270.
Resende T, Pieters M, Vannucci F. 2017. *Mycoplasma hyorhinis* associated with conjunctivitis in pigs. In Proc Annu Meet Am Assoc Swine Vet, pp. 45–46.
Rikihisa Y, Kawahara M, Wen B, et al. 1997. *Clin Microbiol* 35:823–829.
Ritzmann M, Grimm J, Heinritzi K, et al. 2009. *Vet Microbiol* 133:84–91.
Ro LH, Ross RF. 1983. *Am J Vet Res* 44:2087–2094.
Roberts ED, Switzer SD, Ramsey FK. 1963. *Am J Vet Res* 24:9–18.
Robinson LB, Wichelhausen RH. 1956. *Science* 124:1147–1148.
Robinson M, Buchtmann K, Jenkins C, et al. 2013. *Open Biol* 3:130017.
Rodriguez F, Ramirez G, Sarradell J, et al. 2004. *J Comp Pathol* 130:306–312.

Rodríguez F, Batista M, Hernández J, et al. 2016. *J Comp Pathol* 154:165–168.
Roos L, Pieters M. 2017. *Mycoplasma hyorhinis* and *Mycoplasma hyosynoviae* dual colonization of dams and piglets prior to weaning. In Proc Annu Meet Am Assoc Swine Vet, p. 47.
Roos LR, Fano E, Homwong N, et al. 2016. *Vet Microbiol* 184:51–58.
Rosengarten R, Wise KS. 1990. *Science* 247:315–318.
Rosengarten R, Wise KS. 1991. *J Bacteriol* 173:4782–4793.
Ross RF, Karmon JA. 1970. *J Bacteriol* 103:707–713.
Ross RF, Spear ML. 1973. *Am J Vet Res* 34:373–378.
Ross RF, Dale SE, Duncan JR. 1973. *Am J Vet Res* 34:367–372.
Rovira A, Clavijo MJ, Oliveira S. 2010. *Act Sci Vet* 38(Suppl 1):s9–s15.
Ruiz AR, Utrera V, Pijoan C. 2003. *J Swine Health Prod* 11:131–135.
Sarradell J, Andrada M, Ramírez AS, et al. 2003. *Vet Pathol* 40:395–404.
Schreiner S, Hoelzle K, Hofmann-Lehmann R, et al. 2012a. *Vet Microbiol* 160:227–232.
Schreiner S, Sokoli A, Felder K, et al. 2012b. *Vet Microbiol* 156:88–95.
Schultz KK, Strait EL, Erickson BZ, et al. 2012. *Vet Microbiol* 158:104–108.
Schwartz J, Bruner L, Evelsizer B, et al. 2014. Dynamics of *Mycoplasma hyosynoviae* detection and clinical presentation. In Proc Annu Meet Am Assoc Swine Vet, pp. 115–116.
Seo HW, Park SJ, Park C, et al. 2014. *Vaccine* 32:2480–2486.
Seymour L, Deutscher A, Jenkins C, et al. 2010. *J Biol Chem* 285:33971–33978.
Seymour L, Falconer L, Deutscher A, et al. 2011. *J Biol Chem* 286:10097–10104.
Seymour L, Jenkins C, Deutscher A, et al. 2012. *Cell Microbiol* 14:81–94.
Shin JH, Joo HS, Lee WH, et al. 2003. *J Vet Med Sci* 65:501–509.
Sibila M, Calsamiglia M, Segalés J, et al. 2004a. *Vet Rec* 155:57–58.
Sibila M, Calsamiglia M, Vidal D, et al. 2004b. *Can J Vet Res* 68:12–18.
Sibila M, Nofrarias M, Lopez-Soria S, et al. 2007a. *Vet Microbiol* 121:352–356.
Sibila M, Nofrarias M, Lopez-Soria S, et al. 2007b. *Vet Microbiol* 122:97–107.
Sibila M, Bernal R, Torrents D, et al. 2008. *Vet Microbiol* 127:165–170.
Sibila M, Fort M, Nofrarias M, et al. 2012. *J Comp Pathol* 147:285–295.
Siqueira F, Thompson C, Virginio V, et al. 2013. *BMC Genomics* 14:175.
Sokoli A, Groebel K, Hoelzle K, et al. 2013. *Vet Res* 44:6.
Song Q, Zhang W, Song W, et al. 2014. *Prev Vet Med* 117:215–221.
Sorensen V, Ahrens P, Barfod K, et al. 1997. *Vet Microbiol* 54:23–34.
Stadler J, Jannasch C, Mack S, et al. 2014. *Vet Microbiol* 172:294–300.
Stakenborg T, Vicca J, Butaye P, et al. 2005a. *Microb Drug Resist* 11:290–294.
Stakenborg T, Vicca J, Butaye P, et al. 2005b. *Vet Microbiol* 109:29–36.
Stakenborg T, Vicca J, Butaye P, et al. 2006a. *Vet Res Commun* 30:239–247.
Stakenborg T, Vicca J, Maes D, et al. 2006b. *J Microbiol Methods* 66:263–275.
Stemke GW, Phan R, Young TF, et al. 1994. *Am J Vet Res* 55:81–84.
Strait EL, Madsen ML, Minion FC, et al. 2008. *J Clin Microbiol* 46:2491–2498.
Strait E, Rapp-Gabrielson V, Erickson B, et al. 2008a. *J Swine Health Prod* 16:200–206.
Strait E, Hawkins P, Wilson W. 2012. *JAVMA* 241:1666–1667.
Suter M, Kobisch M, Nicolet J. 1985. *Infect Immun* 49:615–620.
Switzer WP. 1955. *Am J Vet Res* 16:540–544.
Tacchi J, Raymond B, Haynes P, et al. 2016. *Open Biol* 6:150210.
Tajima M, Yagihashi T. 1982. *Infect Immun* 37:1162–1169.
Tajima M, Yagihashi T, Nunoya T, et al. 1984. *Am J Vet Res* 45:1928–1932.
Tavío M, Poveda C, Assunção P, et al. 2014. *Vet Rec* 175:539.
Taylor MA, Wise KS, McIntosh MA. 1984. *Isr J Med Sci* 20:778–780.
Taylor MA, Wise K, McIntosh MA. 1985. *Infect Immun* 47:827–830.
Ter Laak EA, Pijpers A, Noordergraaf JH, et al. 1991. *Antimicrob Agents Chemother* 35:228–233.
Thacker B, Thacker BJ, Boettcher TB, et al. 1998. *J Swine Health Prod* 6:107–112.
Thacker E, Halbur P, Ross R, et al. 1999. *J Clin Microbiol* 37:620–627.
Thacker B, Thacker E, Halbur P, et al. 2000a. The influence of maternally-derived antibodies on *Mycoplasma hyopneumoniae* infection. In Proc 16th Congr Int Pig Vet Soc, p. 454.
Thacker EL, Thacker BJ, Kuhn M, et al. 2000b. *Am J Vet Res* 61:1384–1389.
Thacker E, Thacker B, Janke B. 2001. *J Clin Microbiol* 39:2525–2530.
Thacker B, Thacker BJ, Wolff T. 2006. *J Swine Health Prod* 14:140–144.
Thanawongnuwech R, Thacker EL. 2003. *Viral Immunol* 16:357–367.
Thanawongnuwech R, Thacker B, Halbur P, et al. 2004. *Clin Diagn Lab Immunol* 11:901–908.
Thongkamkoon P, Narongsak W, Kobayashi H, et al. 2013. *J Vet Med Sci* 75:1067–1070.
Timenetsky J, Santos LM, Buzinhani M, et al. 2006. *Braz J Med Biol Res* 39:907–914.

Tocqueville V, Ferré S, Nguyen NHP, et al. 2014. *J Clin Microbiol* 52:1664–1671.
Toledo M, Leite A, Gonçalves R, et al. 2016. *Braz J Vet Parasitol* 25:414–417.
Uilenberg G, Thiaucourt F, Jongejan F. 2004. *Exp Appl Acarol* 32:301–302.
Underdahl N, Kennedey G, Ramos J. 1980. *Can Vet J* 21:258–261.
Van Alstine W, Stevenson G, Kanitz C. 1996. *Vet Microbiol* 49:297–303.
Vangroenweghe F, Labarque G, Piepers S, et al. 2015. *Vet J* 205:93–97.
Vicca J, Maes D, Thermote L, et al. 2002. *J Vet Med B Infect Dis Vet Public Health* 49:349–353.
Vicca J, Stakenborg T, Maes D, et al. 2003. *Vet Microbiol* 97:177–190.
Vicca J, Maes D, Jonker L, et al. 2005. *Vet Rec* 156:606–610.
Vicca J, Maes D, Stakenborg T, et al. 2007. *Microb Drug Resist* 13:166–170.
Villarreal I, Maes D, Meyns T, et al. 2009. *Vaccine* 27:1875–1879.
Villarreal I, Maes D, Vranckx K, et al. 2010. *Vaccine* 29:1731–1735.
Villarreal I, Meyns T, Haesebrouck F, et al. 2011. *Vet J* 188:48–52.
Vranckx K, Maes D, Calus D, et al. 2011. *J Clin Microbiol* 49:2020–2023.
Vranckx K, Maes D, Del Pozo Sacristán R, et al. 2012. *Vet Microbiol* 156:315–321.
Williams PP. 1978. *Antimicrob Agents Chemother* 14:210–213.
Wilton J, Jenkins C, Cordwell SJ, et al. 2009. *Mol Microbiol* 71:566–582.
Woolley LK, Fell SA, Gonsalves JR, et al. 2012. *Vaccine* 32:4333–4341.
Woolley LK, Fell SA, Gonsalves JR et al. 2014. *Vaccine* 32:4333–4341.
Wu CC, Shryock TR, Lin LT, et al. 2000. *Vet Microbiol* 76:25–30.
Xiong Q, Wang J, Ji Y, et al. 2016a. *Vet Microbiol* 186:82–89.
Xiong Q, Zhang B, Wang J et al. 2016b. *Vet Microbiol* 197:39–46.
Yazawa S, Okada M, Ono M, et al. 2004. *Vet Microbiol* 98:221–228.
Yogev D, Watson-McKwon R, Rosengarten R, et al. 1995. *J Bacteriol* 177:5636–5643.
Zachary JF, Basgall EJ. 1985. *Vet Pathol* 22:164–170.
Zimmerman W, Odermatt W, Tschudi P. 1989. *Schweiz Arch Tierheilkd* 1989:179–191.
Zinn GM, Jesse GW, Dobson AW. 1983. *J Am Vet Med Assoc* 182:369–371.

57

Pasteurellosis

Karen B. Register and Susan L. Brockmeier

Relevance

The importance of *Pasteurella multocida* as a respiratory disease agent in pigs has been recognized for more than 130 years, yet this bacterium continues to significantly impact swine health worldwide as a cause of progressive atrophic rhinitis (PAR) and pneumonia.

Atrophic rhinitis in pigs, characterized by stunted development or total disappearance of the nasal turbinates, was first reported in 1830 in Germany (Franque 1830). Following debate for more than a century regarding the precise etiology of the disease, it is now known that a severe and often irreversible form, referred to as progressive atrophic rhinitis, is caused by toxigenic strains of *P. multocida* either alone or in combination with *Bordetella bronchiseptica* (de Jong and Nielsen 1990). PAR may negatively affect growth rate and the efficiency of feed conversion (Pedersen and Barfod 1981; Riising et al. 2002), and moderate to severe outbreaks may have a substantial economic impact (Muirhead 1979; Pedersen and Nielsen 1983). *B. bronchiseptica* alone may also cause rhinitis and turbinate atrophy in young pigs accompanied by minor effects on growth, but this reversible condition, known as nonprogressive atrophic rhinitis (NPAR), is distinct from PAR (see Chapter 49).

Pneumonic pasteurellosis is a purulent bronchopneumonia that results from opportunistic *P. multocida* infection of the lung following primary infections by predisposing bacterial and viral pathogens. *P. multocida* is among the most common and costly components of porcine respiratory disease complex (PRDC). In the United States pneumonia is the leading cause of death in nursery, grower/finisher, and wean-to-finish pigs (USDA 2015). Reduced weight gain and the cost of treating sick animals add further to the economic impact. *P. multocida* is among the bacterial agents most frequently isolated from pneumonic lungs with a particularly high prevalence in finishing pigs (Choi et al. 2003; Fablet et al. 2012; Opriessnig et al. 2011; Pors et al. 2011).

Sporadic outbreaks of fatal acute septicemia in farmed and wild pigs have also been attributed to *P. multocida* in Europe, Asia, and Australia (Cameron et al. 1996; Cardoso-Toset et al. 2013; Kalorey et al. 2008; Mackie et al. 1992; Risco et al. 2013; Townsend et al. 1998b; Ujvári et al. 2015).

Etiology

Pasteurella multocida includes three subspecies: *P. multocida*, *P. septica*, and *P. gallicida*. The vast majority of swine isolates appear to be *P. multocida* subsp. *multocida*, but other subspecies are occasionally reported (Blackall et al. 2000; Bowles et al. 2000; Cameron et al. 1996; Davies et al. 2003; Varga et al. 2007).

Pasteurella multocida is a nonmotile gram-negative rod or coccobacillus approximately 1.0–2.0 µm in length. Initial or low-passage isolates may exhibit a distinct bipolar staining not usually observed following serial subculture. The bacterium is a facultative anaerobe and grows well at 98.6 °F (37 °C) in most enriched media. On blood agar plates it forms grayish, nonhemolytic colonies, often mucoid, with a characteristic "sweetish" odor. It does not grow on MacConkey agar, is oxidase and catalase positive, and produces indole.

Five capsular serotypes – A, B, D, E, and F – are recognized (Carter 1955; Rimler and Rhoades 1987) with A and D comprising most swine isolates. A small proportion of isolates from the respiratory tract are noncapsulated and, therefore, untypable. Serotype A is most commonly cultured from pneumonic lungs, while most PAR isolates are serotype D, but either serotype may be isolated from either condition, and serotype F has also rarely been recovered (Bethe et al. 2009; Davies et al. 2003; Ewers et al. 2006; García et al. 2011; Peng et al.

Diseases of Swine, Tenth Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson.

2017; Varga et al. 2007). The most prevalent capsular serotype associated with acute septicemic pasteurellosis in swine is B, but types D and A have also been reported (Cameron et al. 1996; Kalorey et al. 2008; Mackie et al. 1992; Townsend et al. 1998b).

In addition to capsular serotyping, an additional somatic serotyping method was later developed to further distinguish isolates on the basis of lipopolysaccharide (LPS) antigens (Heddleston et al. 1972). There are 16 known somatic serotypes, designated 1–16. Historically, types 3, 5, and 12 predominated among swine isolates, but present-day information is sparse. The combined results of capsular and somatic serotyping have traditionally been the basis for characterization of isolates, but the difficulty of generating consistent, high-quality, and readily available antisera led to the development of PCR assays that type isolates on the basis of differences in the organization of the genes responsible for capsule and LPS synthesis (Harper et al. 2015; Townsend et al. 2001).

A classification scheme based on carbohydrate fermentation and enzyme production is also sometimes used, with most pig isolates represented by 2 of the 14 defined biovars (García et al. 2011). Alternative typing techniques based on pattern analysis of genomic DNA fragments or PCR amplicons have also been employed (Dziva et al. 2008). A significant limitation of those methods is the lack of standardization and the associated difficulty in comparing data across laboratories.

Multilocus sequence typing (MLST), based on DNA sequences obtained from short fragments of a defined set of housekeeping genes, has been used to investigate the population structure and evolution of *P. multocida* (Bisgaard et al. 2013). MLST provides definitive and objective results that are easily evaluated, compared, and archived. Because relatively few isolates from pigs have been analyzed, it is yet unclear whether the method is sufficiently discriminatory for use as an epidemiologic tool in swine.

Genome sequences for nearly 100 isolates of *P. multocida* are now available, but only a few are of swine origin, representing capsular types A, D, and F (Okay and Kurt Kızıldoğan 2015; Peng et al. 2017). Comparative analyses suggest that allelic heterogeneity and differences in gene complement are more closely tied to disease syndrome than to host specialization. Insights provided by comparative genomics with respect to disease in swine will be relatively limited until genomes from additional isolates that more fully represent the diversity of strains infecting pigs are sequenced.

Public health

Pasteurella multocida is an important zoonotic agent and is responsible for most human infections related to animal bites or scratches. Dogs and cats are the predominant source, but infection following bites from pigs, rabbits, rats, and various wild animals has been reported (Wilson and Ho 2013). Human pasteurellosis most often presents as skin or soft tissue infection, typically with rapid onset, characterized by inflammation, swelling, and purulent exudate. More serious manifestations generally limited to immunocompromised patients include septicemia, osteomyelitis, endocarditis, pneumonia, meningitis, and peritonitis.

Pasteurella multocida is not a usual constituent of the human upper respiratory tract, but strains genetically identical to those found in swine are frequently isolated from pig farmers and from inhabitants of regions with intensive pig breeding (Donnio et al. 1999; Marois et al. 2009). Water from scalding tanks is a potential source of exposure for abattoir workers (Marois et al. 2008). Most human carriers remain healthy, but *P. multocida* may also be associated with acute or chronic respiratory disease. It has been proposed that pneumonic pasteurellosis be considered an occupational disease. Appropriate precautions should be observed by immunocompromised persons who have contact with swine infected with *P. multocida*.

Epidemiology

Pasteurella multocida has been isolated throughout the world from a wide array of wild and domesticated mammals and birds, including aquatic mammals (Smith et al. 1978). It causes acute or chronic disease of importance in poultry, cattle, water buffalo, swine, sheep, and rabbits. *P. multocida* often occurs as part of the normal flora, and clinically inapparent infections that spark disease outbreaks in immunologically naïve animals are common in many hosts. It has no known environmental reservoir.

The epidemiology of *P. multocida* in swine is not well understood. In many herds the organism is detectable in the nose and tonsils of healthy animals and may persist for months or even years with little evidence of disease. Although the bacterium may occasionally be spread via aerosols, nose-to-nose contact is the common route of infection. Introduction of *P. multocida* into a herd is usually by introduction of infected swine. Spread within a herd occurs rapidly both vertically from infected dam to suckling piglets and horizontally between infected and uninfected animals (Dritz et al. 1996; Fablet et al. 2011; Zhao et al. 1993). Spread related to contaminated fomites or intermediate hosts has been suggested (Desrosiers 2011; Goodwin et al. 1990), and there is some evidence consistent with occasional interspecies transmission of avian, bovine, ovine, and porcine strains (Davies et al. 2004). Rodents, cats, dogs, and other hosts that commonly carry *P. multocida* should be considered possible sources of exposure for pigs. Whether healthy human carriers can transmit *P. multocida* to swine is unknown.

Molecular typing techniques have been used to better understand the epidemiology of *P. multocida* in pigs, but comparisons among studies are problematic because there is no widely adopted and standardized method and many studies fail to provide a quantitative measure of diversity. Among pneumonic isolates, one or a few strains often predominate in closed herds or in those with minimal introductions, although strains differ between operations (Blackall et al. 2000; Marois et al. 2009; Rúbies et al. 2002; Zhao et al. 1993). Multiple strains are more likely to be involved in herds where pig movement occurs (Bowles et al. 2000). It has been hypothesized that highly pathogenic clones preferentially cause pneumonia, but this seems doubtful since no traits associated exclusively with virulence have been identified and commensal isolates from healthy and diseased pigs possess a similarly limited degree of genetic heterogeneity (Bethe et al. 2009; Ewers et al. 2006). Only a few investigations using DNA-based typing methods have evaluated strains associated with PAR, and these reveal relatively limited genetic diversity. Results of restriction endonuclease analysis and ribotyping demonstrated that multiple PAR strains may be found within a herd and that single strains may be found in more than one herd (Bethe et al. 2009; Gardner et al. 1994; Harel et al. 1990).

Pasteurella multocida survives for approximately 1 week at 39 °F (4 °C) following growth in liquid or on solid medium but may remain viable for up to several months when stored at 59 °F (15 °C) or 98.6 °F (37 °C). It is culturable for up to 6 days in pig slurry and more than 49 days in nasal washings obtained from piglets, with maximal survival at 59 °F (15 °C) or higher (Thomson et al. 1992). Aerosolized organisms suspended in nasal washings remain viable for at least 45 minutes. The half-life of *P. multocida* in a rotating aerosol chamber at 73 °F (23 °C) and 75% relative humidity is 21 minutes (Müller et al. 1992).

Several disinfectants suitable for farm use are bactericidal for *P. multocida*, some under high organic matter conditions (Thomson et al. 2007). The bacterium is inactivated by overnight incubation at 39 °F (4 °C) in 0.5% phenol or 0.2% formalin or, when not protected by organic material, heating to 140 °F (60 °C) for 10 minutes.

Pathogenesis

Colonization

Pasteurlla multocida poorly colonizes the swine respiratory tract in the absence of preexisting damage to the mucosa (Pedersen and Elling 1984). *In vitro* studies using swine turbinate explants or epithelial cells from the nasal cavity or trachea consistently demonstrate little or no attachment (Chung et al. 1990; Frymus et al. 1986; Jacques et al. 1988; Nakai et al. 1988).

Pasteurella multocida can bind to components of porcine respiratory tract mucus (Jacques et al. 1993; Letellier et al. 1991). When mucociliary clearance is compromised, interaction of bacteria with extracellular mucus may facilitate colonization of the mucosal epithelium. Attachment of *P. multocida* to porcine tracheal rings previously infected with *B. bronchiseptica* increased by several orders of magnitude as compared with tracheal rings infected with *P. multocida* alone (Dugal et al. 1992). Enhanced binding was credited to *B. bronchiseptica*-induced mucus accumulation and ciliostasis. This phenomenon was observed with numerous *P. multocida* isolates from healthy and diseased pigs representing capsular type A and type D with or without toxin production.

LPS, a major component of the *P. multocida* outer membrane, has been implicated in attachment. LPS purified from a toxigenic type D swine isolate binds to porcine respiratory tract mucus and blocks adherence of the bacterium to porcine tracheal rings (Jacques et al. 1993). Elimination of the capsule by serial passage *in vitro* significantly increased the number of adherent organisms to trachea, suggesting that the type D capsule may interfere with binding. The authors hypothesized that during the early stages of infection, organisms express a minimal amount of capsular material, thereby exposing otherwise masked outer membrane components (LPS and perhaps others) involved in attachment. A later study showed that cells grown under iron-restricted conditions, such as those encountered *in vivo*, are covered by a thinner layer of capsular material and display increased adherence to porcine respiratory tract mucus and to frozen sections of porcine lung and trachea as compared with cells cultivated under iron-replete conditions (Jacques et al. 1994).

Homologues of the outer membrane proteins OmpA and OmpH, known or putative adhesins in other pathogens, have been identified in swine isolates of *P. multocida* (Davies et al. 2003; Lugtenberg et al. 1986; Marandi and Mittal 1996). Whether they similarly function as adhesins during colonization of the swine respiratory tract is unknown.

Many isolates of *P. multocida* have hemagglutinating activity, but there is no correlation with *in vitro* binding or *in vivo* colonization (Fortin and Jacques 1987; Pijoan and Trigo 1990; Vena et al. 1991). Likewise, fimbriae are produced by many toxigenic and nontoxigenic capsular type A and D strains but appear to play no role in adherence to cells or tissues of swine origin (Isaacson and Trigo 1995; Pijoan and Trigo 1990).

Although there is a general association of toxigenic type D strains with PAR in the upper respiratory tract and of nontoxigenic type A strains with pneumonic

pasteurellosis in the lower respiratory tract, there is little evidence that strains of different capsular types employ distinct adherence strategies (Dugal et al. 1992; Frymus et al. 1986; Letellier et al. 1991; Pijoan and Trigo 1990; Vena et al. 1991). Neither does there appear to be a relationship between toxigenicity and differential attachment. Interestingly, in a study of 158 swine isolates, Davies et al. (2003) found that most pneumonic isolates share outer membrane profiles distinct from those shared among PAR isolates, irrespective of capsular type. However, it is unknown whether any of the discriminatory outer membrane proteins has a role in attachment or colonization.

The tonsil, particularly the tonsillar crypt, appears to be the preferred habitat of *P. multocida* in swine and may protect bacteria from inflammatory cells or act as a physical barrier to removal by swallowing (Ackermann et al. 1994). Colonization of the tonsil by type A and type D strains in the absence of prior mucosal damage and persistence for up to 60 days post infection has been reported in experimentally infected piglets (Ono et al. 2003; Pijoan and Trigo 1990). The bacterial products essential for colonization of this site are unknown, but the tonsil may serve as a reservoir from which strains capable of causing PAR or pneumonia subsequently spread when innate defenses of the upper or lower respiratory tract are weakened or incapacitated.

Progressive atrophic rhinitis

The common predisposing factor in pigs naturally affected by PAR is prior infection with *B. bronchiseptica* (Pedersen and Barfod 1981). The mechanism facilitating colonization by *P. multocida* is unproven, but a tracheal cytotoxin released by *B. bronchiseptica* that causes ciliostasis and destruction of the mucosal epithelium is likely of primary importance (Dugal et al. 1992; Flak et al. 2000). Other toxins and effectors produced by *B. bronchiseptica* also probably play a role (see Chapter 49).

Elaboration by *P. multocida* of a 146 kDa protein toxin, referred to as *P. multocida* toxin (PMT), is the essential virulence factor for the pathogenesis of PAR. PMT produces progressive snout shortening and turbinate atrophy when given to pigs intranasally (Il'ina and Zasukhin 1975) and by a variety of parenteral routes (Rutter and Mackenzie 1984). It produces lesions in the turbinates, liver, and urinary tract characterized by degenerative and hyperplastic changes. The toxin interferes with normal remodeling and formation of bone in the turbinates (Dominick and Rimler 1988; Foged et al. 1987; Martineau-Doizé et al. 1990) and can decrease physeal area in the long bones of pigs (Ackermann et al. 1996), which perhaps contributes to slow growth associated with PAR. PMT may have direct access to the turbinates when toxigenic strains are growing in the nasal cavity, but the capacity to act systemically suggests that its effects may also be exerted by bacteria colonizing the tonsil or other anatomical sites.

The structure of PMT and its molecular mechanism of action have been intensively investigated (reviewed in Orth and Aktories 2012). It interferes with G-protein- and Rho-dependent signaling pathways and stimulates mitogenesis. The biologically active region has been localized to the C-terminus, while a cell-binding and/or internalization domain resides in the N-terminus. PMT is encoded by the *toxA* gene (Petersen and Foged 1989), which has a G + C content significantly different than that of the *P. multocida* genome, indicating that it may have been horizontally acquired. Further study by Pullinger et al. (2004) revealed that the gene is located within an inducible prophage. PMT lacks a typical signal sequence and is not secreted during growth *in vitro*, leading to the suggestion that, like some other bacterial toxins, phage-induced cell lysis triggered by contact with host cells or by some environmental factor encountered *in vivo* is the mechanism of export. Phage-mediated transduction could potentially lead to acquisition and expression of *toxA* by nontoxigenic strains of *P. multocida*, or even other bacteria, but whether this occurs is not known.

Several environmental, management, and husbandry factors can influence the occurrence and presentation of PAR. More severe disease is generally associated with intensive indoor production systems that have high stocking density, poor hygiene, and poor ventilation. Exposure to high levels of dust and ammonia may facilitate colonization of the upper respiratory tract by *P. multocida* and/or exacerbate disease (Andreasen et al. 2000; Hamilton et al. 1999). Continual pig throughput and frequent moving and mixing of pigs are also predisposing factors.

Pigs infected within the first few weeks of life with toxigenic *P. multocida* are the most severely affected with PAR, but mild to moderate turbinate lesions occur in those infected as late as 16 weeks of age (Rutter et al. 1984). Apparently healthy 3-month-old pigs can develop PAR when introduced into a production unit where severe disease is occurring (Nielsen et al. 1976). Age-dependent disease severity may be partly related to changes in the amount and type of mucins present in the nasal cavity and altered cellular distribution within the nasal epithelium that occur as pigs mature (Larochelle and Martineau-Doizé 1990, 1991). The impact of aerial pollutants may also be greatest in young pigs (Robertson et al. 1990).

Feed consumption may be influenced by PAR, since piglets with an acute rhinitis may accept feed less readily and become stunted and weak. Growing pigs with conchal damage may also have reduced feed intake.

Pneumonia

The virulence factors of *P. multocida* that contribute to the development of pneumonia are unknown and may differ depending on the number and identity of other agents present in the lung. Studies comparing the prevalence of potential virulence genes in swine isolates from the nasal cavity of healthy animals with those from pneumonic lungs failed to reveal a specific genotype correlated with virulence (Bethe et al. 2009; Ewers et al. 2006). There is no convincing evidence in support of a role for PMT, but some investigators have reported an unusually high percentage of toxigenic strains from the lung (Høie et al. 1991; Iwamatsu and Sawada 1988), sometimes preferentially associated with acute cases (Kielstein 1986). Nonetheless, nontoxigenic strains continue to comprise the majority of lung isolates (Bethe et al. 2009; García et al. 2011).

Coinfection with other respiratory disease agents is the most significant factor contributing to swine pneumonic pasteurellosis. Implicated agents include *Mycoplasma hyopneumoniae* (Amass et al. 1994; Ciprián et al. 1988) and pseudorabies virus (Carvalho et al. 1997; Fuentes and Pijoan 1987); a synergistic effect with a hog cholera vaccine has also been demonstrated (Pijoan and Ochoa 1978). Coinfection with *Actinobacillus pleuropneumoniae* permits colonization of the lung by *P. multocida*, perhaps by interfering with the ability of porcine alveolar macrophages to phagocytose and kill the bacterium (Chung et al. 1993), but the ensuing disease may be no more severe than that resulting from only *A. pleuropneumoniae* (Little and Harding 1980). Porcine reproductive and respiratory syndrome virus (PRRSV) alone appears not to interact with *P. multocida*, but prior infection with both PRRSV and *B. bronchiseptica* leads to colonization of the lung and induction of pneumonia by nontoxigenic *P. multocida* upon subsequent exposure (Brockmeier et al. 2001; Carvalho et al. 1997). Prior infection with only *B. bronchiseptica* results in colonization of the nasal cavity and tonsil without overt disease, and this could be a means by which healthy carrier pigs arise undetected. Long-term carriage of nontoxigenic strains in the tonsil may lead to pneumonia in the face of other predisposing factors that arise at a later time.

The capsule may contribute to virulence in swine pneumonic pasteurellosis, but its precise role is not fully understood. Swine alveolar macrophages poorly phagocytose encapsulated organisms (Chung et al. 1993; Fuentes and Pijoan 1986), and the capsule also interferes with uptake by swine neutrophils (Rimler et al. 1995). Yet, an antiphagocytic role is called into question by the demonstration that little capsular material is produced under iron-limiting growth conditions expected to mimic the environment *in vivo* (Jacques et al. 1994). The degree to which these data can be extrapolated is unclear since little is known about conditions that exist in the microenvironments to which *P. multocida* is exposed during various stages of pulmonary infection and disease. Growth conditions may vary significantly within different tissues or even within different substructures of a single tissue and further depend on the degree of host cell damage locally present.

Some strains of *P. multocida* produce pleuritis and abscessation (Pijoan and Fuentes 1987; Pors et al. 2011). The factors that distinguish these strains from less virulent pneumonic strains are not defined. However, there is some suggestion that PMT may be involved in the formation of pulmonary abscesses (Ahn et al. 2008; Iwamatsu and Sawada 1988; Kielstein 1986).

Large swine herds with high stocking density and poor air quality are associated with a higher prevalence of pneumonia (Done 1991). Excessive aerial ammonia was reported to facilitate pulmonary infection of unweaned piglets with *P. multocida* (Neumann et al. 1987). Other investigators have reported little effect of ammonia on the development of lung lesions in pigs coinfected with *M. hyopneumoniae* and *P. multocida* but noted lower slaughter weights in those exposed to higher concentrations (Andreasen et al. 2000; Diekman et al. 1993). Oral exposure to endotoxin or fumonisins has also been shown to potentiate pneumonia in pigs infected with *P. multocida*, with or without *B. bronchiseptica* (Halloy et al. 2005a,b; Pósa et al. 2011).

Septicemic pasteurellosis

On the basis of several genetic and phenotypic characteristics, capsular type B *P. multocida* isolates associated with swine septicemic pasteurellosis appear to be more closely related to isolates causing hemorrhagic septicemia in other hosts than to isolates from swine PAR or pneumonia (Cardoso-Toset et al. 2013; Townsend et al. 1998b; Ujvári et al. 2015). Hematogenous spread of type A or type D isolates may, in some instances, be an extension of pneumonic pasteurellosis (Pors et al. 2011). There is little known regarding the identity or role of virulence factors potentially contributing to systemic spread and related disease.

Clinical signs

Progressive atrophic rhinitis

Sneezing in baby pigs is often the first clinical sign of PAR but also results from infection with other pathogens that produce acute rhinitis. Pigs with PAR may continue to sneeze, snuffle, and snort throughout the growing period, and a variable amount of serous to mucopurulent nasal and ocular discharge may be observed. Tear staining that

radiates from the medial canthus of the eye as a result of nasolacrimal duct occlusion may be present in either NPAR or PAR, but epistaxis is typically observed only in PAR. Also specific to PAR is ensuing snout deformation that becomes apparent when pigs are 4–12 weeks of age. The most common visible manifestation of PAR is brachygnathia superior, in which the upper jaw is shortened in relation to the lower, giving the nose a pushed-up appearance with the skin on the dorsum typically thrown into folds (Figure 57.1). Lateral deviation of the snout may be evident when more extensive deformation of the bone occurs on one side of the head than the other (Figure 57.2). This may vary in severity from a barely perceptible misalignment to obvious twisting. The prevalence of snout distortion varies among outbreaks, and not all pigs with significant turbinate atrophy develop marked distortions. In severe outbreaks of PAR, growth retardation and a reduction in the efficiency of feed utilization may occur.

Pneumonia

Pneumonic pasteurellosis most commonly occurs in grower- and finisher-age swine where it exacerbates PRDC that is initiated by one or more primary respiratory pathogens. These polymicrobial disease processes typically cause high morbidity and variable mortality and can greatly lengthen the time to market and increase the number of culls. Clinical signs can vary depending on the pathogens involved and stage or severity of the disease but often include coughing, intermittent fever, depression, anorexia, reduced growth rate, labored breathing or thumping, and in severe cases cyanosis or blue discoloration, especially in the tips of the ears. More severe disease and clinical signs may be associated with certain strains of *P. multocida* that produce abscesses and pleuritis.

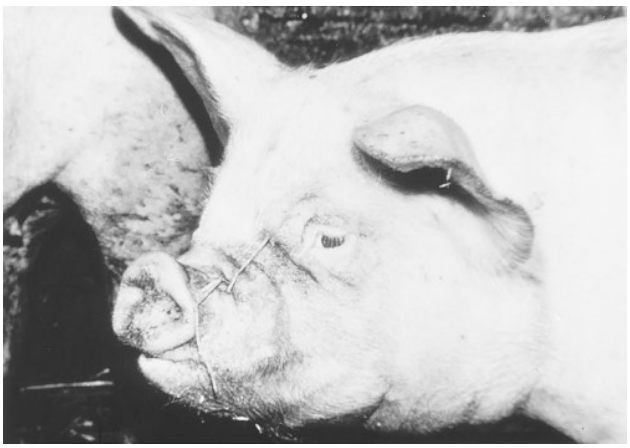


Figure 57.1 A 17-week-old pig with clinical progressive atrophic rhinitis showing marked brachygnathia superior, wrinkling of the skin on the dorsum of the nose, and tear staining.

Figure 57.2 The head of a 15-week-old pig with clinical progressive atrophic rhinitis showing severe lateral deviation of the snout. The anatomy of the skull is distinctly abnormal due to a failure of normal bone development.

Septicemic pasteurellosis

Septicemic pasteurellosis typically has a sudden onset and acute progression of disease with high morbidity and mortality. Clinical signs include high fever, severe dyspnea, cyanosis of the ventrum and ears, anorexia, weakness, and prostration. One of the most striking features is edema and hemorrhage of the ventral neck that may become necrotic (Cardoso-Toset et al. 2013; Ujvári et al. 2015). Clinical signs consistent with pneumonic pasteurellosis have also been noted in some cases.

Lesions

Progressive atrophic rhinitis

The gross lesions of PAR are restricted to the nasal cavity and adjacent structures of the skull although in advanced cases pigs may also be stunted. Atrophy of the ventral and dorsal turbinates, present in variable degrees, is the hallmark lesion. In mild to moderate cases, the ventral scrolls are by far the most consistently and extensively affected; severe cases may progress to total loss of the turbinates and deviation of the nasal septum (Figures 57.3–57.7). Mucopurulent exudate may be found in the nasal cavity, occasionally with associated

Figure 57.3 Cross section of the snout of an 18-week-old pig showing normal anatomy of the turbinates.

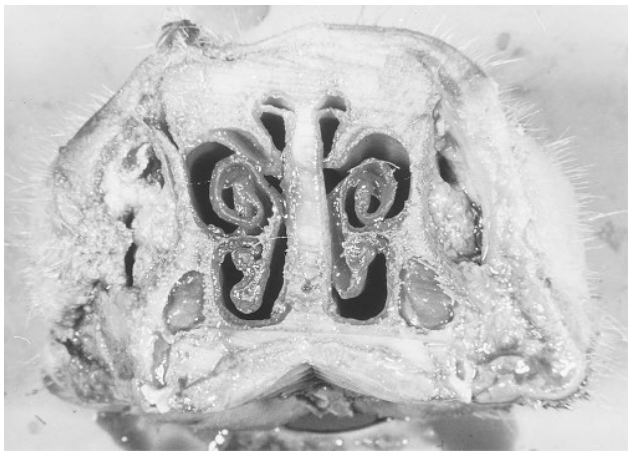


Figure 57.4 Cross section of the snout of an 18-week-old pig. Slight distortion of the ventral scrolls of the turbinates is present, a common finding.

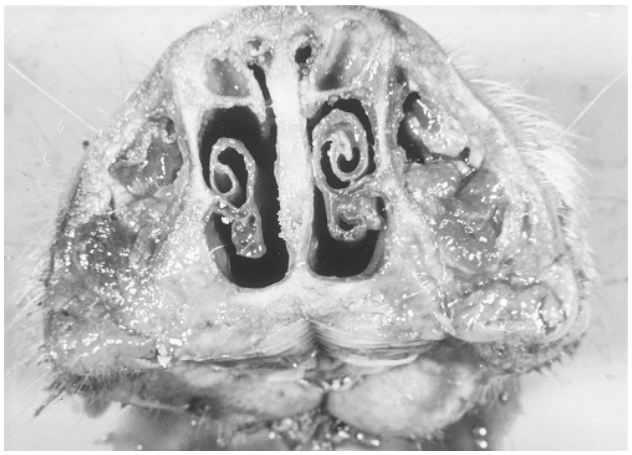


Figure 57.5 Cross section of the snout of an 18-week-old pig showing modest but definite turbinate atrophy.

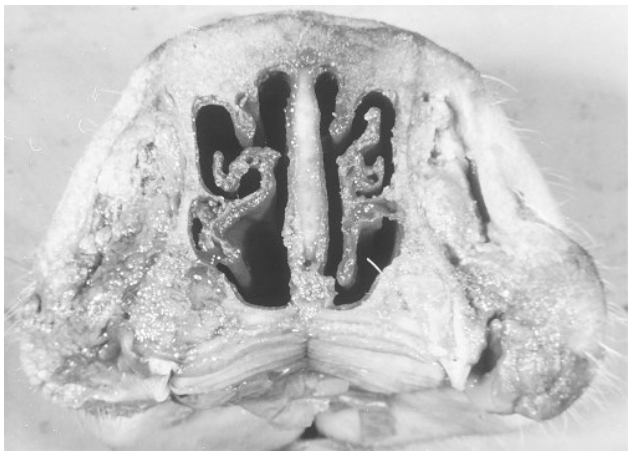


Figure 57.6 Cross section of the snout of an 18-week-old pig showing severe bilateral turbinate atrophy.

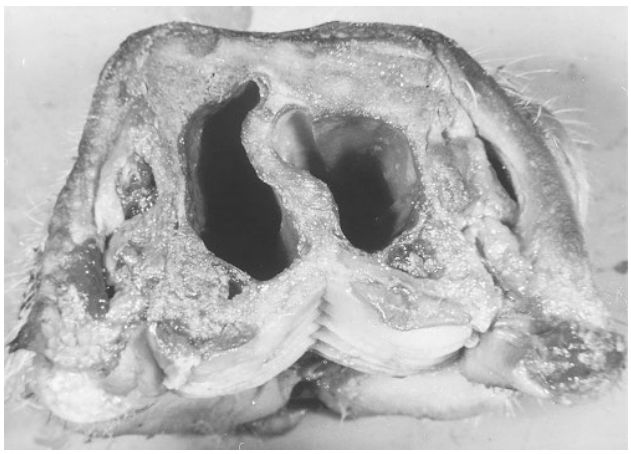


Figure 57.7 Cross section of the snout of a 22-week-old pig showing total atrophy of all turbinate structures with severe bending of the nasal septum.

hemorrhage. Many studies suggest that lesions of PAR are irreversible, but Magyar et al. (2013) have recently reported that turbinate structure may return to normal by approximately 18 weeks post infection in high health status pigs.

Depending on the stage of disease at the time of necropsy, acute, subacute, or chronic histologic changes may be observed. The principal microscopic lesion of PAR is replacement of the bony trabeculae of the nasal turbinates with fibrous connective tissue (Pedersen and Elling 1984). Increased numbers of osteoclasts are observed contributing to this process. The degree of epithelial damage or inflammation depends on the stage of disease and whether coinfection with *B. bronchiseptica* is present (Duncan et al. 1966; Elling and Pedersen 1985). Subacute cases in conventional pigs will show various mixtures of degenerative, inflammatory, dystrophic, and reparative processes.

Pneumonia

Pneumonic lesions vary since *P. multocida* is often only one component of a complex infection. Macroscopic lesions include typical red-to-gray firm consolidation with a cranial ventral well-demarcated lobular distribution and purulent exudate in lumens of conducting airways and alveoli characteristic of acute to chronic bacterial bronchopneumonia (Figure 57.8). Abscesses, pleuritis with visceral and parietal pleural adhesions, and pericarditis also occasionally occur (Figures 57.9 and 57.10). Microscopic lesions consist of suppurative bronchopneumonia characterized by neutrophil infiltration of bronchial and alveolar spaces and interstitial thickening (Pors et al. 2011). Fibrinosuppurative pleuritis and fibrous encapsulation of necrosuppurative areas will be seen in cases where pleuritis and abscesses are present. Additional lesions may be apparent depending on the other agents involved. For example, peribronchiolar lymphocyte infiltration may be seen when *Mycoplasma* is present, while interstitial pneumonia or necrotizing bronchiolitis may be features when PRRSV or swine influenza virus is a component.

Figure 57.8 Pneumonic pasteurellosis. Lung consolidation is anteroventral with a clear demarcation line between affected and healthy tissue.

Figure 57.9 Pleural adhesions of lung to the thoracic wall in a case of pleuritic pasteurellosis. Note that the pleura has a translucent, dry aspect.

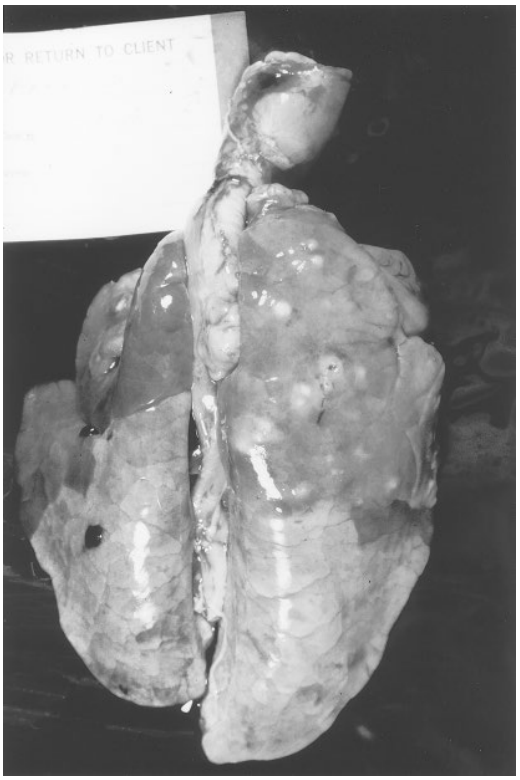


Figure 57.10 Lung from a case of pleuritic pasteurellosis. Note anteroventral well-demarcated lesions with multiple abscesses and extensive interlobar adhesion.

Septicemic pasteurellosis

Subcutaneous hemorrhagic edema is often seen in cases involving capsular type B strains (Cardoso-Toset et al. 2013; Ujvári et al. 2015). Other lesions associated with septicemic pasteurellosis include edema of the pharynx and trachea, pulmonary edema and hemorrhage with or without pulmonary consolidation, enlarged edematous and hemorrhagic lymph nodes, and congestion and

hemorrhage of the visceral organs, occasionally with fibrin deposition in the peritoneal and pleural cavities (Mackie et al. 1992). Microscopically, intravascular thrombosis and changes consistent with widespread vascular damage, such as edema, hemorrhage, and necrosis, are present in tissues, and bacteria can often be observed in the vessels and throughout affected tissues. Purulent inflammatory exudates in multiple organs and fibrinous serositis may also be present.

Diagnosis

Progressive atrophic rhinitis

A definitive diagnosis of PAR depends on clinical and pathological observations and demonstration of toxigenic *P. multocida*. A preliminary diagnosis of PAR can be made based on a pattern of typical clinical signs. Animals showing lateral deviation of the snout and/or marked brachygnathia superior almost always have pronounced turbinate atrophy, but snout deformation alone is not pathognomonic. When these signs are not apparent or are of decreasing prevalence (e.g. following treatment), it is not possible for even experienced observers to assess the extent of turbinate atrophy in the live animal. Radiography (Done 1976) and computed tomography (Magyar et al. 2003) can provide objective observations from live animals and are noninvasive but require that pigs be sedated or physically immobilized. Tomography reveals more subtle changes that may not be apparent by radiography.

The prevalence and severity of turbinate atrophy are best estimated by examination of nasal turbinates during regular slaughter checks. Snouts should be transversely sectioned at the level of the first/second upper premolar; sectioning cranial to this may give a false-positive result. Atrophy may be scored subjectively by various systems (Cowart et al. 1990). Although there may be considerable interobserver variation within a system, subjective scoring is highly useful for monitoring herd status and evaluating treatment. Objective methods better suited for data analysis are also available (Gatlin et al. 1996). Samples from the tonsils and nasal cavity should be evaluated for the presence of toxigenic *P. multocida* to ensure a proper diagnosis. When severe PAR is first detected, infection may have actually occurred weeks or months earlier, and evidence of toxigenic *P. multocida* may be difficult to obtain. In such cases it is recommended to also examine and culture less severely affected (usually younger) pigs.

Tonsil swabs or biopsies provide the highest isolation rates for *P. multocida* (Ackermann et al. 1994), but nasal swabs suffice. Live pigs should be adequately restrained, and the external nares cleaned. Swabs with flexible shafts are preferred to avoid breakage in the event of sudden movements of the animal. Mini-tipped swabs are available that facilitate sampling in young pigs. The surface of the tonsil should be swabbed, or, when sampling the nasal cavity, a single swab should be inserted with slight rotation deep into both sides of the nasal cavity. Swabs should be transported to the laboratory within 24 hours, preferably in a transport medium under cooled conditions (39–46 °F [4–8 °C]). Nutrient transport media that support the growth of fast-growing contaminants are best avoided; sterile phosphate-buffered saline is preferable.

Toxigenic and nontoxigenic strains of *P. multocida* share many cross-reacting antigens, and there are no satisfactory serological tests for identification of animals infected with only toxigenic strains. PMT-specific enzyme-linked immunosorbent assays (ELISAs) designed for use with the bacterium (Bowersock et al. 1992; Foged et al. 1988) can be adapted for use with serum, but in naturally infected pigs PMT is a weak immunogen and PMT-specific antibodies are often undetectable. Use of toxoid-containing vaccines limits the diagnostic value of this approach to herds with no history of vaccination or to detection of vaccine response in immunized herds.

Sneezing in young pigs occurs in active PAR, but also during infection with *B. bronchiseptica* or porcine cytomegalovirus, both widely prevalent. Brachygnathia superior develops naturally in certain lines of the Large White/Yorkshire breed but can generally be distinguished from PAR by the absence of turbinate atrophy. Sows and gilts kept in stalls often bite, chew, or play with bars or drinkers, and this can give rise to asymmetric bone development causing protrusion of the lower jaw or mandibular misalignment. These conditions can be confused with the facial deformity of PAR, especially in the older pig, but careful inspection should reveal that the lower jaw is abnormally placed rather than that the snout is shortened or laterally deviated. The presence of mild turbinate atrophy in a herd may represent either NPAR or developing PAR. Definitive diagnosis requires culture.

Pneumonia

Lung lesions caused by *P. multocida* are not pathognomonic and cannot be used as the only criteria to establish a definite diagnosis. The history of the outbreak, histopathology, and isolation of the organism should be used to confirm the original presumptive diagnosis.

Optimal specimens include swabs of tracheobronchial exudate and affected lung tissue obtained from the border area between affected and normal tissue. Swabs should be immersed in an appropriate transport medium, such as Stuart's. Lung samples should be obtained as

aseptically as possible. All samples should be refrigerated (but not frozen) until cultured. There is no serologic test routinely available to assist in the diagnosis of *P. multocida* lung infection.

Differential diagnosis should include other causes of purulent bronchopneumonia or pleuropneumonia such as *B. bronchiseptica*, *M. hyopneumoniae*, *Streptococcus suis*, *Haemophilus parasuis*, *Trueperella pyogenes*, *Salmonella choleraesuis*, *Actinobacillus suis*, and *A. pleuropneumoniae*. Accurate clinical differentiation is often difficult, requiring bacterial culture, histopathology, and other testing as needed for a definitive diagnosis.

Septicemic pasteurellosis

Diagnosis depends on detection of *P. multocida* in blood and affected tissues, the presence of multifocal thrombosis and necrosis, and the absence of other agents causing sudden death.

Identification and characterization of *p. Multocida*

Culture and phenotypic methods

Pasteurella multocida grows readily on blood agar, but nonselective media are appropriate only when specimens are obtained aseptically from a normally sterile site, for example, the lung. For most specimens a selective medium is preferred to prevent masking of colonies by overgrowth of other bacteria often present in higher numbers. Various formulations have been used, but comparison of studies in the literature indicates that the highest isolation rates are obtained with modified Knight medium (Lariviere et al. 1993) or blood agar plates containing certain antimicrobials (Ackermann et al. 1994). A selective medium for simultaneous isolation of both *P. multocida* and *B. bronchiseptica* from swine has also been described (de Jong and Borst 1985). Once isolated, conventional biochemical tests can be used to identify suspect colonies, although reliability varies among methods and PCR (see below) may be superior (García et al. 2011; Townsend et al. 1998a).

Capsular typing of *P. multocida* is useful for epidemiologic purposes. Serotyping by indirect hemagglutination has traditionally been used (Carter 1955), but the hyaluronidase test (Carter and Rundell 1975) and acriflavine test (Carter and Subronto 1973) are simpler methods for the detection of type A and D strains, respectively.

Classification of *P. multocida* as PMT positive or negative is critical for a diagnosis of PAR and may also be informative in other disease manifestations. Tests based on dermonecrotic or lethal effects of PMT toxin in rodents were initially used, but toxigenicity can be more easily and humanely demonstrated *in vitro* by assessing cytopathic effects in embryonic bovine lung cells or Vero cells (Chanter et al. 1986; Pennings and Storm 1984). ELISAs based on the use of PMT-specific monoclonal antibodies have now generally replaced biological assays and are more rapid, sensitive, and specific (Bowersock et al. 1992; Foged et al. 1988).

DNA-based methods

Several species-specific PCRs for detection of *P. multocida* have been developed, but relatively few have been evaluated for use with swine isolates (Dziva et al. 2008). On the basis of published reports, the PCR of Townsend et al. (1998a), which targets the predicted esterase/lipase gene *kmt1*, appears to be most widely used and offers both high specificity and sensitivity. The *kmt1* gene has also been successfully used as a target for loop-mediated isothermal amplification (Sun et al. 2010). This easy-to-perform method has the potential to be used as a screening assay in the field since a thermal cycler is not required and the amplification products can be visualized directly without the need for electrophoresis.

A multiplex capsular PCR (Townsend et al. 2001) has largely replaced serological capsular typing methods. It has a high correlation with serological results, with the exception of antigenically related types A and F for which PCR typing was shown to be more accurate. Because there is often a reduction or complete loss of capsular material when *P. multocida* is passaged on media, fewer isolates may be untypable by PCR than by serology (Arumugam et al. 2011). However, false-positive PCR results may arise from strains that fail to synthesize a capsule due to point mutations or other minor changes in the DNA sequences targeted by the primers.

A number of PCRs perform well for detection of toxigenic swine isolates (Kamp et al. 1996; Lichtensteiger et al. 1996; Nagai et al. 1994). A multiplex PCR for simultaneous identification of *B. bronchiseptica* and both toxigenic and nontoxigenic *P. multocida* is also useful for investigating the etiology of atrophic rhinitis or pneumonia (Register and DeJong 2006).

Immunity

Progressive atrophic rhinitis

PMT is the major protective antigen in PAR, and immunization with PMT toxoid alone alleviates the associated lesions (Foged et al. 1989). Antibody appears to be important in immune defense as evidenced by passive transfer experiments (Chanter and Rutter 1990) and protection of piglets via antibody passively acquired from vaccinated sows (Foged et al. 1989). It is important to provide maternal colostral protection of piglets since exposure most often occurs during the first few weeks of life. Bacterins made from toxigenic strains of

P. multocida elicit antibacterial antibodies but vary in efficacy since they often fail to induce adequate toxin-specific antibodies (Chanter and Rutter 1990). Therefore, vaccines with added toxoid are superior to bacterins alone. Because PMT is difficult to purify and attenuate in large quantities, methods of producing recombinant detoxified PMT have been developed that rely on either truncation (Nielsen et al. 1991) or genetic modification via amino acid substitution at two key sites for detoxification (To et al. 2005). Vaccines for PAR often contain both *B. bronchiseptica* and *P. multocida* to protect against the combined effects of these pathogens. Vaccination does not provide sterilizing immunity but will reduce the pathogen load and significantly diminish or abolish clinical disease and lesions associated with PAR (Riising et al. 2002). A live attenuated strain of *Salmonella typhimurium* engineered to express antigens from PMT and other putative virulence factors of *P. multocida* and *B. bronchiseptica* has recently been developed as a potential vaccine for pneumonic pasteurellosis and PAR (Hur et al. 2014). A protective immune response was achieved in mice following intranasal administration, but the vaccine has yet to be evaluated in pigs.

Pneumonia

Identification of immunogens or immune responses important for protection against pneumonic pasteurellosis in swine has been elusive. Studies comparing parenteral and aerosol delivery of vaccines indicate that a respiratory mucosal immune response is important for clearance of the organism and protection from pulmonary lesions (Müller et al. 2000). Much of the research devoted to vaccines for pneumonic pasteurellosis has been performed in hosts other than swine. The absence of a reliable pulmonary disease model in pigs makes vaccine efficacy difficult to assess. Maternally derived immunity is probably not as important as an adaptive immune response for protection against pneumonia caused by *P. multocida* since this is typically a disease of older swine. Overall, the effectiveness of vaccines in controlling pneumonic pasteurellosis in swine is questionable.

Prevention and control

Progressive atrophic rhinitis

Effective treatment of PAR requires a selected combination of management, environmental, chemotherapeutic, and vaccination procedures. No single approach is equally applicable to all affected herds. The overall aims of treatment are threefold. The first aim is to reduce the prevalence and load of *P. multocida* in young pigs, with or without *B. bronchiseptica*, by sow vaccination, medication of feed, and antibiotic treatment of piglets. The second aim is to treat growing pigs suffering from acute rhinitis in order to reduce the burden of infection and severity of the hypoplastic changes as well as to maintain efficient growth and feed utilization. The final aim is to manipulate housing, ventilation, and management to improve the overall environment.

To reduce vertical transmission from infected dams to suckling offspring, the sow's feed can be medicated during the final month of gestation. Sulfonamides and tetracyclines are most widely used. Increasing resistance to some sulfonamides has been reported for both *B. bronchiseptica* and *P. multocida*, as has an increase in resistance of *P. multocida* to oxytetracycline (El Garch et al. 2016; Furian et al. 2016). Thus, determining the antibiotic susceptibility profile for isolates from a particular herd is prudent. Suckling piglets are best medicated by strategic injections of antibacterial agents in therapeutic dosages through the first 3–4 weeks of life. Drugs used commonly for treatment of *P. multocida* are ampicillin, ceftiofur, enrofloxacin, and tulathromycin. However, because of resistance, ampicillin and ceftiofur should not be the first choice if *B. bronchiseptica* is a factor in PAR. PAR in weaned pigs that leads to marked turbinate atrophy at slaughter can be controlled to some extent by medication of rations or drinking water with tetracyclines, tilmicosin, and trimethoprim/sulfa. Such medication also assists in the maintenance of growth and feed efficiency in the face of active PAR. Medication is most effective when environment and management are improved and vaccination is employed.

Sow vaccination induces a significant degree of passive colostral protection against PAR (Riising et al. 2002). Vaccines often contain a combination of *B. bronchiseptica* and *P. multocida* bacterins. PMT is an important component of vaccines for PAR, and those with added PMT toxoid offer superior protection (Foged et al. 1989; Hsuan et al. 2009; Nielsen et al. 1991; To et al. 2005). Two doses given to sows, the first at 4–8 weeks prior to initial farrowing and the second at 2–4 weeks prior, followed by revaccination 2 weeks before each subsequent farrowing, is an effective means for controlling disease. Vaccination of nonimmune piglets from unvaccinated dams at 1 and 4 weeks of age can be of value; however, passive antibody from the sow can interfere with parenteral vaccination of the piglet. Vaccination of older pigs undoubtedly produces an active humoral response, but its value is debatable since the main effects of the infection occur in younger animals.

Medication and vaccination should never be introduced without concurrent attempts to improve management and husbandry. All-in/all-out systems are favored for farrowing, weaner, and preferably fattener units. Stocking density should be reduced, strict hygiene measures should be implemented, and

ventilation rates should be maintained to reduce airborne bacteria, noxious gases, and dust. The age of the sow herd can be allowed to rise to avoid the introduction of large numbers of infected gilts. Steps should also be taken to reduce stress in young pigs, including minimization of temperature variations, chilling, and drafts. Infection can be eradicated by depopulation and restocking, and clean herds may be maintained free from PAR by isolation, herd monitoring, and the use of clean breeding stock.

Pneumonia

Treatment of pulmonary *P. multocida* infections with antibiotics is challenging because of the difficulty of achieving therapeutic concentrations in consolidated pneumonic lungs. Parenteral antibiotics are preferable including ampicillin, ceftiofur, enrofloxacin, and tulathromycin. In-feed antibiotics, such as chlortetracycline and tilmicosin, are best used on a preventative basis.

Under field conditions the effectiveness of vaccination against pneumonia with *P. multocida* is dubious. Since pneumonic pasteurellosis is often the final stage of enzootic pneumonia or PRDC, which are polymicrobial infections, control of the primary pathogens such as *M. hyopneumoniae*, *B. bronchiseptica*, or PRRSV through vaccination, medication, or management practices may be the most efficient method of controlling the disease. Management changes that reduce the spread of the pathogens involved may have value in decreasing the incidence of pneumonia. These include segregated early weaning, all-in/all-out production, limiting the introduction of outside pigs and determining the health status of the farm from which they are purchased, minimizing mixing and sorting, reducing the size of buildings and pens in which pigs are housed, and reducing animal density.

References

Ackermann MR, DeBey MC, Register KB, et al. 1994. *J Vet Diagn Invest* 6:375–377.

Ackermann MR, Register KB, Stabel JR, et al. 1996. *Am J Vet Res* 57:848–852.

Ahn KK, Lee YH, Ha Y, et al. 2008. *J Comp Pathol* 139:51–53.

Amass SF, Clark LK, van Alstine WG, et al. 1994. *J Am Vet Med Assoc* 204:102–107.

Andreasen M, Bækbo P, Nielsen JP. 2000. *J Vet Med B Infect Dis Vet Public Health* 47:161–171.

Arumugam ND, Ajam N, Blackall PJ, et al. 2011. *Trop Biomed* 28:55–63.

Bethe A, Wieler LH, Selbitz HJ, et al. 2009. *Vet Microbiol* 139:97–105.

Bisgaard M, Petersen A, Christensen H. 2013. *Microbiology* 159:580–590.

Blackall PJ, Fegan N, Pahoff JL, et al. 2000. *Vet Microbiol* 72:111–120.

Bowersock TL, Hooper T, Pottenger R. 1992. *J Vet Diagn Invest* 4:419–422.

Bowles RE, Pahoff JL, Smith BN, et al. 2000. *Aust Vet J* 78:630–635.

Brockmeier SL, Palmer MV, Bolin SR, et al. 2001. *Am J Vet Res* 62:521–525.

Cameron RDA, O'Boyle D, Frost AJ, et al. 1996. *Aust Vet J* 73:27–29.

Cardoso-Toset F, Gómez-Laguna J, Callejo M, et al. 2013. *Vet Microbiol* 167:690–694.

Carter GR. 1955. *Am J Vet Res* 16:481–484.

Carter GR, Rundell SW. 1975. *Vet Rec* 96:343.

Carter GR, Subronto P. 1973. *Am J Vet Res* 34:293–294.

Carvalho LF, Segalés J, Pijoan C. 1997. *Vet Microbiol* 55:241–246.

Chanter N, Rutter JM. 1990. *Vet Microbiol* 25:253–265.

Chanter N, Rutter JM, Luther PD. 1986. *Vet Rec* 119:629–630.

Choi YK, Goyal SM, Joo HS. 2003. *Can Vet J* 44:735–737.

Chung WB, Collins MT, Bäckström LR. 1990. Adherence of *Bordetella bronchiseptica* and *Pasteurella multocida* to swine nasal ciliated epithelial cells *in vitro*. *APMIS* 98:453–461.

Chung WB, Bäckström L, McDonald J, et al. 1993. *Can J Vet Res* 57:190–197.

Ciprián A, Pijoan C, Cruz T, et al. 1988. *Can J Vet Res* 52:434–438.

Cowart RP, Lipsey RJ, Hedrick HB. 1990. *J Am Vet Med Assoc* 196:1262–1264.

Davies RL, MacCorquodale R, Baillie S, et al. 2003. *J Med Microbiol* 52:59–67.

Davies RL, MacCorquodale R, Reilly S. 2004. *Vet Microbiol* 99:145–158.

Desrosiers R. 2011. *Anim Health Res Rev* 12:1–13.

Diekman MA, Scheidt AB, Sutton AL, et al. 1993. *Am J Vet Res* 54:2128–2131.

Dominick MA, Rimler RB. 1988. *Vet Pathol* 25:17–27.

Done JT. 1976. *Vet Rec* 98:23–28.

Done SH. 1991. *Vet Rec* 128:582–586.

Donnio PY, Allardet-Servent A, Perrin M, et al. 1999. *J Med Microbiol* 48:125–131.

Dritz SS, Chengappa MM, Nelssen JL, et al. 1996. *J Am Vet Med Assoc* 208:711–715.

Dugal F, Bélanger M, Jacques M. 1992. *Can J Vet Res* 56:260–264.

Duncan JR, Ross RF, Switzer WP, et al. 1966. *Am J Vet Res* 27:457–466.

Dziva F, Muhairwa AP, Bisgaard M, et al. 2008. *Vet Microbiol* 128:1–22.

El Garch F, de Jong A, Simjee S, et al. 2016. *Vet Microbiol* 194:11–22.

Elling F, Pedersen KB. 1985. *Vet Pathol* 22:469–474.

Ewers C, Lübke-Becker A, Bethe A, et al. 2006. *Vet Microbiol* 114:304–317.
Fablet C, Marois C, Kuntz-Simon G, et al. 2011. *Vet Microbiol* 147:329–339.
Fablet C, Marois C, Dorenlor V, et al. 2012. *Res Vet Sci* 93:627–630.
Flak TA, Heiss LN, Engle JT, et al. 2000. *Infect Immun* 68:1235–1242.
Foged NT, Pedersen KB, Elling F. 1987. *FEMS Microbiol Lett* 43:45–51.
Foged NT, Nielsen JP, Pedersen KB. 1988. *J Clin Microbiol* 26:1419–1420.
Foged NT, Nielsen JP, Jorsal SE. 1989. *Vet Rec* 125:7–11.
Fortin M, Jacques M. 1987. *J Clin Microbiol* 25:938–939.
Franque. 1830. *Dtsch Z Gesammte Tierheilkd* 1:75.
Frymus T, Wittenbrink MM, Petzoldt K. 1986. *J Vet Med B* 33:140–144.
Fuentes M, Pijoan C. 1986. *Vet Immunol Immunopathol* 13:165–172.
Fuentes MC, Pijoan C. 1987. *Am J Vet Res* 48:1446–1448.
Furian TQ, Borges KA, Laviniki V, et al. 2016. *Braz J Microbiol* 47:210–216.
García N, Fernández-Garayzábal JF, Goyache J, et al. 2011. *Vet Rec* 169:362.
Gardner IA, Kasten R, Eamens GJ, et al. 1994. *J Vet Diagn Invest* 6:442–447.
Gatlin CL, Jordan WH, Shryock TR, et al. 1996. *Can J Vet Res* 60:121–126.
Goodwin RF, Chanter N, Rutter JM. 1990. *Vet Rec* 126:452–456.
Halloy DJ, Gustin PG, Bouhet S, et al. 2005a. *Toxicology* 213:34–44.
Halloy DJ, Kirschvink NA, Mainil J, et al. 2005b. *Vet J* 169:417–426.
Hamilton TD, Roe JM, Hayes CM, et al. 1999. *Clin Diagn Lab Immunol* 6:199–203.
Harel J, Côté S, Jacques M. 1990. *Can J Vet Res* 54:422–426.
Harper M, John M, Turni C, et al. 2015. *J Clin Microbiol* 53:477–485.
Heddleston KL, Gallagher JE, Rebers PA. 1972. *Avian Dis* 16:925–936.
Høie S, Falk K, Lium BM. 1991. *Acta Vet Scand* 32:395–402.
Hsuan SL, Liao CM, Huang C, et al. 2009. *Vaccine* 27:2923–2929.
Hur J, Byeon H, Lee JH. 2014. *Can J Vet Res* 78:297–303.
Il'ina ZM, Zasukhin MI. 1975. Sibirskii Nauchno-Issledovatel'skii Veterinaryni Institut. *Omsk* 25:76.
Isaacson RE, Trigo E. 1995. *FEMS Microbiol Lett* 132:247–251.
Iwamatsu S, Sawada T. 1988. *J Jpn Vet Med Asoc* 50:1200–1206.
Jacques M, Parent N, Foiry B. 1988. *Can J Vet Res* 52:283–285.
Jacques M, Kobisch M, Bélanger M, et al. 1993. *Infect Immun* 61:4785–4792.
Jacques M, Bélanger M, Diarra MS, et al. 1994. *Microbiology* 140:263–270.
de Jong MF, Borst GH. 1985. *Vet Rec* 116:167.
de Jong MF, Nielsen JP. 1990. *Vet Rec* 126:93.
Kalorey DR, Yuvaraj S, Vanjari SS, et al. 2008. *Comp Immunol Microbiol Infect Dis* 31:459–465.
Kamp EM, Bokken GC, Vermeulen TM, et al. 1996. *J Vet Diagn Invest* 8:304–309.
Kielstein P. 1986. *J Vet Med B* 33:418–424.
Lariviere S, Leblanc L, Mittal KR, et al. 1993. *J Clin Microbiol* 31:364–367.
Larochelle R, Martineau-Doizé B. 1990. *Acta Anat* 139:214–219.
Larochelle R, Martineau-Doizé B. 1991. *Am J Anat* 191:103–111.
Letellier A, Dubreuil D, Roy G, et al. 1991. *Am J Vet Res* 52:34–39.
Lichtensteiger CA, Steenbergen SM, Lee RM, et al. 1996. *J Clin Microbiol* 34:3035–3039.
Little TW, Harding JD. 1980. *Br Vet J* 136:371–383.
Lugtenberg B, van Boxtel R, Evenberg D, et al. 1986. *Infect Immun* 52:175–182.
Mackie JT, Barton M, Kettlewell J. 1992. *Aust Vet J* 69:227–228.
Magyar T, Kovács F, Donkó T, et al. 2003. *Acta Vet Hung* 51:485–491.
Magyar T, Donkó T, Repa I, et al. 2013. *BMC Vet Res* 9:222.
Marandi M, Mittal KR. 1996. *Vet Microbiol* 53:303–314.
Marois C, Cariolet R, Morvan H, et al. 2008. *Vet Microbiol* 129:325–332.
Marois C, Fablet C, Gaillot O, et al. 2009. *J Appl Microbiol* 107:1830–1836.
Martineau-Doizé B, Frantz JC, Martineau GP. 1990. *Anat Rec* 228:237–246.
Muirhead MR. 1979. *Br Vet J* 135:497–508.
Müller W, Schneider J, Von Dossow A, et al. 1992. *Monatsh Vet Med* 47:253–256.
Müller G, Köhler H, Diller R, et al. 2000. *Vaccine* 19:751–757.
Nagai S, Someno S, Yagihashi T. 1994. *J Clin Microbiol* 32:1004–1010.
Nakai T, Kume K, Yoshikawa H, et al. 1988. *Infect Immun* 56:234–240.
Neumann R, Mehlhorn G, Buchholz I, et al. 1987. *J Vet Med B* 34:241–253.
Nielsen NC, Riising HJ, Bille N. 1976. Experimental reproduction of atrophic rhinitis in pigs reared to slaughter weight. In Proceedings, International Pig Veterinary Science Congress, p. 202.
Nielsen JP, Foged NT, Sørensen V, et al. 1991. *Can J Vet Res* 55:128–138.
Okay S, Kurt Kızıldoğan A. 2015. *Gene* 567:58–72.
Ono M, Okada M, Namimatsu T, et al. 2003. *J Comp Pathol* 129:251–258.
Opriessnig T, Giménez-Lirola LG, Halbur PG. 2011. *Anim Health Res Rev* 12:133–148.
Orth JH, Aktories K. 2012. *Curr Top Microbiol Immunol* 361:73–92.
Pedersen KB, Barfod K. 1981. *Nord Vet Med* 33:513–522.
Pedersen KB, Elling F. 1984. *Comp Pathol* 94:203–214.

Pedersen KB, Nielsen NC, eds. 1983. Atrophic rhinitis of pigs. In Commission European Communities Report, EUR 8643 EN. Luxembourg, p. 205.
Peng Z, Liang W, Wang Y, et al. 2017. *Vet Microbiol* 198:23–33.
Pennings AM, Storm PK. 1984. *Vet Microbiol* 9:503–508.
Petersen SK, Foged NT. 1989. *Infect Immun* 57:3907–3913.
Pijoan C, Fuentes M. 1987. *J Am Vet Med Assoc* 191:823–826.
Pijoan C, Ochoa G. 1978. *J Comp Pathol* 88:167–170.
Pijoan C, Trigo F. 1990. *Can J Vet Res* 54(Suppl): S16–21.
Pors SE, Hansen MS, Bisgaard M, et al. 2011. *Vet Microbiol* 150:160–166.
Pósa R, Donkó T, Bogner P, et al. 2011. *Can J Vet Res* 75:176–182.
Pullinger GD, Bevir T, Lax AJ. 2004. *Mol Microbiol* 51:255–269.
Register KB, DeJong KD. 2006. *Vet Microbiol* 117:201–210.
Riising HJ, van Empel P, Witvliet M. 2002. *Vet Rec* 150:569–571.
Rimler RB, Rhoades KR. 1987. *J Clin Microbiol* 25:615–618.
Rimler RB, Register KB, Magyar T, et al. 1995. *Vet Microbiol* 47:287–294.
Risco D, Fernández-Llario P, Cuesta JM, et al. 2013. *J Vet Diagn Invest* 25:791–794.
Robertson JF, Wilson D, Smith WJ. 1990. *Anim Prod* 50:173–182.
Rúbies X, Casal J, Pijoan C. 2002. *Vet Microbiol* 84:69–78.
Rutter JM, Mackenzie A. 1984. *Vet Rec* 114:89–90.
Rutter JM, Taylor RJ, Crighton WG, et al. 1984. *Vet Rec* 115:615–619.
Smith AW, Vedros NA, Akers TG, et al. 1978. *J Am Vet Med Assoc* 173:1131–1133.
Sun D, Wang J, Wu R, et al. 2010. *Vet Res Commun* 34:649–657.
Thomson CM, Chanter N, Wathes CM. 1992. *Appl Environ Microbiol* 58:932–936.
Thomson JR, Bell NA, Rafferty M. 2007. *Pig J* 60:15–25.
To H, Someno S, Nagai S. 2005. *Am J Vet Res* 66:113–118.
Townsend KM, Frost AJ, Lee CW, et al. 1998a. *J Clin Microbiol* 36:1096–1100.
Townsend KM, O'Boyle D, Phan TT, et al. 1998b. *Vet Microbiol* 63:205–215.
Townsend KM, Boyce JD, Chung JY, et al. 2001. *J Clin Microbiol* 39:924–929.
Ujvári B, Szeredi L, Pertl L, et al. 2015. *Acta Vet Hung* 63:141–156.
USDA. 2015. Swine 2012, Part I: Baseline reference of swine health and management in the United States, 2012. Fort Collins, CO: USDA-APHIS-VS, CEAH, #663.0814.
Varga Z, Sellyei B, Magyar T. 2007. *Acta Vet Hung* 55:425–434.
Vena MM, Blanchard B, Thomas D, et al. 1991. *Ann Rech Vet* 22:211–218.
Wilson BA, Ho M. 2013. *Clin Microbiol Rev* 26:631–655.
Zhao G, Pijoan C, Murtaugh MP. 1993. *Can J Vet Res* 57:136–138.

58

Proliferative Enteropathy

Fabio A. Vannucci, Connie J. Gebhart, and Steven McOrist

Relevance

Proliferative enteropathy (PE), also known as ileitis, is an infectious disease caused by the obligate intracellular bacterium *Lawsonia intracellularis*. It is characterized by thickening of the intestinal mucosa due to intestinal crypt epithelial cell proliferation (McOrist et al. 1995a). Lesions are most often in the ileum but may also be in the jejunum and colon. Crypt epithelial cell proliferation is directly associated with intracytoplasmic *L. intracellularis* infection and replication. Mild to severe diarrhea and weight loss are the major clinical signs observed in clinically affected animals. Subclinical infection (i.e. lacking diarrhea) is common in which infected pigs have less extensive lesions that are associated with a commensurate retardation in growth rates (Jacobson et al. 2003).

Two major clinicopathologic forms of PE have been recognized, acute and chronic (Rowland and Lawson 1975). Proliferative hemorrhagic enteropathy (PHE) is an acute form of disease that may be associated with bloody diarrhea and high mortality, and that is characterized by mucosal proliferation and hemorrhage. Porcine intestinal adenomatosis (PIA) is uncomplicated chronic PE characterized by marked corrugated thickening of the mucosa. Chronic PE that is complicated by opportunistic bacteria resulting in fibrinonecrotic exudate adherent to thickened corrugated mucosa is known as necrotic enteritis (NE).

PE is a common and an important enteric disease worldwide. Globally, it is estimated that 96% of farm sites are infected, with approximately 30% of weaner-to-finisher pigs having lesions of variable severity and duration (McOrist et al. 2003; Stege et al. 2000). Biester and Schwarte (1931) first described lesions of PE in pigs. By studying field outbreaks of PE, Rowland and Lawson (1974) found that intracellular bacteria were consistently observed within the abnormal proliferating mucosal epithelial cells by silver staining or ultrastructural methods. The identity of these bacteria and their etiologic role in PE were resolved in 1993 with successful co-culture of the intracellular organism in a rat enteric cell line and subsequent reproduction of the disease by inoculation of pigs with a pure culture of the organism (Lawson et al. 1993; McOrist et al. 1993). Its taxonomic position was clarified by phenotypic and genetic characterization (Gebhart et al. 1993), a new genus and species were established, and the organism was named *Lawsonia intracellularis* (McOrist et al. 1995a).

Economic losses due to PE have been estimated from its negative impacts on slaughter weight, feed conversion efficiency, space utilization, breeding problems and morbidity–mortality effects, totaling from US $1 to $5 per affected growing pig (McOrist 2005; McOrist et al. 1997b; Veenhuizen et al. 2002). The impact is likely higher since estimates are based on clinical cases and do not include subclinical cases.

Etiology

Lawsonia intracellularis is the sole species in the bacterial genus *Lawsonia* (McOrist et al. 1995a) and the only cause of PE. It is an obligate intracellular bacterium that grows *in vivo* in the cytoplasm of intestinal epithelial cells and *in vitro* in cultures of intestinal and fibroblast-derived cell lines (Guedes and Gebhart 2003a; Lawson et al. 1993). It has not been cultivated in cell-free media probably due to unique metabolic requirements that are not well understood (Schmitz-Esser et al. 2008).

Lawsonia intracellularis is an obligately intracellular, gram-negative, non-spore-forming, microaerophilic, curved or vibroid-shaped rod (Figure 58.1). It is 1.25–1.75 µm in length and 0.25–0.43 µm in width and has a trilaminar outer envelope (McOrist et al. 1995a). It has a unipolar flagellum and exhibits darting motility *in vitro* upon escape from infected enterocytes (Lawson and Gebhart 2000). *In vivo* it locates in the apical cytoplasm of intestinal epithelial cells and is commonly associated

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

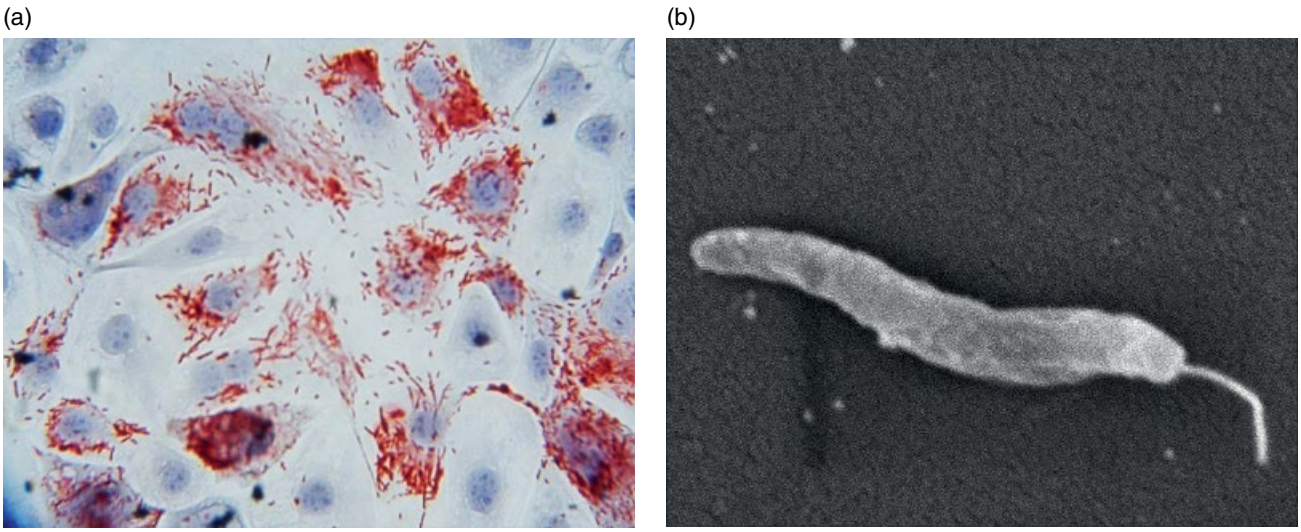


Figure 58.1 *Lawsonia intracellularis*. (a) Immunocytochemistry. Co-culture of intestinal porcine enterocyte cells (IPEC-J2) in adherent monolayer and intracytoplasmic *L. intracellularis*. (b) Scanning electron microscopy. Vibroid-shaped rod morphology and unipolar flagellum of *L. intracellularis*.

with free ribosomes and mitochondria (Johnson and Jacoby 1978).

Lawsonia intracellularis is classified as a member of the family *Desulfovibrionaceae* in the delta subdivision of *Proteobacteria* (Gebhart et al. 1993). Based on the DNA sequence of the 16S ribosomal gene, it is most closely related to *Bilophila wadsworthia*, a free-living anaerobic human pathogen (Sapico et al. 1994), and to *Desulfovibrio desulfuricans*, a sulfate-reducing bacterium (Gebhart et al. 1993). Evolutionary analysis suggests that *L. intracellularis* has gained cell-dependent respiration and lost its sulfate reduction capacity (Schmitz-Esser et al. 2008). The bacterium has a small chromosome and three plasmids, totaling 1.72 million bp and 1324 protein encoding regions. It possesses low G + C% content and significant presence of heat shock proteins that are common in other symbiont intracellular bacteria (Dale et al. 1998).

Since the first reports of cultivation and maintenance of *L. intracellularis in vitro* (Lawson et al. 1993), various cell lines have been shown to support growth, including insect and avian cells (Guedes et al. 2002a; McOrist et al. 1995b). Regardless of the cell lineage, the bacteria require dividing cells in a specific microaerophilic atmosphere for cultivation (Lawson et al. 1993; Vannucci et al. 2012c). Culture attempts have regularly been more successful from PHE cases than from chronic PE cases, likely due to the lower numbers of contaminating enteric organisms present in the affected ilea. Cultivation of *L. intracellularis* has been conventionally achieved in a static adherent monolayer in a humidified tri-gas incubator with 83.2% nitrogen, 8.8% carbon dioxide, and 8% oxygen at 37 °C (Lawson et al. 1993; McOrist et al. 1995b). Suspension cultures have also been used for large-scale cultivation for vaccine production (Kroll et al. 2004).

Clinical disease and lesions typical of PE have been reproduced reliably in pigs given oral inocula consisting of pure cultures of *L. intracellularis* derived at low passages (4–20 times) in cell culture (Guedes and Gebhart 2003b; McOrist et al. 1993, 1996b; Vannucci et al. 2013a), but not when given cultures passed 40 times *in vitro* (Vannucci et al. 2013a). It is therefore suggested that attenuation of virulence factors within *L. intracellularis* occurs between passages 20 and 40, probably due to gene deletions or mutation. However standard DNA-based typing techniques such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and variable number tandem repeat (VNTR) have shown identical genotypes in homologous isolates at low and high passages, so the exact type of gene modification in high-passage isolates is not known.

Public health

There is no evidence of *L. intracellularis* infection in humans. A surveillance study in children living on European pig farms failed to find *L. intracellularis* DNA in their fecal samples (Jacobson et al. 2007). Investigations of Crohn's disease and other mucosal inflammatory conditions of humans have consistently failed to find typical PE lesions or *L. intracellularis* (Crohn and Turner 1952; Michalski et al. 2006).

Epidemiology

PE is endemic in domesticated swine herds and has also been described in feral swine populations worldwide (Baroch et al. 2015; McGregor et al. 2015; Tomanova et al. 2002; Yeh 2014; Zlotowski et al. 2008). Epidemiological modeling has assessed the risk of introduction of PE from feral swine into commercial herds as being greatest in free-range production systems (Pearson et al. 2016), but transmission has not been documented.

Lawsonia intracellularis infection and proliferative intestinal lesions have been occasionally reported in other species, including hamsters (Cooper et al. 1997b), rabbits (Umemura et al. 1982), ferrets (Fox et al. 1994), guinea pigs (Elwell et al. 1981), foxes (Landsverk 1981), dogs (Collins et al. 1983), rats (Vandenberghe and Marsboom 1982), mice (Abshier et al. 2001), sheep (Cross et al. 1973), deer (Drolet et al. 1996), emus (Lemarchand et al. 1997), ostriches (Cooper et al. 1997b), turkeys (Moura-Alvarez et al. 2014), chickens (Ohta et al. 2016), and nonhuman primates (Klein et al. 1999). The presence of *L. intracellularis* in fecal samples of various wild species such as jackrabbits, skunks, opossums, raccoons, and coyotes has also been reported (Hossain et al. 2016; Pusterla et al. 2008). While PE has been only sporadically described in a wide range of species, patterns of disease similar to those observed in pigs have been widely reported in laboratory hamsters and have increasingly been reported in young foals (Pusterla et al. 2009).

The role of other animal species as potential sources of *L. intracellularis* for infection and disease in swine is unclear. Very high degrees (>99%) of similarity among isolates from different animal species have been demonstrated based on 16S ribosomal DNA sequences (Cooper et al. 1997a). However, there is some data that suggest the potential for animal species-adapted subtypes of *L. intracellularis* (Vannucci et al. 2012b). Typical proliferation of *L. intracellularis*-infected intestinal epithelial cells has been reproduced in a variety of animal species by inoculation with species-specific *L. intracellularis* isolates (Jacoby 1978; McOrist et al. 1993; Pusterla et al. 2010). However the clinicopathology of disease varies between some animal species (Deprez et al. 2005; Duhamel and Wheeldon 1982; Frazer 2008; Jacoby et al. 1975; Vannucci et al. 2010). Differences in virulence have been reported when one animal species is inoculated with an *L. intracellularis* isolate from another species (Jasni et al. 1994a; Murakata et al. 2008; Vannucci et al. 2012b; Viott et al. 2013). Recently a genomic island associated with the presence of a prophage has been detected in a non-extensive sampling of porcine but not equine isolates (Vannucci et al. 2013c), confirming a potential difference in genotypes of *L. intracellularis* isolated from these animal species. Taken together, these data suggest that there may be species-adapted genotypes or subspecies of *L. intracellularis*. More work is needed to determine the extent of interspecies differences in *L. intracellularis* and the potential impact on cross-species transmission and disease.

High prevalence of *L. intracellularis* is reported in commercial swine herds mainly by serology and fecal PCR. Transmission is by the fecal–oral route from infected pigs or contaminated environs to noninfected pigs. The infectious dose is relatively low (Guedes et al. 2003; McOrist et al. 1993), and fecal excretion may be high in infected pigs (Guedes et al. 2002a; Smith and McOrist 1997). Disease manifests differently between farms, based on management systems as they impact primarily dose and immunity in exposed pigs. On farms with a continuous pig flow between different pig ages/farm areas/housing locations, infection usually occurs a few weeks after weaning, presumably when maternal antibodies fade. This dynamic can be delayed by the use of effective antimicrobials in the first weeks post weaning. In these cases, clinical disease is usually later in the growing and finishing phases. Once infection starts in a population, it amplifies through fecal–oral cycling (Stege et al. 2004). When organic bedding is used and/or there is group housing of breeding animals, infection may amplify faster.

An intermittent pattern of PE occurs in weaner and grower/finishers on farms with periodic oral antibiotic usage. On farm systems with distinct separation of groups of post weaning and breeding pigs by age and site (all-in/all-out multisite systems), *L. intracellularis* infection is usually delayed in grower/finishers until they are 14–20 weeks of age and occurs rarely in breeding stock (Bronsvoort et al. 2001; Marsteller et al. 2003). This pattern is accentuated in the United States, where weaner–nursery pigs may receive quinoxaline antimicrobials, forestalling early infections. Application of strict disinfection protocols and all-in/all-out procedures are critical to decrease the prevalence of PE (Dors et al. 2015).

It is likely that the environment of most pig farms contains a sustained level of *L. intracellularis* in the residual fecal material or organic bedding in buildings or on equipment or other fomites. *L. intracellularis* can remain viable in feces at 5–15 °C (41–59 °F) for 2 weeks (Collins et al. 2000). This may act to reintroduce the infection to new groups of pigs. The infection can build up slowly or quickly, with variations in the time of disease onset on different farms and importantly on the same farm between different groups in the same building or pens (Hammer 2004; Philips et al. 1998). Transmission of fecal matter from contaminated areas to other areas of a farm, such as those containing breeding animals, would be expected to occur more commonly on single-site farms. Modes of this transmission probably include transfer of feces on boots or other fomites as well as possible transfer via insects and rodents in contact with pig feces

(Friedman et al. 2008). A recent study experimentally demonstrated subclinical infection in pigs exposed to fecal samples from infected mice (Gabardo et al. 2016). Therefore, rodents in particular may have a critical role in introduction and maintenance of *L. intracellularis* in swine herds (Backhans et al. 2013; Collins et al. 2011). Application of rodent control strategies is an important factor in controlling PE. Studies tracking PE infection on breeding farms have indicated that infected gilts or sows do not readily transmit the infection to their progeny in the farrowing area (Guedes et al. 2002a; Jacobson et al. 2010).

Acute and chronic PE continue to be an important problem in herds with high health status. The absence of clinical PE in conventional swine herds, even over a period of years, is no guarantee of freedom from *L. intracellularis* infection. Animals from such herds may be responsible for the introduction of PE into a hitherto uncontaminated environment or naïve population, often followed by an explosive outbreak of acute hemorrhagic PE and later by endemic chronic PE. Examples where this has typically occurred include boar and gilt performance testing stations, gilts within breeding programs that involve transportation to new units, and the movement and mixing of boars and gilts into breeding groups (Friendship et al. 2005). However, these outbreaks have become noticeably less common following the wide usage of a live attenuated *Lawsonia* vaccine in breeding-age pigs prior to mixing or moving.

Pathogenesis

Experimental infections using pure cultures of porcine-origin *L. intracellularis* as oral inocula for conventional pigs or gnotobiotic pigs pre-dosed with a minimal bacterial flora of nonpathogenic enteric species have resulted in reproduction of the specific and characteristic lesions of PE (McOrist et al. 1993, 1994). Pathogenesis of PE has been studied using *in vitro* and *in vivo* experimental models. However, the typical proliferation of *L. intracellularis*-infected intestinal epithelial cells observed *in vivo* has not been reproduced in any cell type *in vitro* (Lawson et al. 1993; Vannucci et al. 2012c). Therefore, comprehensive studies on the progression of lesions of PE have been conducted in experimental infection models primarily in pigs and hamsters (Guedes and Gebhart 2003a; Jasni et al. 1994b).

Exposure of pigs to crude or partially filtered, homogenized diseased mucosa resulted in reproduction of specific intestinal lesions and clinical disease in some early disease reproduction trials (Mapother et al. 1987; McOrist and Lawson 1989; Roberts et al. 1977). The difficulties in routine culture of *L. intracellularis* have resulted in this strategy continuing as a model for reproduction of PE in conventional pigs (Boutrup et al. 2010; Guedes and Gebhart 2003b; Winkelman et al. 2002).

Naïve pigs of a wide age range (neonates to grower/finishers) are susceptible to oral challenge. In typical oral inoculation studies in naïve pigs inoculated with approximately 10^8 *L. intracellularis* bacteria/mL, infection, proliferative lesions, and fecal shedding begin at 1 week, peak at 3 weeks, and persist for approximately 4 weeks in most pigs (Figure 58.2). In some, fecal shedding may persist for 12 weeks (Guedes et al. 2002a; Smith and McOrist 1997). Lesions in the large intestines appear 1–2 weeks after those in the small intestines (Guedes and Gebhart 2003b).

Lawsonia intracellularis organisms have the ability to infect mature enterocytes as well as immature crypt epithelial cells. They are first observed in the cytoplasm of mature enterocytes on the tips of small intestinal villi 12 hours following oral inoculation but are observed as a persistent infection in the cytoplasm of the poorly differentiated crypt cells from 5 to 28 days post infection (Boutrup et al. 2010). These infected crypt epithelial cells continue to proliferate and fail to mature, resulting in the characteristic adenomatous proliferation of an immature mucosa that is the hallmark of PE. In general, no significant inflammatory response occurs while the bacteria persist within the intestinal epithelium. Loss of body protein and amino acids into the intestinal lumen and the reduced nutrient absorption by the intestinal mucosa lacking mature enterocytes are the likely causes of the reduction in weight gain and feed conversion efficiency seen in pigs and hamsters affected with chronic uncomplicated PE lesions (Gogolewski et al. 1991; Rowan and Lawrence 1982; Vannucci et al. 2010).

During the first 12 hours after infection, before the bacteria reach the enterocyte, *L. intracellularis* must survive low gastric pH. Functional analysis of the *L. intracellularis* genome revealed the genes of two systems known to play a crucial role in the maintenance of pH homeostasis, the glutamate decarboxylase (GAD) system and the F0F1-ATPase operon (Vannucci et al. 2013c). There is currently no mechanistic evidence demonstrating the activity of these acid-tolerant systems in *L. intracellularis*; however, their roles in responding to acid stress has been well described among other enteric pathogens (Bearson et al. 1997).

Survival of *L. intracellularis* in the intestinal microenvironment is influenced by the intestinal microbiota. Germ-free pigs are not susceptible to infection using pure cultures of *L. intracellularis* (McOrist et al. 1994), whereas pigs exposed to intestinal material from infected animals develop the disease (McOrist and Lawson 1989). Diet composition may also influence infection in hamsters and pigs (Boesen et al. 2004; Jacoby and Johnson 1981). Pelleted diets are associated with higher levels of *L. intracellularis* in ileal microbiota compared with

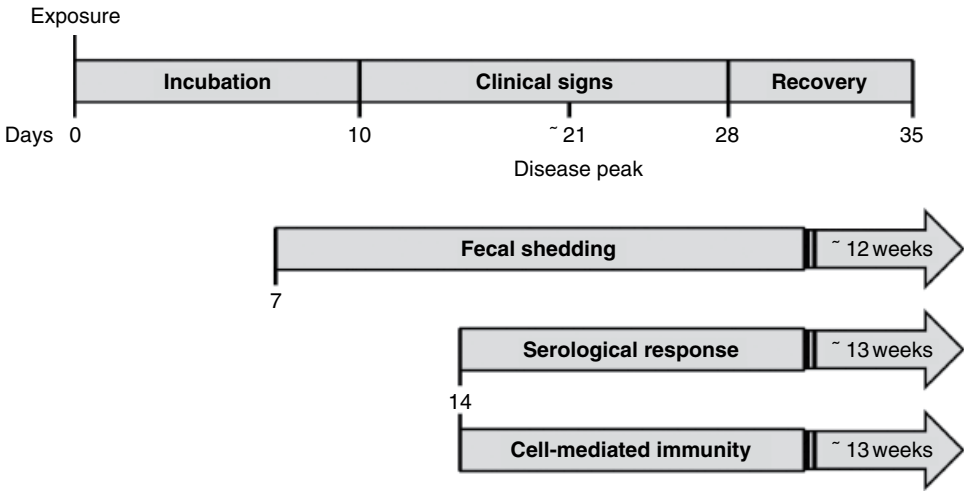


Figure 58.2 Porcine proliferative enteropathy. Typical clinical course, fecal shedding, and immune response of proliferative enteropathy in naïve pigs experimentally infected with *L. intracellularis*. *Source:* Adapted from Gebhart and Guedes (2010). Reproduced with permission of John Wiley and Sons.

non-pelleted diets (Mølbak et al. 2008). The mechanisms by which diet or intestinal microbiota influences *L. intracellularis* remain unclear.

Internalization of *L. intracellularis* has been described *in vitro*. The bacterium is found in close association with the cell surface after 10 minutes, internalized within transient membrane-bound vacuoles by 3 hours, and released from the vacuoles to live and proliferate in the apical cytoplasm thereafter (Johnson and Jacoby 1978; McOrist et al. 1995b). Inhibitors of host cell growth reduce bacterial multiplication *in vitro*, suggesting that actively dividing cells enhance bacterial replication (Lawson et al. 1995).

Virulence factors associated with adhesion and entry into enterocytes have not been characterized. However, bacterial invasion does not depend on the viability of the *L. intracellularis* cells. Cell entry may be partially mediated or enhanced by a type III secretion system, of which the genes and associated proteins are characterized and conserved among *L. intracellularis* isolates (Alberdi et al. 2009).

The microaerophilic properties of *L. intracellularis* and its proximity to the host mitochondria create a paradox for intracellular survival of the bacterium due to the oxidative phosphorylation supported by the continuous transport of oxygen through the mitochondrial membrane (Koyama et al. 1989). In order to survive in this microenvironment, *L. intracellularis* harbors a sophisticated oxidative protection mechanism involving Cu–Zn superoxide dismutase and dioxygenases (Vannucci et al. 2012a, 2013b). Additionally *L. intracellularis* seems to take advantage of this oxidative intracellular microenvironment while utilizing the host energy by expressing ATP/ADP translocase (Schmitz-Esser et al. 2008). This enzyme catalyzes the exchange of bacterial ADP for host ATP and allows the bacterium to exploit its hosts' energy pool, a process referred to as energy parasitism. Intracellular survival and/or pathogenesis of *L. intracellularis* may also be aided by high levels of *in vivo* expression of genes sharing homology with the *Salmonella* pathogenicity island 2 (Vannucci et al. 2013b), known to be pivotal to the intracellular survival and virulence of *Salmonella* in mammals.

Lesions of PE are restricted to the intestinal epithelium, and the dissemination of active infection to other organs has not been reported. The primary mode of cell-to-cell transmission of *L. intracellularis* is by the proliferation of *L. intracellularis*-infected crypt cells, resulting in infected progeny cells that continue the cycle (Jacoby and Johnson 1981; Vannucci and Gebhart 2014). Detection of bacterial antigens in mesenteric lymph nodes and tonsillar crypt cells has been demonstrated but attributed to the carriage of *L. intracellularis* antigens by infected macrophages (Gebhart and Guedes 2010). Movement of *L. intracellularis* through the lamina propria has been speculated as an additional route for spreading infection from crypt to crypt in the intestinal tract (Boutrup et al. 2010; Jensen et al. 2006).

The intriguing mechanisms whereby intracytoplasmic *L. intracellularis* causes infected cells to fail to mature and to undergo mitosis and form the hyperplastic to adenomatous crypts are not fully understood. Activation of DNA transcription, protein biosynthesis, and genes acting on the G_1 phase of the host cell cycle have been identified in infected cells *in vivo* and are potentially involved on the pathogenesis of PE (Vannucci et al. 2012a). Cell proliferation during *L. intracellularis* infection showed no association with an observed concurrent reduction of apoptosis (Guedes et al. 2017).

The dominance of the immature phenotype of proliferating crypt epithelial cells in PE, with their characteristic morphology and staining, means that fewer membrane transporters related to digestion and nutrient acquisition are operative (Jacobson et al. 2011; Vannucci et al. 2013a). This lack of membrane transporters in *Lawsonia*-infected enterocytes includes those involved in the absorption of carbohydrates, amino acids, lipids, and vitamin B_{12}. Thus reduced nutrient absorption by the immature intestinal mucosa is likely a significant cause of reduction in weight gain and feed conversion efficiency seen in PE-affected pigs (Gogolewski et al. 1991; Grützner et al. 2015; Rowan and Lawrence 1982; Vannucci et al. 2010).

Degenerative, necrotic, and reparative changes may superimpose the primary lesion of crypt epithelial proliferation over time. The acute form of PE, PHE, is marked by severe bleeding into the lumen of the intestine, but with typical underlying cell proliferation typical of PE. Hemorrhage occurs concurrently with the widespread degeneration and desquamation of many epithelial cells and leakage from the capillary bed. The pathogenesis of this more acute lesion is not well characterized.

Lawsonia intracellularis may also modulate the immune response, facilitating chronic infection of intestinal epithelial cells (see discussion in immunology section below).

Clinical signs

Three clinically different forms of PE are observed most commonly: acute, chronic, and subclinical. The acute form (PHE) occurs more commonly in young adults 4–12 months of age, such as breeding gilts. Clinical presentation is mainly characterized by sudden death associated with anemia and hemorrhagic diarrhea. Black tarry feces are often the first visible clinical sign and these may become loose. However, some animals die without fecal abnormality and show only marked pallor. Around half of the animals clinically affected may die with the remainder recovering over some weeks. Pregnant animals that are clinically affected may abort within 6 days of the onset of clinical signs (McOrist et al. 1999).

The chronic form of PE (PIA) is the most common and is observed predominantly in weaned pigs 6–20 weeks of age. Affected pigs have mild to moderate loose-to-watery grey-green diarrhea, variable anorexia, and failure to sustain growth despite normal feed intake. Fecal blood and mucus are not observed. Within the same groups, there are typically subclinically affected pigs that have normal stools, reduced weight gain, and less severe microscopic mucosal proliferative lesions.

Severe chronic cases can be complicated by opportunistic bacterial infections resulting in NE. These animals display a remarkable loss of condition and persistent diarrhea, sometimes with liquid feces containing fibrinonecrotic casts. Necrotic enteritis occurs more often in conditions that facilitate fecal–oral cycling, such as use of straw bedding or solid flooring with poor sanitation, thus increasing the likelihood of complicating opportunistic diseases such as enteric salmonellosis.

In most cases of uncomplicated PE, recovery occurs around 5 weeks after the onset of clinical signs with a return of appetite and growth rate to normal levels. Although these pigs usually progress to slaughter weight despite having PE lesions (Suto et al. 2004), average daily weight gain and feed efficiency are typically reduced, and days to market are increased. Chronically affected pigs have an average reduction in daily weight gain of 6–20% (Gogolewski et al. 1991; McOrist et al. 1996b, 1997a).

Lesions

Thickening of the intestinal mucosa due to crypt epithelial cell proliferation is the primary and most remarkable gross lesion of PE. In chronically affected growing pigs (PIA), gross lesions are most commonly observed in the terminal ileum and cecum, and with increasing severity, they typically extend to include the jejunum, cecum, and spiral colon. In mild cases, the earliest distinguishable gross lesions are often in the terminal ileum, approximately 10 cm oral from the ileocecal valve. Uncommonly, lesions may be localized to only one or more foci in the jejunum. Regardless of location, affected mucosa is variably thickened and thrown into longitudinal-to-transverse folds resembling cerebral cortex (i.e. cerebriform) with a moist but not mucoid surface (Figure 58.3). In severe areas, the intestinal wall is obviously thickened when viewed externally, and the overall intestinal diameter is increased. In some cases, there may be sparse multifocal adherent fibrinonecrotic mucosal exudate. Careful examination is required since contracted intestines due to post mortem peristalsis may result in relative thickening of the mucosa that can be easily misinterpreted as mild PE. Additionally, nonspecific submucosal edema found in many types of enteritis can enhance the normal mucosal folds, especially overlying Peyer's patches, and also be misinterpreted as mild PE.

Necrotic enteritis may be observed as a loosely or tightly adherent fibrinonecrotic membrane on the surface of the proliferated intestinal mucosa (Figure 58.3). Occasionally, this membrane may detach and be found intact (a cast) or in pieces in the contents of the large intestines or in diarrheic feces.

In acute hemorrhagic cases (PHE), the intestine is dilated and the wall is thickened by serosal edema and mucosal proliferation. The lumen of the ileum usually contains one or more formed blood clots combined with fibrinonecrotic debris (fibrinohemorrhagic cast;

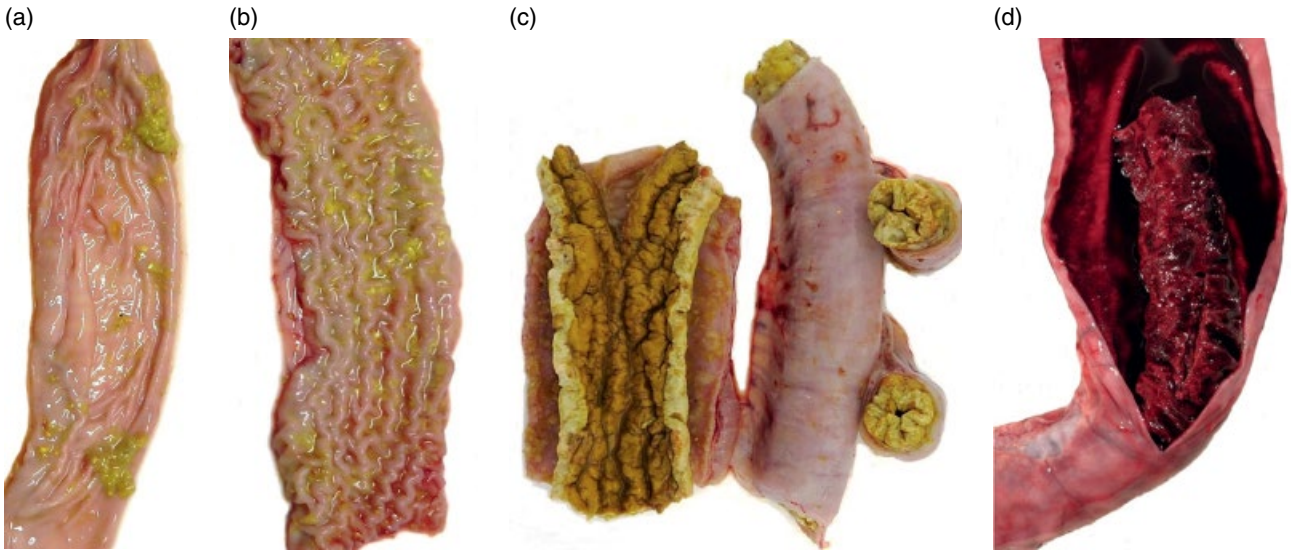


Figure 58.3 Porcine proliferative enteropathy, macroscopic lesions. (a) Normal small intestine. (b) Typical chronic porcine intestinal adenomatosis (PIA) characterized by thickening and corrugation of the intestinal mucosa. (c) Necrotic enteritis due to opportunistic bacterial aggravation of the chronic intestinal adenomatosis. (d) Proliferative hemorrhagic enteropathy (PHE) with fibrinohemorrhagic cast in the intestinal lumen.

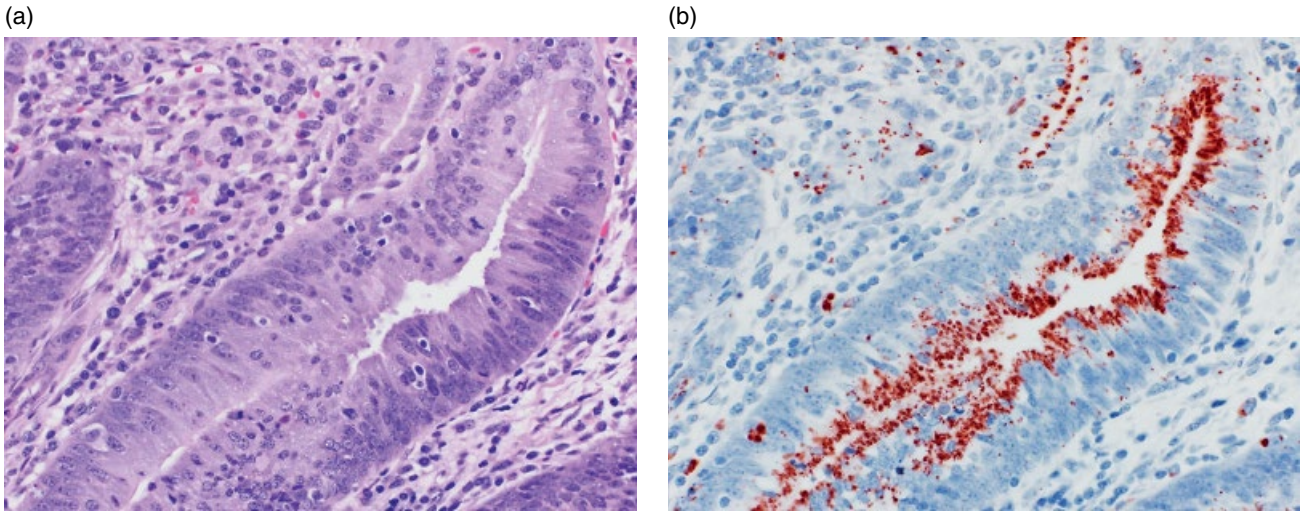


Figure 58.4 Porcine proliferative enteropathy, microscopic lesions. (a) H&E stain. Marked enlargement of an affected intestinal crypt composed of multiple epithelial layers and lacking goblet cells (400× magnification). (b) Immunohistochemistry. Affected intestinal crypt showing large numbers of *L. intracellularis*-specific antigen (maroon staining) predominantly in the apical cytoplasm of proliferative enterocytes (400× magnification).

Figure 58.3) (Lawson et al. 1979; Rowland and Lawson 1975). The rectum may contain black tarry feces of mixed blood and digesta. The mucosa of the affected portion of intestine has few lesions except proliferation typical of chronic PE (PIA). No petechial hemorrhages, erosions or ulcers typically are observed.

The hallmark microscopic lesion in all forms of PE is mucosal thickening produced by proliferation of immature crypt epithelial cells that form hyperplastic and dysplastic crypts (Lomax and Glock 1982; Rowland and Lawson 1974). The normal single layer of simple columnar crypt epithelial cells is replaced by 2 or more irregular pseudostratified layers containing numerous mitotic figures (Figure 58.4). This results in enlarged crypts with irregular outlines that are often branched and that lack goblet cells present in crypts of normal swine intestine. Reappearance of goblet cells in deep glands is an indication of impending resolution. Infiltration of inflammatory cells is minimal or absent and only evident in late-stage lesions or in lesions complicated by other opportunistic infections (McOrist et al. 2006).

Specific immunostains or electron microscopy of affected intestinal sections reveals intracellular *L. intracellularis*, often in considerable numbers, lying in the apical cytoplasm of the affected crypt epithelial cells (Figure 58.4). In recovering lesions, the organisms may be either in extruded degenerate cells in the lumen or in cytoplasmic vacuoles within activated macrophages in the lamina propria. Recovering lesions of PE are notable for resumption of development of a population of mature epithelium through the crypt–villus axis, reappearance of goblet cells, and reestablishment of normal mucosal thickness and morphology (McOrist et al. 1996a).

In cases of NE, the superficial mucosa is effaced by coagulative necrosis, fibrin, and degenerative inflammatory cells. Typical proliferated *L. intracellularis*-infected crypt epithelium can be observed in deeper non-necrotic mucosa. Histological lesions in acute hemorrhagic cases (PHE) are characterized by variably severe mucosal proliferation typical of PE, fibrinohemorrhagic luminal clots or fibrinonecrotic and hemorrhagic luminal casts, and accumulation of bloody cellular debris containing numerous *L. intracellularis* organisms on the surface of the affected mucosa and in the lumen of affected intestinal crypts. In most cases, relatively minimal mucosal hemorrhage is observed relative to the large amount of accumulated blood in the intestinal lumen.

Diagnosis

The clinical diagnosis of PE has been historically based on clinical and pathological characteristics of the *L. intracellularis* infection. The differential diagnosis for clinical cases of PE varies with the particular form of the disease.

Pigs affected with PHE may have acute red-tinged loose-to-watery diarrhea or may be found dead with no prior clinical signs detected and with fecal staining of the skin in the perianal region. Differential clinical diagnosis should include acute swine dysentery (Chapter 62), gastric ulceration, and hemorrhagic bowel syndrome (Chapter 15).

Pigs affected with PIA predominantly exhibit diarrhea, anorexia, and poor growth. This form of PE is most likely confused clinically with endemic forms of coronaviruses (Chapter 31), rotaviruses (Chapter 43), brachyspiral colitis (Chapter 62), enteric salmonellosis (Chapter 59), porcine circovirus-associated enteritis (Chapter 30), enterotoxigenic colibacillosis (Chapter 52), and some nutritional diarrheas (Chapter 15). Porcine circovirus infections may produce grossly evident mucosal thickening of the intestines, but distinctive systemic lesions of PCVAD and granulomatous (non-adenomatous) enteritis differentiate it from PE (Jensen et al. 2006).

Confirmation of PE has typically relied on the observation of characteristic gross and microscopic lesions with detection of *L. intracellularis* infection by one or more methods. The difficulty in routinely culturing *L. intracellularis* has led to several alternative methods for confirmation of *L. intracellularis* infection. Immunohistochemistry or, less commonly, *in situ* hybridization can confirm the organism by visualization within the microscopic lesions. PCR assays have been widely used to confirm *L. intracellularis* DNA in fecal or intestinal mucosal samples (Jacobson et al. 2004; Jones et al. 1993).

Immunohistochemical staining using *L. intracellularis*-specific antibody has been the gold standard for specific identification of the bacterium in PE lesions (Guedes and Gebhart 2003a; Ladinig et al. 2009; McOrist et al. 1987). Immunohistochemistry can also be used to estimate the level of infection based on the amount of labeled *L. intracellularis*-specific antigen present in the intestinal mucosa (Guedes and Gebhart 2003a).

In situ hybridization assays can also specifically detect *L. intracellularis* nucleic acids in PE lesions (Gebhart et al. 1994; Jensen et al. 2010; Weissenböck et al. 2007); however, they are uncommonly used for routine diagnostics. Silver staining techniques are nonspecific in that they allow visualization of intracellular bacteria typical of *L. intracellularis* within lesions of PE, but they do not allow specific identification. Given the unique features of PE and the unique intracellular location of *L. intracellularis*, visualization with silver staining in typical PE lesions is essentially diagnostic. Where electron microscopic facilities are available, the presence of typical curve-shaped intracellular organism can be confirmed.

The sensitivity of PCR techniques for *L. intracellularis* has increased considerably over the last decade mainly due to development of real-time assays, including commercial kits with multiple sequence targets, and optimization of DNA extraction methods. Many PCR assays can detect as low as 100 organisms per gram of feces. Because of this high sensitivity, the mere demonstration of *L. intracellularis* in feces by PCR may not indicate economically significant PE at a herd level. Caution should also be exercised when interpreting negative IHC results since they are often based on microscopic evaluation of few sections of the small intestine. Considering the inexact segmental distribution of the PE lesions, examination of more segments of intestine may well demonstrate *L. intracellularis* infection within typical localized microscopic lesions.

The *antemortem* diagnosis of chronic or subclinical PE, without the aid of histopathology, can be challenging (Jacobson et al. 2003). A comprehensive investigation focusing on apparent wasting animals with intermittent diarrhea and variable weight gain is warranted along with serial fecal sampling for PCR testing from suspect groups. Records should be carefully examined to detect changes in average weight gain and feed conversion efficiency in the post weaned group (Gogolewski et al. 1991;

Roberts et al. 1979). A correlation should be evident between levels of *L. intracellularis* fecal shedding and diarrheic disease and growth performance. As an example, Burrough et al. (2015) published findings of a study that sought to correlate results of a semiquantitative real-time PCR test for *L. intracellularis* run on feces of diarrheic pigs with the presence of typical PE lesions and positive IHC tests. The authors found that Ct values of 29–32 or lower in the evaluated PCR test represented approximately 10^4 organisms/g of feces or more and that these Ct values were predictive of finding lesions of PE and detection of *L. intracellularis* by IHC. Given this correlation, Ct values below 30 in this particular test on fecal samples from diarrheic pigs could be interpreted as indicating that *L. intracellularis* is at least partially the cause of diarrhea and that the sampled pig could reasonably be expected to have typical lesions. These findings would need to be confirmed on an appropriate number of animals if a conclusion were to be made about the population as a whole.

The challenge in measuring the economic impact of subclinical cases of PE has motivated discussions questioning their importance, especially after observing positive fecal PCR tests from apparently healthy animals. This debate is primarily fueled by the growing number of countries adopting policies to restrict the usage of antimicrobials in swine production. It has been questioned whether the simple detection of *L. intracellularis* in feces should justify antimicrobial therapy. Consequently there is increasing concern to better define a protocol for therapeutic intervention for PE control. A PCR threshold has been investigated as an indicator of clinically significant fecal shedding that would merit treatment (Pedersen et al. 2012). Regardless of specific levels of fecal shedding, increasing numbers of *L. intracellularis* in feces of individuals over time have been strongly correlated with a decreasing average daily gain. More specifically, a $\log_{10}$ unit increase in *L. intracellularis* fecal load (around 3 Ct values) increases by 2 the odds ratio of a pig having a low growth rate (Johansen et al. 2013).

The use of oral fluid samples has been explored for detection of *L. intracellularis* DNA by PCR (Frana et al. 2014; LaVigne et al. 2013) and antibodies (IgA and IgG) by immunoperoxidase monolayer assay (IPMA) (Gabardo et al. 2014) and blocking ELISA (Johnson et al. 2011). Detection of *L. intracellularis* by PCR from oral fluids results from the behavior of pigs in which infected fecal matter is transported through the animal's mouth from the ground to the ropes used for oral fluid collection. These samples then represent a pool of feces from animals grouped in a pen. Therefore, a potential reduction on the sensitivity should be considered due to the dilution factor intrinsic to the pooling approach. The IPMA technique for IgG and IgA detection from oral fluids has shown high specificity and moderate sensitivity (Gabardo et al. 2014). Based on the endemic scenario of PE in swine herds worldwide, the diagnostic value of positive results by either PCR or serology in oral fluids is questionable. However, these diagnostic tools may be useful for diagnosis in herds with high health status or in naïve populations.

Serological tests for detection of IgG specific for *L. intracellularis* using whole bacterial antigen such as indirect immunofluorescence assay (Knittel et al. 1998) and IPMA (Guedes et al. 2002b) have been well validated. Several ELISA assays have been described incorporating differing antigen extracts (Boesen et al. 2005; Collins et al. 2012; Kroll et al. 2005; Watarai et al. 2004). Serologic assays have documented systemic IgM and IgG responses to *L. intracellularis*. Local intestinal IgA response has been detected by IPMA from intestinal lavage (Guedes and Gebhart 2010). Antibody responses usually have a good correlation with the presence of lesions. But exposure may not necessarily induce significant seroconversion in all cases. Although blood collection can be more time consuming than feces collection, the serological tests are cheaper to perform than individual fecal PCR tests.

Immunity

Humoral and cell-mediated immune responses are first detected two weeks after challenge of pigs with pure culture of *L. intracellularis* and persist in some animals for 13 weeks after exposure (Figure 58.2; Guedes and Gebhart 2003b). Macrophage ingestion of *L. intracellularis* in developing lesions probably leads to a predominant typical Th1 type immune cell response in the lamina propria (MacIntyre et al. 2003; McOrist et al. 1992). Serological IgG peaks near the end of the third week after infection and then drops gradually (Knittel et al. 1998). Naturally infected pigs showed a large accumulation of total IgA in the apical cytoplasm of proliferating enterocytes (McOrist et al. 1992). *Lawsonia*-specific IgA was detected in intestinal lavage of experimentally infected pigs three weeks after infection (Guedes and Gebhart 2010). Pigs rechallenged with *L. intracellularis* after recovering from previous exposure were protected from recolonization and clinical disease (Collins and Love 2007). *Lawsonia*-specific immune response appears to correlate with long-lasting (at least 10 weeks) protective immunity to reinfection (Cordes et al. 2012; Kroll et al. 2004; Riber et al. 2011). Progeny from acutely affected breeding stock is not fully protected from PE (Guedes et al. 2002a; Jacobson et al. 2010). The cell-mediated immune response mediated through CD8+ effector T cells is likely primarily responsible for protective immunity (Cordes et al. 2012).

Some data indicates that *L. intracellularis* modulates the immune response, enabling persistent infection of crypt epithelial cells. Early observations that well-developed typical PE lesions lacked significant inflammatory or immune cellular infiltrates led to the suggestion of immunomodulation (Rowland and Rowntree 1972). This has been confirmed by numerous *in vivo* studies that have consistently shown downregulation of genes related to the immune response, including those typically induced by intracellular pathogens such as tumor necrosis factor (Jacobson et al. 2011; Vannucci et al. 2012b). Reduction in the numbers of T cells and B cells in heavily infected pigs has also been demonstrated (MacIntyre et al. 2003).

Prevention and control

Improved farm hygiene measures will reliably reduce levels of PE. Quaternary ammonium-based compounds have effective anti-*Lawsonia* disinfectant activities (Collins et al. 2000; Wattanaphasak et al. 2010), but isolates appeared somewhat resistant to phenolic or iodine-based mixtures. Rigorous washing and cleaning of feces from all parts of pig pens, facilities, boots, and equipment on both single-site and multisite farms, as well as insect and rodent control, are likely to be more effective at reduction of PE than reliance on slatted floors and sunken pits for feces removal (Bronsvoort et al. 2001; Smith et al. 1998).

Limited *in vitro* studies have compared the minimum inhibitory concentrations of different classes of antimicrobials with potential activity against *L. intracellularis* (McOrist et al. 1995c; Wattanaphasak et al. 2009; Yeh et al. 2011). These *in vitro* evaluations corroborate results of controlled *in vivo* studies in commercial herds. Overall, macrolides and pleuromutilins are the most effective antibiotics when given at an adequate dosage rate per kg of body weight (McOrist et al. 1996b, 1997a; Pommier et al. 2008; Schwartz et al. 1999; Walter et al. 2001). In the United States quinoxalines (such as carbadox) are also available and effective. Acquired resistance by *L. intracellularis* to these active drug groups has not been demonstrated (McOrist 2000). Although tetracycline demonstrated only intermediate effectiveness *in vitro* (Wattanaphasak et al. 2009), medication via water using tetracycline and its derivative, doxycycline, demonstrated reduction in fecal shedding of *L. intracellularis* under field conditions (Larsen et al. 2016; Weber et al. 2017).

Apparent medication failures with antimicrobials known to be effective are most likely due to subtherapeutic dosages. Antimicrobials known to be ineffective against *L. intracellularis* in clinical PE include penicillins, bacitracin, and aminoglycosides such as neomycin, virginiamycin, and ionophores. Non-antibiotic therapies such as copper or zinc compounds or feed acidifiers are generally ineffective.

Effective medication protocols vary depending on the time course of the disease and the age of pigs involved. Controlled field trials suggest that incorporation of in-feed or water-soluble antibiotics for control achieves best results if given early in the course of infection. Treatment of PHE in breeding herds requires a vigorous approach, medicating both clinically affected and in-contact animals (which may include whole herd treatment). A preferred strategy would be tiamulin (120 ppm), tylosin (100 ppm), or tylvalosin (100 ppm) for 14 days, delivered orally via a water-soluble formulation or an in-feed premix or by intramuscular injection of an equivalent dose. Severe PIA manifested as wasting pigs will often be moderated by the use of tiamulin, tylosin, tylvalosin, or carbadox. The removal of affected animals to separate pens, with supportive therapy, may also limit losses.

Medication of older pigs (i.e. breeding stock) is not likely to eliminate the infection. Partial depopulation and medication-based eradication attempts have been largely unsuccessful. Since PE can vary in the time of onset on different farms and between batches of pigs within the same farm, in-feed antimicrobials added too late may not adequately reduce the clinical signs or improve performance (Hammer 2004). Conversely, antimicrobials added too early may prevent exposure and subsequent development of active immunity. These pigs may remain naïve and susceptible to exposure later and thus experience the acute hemorrhagic form (PHE).

A modified live vaccine has been widely used with demonstrated effectiveness in preventing PE. It has provided protection against subsequent challenge with virulent heterologous *L. intracellularis* when administrated either by individual oral drench or drinking water delivery to a group of pigs (Kroll et al. 2004). Vaccinated animals showed reduced PE lesions and fecal shedding of *L. intracellularis* along with better cell-mediated immune responses upon challenge (Nogueira et al. 2013; Riber et al. 2015). Specific IgG and IgA responses and cytokine production in the ileal mucosa were also observed in vaccinated pigs (Nogueira et al. 2015). Field studies confirm that vaccination results in improved rate of gain and economic benefits (i.e. return of investment) (Hardge et al. 2004; McOrist and Smits 2007; Park et al. 2013). Additionally, vaccination has resulted in reduced antibiotic usage in PE-affected herds (Bak and Rathkjen 2009).

A particular concern regarding the use of an avirulent live vaccine is the concurrent administration of some antimicrobials that inactivate the live *L. intracellularis* vaccine. This scenario may prevent production of IFN-γ, important for a specific cell-mediated immune response. Temporary removal of most medications from feed is recommended at least 3 days

before and 3 days after vaccination (Kolb and Sick 2003). The concurrent use of in-feed administration of colistin at 30 g/ton or virginiamycin at 11 g/ton has not demonstrated interference with the effectiveness of the modified live oral vaccine (Beckler et al. 2013; Liesner et al. 2012).

In 2016 an injectable killed vaccine became commercially available in the United States. The label recommends a single intramuscular dose in pigs at 3 weeks of age or older. A study conducted by the manufacturer showed specific serological response (humoral IgG) along with reduced PE lesions and fecal shedding of *L. intracellularis* in vaccinated pigs challenged 20 weeks after vaccination (Roerink et al. 2016). Presently, there is no published information on the effect of this injectable bacterin on growth performance or mucosal immune responses in vaccinated pigs.

Vaccination is particularly important in nucleus herds and for introduction of replacement breeding stock into commercial farms. Acclimation and medication programs, without vaccination, for naïve gilts may result in PE outbreaks.

References

Abshier JM, Besh-Williford CL, Franklin CL, et al. 2001. Spontaneous infection of *Lawsonia intracellularis*-like bacteria in the mouse. In Proceedings, Conference of Research Works in Animal Disease, Saint Louis, MO, p. 49.

Alberdi MP, Watson E, McAllister GEM, et al. 2009. *Vet Microbiol* 139:298–303.

Backhans A, Jacobson M, Hansson I, et al. 2013. *Epidemiol Infect* 141:1885–1891.

Bak H, Rathkjen PH. 2009. *Acta Vet Scand* 51:1.

Baroch JA, Gagnon CA, Lacouture S, et al. 2015. *Can J Vet Res* 79:74–78.

Bearson S, Bearson B, Foster JW. 1997. *FEMS Microbiol Lett* 147:173–180.

Beckler DC, Segal MU, Weiss DL, et al. 2013. *J Swine Health Prod* 21:253–260.

Biester HE, Schwarte LH. 1931. Intestinal adenoma in swine. *Am J Pathol* 7:175–185.

Boesen HT, Jensen TK, Schmidt AS, et al. 2004. *Vet Microbiol* 103:35–45.

Boesen HT, Jensen TK, Moller K, et al. 2005. *Vet Microbiol* 109:105–112.

Boutrup TS, Boesen HT, Boye M, et al. 2010. *J Comp Pathol* 143:101–109.

Bronsvoort M, Norby B, Bane DP, et al. 2001. Management factors associated with seropositivity to *Lawsonia intracellularis* in US swine herds. *J Swine Health Prod* 9: 285–290.

Burrough ER, Rotolo ML, Gauger PC, et al. 2015. *J Swine Health Prod* 23:204–207.

Collins AM, Love RJ. 2007. *Vet Microbiol* 120:381–386.

Collins JE, Libal MC, Brost D. 1983. *J Am Vet Med Assoc* 183:886–889.

Collins AM, Love RJ, Pozo J, et al. 2000. *J Swine Health Prod* 8:211–215.

Collins AM, Fell S, Pearson H, et al. 2011. *Vet Microbiol* 150:384–388.

Collins A, Goncalves J, Fell S, et al. 2012. *Aust Vet J* 90:97–99.

Cooper DM, Swanson DL, Barns SM, et al. 1997a. *Int J Syst Bacteriol* 47:635–639.

Cooper DM, Swanson DL, Gebhart CJ. 1997b. *Vet Microbiol* 54:47–62.

Cordes H, Riber U, Jensen TK, et al. 2012. *Vet Res* 43:9.

Crohn BB, Turner DA. 1952. *Gastroenterology* 20:350–351.

Cross RF, Smith CK, Parker CF. 1973. *J Am Vet Med Assoc* 162:564–566

Dale CJH, Moses EJ, Ong CC, et al. 1998. *Microbiology* 144:2073–2084.

Deprez P, Chiers K, Gebhart CJ, et al. 2005. *Lawsonia intracellularis* infection in a 12-month-old colt in Belgium. *Vet Rec* 157:774–776.

Dors A, Pomorska-Mól M, Czyżewska E, et al. 2015. *Pol J Vet Sci* 18:825–831.

Drolet R, Larochelle D, Gebhart CJ. 1996. *J Vet Diagn Invest* 8:250–253.

Duhamel GE, Wheelson EB. 1982. *Vet Pathol* 19:447–450.

Elwell MR, Chapman AL, Frenkel JK. 1981. *Vet Pathol* 18:136–139.

Fox JG, Dewhirst FE, Fraser GJ, et al. 1994. *J Clin Microbiol* 32:1229–1237.

Frana T, Warneke H, Stensland BS, et al. 2014. Comparative detection *of Lawsonia intracellularis, Salmonella and Brachyspira* from oral fluids and feces. In Proceedings of the American Association of Swine Veterinarians, Dallas, TX, pp. 67–70.

Frazer ML. 2008. *J Vet Intern Med* 22:1243–1248.

Friedman M, Bednar V, Klimes J, et al. 2008. *Lett Appl Microbiol* 47:117–121.

Friendship RM, Corzo CA, Dewey CE, et al. 2005. *J Swine Health Prod* 13:139–142.

Gabardo, MP, Resende TP, Otoni LVA, et al. 2014. Detection of *Lawsonia intracellularis* antibodies in oral fluid samples from experimentally challenged swine. In Proceedings of the Allen D. Leman Swine Conference, St. Paul, MN, p. 56.

Gabardo M, Sato JP, Daniel A, et al. 2016. Involvement of Mice (*Mus musculus*) in the epidemiology of porcine proliferative enteropathy. In Proceedings of the 24th International Pig Veterinary Society Congress, Dublin, Ireland, p. 136.

Gebhart CJ, Guedes RMC. 2010. *Lawsonia intracellularis*. In Gyles CL, Prescott JF, Songer JG, et al., eds. *Pathogenesis of Bacterial Infections in Animals*. Ames, IA: Blackwell Publishing, pp. 503–509.

Gebhart CJ, Barns SM, McOrist S, et al. 1993. *Int J Syst Bacteriol* 43:533–538.
Gebhart CJ, McOrist S, Lawson GH, et al. 1994. *Vet Pathol* 31:462–467.
Gogolewski RP, Cook RW, Batterham ES. 1991. *Aust Vet J* 68:406–408.
Grützner N, Gebhart CJ, Lawhorn BD, et al. 2015. *Vet J* 203:320–325.
Guedes RMC, Gebhart CJ. 2003a. *Vet Microbiol* 91:135–145.
Guedes RMC, Gebhart CJ. 2003b. *Vet Microbiol* 93:159–166.
Guedes RMC, Gebhart CJ. 2010. *Can J Vet Res* 74:97–101.
Guedes RMC, Gebhart CJ, Armbruster G, et al. 2002a. *Can J Vet Res* 66:258–263.
Guedes RMC, Gebhart CJ, Winkelman NL, et al. 2002b. *Can J Vet Res* 66:99–107.
Guedes RMC, Winkelman NL, Gebhart CJ. 2003. *Vet Rec* 153:432–433.
Guedes RMC, Machuca MA, Quiroga MA, et al. 2017. *Vet Pathol* 54:620–628.
Hammer JM. 2004. *J Swine Health Prod* 12:29–33.
Hardge T, Nickoll E, Grunert H, et al. 2004. *Pig J* 54:17–34.
Hossain MM, Oh Y, Cho HS. 2016. *J Wildl Dis* 52:803–808.
Jacobson M. Segarstad CH, Gunnarsson A, et al. 2003. *Res Vet Sci* 74:163–169.
Jacobson M, Aspan A, Konigsson MH, et al. 2004. *Vet Microbiol* 102:189–201.
Jacobson M, Råsbäck T, Flöstrup H, et al. 2007. *Epidemiol Infect* 135:1043–1045.
Jacobson M, Aspan A, Nordengrahn A, et al. 2010. *Vet Microbiol* 142:317–322.
Jacobson M, Andersson M, Lindberg R, et al. 2011. *Vet Microbiol* 153:307–314.
Jacoby RO. 1978. *Am J Pathol* 91:433–450.
Jacoby RO, Johnson EA. 1981. Transmissible ileal hyperplasia. *Adv Exp Med Biol* 134:267–289.
Jacoby RO, Osbaldiston GW, Jonas AM. 1975. *Lab Anim Sci* 25:465–473.
Jasni S, McOrist S, Lawson GHK. 1994a. *Vet Microbiol* 41:1–9.
Jasni S, McOrist S, Lawson GH. 1994b. *Res Vet Sci* 56:186–192.
Jensen TK, Vigre H, Svensmark B, et al. 2006. *J Comp Pathol* 135:176–182.
Jensen TK, Boesen HT, Vigre H, et al. 2010. *J Comp Pathol* 142:1–8.
Johansen M, Nielsen M, Dahl J, et al. 2013. *Prev Vet Med* 108:63–72.
Johnson EA, Jacoby RO. 1978. Transmissible ileal hyperplasia of hamsters. II. Ultrastructure. *Am J Pathol* 91:451–468.
Johnson J, Olsen C, Schwartgz K, et al. 2011. Feasibility of oral fluids for detection of antibody response to porcine parvovirus, *leptospira*, swine influenza, *lawsonia,* or *salmonella* in adult swine. In Proceedings of the Annual Meeting by American Association of Swine Veterinarians, Phoenix, AZ, pp. 1327–1330.
Jones GF, Ward GE, Murtaugh MP, et al. 1993. *J Clin Microbiol* 31:2611–2615.
Klein EC, Gebhart CJ, Duhamel GE. 1999. *J Med Primatol* 28:11–18.
Knittel JP, Jordan DM, Schwartz KJ, et al. 1998. *Am J Vet Res* 59:722–726.
Kolb J, Sick F. 2003. Summary of field trials implementing Enterisol® Ileitis against ileitis. In Proceedings of the Annual Meeting by American Association of Swine Veterinarians, Orlando, FL, pp. 243–244.
Koyama T, Kinjo M, Araiso T. 1989. *Adv Exp Med Biol* 248:763–767.
Kroll J, Roof MB, McOrist S. 2004. *Am J Vet Res* 65:559–565.
Kroll JJ, Eichmeyer MA, Schaeffer ML, et al. 2005. *Clin Diagn Lab Immunol* 12:693–699.
Ladinig A, Sommerfeld-Stur I, Weissenbock H. 2009. *J Comp Pathol* 140:140–148.
Landsverk T. 1981. *Vet Pathol* 18:275–278.
Larsen I, Hjulsager CK, Holm A, et al. 2016. *Prev Vet Med* 123:52–9.
Lavigne KA, Pierdon KM, Armbruster GA, et al. 2013. Comparison of sampling methods for *Lawsonia intracellularis* testing using qpcr. In Annual Meeting by American Association of Swine Veterinarians, San Diego, CA, pp. 71–74.
Lawson GH, Gebhart CJ. 2000. *J Comp Pathol* 122:77–100.
Lawson GHK, Rowland AC, Roberts L, et al. 1979. *Res Vet Sci* 27:46–51.
Lawson GHK, McOrist S, Sabri J, et al. 1993. *J Clin Microbiol* 31:1136–1142.
Lawson GHK, Mackie RA Smith DGE, et al. 1995. *Vet Microbiol* 45:339–350.
Lemarchand TX, Tully TN, Shane SM, et al. 1997. *Vet Pathol* 34:152–156.
Liesner BG, Husa J, Saltzman R. 2012. Compatibility of Enterisol Ileitis oral Lawsonia intracellularis vaccine for swine when given concurrently with oral colistin sulfate. In Proceedings of the International Pig Veterinary Society, Jeju, South Korea, p. 663.
Lomax LG, Glock RD. 1982. *Am J Vet Res* 43:1608–1614.
MacIntyre N, Smith DGE, Shaw DJ, et al. 2003. *Vet Pathol* 40:421–432.
Mapother ME, Joens LA, Glock RD. 1987. *Vet Rec* 121:533–536.
Marsteller TA, Armbruster G, Bane DP, et al. 2003. *J Swine Health Prod* 11:127–130.
McGregor GF, Gottschalk M, Godson DL, et al. 2015. *Can Vet J* 56:839–844.
McOrist S. 2000. *Trends Microbiol* 8:483–487.
McOrist S. 2005. *Vet J* 170:8–9.
McOrist S, Lawson GHK. 1989. *Res Vet Sci* 46:27–33.
McOrist S, Smits RJ. 2007. *Vet Rec* 161:26–28.
McOrist S, Boid R, Lawson GHK, et al. 1987. *Vet Rec* 121:421–422.
McOrist S, MacIntyre N, Stokes CR, et al. 1992. *Infect Immun* 60:4184–4191.

McOrist S, Jasni S, Mackie RA, et al. 1993. *Infect Immun* 61:4286–4292.
McOrist S, Mackie RA, Neef N, et al. 1994. *Vet Rec* 134:331–332.
McOrist S, Gebhart CJ, Boid R, et al. 1995a. *Int J Syst Bacteriol* 45:820–825.
McOrist S, Jasni S, Mackie RA, et al. 1995b. *Res Vet Sci* 59:255–260.
McOrist S, Mackie RA, Lawson GH. K. 1995c. *J Clin Microbiol* 33:1314–1317.
McOrist S, Roberts L, Jasni S, et al. 1996a. *J Comp Pathol* 115:35–45.
McOrist S, Smith SH, Shearn MFH, et al. 1996b. *Vet Rec* 139:615–618.
McOrist S, Morgan J, Veenhuizen MF, et al. 1997a. *Am J Vet Res* 58:136–139.
McOrist S, Smith SH, Green LE. 1997b. *Vet Rec* 140:579–581.
McOrist S, Smith SH, Klein T. 1999. *Vet Rec* 144:202–204.
McOrist S, Barcellos DE, Wilson RJ. 2003. *Pig J* 51:26–35.
McOrist S, Gebhart CJ, Bosworth BT. 2006. *Can J Vet Res* 70:155–159.
Michalski CW, Di Mola FF, Kummel K, et al. 2006. *BMC Microbiol* 6:81.
Mølbak L, Johnsen K, Boye M, et al. 2008. *Vet Microbiol* 128:96–107.
Moura-Alvarez J, Nuñez LF, Astolfi-Ferreira CS, et al. 2014. *Tropl Anim Health Prod* 46:1051–1058.
Murakata K, Sato A, Yoshiya M, et al. 2008. *J Comp Pathol* 139:8–15.
Nogueira MG, Collins AM, Donahoo M, et al. 2013. *Vet Microbiol* 164:131–138.
Nogueira MG, Collins AM, Dunlop RH, et al. 2015. *Aust Vet J* 93: 124–126.
Ohta T, Kimura K, Katsuda K, et al. 2016. *J Comp Pathol* S0021-9975:30494-73049.
Park S, Lee J-B, Kim K-J, et al. 2013. *Clin Exp Vaccine Res* 2:135–139.
Pearson HE, Toribio JA, Lapidge SJ, et al. 2016. *Prev Vet Med* 123:39–51.
Pedersen KS, Ståhl M, Guedes RM, et al. 2012. *BMC Vet Res* 8:198.
Philips RC, Geiger JO, Karhoff K. 1998. Evaluation of the use of Lawsonia intracellularis indirect fluorescent antibody test (IFA) in a large production system. In Proceedings of the Allen D. Leman Conference, St. Paul, MN, pp. 5–6.
Pommier P, Keita A, Pagot E, et al. 2008. *Revue Méd Vét* 159:579–582.
Pusterla N, Mapes S, Rejmanek D, et al. 2008. *J Wildl Dis* 44:992–998.
Pusterla N, Jackson R, Wilson R, et al. 2009. *Vet Microbiol* 136:173–176.
Pusterla N, Wattanaphansak S, Mapes S, et al. 2010. *J Vet Intern Med* 24:622–627.
Riber U, Cordes H, Boutrup TS, et al. 2011. *Vet Microbiol* 149:406–414.
Riber U, Heegaard PM, Cordes H, et al. 2015. *Vaccine* 33:156–162.
Roberts L, Rowland AC, Lawson GHK. 1977. *Vet Rec* 100:12–13.
Roberts L, Lawson GHK, Rowland AC, et al. 1979. *Vet Rec* 104:366–368.
Roerink F, Morgan C, Knetter S, 2016. Porcilis® Ileitis: 20–week duration of immunity against Lawsonia intracellularis challenge. In Proceedings of the Annual Meeting by American Association of Swine Veterinarians, New Orleans, LA, p. 144.
Rowan TG, Lawrence TLJ. 1982. *Vet Rec* 110:306–307.
Rowland AC, Lawson GHK. 1974. *Res Vet Sci* 17:323–330.
Rowland AC, Lawson GHK. 1975. *Vet Rec* 97:178–180.
Rowland AC, Rowntree PGM. 1972. *Vet Rec* 91:235–241.
Sapico FL, Reeves D, Wexler HM, et al. 1994. *J Clin Microbiol* 32:2510–2513.
Schmitz-Esser S, Haferkamp I, Knab S, et al. 2008. *J Bacteriol* 190: 5746–5752.
Schwartz K, Knittel J, Walter D, et al. 1999. *J Swine Health Prod* 7:5–11.
Smith SH, McOrist S. 1997. *Res Vet Sci* 62:6–10.
Smith SH, McOrist S, Green LE. 1998. *Vet Rec* 142:690–693.
Stege H, Jensen TK, Moller K, et al. 2000. *Prev Vet Med* 46:279–292.
Stege H, Jensen TK, Moller K, et al. 2004. *Vet Microbiol* 104:197–206.
Suto A, Asano S, Goto Y, et al. 2004. *Jpn J Vet Med Sci* 66:547–549.
Tomanova K, Bartak P, Smola J. 2002. *Vet Rec* 151:765–767.
Umemura T, Tsuchitani M, Totsuka M, et al. 1982. *Vet Pathol* 19:326–329.
Vandenberghe J, Marsboom R. 1982. *Vet Rec* 111:416–417.
Vannucci FA, Gebhart CJ. 2014. *Vet Pathol* 5:465–477.
Vannucci FA, Borges EL, Vilaça de Oliveira JS, et al. 2010. *Vet Microbiol* 145:286–291.
Vannucci FA, Foster DN, Gebhart CJ. 2012a. *PLoS One* 7:e46708.
Vannucci FA, Pusterla N, Mapes SM, et al. 2012b. *Vet Res* 43:53.
Vannucci FA, Wattanaphansak S, Gebhart CJ. 2012c. *J Clin Microbiol* 50:1070–1072.
Vannucci FA, Beckler D, Pusterla N, et al. 2013a. *Vet Microbiol* 162:265–269.
Vannucci FA, Foster DN, Gebhart CJ. 2013b. *BMC Genomics* 14:421.
Vannucci FA, Kelley MR, Gebhart CJ. 2013c. *Vet Res* 44:49.
Veenhuizen MF, Elam TE, Soenksen N. 2002. *Comp Contin Educ Pract Vet* 24:S10–S15.
Viott AM, França SA, Vannucci FA, et al. 2013. *Pesq Vet Bras* 33:372–378.
Walter D, Knittel J, Schwartz K, et al. 2001. *J Swine Health Prod* 9:109–115.

Watarai M, Yamato Y, Horiuchi N, et al. 2004. *Jpn J Vet Med Sci* 66:735–737.
Wattanaphasak S, Singer RS, Gebhart CJ. 2009. *Vet Microbiol* 134:305–310.
Wattanaphasak S, Singer RS, Gebhart CJ. 2010. *J Swine Health Prod* 18:11–17.
Weber NR, Pedersen KS, Hansen CF, et al. 2017. *Prev Vet Med* 137:69–76.
Weissenböck H, Mrakovcic M, Ladinig A, et al. 2007. *J Vet Diagn Invest* 19:282–285.
Winkelman NL, Crane JP, Elfring GD, et al. 2002. *J Swine Health Prod* 10:106–110.
Yeh JY. 2014. *BMC Vet Res* 10:5.
Yeh J-Y, Lee J-H, Yeh H-R, et al. 2011. *Antimicrob Agents Chemother* 55:4451–4453.
Zlotowski P, Correa AMR, Borba MR, et al. 2008. *Cienc Rural* 38:2540–2544.

59

Salmonellosis

Ronald W. Griffith, Steven A. Carlson, and Adam C. Krull

Relevance

Members of the genus *Salmonella* are notorious for their ability to infect a broad range of hosts. Taylor and McCoy (1969) observed that salmonellae have been isolated from virtually all vertebrate hosts from which they have been sought. Many of the more than 2400 *Salmonella* serotypes have a broad host range, but several serotypes are quite adapted to a single host species including *S.* Typhi (humans), *S.* Dublin (bovine), *S.* Gallinarum (poultry), and *S.* Choleraesuis (swine).

Salmonella infections of swine are of concern for two major reasons. The first is clinical disease in pigs (salmonellosis), and the second is that swine can be infected with a broad range of *Salmonella* serotypes that can potentially contaminate pork products and pose a threat to human health.

Etiology

There has been considerable disagreement over the nomenclature of the genus *Salmonella*, and several proposals for altering it have been made. Convention has been to refer to each of the over 2400 distinct serotypes as though it is a species. *Salmonella enterica* has been designated as the type species for the genus *Salmonella*, and approximately 60% of all *Salmonella* serotypes belong in the subspecies *enterica* including the vast majority of pathogens. Not italicizing the serovar names and capitalizing the first letter in recognition that they are not species names has been widely accepted. For simplicity, serovar names will be shortened in this chapter to *S.* Choleraesuis, *S.* Typhimurium, etc.

Disease in swine has historically been almost always caused by either *S.* Choleraesuis variety Kunzendorf or by *S.* Typhimurium. The former produces septicemia with lesions in a variety of organs and has historically been and may continue in some parts of the world to be the most frequent serotype causing disease in swine (Lawson and Dow 1966; Levine et al. 1945; Mills and Kelly 1986; Morehouse 1972; Schwartz and Daniels 1987; Wilcock et al. 1976). An emerging concern is illustrated by a recent study that identified a multiple antibiotic resistant isolate of *S.* Choleraesuis exhibiting hypervirulence after the bacterium has escaped from waterborne protozoa (Xiong et al. 2010). However, the National Animal Disease Laboratories (NADL) report that *S.* Choleraesuis is no longer in the top 10 serovars identified by their laboratory from swine in the United States (personal communication, 2017).

Salmonella Choleraesuis *had diminished and S.* Typhimurium had emerged as the most frequently isolated serotype from diseased swine in North America at the time of publication of the previous (10th) edition of *Diseases of Swine* (Foley et al. 2008). *S.* Typhimurium causes diarrheal disease as a consequence of enterocolitis. Disease caused by *S.* Typhimurium occurs with greater than expected frequency in what could be considered unusually clean herds such as purebred breeding herds (Gooch and Haddock 1969; Heard et al. 1968; Lynn et al. 1972) because of introduction into a previously immunologically naïve population. This organism is also frequently isolated as a sequel to other enteric or debilitating diseases. As with *S.* Choleraesuis, some multiresistant *S.* Typhimurium can be hypervirulent following exposure to common waterborne protozoa (Xiong et al. 2010).

Salmonella I 1,4,[5],12 : i:- is antigenically similar to *S.* Typhimurium but lacks the second-phase flagellar antigens (Moreno Switt et al. 2009; Soyer et al. 2009). It is considered to be a serologic variant of *S.* Typhimurium. It has been identified for many years but has been increasingly seen as a cause of clinical disease in humans and animals. In 2012 and 2013, this variant was ranked by NADL in the top five causing clinical disease in swine. In 2014–2016, this organism was the leading cause of clinical salmonellosis in swine in the United States.

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Clinical disease and lesions caused by infection with this serovar appear to be identical to those caused by infection with *S.* Typhimurium (Krull 2017, unpublished data). There has also been a steady increase over time in the percentage of isolates with ampicillin, streptomycin, sulfonamide, and tetracycline (ASSuT) antimicrobial resistance. Approximately 38% of isolates at the Iowa State University Veterinary Diagnostic Laboratory in 2016 expressed ASSuT resistance (Krull 2017, unpublished data).

Localized epidemics of disease caused by the biochemically atypical *S.* Typhisuis have been historically reported in the American Midwest (Andrews 1976; Barnes and Bergeland 1968) and Europe (Barnes and Sorensen 1975). This organism grows poorly in standard selective media for *Salmonella* isolation, but the disease produced by *S.* Typhisuis is so characteristic that outbreaks are not likely to remain unnoticed (Barnes and Bergeland 1968). This serotype was demonstrated to be able to subsist on antibiotics as a sole carbon source (Barnhill et al. 2010), a concern given the potential for antibiotics present in manure lagoons.

Other serotypes are occasional causes of disease in swine but are usually transient and associated with predisposing factors including debilitation, immunocompromise, other intestinal disturbances, or circumstances, which allow immunologically naïve pigs to be exposed to very large doses. *S.* Heidelberg has been associated with post weaning diarrhea with mild lesions more typical of enterotoxigenic diarrheal disease than of the typical fibrinonecrotic enterocolitis observed with *S.* Choleraesuis or *S.* Typhimurium (Reed et al. 1985). *S.* Dublin (Lawson and Dow 1966; McErlean 1968) and *S.* Enteritidis (Reynolds et al. 1967) have been rarely reported as causes of meningitis in suckling pigs.

Public health

Non-typhoidal salmonellosis is a worldwide health problem and is the leading cause of foodborne illnesses in the United States and many parts of the world. The topic has been recently reviewed (Doyle et al. 2009; Foley et al. 2008; Foley and Lynne 2008). Although less common than contaminated food, direct contact with clinically or subclinically infected animals can also be a source of human infection (Hoelzer et al. 2011). Among the top 20 *Salmonella* isolated from diseased humans, 5 are commonly isolated from swine (i.e. *Salmonella* I 1,4,[5],12 : i:-, *S.* Typhimurium, *S.* Heidelberg, *S.* Agona, and *S.* Infantis). Even so, human salmonellosis is more commonly acquired in the United States through consumption of contaminated poorly cooked poultry, eggs, or beef than by consumption of pork or direct contact with pigs. *S.* Choleraesuis is an uncommon human pathogen in the United States, but the non-Kunzendorf variety is a cause of human disease in other countries.

Non-typhoidal human salmonellosis usually manifests as self-limiting diarrhea, abdominal cramps, vomiting, and fever that begins 6–12 hours after oral exposure and resolves after 2–7 days. In a low percentage of affected individuals, septicemic disease develops, leading to osteomyelitis, pneumonia, or meningitis that require antibiotic therapy. An exception is *S. Choleraesuis* that consistently produces severe septicemic disease. Mortality may result from dehydration or effects of septicemic disease. Severe disease and mortality are most common in infants, the elderly, or immunocompromised individuals.

Epidemiology

The reservoir for salmonellae is the intestinal tract of warm- and cold-blooded animals. Salmonellae have virtually all of the attributes necessary to ensure wide distribution, including abundant reservoir hosts, efficient fecal shedding from carrier animals, persistence within the environment, and the effective use of transmission vectors (feed, fomites, vehicles, etc.). Inapparent, long-term carriers that can shed salmonellae in feces continuously or intermittently, often in high numbers, are common in most host species. Shedding of the organism can be exacerbated by a long list of stressors, including commingling of pigs, transportation, concurrent diseases, antibiotic therapy, and food deprivation.

Infection of swine with persistent shedding by one or more serotypes is common, but primary clinical disease caused by serotypes other than *S.* Typhimurium or the *S.* I 1,4,[5],12 : i:- variant is uncommon. Put differently, clinically normal swine may be infected and shed a variety of serotypes that pose little threat to pigs but may pose risk for humans through direct exposure or contamination of pork products. Thus, shedding of salmonellae by clinically and subclinically infected pigs has implications both for contamination of pork carcasses and foodborne human illness and for the development of clinical salmonellosis in swine populations.

Salmonellae in pork

Although much of the *Salmonella* contamination of pork products occurs by fecal contamination during slaughter within abattoirs, infected pigs leaving the farm are considered the original source of abattoir contaminations. The stress of transport and feed deprivation increases shedding from inapparent carriers, which then contaminate the environment of the truck and abattoir (Isaacson et al. 1999; Williams and Newell 1970). The prevalence of infection within the group continues to increase with

increasing length of stay in the pens prior to slaughter, rising by about 50% for each 24 hour period (Craven and Hurst 1982; Morgan et al. 1987).

Although *Salmonella* contamination of poultry and beef products exceeds that of pork, *Salmonella* control programs in swine will continue to be a primary focus of food safety initiatives. *Salmonella* reduction programs are becoming commonplace in some regions, with long-range goals to include the production and marketing of salmonellae-free pork products. Numerous dynamic programs are in place utilizing hazard analysis and critical control point principles. Those programs that have been in place for a sufficient period of time, such as the Danish program, have significantly reduced the rate of *Salmonella* contamination in pork products (Nielsen et al. 1995). Fortunately, most of the methods useful for preharvest *Salmonella* reduction in swine populations are related to sound management practices that also improve the overall health of a swine operation. The Danish program, which uses serology to identify *Salmonella*-infected herds and therefore control the introduction of shedding swine into the abattoir, has not been as successful as originally hoped.

Salmonellosis in swine

Most salmonellosis outbreaks occur in intensively reared weaned pigs, and although disease in adults and suckling pigs is infrequent, infection is not (Gooch and Haddock 1969; Wilcock et al. 1976). The low frequency of salmonellosis in suckling pigs presumably results from lactogenic immunity, since naïve neonatal swine are susceptible to oral challenge with salmonellae and develop a disease comparable with that in weaned pigs (Wilcock 1978). Disease occurs worldwide but varies markedly in estimated prevalence, morbidity, and mortality.

Host-adapted *S.* Choleraesuis is isolated almost exclusively from diseased swine and is usually manifested as septicemia. Midwestern US veterinary diagnostic laboratories and veterinarians reported an increasing frequency of salmonellosis due to *S.* Choleraesuis from 1981 to 1990 and a decreasing frequency from 1991 to 2016 (Krull 2017, unpublished data; Schwartz 1997). The decrease starting in the mid-1990s is likely due to improvements in swine management and husbandry and the advent of efficacious attenuated live vaccines. Immunization of swine against *S.* Choleraesuis is no longer a routine practice in many operations.

Salmonella Typhimurium and its variant *S.* I 1,4,[5],12 : i:- have worldwide distribution, are not host specific, and have become the most frequently isolated serotypes from clinically ill pigs in the United States (Foley et al. 2008; Krull et al. 2017, unpublished data). Clinically ill pigs develop enterocolitis and exhibit diarrhea and dehydration. Disease most commonly develops in pigs with concurrent debilitating diseases, in conditions of poor hygiene that allow exposure to high doses of the organism, or in immunologically naïve pigs. The latter are frequently encountered in modern production systems utilizing age-segregated production.

Caution should be exercised before incriminating other serotypes as primary pathogens. Most other serotypes are transient, sporadic causes of disease, and often disease cannot be reproduced experimentally without unique qualifying criteria. *S.* Heidelberg has been implicated in watery diarrhea in recently weaned pigs thought to be caused by an enterotoxin-mediated secretory mechanism and associated with only mild catarrhal enterocolitis (Reed et al. 1985). This is in sharp contrast to the severe lesions of fibrinonecrotic enterocolitis observed in *S.* Typhimurium-associated disease.

Sources of infection

The number of potential sources of *Salmonella* infection for a population of swine is seemingly endless. A task force study in the United States did not reach a consensus as to the most important source of salmonellae for pigs (Bixler 1978), in large part due to the diversity and the biology of the genus *Salmonella*. *S.* Choleraesuis used to be a frequent isolate from clinically ill pigs, but was always a very infrequent isolate from pig feeds or non-porcine *Salmonella* reservoirs. Vertical (dam to offspring) and horizontal transmission both occurred. Pigs may also be the likely source for *S.* Derby, which seems to be very common in some abattoir surveys of swine.

The source of infection for other serotypes is less clear since the host and vector range for salmonellae is broad and they have amazing capability to persist in environments outside the host. There are generally low numbers of various *Salmonella* serotypes present in feed, water, or litter contaminated by birds, rodents, or other animals. Evidence linking sources of contamination to primary clinical outbreaks, without other concurrent diseases or predisposing conditions, is generally lacking. Feed containing ingredients of animal origin is widely accepted as a source of *Salmonella* infection to herds, but it should be emphasized that ingredients of vegetable origin can also be a source of salmonellae-contaminated feed. Water is not as likely a source of infection unless surface water is used for consumption or pigs have access to recycled lagoon water. Birds, insects, rodents, and pets can all act as carriers, as can bedding and litter (Allred et al. 1967; Nape and Murphy 1971; Williams et al. 1969). The isolation of salmonellae from feed was significantly associated with the lack of bird proofing, with on-farm feed preparation, and with the housing of pigs in facilities other than total confinement, for all stages of production.

Transmission, shedding, and carrier state

Definitive statements regarding the transmission, shedding, and carrier states of salmonellae are difficult due to the dynamic and complex relationships between salmonellae, hosts, and environment. *Salmonella* transmission and shedding within differing populations of animals in an endless variety of environmental, feeding, and management situations result in countless unique situations that cannot be experimentally reproduced.

In general, fecal–oral transmission is the most likely mode of transmission of virulent salmonellae. *Salmonella* can be recovered from the intestinal tract of pigs within several minutes of oral exposure. Transmission can occur from pig to pig, contaminated environment to pig, or dam to offspring. Oropharyngeal secretions may contain salmonellae, largely due to the fact that tonsils become rapidly contaminated with salmonellae following oral transmission. This may allow nose-to-nose transmission. Aerosolized secretions, feces, or contaminated dust particles make the potential for aerosol transmission for short distances quite real. In fact, experimentally esophagostomized pigs can acquire systemic *Salmonella* following aerosol introduction of the pathogen (Fedorka-Cray et al. 1995).

Salmonella infection of swine herds is much more common than disease. Longitudinal studies in the Netherlands suggest that about 25% of herds are never infected, 24% are constantly infected, and 50% are infected most of the time. There appear to be infection cycles, with the endemic salmonellae having an ecological advantage over newly introduced *Salmonella* serotypes. Infection occurs in the first weeks after arrival or commingling and reaches a maximum of 80–100% prevalence within another 2–3 weeks. About 5–30% of the pigs are still excreting salmonellae at the end of the finishing period. In 2006, the National Animal Health Monitoring System (NAHMS) reported that 52.6% of herds sampled across the United States were *Salmonella* positive, up from data obtained in 1995 (38.2%) and 2000 (32.8%). The 2012 NAHMS survey reported on the percentage of grower/finisher sites listing salmonellosis as a disease problem. Grower/finishers with <2000 hogs reported 3.1% of sites with salmonellosis. Grower/finishers with >5000 head reported 18.8% of sites with salmonellosis.

During acute disease, pigs will shed up to 10^6 *S.* Choleraesuis per gram feces (Smith and Jones 1967) or 10^7 *S.* Typhimurium per gram of feces (Gutzmann et al. 1976). The minimum disease-producing dose of either serotype has not been established in field situations, but disease is difficult to reproduce experimentally at low doses. There is one report of moderate disease following oral inoculation of 10^6 microbes (Dawe and Troutt 1976), but most authors report successful experimental disease production with doses of 10^8–10^{11} organisms unless pigs are artificially stressed by injection of dexamethasone or in some other manner. Pigs infected with 10^3 organisms remained clinically normal, but uninoculated pigs in the same pen did become clinically ill (Gray et al. 1996). It is likely that dose (and perhaps virulence) is magnified when pigs are infected and sequential (pig-to-pig) transmission occurs in field situations so that the initial infective dose in the field is considerably less than that required in experimental situations. High animal density, stress of transport, and intercurrent nutritional or infectious disease are assumed to increase the shedding by carriers as well as the susceptibility of exposed pigs. Pigs with nondetectable shedding of salmonellae can begin shedding within hours of an applied stress. The transmission demonstrated between feeder pigs also occurs between pigs during market transport and lairage at abattoirs, with infection rates proportional to time spent in transport and lairage (Hurd et al. 2001a,b). It is likely that catecholamines are released in association with stress, resulting in decreased gastric acid production and increased intestinal motility. Increases in stomach pH increase the likelihood that salmonellae will survive passage through the stomach and will access and replicate in the intestine and colon.

Outbreaks of salmonellosis are usually characterized by spread from pen to pen. Situations of spread from pen to distant pen are likely because of vectors or caretaker transmission. When all animals sicken simultaneously, a common source such as feed, bedding, water, or a contaminated environment should be suspected. *Salmonella* infections tend to be more prevalent in continuous-flow systems than in barns managed by principles of all-in/all-out. Prevalence is also higher in barns with open flush gutters than in those with slotted floors, with the highest rates of infection observed in outdoor finishing systems (Davies et al. 1997).

Numerous studies in a variety of host species with a variety of serotypes have demonstrated prolonged carrier states following infection. The pattern of shedding and the duration of the carrier state after clinically apparent disease have been studied only in group-housed pigs with no barrier to repeated reinfection (Wilcock and Olander 1978; Wood et al. 1989). After experimental infection, *S.* Typhimurium was isolated from feces daily during the first 10 days postinfection and frequently during the next 4–5 months. When slaughtered 4–7 months after initial infection, over 90% of pigs were positive for *S.* Typhimurium in the mesenteric lymph node, tonsil, cecum, or feces (Fedorka-Cray et al. 1994; Wilcock and Olander 1978; Wood et al. 1989; Wood and Rose 1992). *S.* Newport has been shown to persist in mesenteric lymph nodes for 28 weeks. Infection of individual animals may be relatively short lived (less than 8 weeks), but organisms may circulate

within a population and between pigs and the environment for extended periods of time. *S.* Choleraesuis has been shown to persist for at least 3 months in wet feces and 6 months in desiccated feces.

The influence of antibiotics on the frequency and duration of shedding of salmonellae in pigs has received little attention. In human enteric salmonellosis, the use of antibiotics has long been recognized to prolong the carrier state (Aserkoff and Bennett 1969; Dixon 1965). In pigs with enterocolitis, antibiotics do not reduce the duration or the magnitude of fecal shedding, but neither are they reported to prolong or intensify shedding (Degeeter et al. 1976; Finlayson and Barnum 1973; Gutzmann et al. 1976; Jacks et al. 1988; Jones et al. 1983; Wilcock and Olander 1978). In contrast, vigorous antibacterial therapy early in the course of septicemia caused by *S.* Choleraesuis may significantly reduce the magnitude and duration of fecal shedding (Jacks et al. 1981).

Pathogenesis

The clinical and pathological features of *Salmonella* infections are extremely variable. Severity is influenced by serotype, virulence, natural and acquired host resistance, and route and quantity of the infective dose. Over 200 virulence factors have been associated with salmonellae, but few have been completely characterized. Generally, those that promote virulence in pathogenic salmonellae are involved in adhesion, invasion, cytotoxicity, and resistance to intracellular killing, often working in combination to promote disease.

Although large doses (greater than 10^7 microbes) are required to induce disease experimentally, intraluminal replication may be important with small inocula. Disease is facilitated by factors such as peristaltic impairment, interference with intestinal flora, and elevation of gastric pH (Clarke and Gyles 1993). Replication to about 10^7 organisms per gram of intestinal content is required for lesion production in pigs infected with *S.* Typhimurium, a finding that probably also applies to other serotypes causing enterocolitis. Alterations in normal intestinal defenses by antibiotic-induced changes in normal flora or cold-induced alteration in intestinal motility may reduce the amount of replication required for disease or increase the ease of *Salmonella* replication (Bohnhoff et al. 1954).

The ability to invade is a requirement for pathogenesis and is encoded by a serotype-specific plasmid (Helmuth et al. 1985). Removal of this plasmid results in a lack of ability to invade but has no effect on ingestion or killing by murine macrophages, LPS production, or serum resistance (Gulig and Curtiss 1987). During the invasion process there is induction of synthesis of new proteins that enhance intracellular survival (Finlay et al. 1989). While many epithelial cell types (enterocytes, M cells, goblet cells) in the jejunum and ileum may be invaded, the predominant portal of entry into the submucosa may occur at Peyer's patches (Meyerholz et al. 2002; Meyerholz and Stabel 2003; Schauser et al. 2004). *S.* Choleraesuis locates preferentially in the colon on the luminal surface of ileal M cells of Peyer's patches (Pospischil et al. 1990). Invasion into M cells appears to be the preferred route given the shorter glycocalyx that coats these cells. Attachment of the bacteria to epithelial receptors triggers microfilament-controlled uptake, vacuole formation, vacuole transport through the cell cytoplasm, and entry into the lamina propria via exocytosis through the basement membrane (Takeuchi 1967; Takeuchi and Sprinz 1967). Passage through the epithelium causes mild and transient enterocyte damage. Salmonellae can synthesize over 30 proteins that are selectively induced during infection of macrophages, making them facultative intracellular bacteria (similar to *Brucella*, *Mycobacterium*, and *Listeria* species) that can survive within macrophages and neutrophils in the lamina propria (Roof et al. 1992a,b). Spread to mesenteric lymph nodes is rapid, occurring within 2 hours of inoculation of ligated intestinal loops or 24 hours after oral challenge (Reed et al. 1985, 1986). Knockout mice ($CD18^-$) have been used to show that $CD18^+$ phagocytes are important in the dissemination of the organism to the spleen and liver (Vazquez-Torres et al. 1999). Two leading cell candidates for this systemic transport are macrophages and dendritic cells (Vazquez-Torres et al. 2000). Recent *in vitro* work has suggested that dendritic cells are capable of producing tight junction proteins to penetrate the epithelium and sample luminal bacteria including *Salmonella* (Rescigno et al. 2001). Concurrent with bacillary spread is the appearance of an acute predominantly macrophagic inflammatory reaction and prominent microvascular damage with thrombosis within the lamina propria and submucosa. Non-enteric routes of systemic invasion may be important since *S.* Choleraesuis demonstrated primary colonization of the lung within 4 hours after intranasal administration to esophagostomized pigs (Fedorka-Cray et al. 1995; Gray et al. 1995).

Early intestinal inflammation is considered a key feature of pathogenesis for enteric forms of salmonellosis. Neutrophil recruitment and transmigration across the epithelium is considered the most significant component (McCormick et al. 1995). Host-derived caspase-1 can act as a proinflammatory agent by cleaving interleukin-1 beta and interleukin-18 into active molecules (Fantuzzi and Dinarello 1999). SipA, a protein that *Salmonella* injects into host cells, has also been shown to contribute to the inflammatory response by activation of phospholipase C (Lee et al. 2000). *Salmonella*-induced activation

of inflammatory mediators such as nuclear factor-kappa B and phosphokinase C results in basolateral secretion of interleukin-8 and apical secretion of pathogen-elicited epithelial chemoattractant (Eaves-Pyles et al. 1999; Lee et al. 2000). These molecules act as chemokines promoting the transepithelial migration of neutrophils into the intestinal lumen (Gewirtz et al. 1999). In contrast, several studies using rabbits, monkeys, calves, or pigs have demonstrated fluid secretion independent of mucosal necrosis or inflammation (Clarke and Gyles 1987; Giannella et al. 1973; Kinsey et al. 1976; Rout et al. 1974). These studies present evidence that, at least early in the disease, diarrhea is the result of decreased sodium resorption and increased chloride secretion due to cholera-like and Shiga-like enterotoxins. Secretion stimulated by prostaglandins elaborated by endotoxin-stimulated neutrophils may also be important (Stephen et al. 1985). Toxic effects of certain *Salmonella* outer membrane proteins, as well as lipid A associated with the LPS, are also important mediators of cell damage. Survival within phagocytes is an important attribute of virulent salmonellae, the mechanism of which is not clear. Salmonellae that possess smooth LPS, O side chains, and a complete LPS core are more resistant to phagocyte killing.

Mucosal inflammation and necrosis, as well as septicemia, occur in concert with the diarrhea but perhaps independently of this response. Microvascular thrombosis and endothelial necrosis in the submucosa and lamina propria are consistent early lesions in porcine salmonellosis (Brown et al. 2007; Lawson and Dow 1966; Reed et al. 1986; Wilcock et al. 1976), probably in response to locally produced endotoxin. Salmonellae are not directly associated with the damaged vessels but direct the events from the protected intracellular niche of macrophages in the surrounding submucosa or lamina propria (Takeuchi and Sprinz 1967). Mucosal ischemia as a result of the microvascular thrombosis is probably a major contributor to the mucosal necrosis so typical of salmonellosis in all species. The second major contribution to mucosal necrosis is probably from the chemical products of mucosal inflammation. The systemic signs and lesions of septicemic salmonellosis are most commonly attributed to endotoxemia from bacterial dissemination. The complex biology of endotoxin is beyond the scope of this chapter, and readers should consult Cybulsky et al. (1988), Elin and Wolff (1976), or Wolff (1973). Briefly, endotoxin initiates inflammation and fever. Most of the effects are mediated by interleukin-1, a lymphokine produced by macrophages stimulated by the endotoxin (Rubin and Weinstein 1977). Endotoxins have either direct effects on tissue or effects via an array of cytokine mediators that are likely a result of interactions with Toll-like receptor 4 (McGettrick and O'Neill 2010).

Clinical signs

Salmonella typhimurium and the *S.* I 1,4,[5],12 : i:- Variant

Clinical signs of *S.* I 1,4,[5],12 : i:- infection in swine are essentially indistinguishable from those of *S.* Typhimurium. The initial clinical sign in pigs is watery yellow diarrhea initially without blood or mucus. The disease may spread rapidly to involve most pigs in a pen within a few days. The initial diarrhea in an individual pig usually lasts 3–7 days, but it typically may recur for second and third bouts, giving the impression of a waxing and waning diarrheal disease of several weeks' duration. Blood may appear sporadically in the feces but rarely with the profuseness typical of swine dysentery or hemorrhagic porcine proliferative enteropathy. Affected pigs are febrile, have decreased feed intake, and are dehydrated, paralleling the severity and duration of the diarrhea. Mortality usually is low and occurs only after several days of diarrhea, presumably as the result of hypokalemia and dehydration. Most pigs make complete clinical recovery, but a portion may remain as carriers and intermittent shedders for at least 5 months. A few pigs may remain unthrifty and chronically waste. Occasional pigs may develop rectal strictures, resulting in obstipation and marked distension of the abdomen. Rectal strictures have been ascribed to defective healing of ulcerative proctitis caused by *S.* Typhimurium (Wilcock and Olander 1977a,b). The stricture reportedly represents fibrosis in an area of persistent ischemia, with the rectum predisposed because of a normally precarious blood supply.

Salmonella choleraesuis

Septicemic salmonellosis caused by *S.* Choleraesuis is most often observed in weaned pigs less than 5 months of age but may be seen occasionally in market swine, suckling piglets, or adult breeding stock. Clinical signs observed are initially from generalized sepsis and later may be from localization in one or more organs/systems. Initially affected animals are inappetent, lethargic, and febrile with temperatures of 40.5–41.6 °C (105–107 °F) and may have a shallow, moist cough with slight expiratory dyspnea. Icterus may be apparent. The first evidence of disease may be finding pigs reluctant to move, huddled in the corner of a pen, or even dead, with cyanosis of extremities and abdomens (Figure 59.1). Diarrhea is not usually a feature of septicemic salmonellosis until the third or fourth day of disease, when watery yellow feces may be seen. Infrequently, nervous signs may be observed that resemble *Streptococcus suis* meningitis, classical swine fever, or pseudorabies (Reynolds et al. 1967; van der Wolf et al. 2001; Wilcock and Olander 1978) as a

Figure 59.1 *Salmonella* Choleraesuis. Lethargic, gaunt, and cyanotic extremities. *Source:* Courtesy of Dr. Greg Stevenson.

result of necrotizing and histiocytic vasculitis leading to encephalitis and/or meningitis. In pregnant sows, abortions may be observed. In most outbreaks, the case fatality rate is high, while morbidity is variable but is usually less than 10%. The duration of the disease in individual pigs, as well as the duration and severity of each epidemic, is unpredictable but will be prolonged without successful intervention.

Other serotypes

Salmonella Typhisuis is an infrequent cause of chronic diarrhea and wasting with characteristic caseating lesions in affected pigs. *S.* Heidelberg has been infrequently associated with outbreaks of acute watery diarrhea in weaned pigs. *S.* Dublin and *S.* Enteritidis rarely cause nervous signs in suckling pigs as a consequence of suppurative meningitis.

Lesions

Salmonella typhimurium

The most consistent gross lesion in pigs suffering from *S.* Typhimurium is enterotyphlocolitis most often involving the ileum, cecum, and spiral colon and occasionally extending to involve the descending colon and rectum. Affected segments typically have thickened edematous walls, and the mucosa is red and roughened with a granular appearance and may have multifocal or coalescing erosions and ulcers that are covered with adherent gray-yellow fibrinonecrotic debris (Figure 59.2). Sharply delineated deep button ulcers may be observed in more chronic lesions (Figure 59.3). Mesenteric lymph nodes, especially ileocecal nodes, are consistently markedly enlarged, congested, and moist (Figure 59.4). Stomach and intestinal contents are usually scant and bile stained.

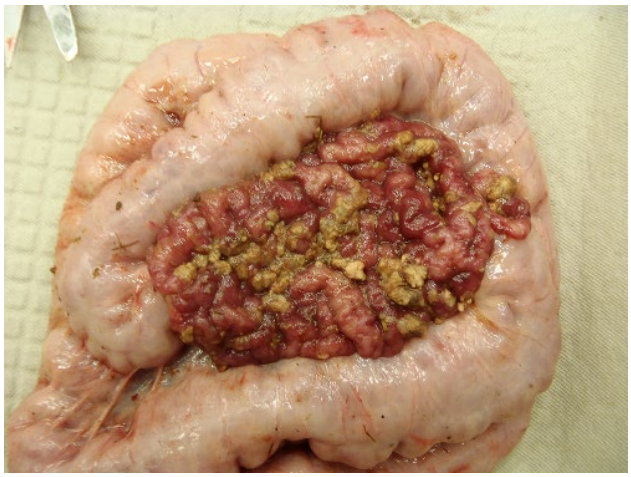


Figure 59.2 *Salmonella* Typhimurium. Spiral colon. Thickened turgid wall and hyperemic and thickened mucosa with adherent yellow-tan fibrinonecrotic exudate. Lesions are typical of acute colitis caused by *S.* Typhimurium, *S.* I 1,4,[5],12 : i:-, and *S.* Choleraesuis. *Source:* Courtesy of Dr. Darin Madson.

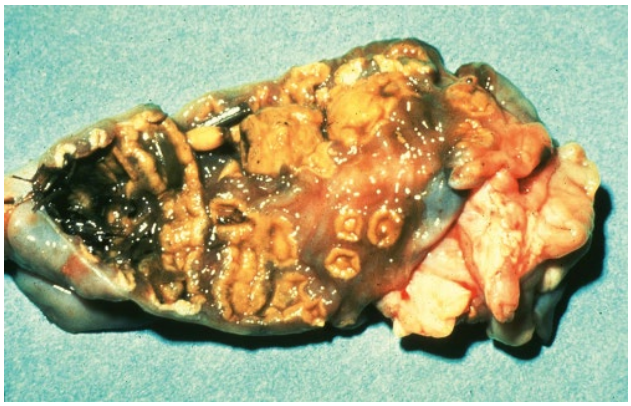


Figure 59.3 *Salmonella* Choleraesuis. Spiral colon. Multifocal-to-coalescing well-demarcated "button" ulcers covered by yellow fibrinonecrotic exudate. Lesions are typical of chronic colitis caused by *S.* Choleraesuis, *S.* Typhimurium, and *S.* I 1,4,[5],12 : i:-. *Source:* Courtesy of Dr. Greg Stevenson.

Often cecal and colonic contents contain black or sand-like gritty material. Of note is that the ileal mucosa in uncomplicated cases of *S.* Typhimurium is usually reddened and slightly roughened with occasional adherent fibrin. It should not be confused with the markedly thickened and necrotic ileal mucosa that was observed in the historic condition known as "necro" and is now known to most commonly be a sequel to proliferative enteropathy (see Chapter 59). Rarely, there is a stricture in the intrapelvic rectum associated with mural fibrosis and distension of the more proximal colon (and sometimes small intestines) with impacted feces.

Microscopic lesions are most consistent in the cecum and spiral colon but may be also in the ileum, descending colon, and rectum and include focal to diffuse necrosis of

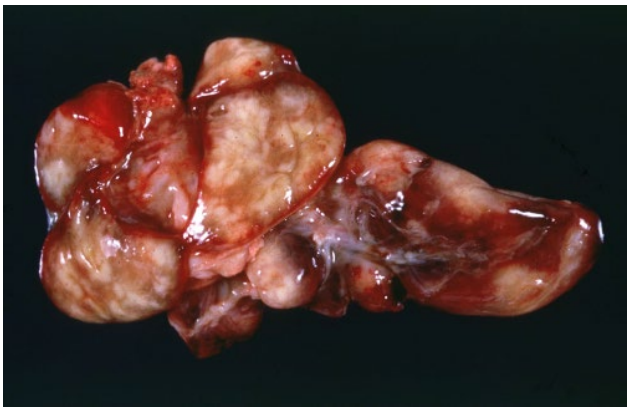


Figure 59.4 *Salmonella* Choleraesuis. Mesenteric lymph node is congested, moist and enlarged typical of *S*. Choleraesuis, *S*. Typhimurium, and *S*. I 1,4,[5],12 : i:-. *Source:* Courtesy of Dr. Greg Stevenson.

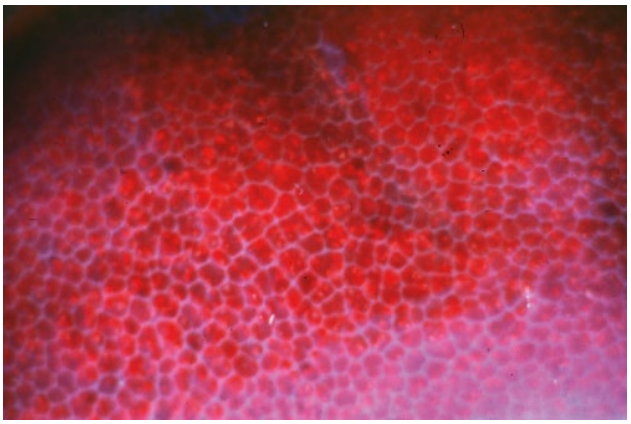


Figure 59.5 *Salmonella* Choleraesuis. Capsular surface of the liver. Visible through the capsule amid the normal lobular pattern of the porcine liver are many small variably sized white-to-pink foci of hepatic parenchymal necrosis. *Source:* Courtesy of Dr. Greg Stevenson.

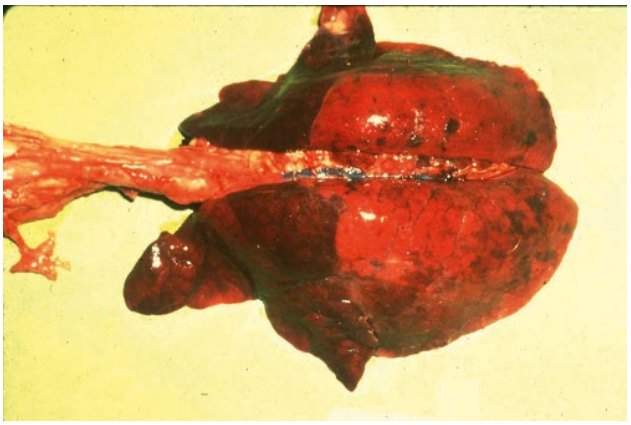


Figure 59.6 *Salmonella* Choleraesuis. Dorsal caudal lung is slightly firm, moist, resilient, and non-collapsing and has interlobular hemorrhagic edema and multifocal ecchymoses typical of acute *S*. Choleraesuis-induced interstitial pneumonia. Cranial ventral lung is dark purple, firm, and non-collapsing with focal fibrinous pleuritis, typical of subacute *S*. Choleraesuis-induced bronchopneumonia. *Source:* Courtesy of Dr. Greg Stevenson.

crypt and surface epithelial cells. The lamina propria and submucosa are typically infiltrated first by neutrophils followed by predominantly macrophages and fewer lymphocytes by the second day. Fibrin thrombi are frequently observed in capillaries of the lamina propria and are less frequent in larger vessels in the submucosa. Necrosis, likely a sequel to infarction, sometimes extends through the mucosa into the submucosa and lymphoid patches, creating grossly visible ulcers. Fibrinonecrotic debris that may contain myriad opportunistic bacteria and numerous *Balantidium coli* organisms is often adherent to the luminal surface of damaged epithelium. Submucosal lymphoid patches may be necrotic in acute disease, but later in pigs dying of the naturally occurring disease, lymphoid hypertrophy or even regenerative hyperplasia is more common. Regional lymph nodes are typically edematous and contain neutrophils acutely and predominantly macrophages by day 2–3 in sinuses. In some nodes, there may be focal necrosis. More complete discussions of lesions are available (Brown et al. 2007; Wilcock et al. 1976).

Salmonella choleraesuis

Gross lesions in pigs dying in the acute phase of septicemia include cyanosis of ears, feet, tail, and ventral abdominal skin (Figure 59.1). Lymph nodes, especially the gastrohepatic and mesenteric, are typically enlarged, moist, and congested (Figure 59.4), and the spleen is enlarged, dark purple, and pulpy. The liver may be slightly enlarged with scattered small 1–2 mm foci of parenchymal necrosis (Figure 59.5), and the wall of the gallbladder may be thickened and edematous. There are often renal cortical petechia and ecchymoses. Acute interstitial pneumonia evidenced by moist, slightly firm, resilient non-collapsing lungs that often have red (hemorrhagic) fluid separating lobules is usually observed (Figure 59.6). The gastric mucosa is often markedly congested. Additionally, in pigs that survive a few days, there may be infarction of the skin on the tips of ears that appears dry and dark purple, sometimes with portions that slough (Figure 59.7). Icterus is inconsistently severe. Bronchopneumonia may be observed as consolidation of cranial ventral lungs with purulent exudate in airways (Figure 59.6). The gastric fundic mucosa may be infarcted, appearing dark purple. Enterocolitis may be present and is identical to that described for *S*. Typhimurium (Figures 59.1 and 59.2).

Microscopic lesions are extensive and involve many organs. The most unique and diagnostic lesions are randomly scattered foci of coagulative necrosis in the liver

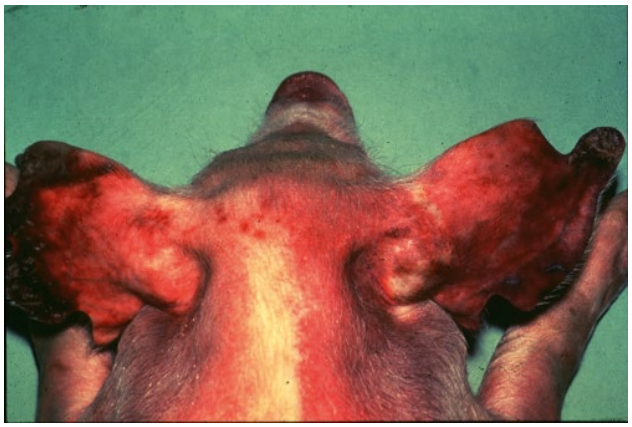


Figure 59.7 *Salmonella* Choleraesuis. Cyanosis of the skin on the snout, shoulders, and legs with ischemic necrosis of skin on the ears evidenced by dark purple-black discoloration. *Source:* Courtesy of Dr. Greg Stevenson.

that are variably infiltrated with neutrophils and histiocytes (Lawson and Dow 1966), so-called paratyphoid nodules. Similar coagulative necrosis may be randomly scattered in spleen or observed in lymph nodes. Other lesions typical of salmonellosis include fibrinoid thrombi in venules of gastric mucosa, in cyanotic skin, in glomerular capillaries, and less regularly in pulmonary vessels. There is hyperplasia of reticular cells of spleen and lymph nodes as well as generalized swelling of endothelial cells and histiocytosis typical of gram-negative sepsis. Diffuse histiocytic interstitial pneumonia or suppurative bronchopneumonia is often observed. Segmental necrotizing vasculitis with perivascular histiocytic infiltrates, sometimes with localized necrotizing encephalitis, is uncommonly observed. Lesions in small and large intestines are the same as for *S.* Typhimurium and are described below. More complete discussions of the pathology of septicemic salmonellosis are available (Brown et al. 2007; Lawson and Dow 1966).

Other serotypes

Lesions observed in wasting pigs infected with *S.* Typhisuis are characteristic (Andrews 1976; Barnes and Bergeland 1968; Fenwick and Olander 1987). Chronic ulcerative colitis is observed with deep multifocal-to-coalescing mucosal ulcers that have caseous necrotic material in their centers. Likewise, regional lymph nodes are enlarged and contain caseous necrotic debris, forming the base. Bronchopneumonia with caseous abscesses, resembling tuberculosis, may also be observed.

Lesions in weaned pigs with diarrhea associated with *S.* Heidelberg are mild or absent. Small and large intestines are typically fluid filled, and the mucosa may have sparse to moderate amounts of adherent mucus. In the few reports of nervous disease in suckling pigs infected with *S.* Dublin or *S. Enteritidis*, the leptomeninges are distended by fibrin and aggregates of neutrophils and fewer macrophages.

Diagnosis

Clinical signs and lesions may lead to a presumptive diagnosis of salmonellosis, but are insufficient for confirmation given the number of other diseases that resemble the various forms of salmonellosis. The clinical signs observed with *S.* Choleraesuis are similar to other septicemias such as caused by *Erysipelothrix rhusiopathiae* (Chapter 53), *S. suis* (Chapter 61), or *Actinobacillus suis* (Chapter 48) or to those observed with classical swine fever (Chapter 39). Lesions in lungs may resemble those of or *Actinobacillus pleuropneumoniae* (Chapter 48). Gross lesions of splenomegaly, hepatomegaly, lymphomegaly, interstitial pneumonia, and focal hepatic necrosis are very suggestive of septicemia by *S.* Choleraesuis, but are not seen in every case and closely resemble lesions of classical swine fever.

Likewise the differential diagnosis of diarrhea in weaned pigs, in addition to *S.* Typhimurium and *S.* I 1,4,[5],12 : i:-, should include swine dysentery (Chapter 62), proliferative enteropathy (Chapter 58), rotaviruses (Chapter 43), coronaviruses (Chapter 31), circovirus type 2 (Chapter 30), colibacillosis (Chapter 52), and trichuriasis (Chapter 67). Combined infections in diarrheal disease are commonplace. Gross lesions in enteric salmonellosis, swine dysentery, and proliferative enteropathy are similar in that all three may present as fibrinonecrotic typhlocolitis. However, presumptive differentiation at necropsy is possible in many cases by recognition of differences in lesion distribution. Salmonellosis is usually in the spiral colon and occasionally small intestine, may be focal or diffuse, may include mucosal ulcers, and always involves marked mesenteric lymphadenopathy. The lesion of swine dysentery is diffuse and shallow (no deep ulceration) and restricted to the cecum and spiral colon. In addition, lymph node enlargement is absent or mild. In proliferative enteropathy, ileal involvement usually overshadows the milder colonic lesions, and the mucosa underlying any fibrinonecrotic exudate is markedly hyperplastic.

The lesions of caseating button ulcers in the spiral colon, caseous lymphadenitis, and bronchopneumonia with caseating abscesses in wasting pigs with chronic diarrhea are strongly presumptive of infection with *S.* Typhisuis. However the intestinal lesions may heal in some pigs, leaving the lymphoid and pulmonary lesions to be distinguished from tuberculosis and infection with *Trueperella pyogenes* (Barnes and Sorensen 1975).

A definitive diagnosis of salmonellosis is confirmed by bacterial culture and identification in conjunction

with demonstration of compatible lesions. The wide distribution of environmental salmonellae and the incidence of subclinical infection and shedding of various *Salmonella* serotypes make culture of intestinal lesions or contents alone unreliable for disease diagnosis. Positive cultural identification should always be supported by appropriate lesions before a diagnosis of salmonellosis is made.

To culture *S.* Choleraesuis from suspected cases of septicemia, samples of lung, liver, or spleen often yield pure cultures of the organism on brilliant green, bismuth sulfite, blood agar, or MacConkey agar. Enrichment techniques are seldom required unless the organs have been contaminated by feces or careless handling or have autolysis, in which case tetrathionate broth at 107.6–109.4 °F (42–43 °C) is the enrichment medium of choice. Selenite broth is inhibitory for *S.* Choleraesuis and should be avoided (Edwards and Ewing 1972). Attempts to isolate salmonellae from animals that have received antimicrobial therapy are often unrewarding. Intestine or feces are not reliable specimens for isolation of *S.* Choleraesuis in pigs with acute septicemia (Schwartz 1991).

In cases of diarrhea in which S. Typhimurium or the I 1,4,[5],12 : i:- variant is suspected, a pool of ileum, colon, and ileocecal lymph node should enable detection of virtually all active or recently recovered cases, although tissues such as tonsil or cecal wall will usually yield positive cultures as well (Wilcock et al. 1976; Wood et al. 1989). From live animals, large (10 g) aliquots of feces or pharyngeal tonsil scrapings are preferable to rectal swabs for isolation, with tetrathionate enrichment the method of choice.

Other tests using more sophisticated technology, including polymerase chain reaction (PCR), are not required for routine diagnosis. PCR currently has value as a screening tool in populations but has a relatively high cost and currently lacks sensitivity without preenrichment. PCR-based detection of salmonellae does not constitute diagnosis of salmonellosis since this assay can detect DNA from dead *Salmonella* and the organism may be present without causing clinical disease.

Serology is becoming increasingly available, usually in the form of an ELISA test. Most tests use surface antigens such as LPS. These tests, some of which use mixed antigens containing both LPS and antigens from several serotypes, thus far appear to lack specificity and sensitivity for individual animal diagnosis but are useful for herd screening (Baum et al. 1996). A mixed ELISA test using meat juice at slaughter to detect antibody to a broad range of serotypes has been useful in categorizing the level of *Salmonella* infection in herds in Denmark (Mousing et al. 1997; Nielsen et al. 1995).

Prevention and control

Prevention

Infection does not necessarily result in disease, and pigs may not sicken with disease until severely stressed long after initial exposure. The control of disease expression rests on efforts to minimize the exposure dose and stress and to maximize pig resistance. Carrier pigs and contaminated feed or environment are the most significant sources of infection to pigs, and pigs are most likely to develop disease during periods of stress or when exposed to massive numbers of salmonellae. The commingling and transport of weanling pigs from different sources to finishing farms enhance activation of latent carriers and ensure exposure of stressed pigs to salmonellae (Allred 1972). The source of host-adapted *S.* Choleraesuis, which is rarely isolated from feed or feed ingredients, would seem to be limited to carrier pigs and facilities previously contaminated with this serotype. The fact that many outbreaks occur in facilities with good sanitation suggests that other stresses probably contribute to occurrence of the disease. Management practices that allow filling of grower and finishing rooms with single-source and single-age pigs are beneficial. Minimizing the variety of stresses often involved in acute outbreaks requires constant attention to details of management and husbandry, including proper animal density, dry, comfortable pens and temperatures, and adequate ventilation. On farms with endemic disease, modifications to the facility and environment as well as implementation of management practices that emphasize all-in/all-out production should precede a prophylactic drug program. Antibiotics are probably useful as aids in preventing occurrence of disease when used prophylactically, but their use will not prevent infection and when relied upon for prevention of disease will eventually fail.

As with other facultative intracellular bacteria, live vaccines that stimulate cell-mediated immunity are the most likely to be protective for salmonellae in swine. Historically, an attenuated live *S.* Choleraesuis vaccine was used widely in the United Kingdom for many years but was withdrawn when *S.* Choleraesuis infection decreased in that country to negligible proportions. Introduction of effective and safe modified live attenuated vaccines for *S.* Choleraesuis has been credited with a major reduction in the occurrence of systemic salmonellosis in North America. The isolates used in these vaccines are either naturally occurring avirulent *S.* Choleraesuis or are derived from repeated passage through porcine neutrophils, the product of which was demonstrated to have been cured of a 50 kb virulence plasmid necessary for intracellular survival (Kramer et al. 1987, 1992; Roof et al. 1992b). When given at weaning, vaccine protected pigs for at least 20 weeks (Roof

and Doitchinoff 1995) against homologous serotypes, with some cross-protection suggested with heterologous serotypes. A vaccine containing *S.* Choleraesuis and *S.* Typhimurium is also available.

Partial protection can be obtained with bacterins, primarily because of the nonspecific mitogenic and immunostimulant effect of LPS (Fenwick et al. 1986) and the immunodominance of *Salmonella* O antigens. Killed vaccines for *S.* Typhimurium are safe, but the bulk of the evidence suggests that they have little efficacy in preventing disease following strong challenge because resistance to disease rests primarily on cell-mediated immunity (Collins 1974; Davies and Kotlarski 1976). Extrapolation of information from experience in humans (Hornick et al. 1970; Welliver and Ogra 1978) and calves (Bairey 1978) suggests, however, that use of a potent killed vaccine may increase the dose necessary to cause disease and may offer some protection from septicemic salmonellosis, in which humoral immunity may play a role.

Monitoring herds for salmonellae has not been commonly practiced. The detection of carrier animals is difficult because of the unpredictability of fecal shedding. The detection of salmonellae by bacterial culture of feces and tonsils of diarrheic pigs in the nursery would likely be the most rewarding for identification of infected herds. However, even repeated negative fecal or tonsillar cultures do not guarantee that a herd or individual is not a *Salmonella* carrier and thus a potential shedder. The use of *Salmonella* serology can determine if an animal has had previous exposure to salmonellae, but this has little relevance to the carrier status or to the probability of shedding. Food safety concerns have stimulated renewed interest in serology as a method to determine the *Salmonella* infection status of groups of market swine. This technology offers the possibility of sensitive and specific methods to identify infected herds, but it is not yet useful for determining the infection status of individual pigs.

Control

With either septicemic or enteric salmonellosis, the goals of treatment in an outbreak of salmonellosis are to minimize the severity of clinical disease, prevent spread of infection and disease, and prevent recurrence of the disease in the herd. With salmonellosis the attainment of these goals is particularly difficult. Salmonellae are often resistant *in vitro* to many antibacterial agents used in swine (Barnes and Sorensen 1975; Blackburn et al. 1984; Krull 2017, unpublished data; Schultz 1989; Schwartz 1997; Wilcock et al. 1976). During clinical disease, the organism inhabits a protected intracellular niche inaccessible to many common antibacterials. The use of various antibiotics to treat enteric salmonellosis is widely advocated (Barnes and Sorensen 1975; Morehouse 1972; Radostits et al. 2007), but much of the information to support this recommendation has been taken from trials designed to test the prophylactic efficacy of drugs, not their therapeutic efficacy. Thus pigs on medicated feed when inoculated orally with salmonellae have the antibiotic already present in the gastrointestinal tract to interact with the salmonellae, resulting in milder disease because of what amounts to a decreased inoculum. In the few trials designed specifically to test antibacterial drug efficacy against clinical enteric salmonellosis, such therapy was considered to have little merit (Gutzmann et al. 1976; Heard et al. 1968; Olson et al. 1977; Wilcock and Olander 1978). Although not therapeutic, oral medications may decrease efficiency of transmission and have a prophylactic effect on pigs not yet affected. Antimicrobials are ordinarily administered at maximum permissible levels in feed or, preferably, water. Ideally, the choice of antibacterial agent should be based on *in vitro* susceptibility testing of isolates from each outbreak. Since medication often must be initiated before such results are available, choices must be based on previous experience and results of controlled trials.

In contrast, vigorous therapy early in the course of septicemia caused by *S.* Choleraesuis has been reported to significantly reduce the duration and severity of disease (Jacks et al. 1981). In that report, therapy was initiated after inoculation but prior to the onset of clinical signs. Evaluation of efficacy under field conditions is difficult because of the unpredictability of the disease and because husbandry changes often accompany the use of antibacterials in an outbreak. Reports and practitioner communications from the American Midwest, however, suggest that visibly affected animals respond to aggressive therapy with parenteral antimicrobials (Schwartz 1991). Mass medication of the population at risk to decrease severity of disease and transmission of salmonellae is also widely practiced. The choice of an appropriate antimicrobial is aided by antibiograms and previous herd experience. In the absence of either, amikacin, gentamicin, apramycin, ceftiofur, and trimethoprim/sulfonamide have been effective *in vitro* against most isolates (Barnes and Sorensen 1975; Mills and Kelly 1986; Schultz 1989; Schwartz 1997; Wilcock et al. 1976). The increase in incidence of ASSuT resistance in *S.* Typhimurium and the *S.* I 1,4,[5],12 : i:- variant increases the need for isolation, identification, and susceptibility testing. Anti-inflammatory agents are sometimes administered to critically ill animals to combat the effects of endotoxin (Schultz 1989; Schwartz and Daniels 1987). Dipyrone was the anti-inflammatory of choice in swine, but this drug is now banned in food-producing animals. Flunixin is approved for use in swine and this drug has potent antiendotoxin effects. Meloxicam is another NSAID that has been used in swine (Pairis-Garcia et al. 2015).

Most *Salmonella* antimicrobial resistance is plasmid mediated. Of concern is the recent emergence of a *S.* Typhimurium definitive type 104 (DT104), isolated primarily from bovine and human populations, that has chromosomally integrated multiple antimicrobial resistance (Low et al. 1997). This isolate has a higher morbidity and mortality in humans than other *S.* Typhimurium organisms and was, until recently, increasing in prevalence in human and bovine populations. Xiong et al. 2010 identified a DT104-like resistance gene cassette in *S.* Choleraesuis exhibiting hypervirulence *in vivo*. However, this isolate has yet to be implicated in field outbreaks of swine salmonellosis. The *S.* I 1,4,[5],12 : i:- variant of *S.* Typhimurium is the result of a full or partial deletion of the fljB gene responsible for expression of flagellar antigen 2 (Foley et al. 2008)

In addition to antimicrobial therapy, the successful treatment of salmonellosis relies heavily on routine husbandry procedures recommended for control of infectious diseases. The diarrheic pig contaminates its environment and is the single most important source of infection for other pigs. Removal and isolation of sick animals, minimizing exposure to infective material by scrupulous pen sanitation, frequent cleaning of water bowls, and restriction of animal or staff movement from potentially contaminated to clean areas are necessary. Efforts to modify management and environment to decrease crowding and stress and increase pig comfort are essential adjuncts to specific therapy.

References

Allred JN. 1972. *J Am Vet Med Assoc* 160:601–602.

Allred JN, Walker JW, Beal VC, et al. 1967. *J Am Vet Med Assoc* 151:1857–1860.

Andrews JJ. 1976. Salmonella typhisuis infection in swine. In Proceedings of the North Central Conference Veterinary Laboratory Diagnostics, p. 7.

Aserkoff B, Bennett JV. 1969. *N Engl J Med* 281:636–640.

Bairey MH. 1978. *J Am Vet Med Assoc* 173:610–613.

Barnes DM, Bergeland ME. 1968. *J Am Vet Med Assoc* 152:1766–1769.

Barnes DM, Sorensen DK. 1975. Salmonellosis. In Dunne HW, Leman AD. *Diseases of Swine*, 4th ed. Ames, IA: Iowa State University Press, pp. 554–564.

Barnhill AE, Weeks KE, Xiong N, et al. 2010. *Appl Environ Microbiol* 76:2678–2680.

Baum DH, Harris DL, Nielsen B, et al. 1996. Comparison of the Danish MIX-ELISA to culture for the detection of *Salmonella* in finishing pigs at or near slaughter. In ISU Swine Research Report, p. 177.

Bixler WB. 1978. FDA salmonella control activities for animal feeds and feed ingredients. In Proceedings of the National Salmonellosis Seminar, Washington, DC.

Blackburn BO, Schlater LK, Swanson MR. 1984. *Am J Vet Res* 45:1245–1249.

Bohnhoff M, Drake BL, Miller CP. 1954. Effect of streptomycin on susceptibility of intestinal tract to *Salmonella* infection. *Proc Soc Exp Biol Med* 86:133–137.

Brown CC, Baker DC, Barker IK. 2007. Alimentary system. In Maxie MG, ed., *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*, Vol. 2. Burlington, MA: Elsevier.

Clarke RC, Gyles CL. 1987. *Am J Vet Res* 48:504–510.

Clarke RC, Gyles CL. 1993. *Salmonella*. In *Pathogenesis of Bacterial Infections in Animals*, 2nd ed. Ames, IA: Iowa State Univ Press, pp. 133–153.

Collins FM. 1974. *Bacteriol Rev* 38:371–402.

Craven JA, Hurst DB. 1982. *J Hyg (Camb)* 88:107–111.

Cybulsky MI, Chan MKW, Movat HZ. 1988. *Lab Invest* 58:365–378.

Davies R, Kotlarski I. 1976. *Aust J Exp Biol Med Sci* 54:207–219.

Davies PR, Morrow WEM, Jones FT, et al. 1997. *J Am Vet Med Assoc* 210:386–389.

Dawe DL, Troutt HF. 1976. Treatment of experimentally induced salmonellosis in weanling pigs with trimethoprim and sulfadiazine. *Proc Int Congr Pig Vet Soc* 4:M4.

Degeeter MH, Stahl GL, Geng S. 1976. *Am J Vet Res* 37:525–529.

Dixon JMS. 1965. *Br Med J* 2:1343–1345.

Doyle ME, Kaspar C, Rachel Klos JA. 2009. White paper on human illness caused by *Salmonella* from all food and non-food vectors. FRI Briefings 2009.

Eaves-Pyles T, Szabo C, Salzman AL. 1999. *Infect Immun* 67:800–804.

Edwards PR, Ewing WH. 1972. *Identification of Enterobacteriaceae*. 3rd ed. Minneapolis, MN: Burgess, pp. 146–207.

Elin RJ, Wolff SM. 1976. *Annu Rev Med* 27:127–141.

Fantuzzi G, Dinarello CA. 1999. *J Clin Immunol* 19:1–11.

Fedorka-Cray PJ, Whipp SC, Isaacson RE, et al. 1994. *Vet Microbiol* 41:333–344.

Fedorka-Cray PJ, Kelley LC, Stabel TJ, et al. 1995. *Infect Immun* 63:2658–2664.

Fenwick BW, Olander HJ. 1987. *Am J Vet Res* 48:1568–1573.

Fenwick BS, Cullor JS, Osburn BI, Olander HJ. 1986. *Infect Immun* 53:296–302.

Finlay BB, Heffron F, Falkow S. 1989. *Science* 243:940–943.

Finlayson M, Barnum DA. 1973. *Can J Comp Med* 37:139–146.

Foley SL, Lynne AM. 2008. *J Anim Sci* 86(14 Suppl):173–187.

Foley SL, Lynne AM, Nayak R. 2008. *J Anim Sci* 86(14 Suppl):149–162.

Gewirtz AT, Siber AM, Madara JL, et al. 1999. *Infect Immun* 67:608–617.

Giannella RA, Formal SB, Dammin GJ, et al. 1973. *J Clin Invest* 52:441–453.

Gooch JM, Haddock RL. 1969. *J Am Vet Med Assoc* 154:1051–1054.
Gray JT, Fedorka-Cray PJ, Stabel TJ, et al. 1995. *Vet Microbiol* 47:43–59.
Gray JT, Stabel TJ, Fedorka-Cray PJ. 1996. *Am J Vet Res* 57:313–319.
Gulig PA, Curtiss R. 1987. *Infect Immun* 55:2891–2901.
Gutzmann F, Layton H, Simkins K, et al. 1976. *Am J Vet Res* 37:649–655.
Heard TW, Jennett NE, Linton AH. 1968. *Vet Rec* 82:92–99.
Helmuth R, Stephan R, Bunge C, et al. 1985. *Infect Immun* 48:175–182.
Hoelzer K, Moreno Switt AI, Wiedmann M. 2011. *Vet Res* 42:34. doi: https://doi.org/10.1186/1297-9716-42-34
Hornick RB, Griesman SE, Woodward TE. 1970. *N Engl J Med* 283:735–746.
Hurd HS, Gailey JK, M cKean JD, et al. 2001a. *Am J Vet Res* 62:1194–1197.
Hurd HS, McKean JD, Wesley IV, et al. 2001b. *J Food Prot* 64:939–944.
Isaacson RE, Firkins LD, Weigel RM, et al. 1999. *Am J Vet Res* 60:1155–1158.
Jacks TM, Welter CJ, Fitzgerald GR, et al. 1981. *Antimicrob Agents Chemother* 19:562–566.
Jacks TM, Frazier E, Judith FR, et al. 1988. *Am J Vet Res* 49:1832–1835.
Jones FT, Langlois BE, Cromwell GL, et al. 1983. *J Anim Sci* 57:279–285.
Kinsey MD, Dammin GJ, Formal SB, et al. 1976. *Gastroenterology* 71:429–434.
Kramer TT, Pardon P, Marly J, et al. 1987. *Am J Vet Res* 48:1072–1076.
Kramer TT, Roof MB, Matheson RR. 1992. *Am J Vet Res* 53:444–448.
Lawson GHK, Dow C. 1966. *J Comp Pathol* 76:363–371
Lee CA, Silva M, Siber AM, et al. 2000. A secreted *Salmonella* protein induces a proinflammatory response in epithelial cells, which promotes neutrophil migration. *Proc Natl Acad Sci USA* 97:12283–12288.
Levine ND, Peterson EH, Graham R. 1945. *Am J Vet Res* 6:241–246.
Low JC, Angus M, Hopkins G, et al. 1997. *Epidemiol Infect* 118:97–103.
Lynn M, Dobson AW, McClune EL, et al. 1972. *Vet Med Samll Anim Clin* 67:1022–1027.
McCormick BA, Miller SI, Carnes D, et al. 1995. *Infect Immun* 63:2302–2309.
McErlean BA. 1968. *Vet Rec* 82:257–258.
McGettrick AF, O'Neill LA. 2010. *Subcell Biochem* 53:153–171.
Meyerholz DK, Stabel TJ. 2003. *Vet Pathol* 40(4):371–375.
Meyerholz DK, Stabel TJ, Ackermann MR, et al. 2002. *Vet Pathol* 39(6):712–720.
Mills KW, Kelly BL. 1986. *Am J Vet Res* 47:2349–2350.
Morehouse LG. 1972. *J Am Vet Med Assoc* 160:594–601.
Moreno Switt AI, Soyer Y, Warnick LD, et al. 2009. *Foodborne Pathog Dis* 6:407–415.
Morgan IR, Krautil FL, Craven JA. 1987. *Epidemiol Infect* 98:323–330.
Mousing J, Thode Jensen P, Bager F, et al. 1997. *Prev Vet Med* 29:247–261.
Nape WF, Murphy C. 1971. *J Am Vet Med Assoc* 159:1569–1572.
Nielsen B, Baggesen D, Bager F, et al. 1995. *Vet Microbiol* 47:205–218.
Olson LD, Rodebaugh DE, Morehouse LG. 1977. *Am J Vet Res* 38:1471–1477.
Pairis-Garcia MD, Johnson AK, KuKanich B, et al. 2015. *J Vet Pharmacol Ther* 38(3):265–270.
Pospischil A, Wood RL, Anderson TD. 1990. *Am J Vet Res* 51:619–624.
Radostits OM, Gay CC, Hinchcliff KW, et al. 2007. *Veterinary Medicine*. Saunders Elsevier, pp. 913–914.
Reed WM, Olander HJ, Thacker HL. 1985. *Am J Vet Res* 46:2300–2310.
Reed WM, Olander HJ, Thacker HL. 1986. *Am J Vet Res* 47:75–83.
Rescigno M, Urbano M, Valzasina B, et al. 2001. *Nat Immunol* 2:361–367.
Reynolds IM, Miner PW, Smith RE. 1967. *Cornell Vet* 58:180–185.
Roof MB, Doitchinoff DD. 1995. *Am J Vet Res* 56:39–44.
Roof MB, Kramer TT, Roth JA, et al. 1992a. *Am J Vet Res* 53:1328–1332.
Roof MB, Kramer TT, Kunesh JP, et al. 1992b. *Am J Vet Res* 53:1333–1346.
Rout WR, Formal SB, Giannella RA, et al. 1974. *Gastroenterology* 67:59–70.
Rubin RH, Weinstein L. 1977. *Salmonellosis*. New York, NY: Stratton Intercontinental Medical Book Corp, p. 25.
Schauser K, Olsen JE, Larsson LI. 2004. *J Med Microbiol* 53(7):691–695.
Schultz RA. 1989. Salmonellosis – The Problem-How Do We Handle It? In Proceedings of the American Association on Swine Production, Des Moines, Iowa.
Schwartz KJ. 1991. *Compend Contin Educ Dent* 13(1):139–148.
Schwartz KJ. 1997. Salmonellosis. In Proceedings of the American Association on Swine Practitioners, Quebec City, Quebec.
Schwartz KJ, Daniels G. 1987. Salmonellosis. In Proceedings of the Minnesota Swine Herd Health Program Conference, University of Minnesota.
Smith HW, Jones JET. 1967. *J Pathol* 93:141–156.
Soyer Y, Moreno Switt A, Davis MA, et al. 2009. *J Clin Microbiol* 47(11) 3546–3556.
Stephen J, Wallis TS, Starkey WG, et al. 1985. Salmonellosis: In retrospect and prospect. In Microbial Toxins and Diarrhoeal Diseases. Ciba Foundation Symposium. London: Pitman.
Takeuchi A. 1967. *Am J Pathol* 50:109–136.
Takeuchi A, Sprinz H. 1967. *Am J Pathol* 51:137–161.
Taylor J, McCoy JH. 1969. *Salmonella* and *Arizona* infections and intoxications. In *Foodborne Infections and Intoxications*. New York: Academic Press, pp. 3–71.

Vazquez-Torres A, Jones-Carlson J, Baumler AJ, et al. 1999. *Nature* 401:804–808.
Vazquez-Torres A, Jones-Carlson J, Baumler AJ, et al. 2000. *Gastroenterology* 118:803–805.
Welliver RC, Ogra PL. 1978. *J Am Vet Med Assoc* 173:560–564.
Wilcock BP. 1978. *Can J Comp Med* 43:100–106.
Wilcock BP, Olander HJ. 1977a. *Vet Pathol* 14:36–42.
Wilcock BP, Olander HJ. 1977b. *Vet Pathol* 14:43–55.
Wilcock BP, Olander HJ. 1978. *J Am Vet Med Assoc* 172:472–477.
Wilcock BP, Armstrong CH, Olander HJ. 1976. *Can J Comp Med* 40:80–88.
Williams LP, Newell KW. 1970. *Am J Public Health* 50:926–929.
Williams LP, Vaughn JP, Scott A, et al. 1969. *J Am Vet Med Assoc* 155:167–174.
van der Wolf PJ, Vercammen TJ, van Exsel AC. et al. 2001. *Vet Q* 23:199–201.
Wolff SM. 1973. *J Infect Dis* 128(Suppl):159–164.
Wood RL, Rose R. 1992. *Am J Vet Res* 53:653–658.
Wood RL, Pospischil A, Rose R. 1989. *Am J Vet Res* 50:1015–1021.
Xiong N, Brewer MT, Day TA, et al. 2010. *Am J Vet Res* 71(10):1170–1177.

60

Staphylococcosis

Timothy S. Frana and Samantha J. Hau

Staphylococci are essentially ubiquitous in pigs and swine facilities. Diseases in pigs are commonly caused by *Staphylococcus hyicus*, the cause of exudative epidermitis (EE), and *Staphylococcus aureus* that causes abscesses and other conditions. Occasionally other staphylococcal species such as *Staphylococcus chromogenes, Staphylococcus epidermidis, Staphylococcus sciuri, Staphylococcus warneri*, and *Staphylococcus xylosus* may be isolated from lesions, but their role as primary pathogens should be assessed with caution.

Staphylococcus hyicus: exudative epidermitis

EE, also known as greasy pig disease, occurs worldwide and is the most common staphylococcal skin disease in swine. EE occurs most frequently and is most severe in pigs from approximately 3–32 days of age where affected pigs develop generalized nonpruritic exudative dermatitis–epidermitis. Affected pigs fail to thrive, and loss of body fluid may lead to dehydration and death. Localized milder forms of EE affecting ear tips, head, flanks, and extremities occur primarily in older animals. *S. hyicus* has also been infrequently associated with polyarthritis and reproductive failure (Duncan and Smith 1992; Hill et al. 1996).

Etiology

Although EE was described nearly 170 years ago by Spinola (1842), it was not until 1953 that the cause was attributed to *Micrococcus hyicus* (Sompolinsky 1953). *M. hyicus* was placed in the *Staphylococcus* genus in 1965 (Baird-Parker 1965) and was later divided into subspecies *S. hyicus* and *S. chromogenes* (Devriese et al. 1978). Elevation in 1986 of *S. hyicus* subsp. *chromogenes* to *S. chromogenes* resulted in *S. hyicus* as a singular species (Hajek et al. 1986). *S. hyicus* is a gram-positive coccus. Colonies of *S. hyicus* on blood agar appear non-hemolytic, creamy white, convex, and circular. Biochemically, *S. hyicus* is catalase positive, oxidase negative, and Voges–Proskauer negative. *S. hyicus* produces DNase, phosphatase, hyaluronidase, gelatinase, and lecithinase. Some strains produce coagulase that is enhanced by porcine plasma (Lammler 1991). Aerobic fermentation of fructose, glucose, lactose, mannose, and trehalose occurs with most strains (Lammler 1991). A small zone of hemolysis occurs on chocolate agar, and a CAMP-like zone of complete lysis in the zone of incomplete lysis of the staphylococcal beta-lysine occurs on sheep blood agar (Lammler 1991). Expression of protein A-like receptors for IgG is common in porcine strains (Lammler 1991). Although *S. hyicus* does not form spores, it is resistant to drying and can remain viable in the environment for long periods.

Strains of *S. hyicus* vary in virulence. Virulence is closely associated with the production of exfoliative toxins (Andresen et al. 1997; Futagawa-Saito et al. 2007; Sato et al. 1991b; Wegener et al. 1993). At least six exfoliative toxins – ExhA, ExhB, ExhC, ExhD, ShetA, and ShetB – are described in *S. hyicus* strains from diseased pigs (Ahrens and Andresen 2004; Andresen 1998; Andresen et al. 1997; Leekitcharoenphon et al. 2016; Sato et al. 2000; Watanabe et al. 2000). These toxins target epidermal cells of the stratum granulosum and stratum spinosum. Prevalence studies indicate that toxigenic *S. hyicus* is found more commonly from EE-affected pigs than healthy pigs (Andresen 2005; Kanbar et al. 2008). However there was no clear pattern as to which toxins are most common in strains causing EE, and regional differences apparently exist.

Several other virulence factors are described for *S. hyicus*. A homologue of *S. aureus* protein A with multiple binding sites for IgG helps the bacterium resist phagocytosis (Rosander et al. 2011). Coagulase aids in the formation of a fibrin clot that can protect the *S. hyicus* from host defenses. Surface fibronectin-binding proteins are

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

likely important in bacterial adhesion to fibronectin (Lammler et al. 1985). Additionally *S. hyicus* produces staphylokinase and excretes lipase that cleave proteins and phospholipids, respectively. The exact role of these factors in enhancing virulence is unclear.

Certain strains of staphylococcal species other than *S. hyicus* appear capable of causing EE in pigs. A strain of *S. sciuri* carrying the ExhC exfoliative toxin gene was reportedly isolated from a diseased piglet with EE (Chen et al. 2007). A toxin similar to ExhB was also identified in a strain of *S. chromogenes*, and this strain was able to induce clinical signs of EE in experimentally inoculated pigs (Andresen et al. 2005). Additionally, methicillin-resistant strains of *S. aureus* have been associated with EE (van Duijkeren et al. 2007). Whether these findings represent emerging EE pathogens or isolated instances of non-*S. hyicus*-induced EE has yet to be determined.

Virulent and avirulent strains of *S. hyicus* can be found on the skin of EE-affected animals as well as healthy pigs (Devriese 1977; Park 1986; Wegener et al. 1993), indicating that cofactors in addition to strain virulence are required for expression of disease.

Public health

Staphylococcus hyicus causes no disease in humans.

Epidemiology

Staphylococcus hyicus is distributed globally and infects most pig herds. In the United States it was reported to cause sickness or mortality in 27.5% of sites with nursery-age pigs (USDA 2007) and 16.5% of pig herds in the United Kingdom (Taylor 2004). *S. hyicus* is considered normal bacterial flora on the skin of adult swine and is present in many herds without causing disease. It can be recovered from multiple skin sites on healthy pigs (Hajsig et al. 1985), most commonly from young pigs, and from the environment in swine facilities of infected herds. Infected pigs are the nearly exclusive source of infection for uninfected individuals and herds. Although *S. hyicus* has been isolated from cattle, goats, horses, and chickens (Birgersson et al. 1992; Devriese et al. 1983; Jarp 1991; Kibenge et al. 1983; Myllys 1995; Takeuchi et al. 1985), these animal species are not considered sources of *S. hyicus* for pigs. Species-specific differences in *S. hyicus* apparently render these strains incapable of causing EE in pigs (Devriese et al. 1978; Shimizu et al. 1987, 1997; Takeuchi et al. 1987).

Transmission is primarily by direct contact and can be lateral, between pigs in the same cohort, or vertical by contact of suckling pigs with their infected dam. Vertical transmission may also occur at birth as pigs contact *S. hyicus* while transiting an infected vagina. *S. hyicus* strains isolated from the skin of piglets within 24 hours after birth were the same type as their dams and identical to strains isolated from the same litter 3 weeks later (Wegener and Skov-Jensen 1992).

Expression of disease in herds infected with virulent strains of *S. hyicus* varies and is impacted by the interaction of other cofactors of disease. These include immune status, genetic susceptibility, and a variety of other factors that have in common damage to the epidermis. These include physical, chemical, or solar trauma, viral infections, ringworm, pityriasis rosea, ectoparasitism, and excessive moisture via poor hygiene or poor ventilation.

Inadequate or absent maternal immunity is likely the most important cofactor in the majority of outbreaks that commonly occur in pigs under 8 weeks of age. EE is observed more frequently in piglets born to gilts in start-up herds or born to gilts introduced to an infected herd or environment without adequate time to develop immunity prior to farrowing. Entire litters or cohorts may be affected with high mortality. In contrast, offspring from infected carriers or animals where *S. hyicus* is endemic are generally unaffected, suggesting transfer of protective maternal immunity. Outbreaks are usually self-limiting and generally last 2–3 months but may persist for 12–18 months. Cessation is likely due to development of acquired immunity in a majority of dams prior to farrowing.

The cofactors operative in outbreaks of EE in older animals in endemically infected herds, presumed to have maternal or acquired immunity, are often less certain. Limited outbreaks may occur when colonization by *S. hyicus* coincides with decay of maternal immunity. In most of these outbreaks, damage to the epidermis by one or more of the aforementioned factors allows virulent strains of *S. hyicus* to penetrate to deeper layers of the epidermis beyond the superficial stratum corneum where it colonizes. Common causes include trauma due to fighting and persistent skin moisture due to poor ventilation and/or hygiene. Epizootic or limited endemic infection with certain viruses that damage skin has also been implicated including porcine parvovirus (Kresse et al. 1985; Whitaker et al. 1990), porcine circovirus 2 (Wattrang et al. 2002), or both (Kim and Chae 2004). Ages and numbers of animals with epidermis so damaged by predisposing cofactors determine the extent of the EE outbreak. In summary, development of EE likely involves interplay of immune status, prior exposure, nutrition, housing conditions, and commitment disease, combined with strain virulence and skin entry site.

Pathogenesis

Exudative epidermitis, as the name implies, is primarily a disease of the epidermis. The epidermis wraps the body in an effective barrier to microbial invasion and fluid

transit, preventing dehydration or overhydration from the ambient environment. The outer layer of the epidermis most responsible for these functions is the stratum corneum. The stratum corneum is composed of dead keratinocytes, essentially bricks of the fibrous protein keratin that are bonded together by a complex hydrophobic lipid mortar. The tensile strength of the epidermis is due to a variety of intercellular adhesive structures, most importantly desmosomes, in the stratum spinosum and stratum granulosum. *S. hyicus* colonizes the surface of the stratum corneum, likely aided by secretion of staphylokinase and lipase that cleave proteins and phospholipids, respectively. Although *S. hyicus* may directly penetrate the skin, a breach of the stratum corneum by one or more cofactors is the most common inciting factor for EE in susceptible pigs. Once *S. hyicus* gains access to the underlying stratum granulosum and stratum spinosum, secreted exfoliative toxins cause extensive damage, enabling *S. hyicus* to proliferate within the epidermis, causing extensive damage.

Exfoliative toxins are the most important virulence factor in the development of EE in pigs. In 1979, Amtsberg demonstrated that a culture filtrate of *S. hyicus* could cause exfoliation in the skin of piglets and suggested this was due to exotoxin production. So far six exfoliative toxins have been identified in *S. hyicus*: EhA, EhB, EhC, EhD, SHETA, and SHETB (Ahrens and Andresen 2004; Andresen 1998; Andresen et al. 1997; Sato et al. 2000; Watanabe et al. 2000). Toxins EhA–EhD and SHETB are similar to serine proteases ETA, ETB, and ETD that are produced by *S. aureus* and cause staphylococcal scalded skin syndrome and bullous impetigo in humans (Ahrens and Andresen 2004). The target for this group of toxins is desmoglein (Dsg1), a component of desmosomes (Fudaba et al. 2005). Cleavage of extracellular domains of Dsg1 leads to cell separation in the stratum granulosum and stratum spinosum, exfoliation of the outer layers of the epidermis, and serous exudation. Subcutaneous injection of purified exfoliative toxins from *S. hyicus* caused exfoliation in piglets and chickens (Sato et al. 1991a; Tanabe et al. 1993). Additionally, piglets experimentally inoculated with toxin-producing strains of *S. hyicus* developed local erythema, exfoliation, exudation, and crusting, while those inoculated with non-toxin-producing strains developed only local erythema that disappeared within 48 hours (Tanabe et al. 1996).

Dissolution of desmosomes in the stratum granulosum causes clefts that soon fill with serum filtrates and neutrophils, resulting in subcorneal vesicles, pustules, and erosions. As bacteria multiply, desmosomes are dissolved deeper in the stratum spinosum, and greater inflammation is induced, resulting in dermal and epidermal edema, intraepidermal leukocytosis, and exudation on skin surfaces. The loss of fluids and electrolytes leads to dehydration and death in severely affected piglets. Older animals may develop subcutaneous abscesses, polyarthritis, and necrosis of the ears and tail.

Clinical signs

Disease is most severe with highest mortality when in young suckling pigs. Piglets as young as 3–4 days may develop severe acute EE. Red glistening moist areas of the skin are first observed on the head and on non-haired skin on the medial aspect of legs. Ulcers may develop on the tongue or in the mouth (Andrews 1979). Lesions rapidly spread to the ventral abdomen, coronets, and then the entire body within 24–48 hours. Pigs are listless and anorexic, but are not pruritic and do not have elevated body temperature. The affected skin is soon covered by a thick tenacious brown crust. Some pigs can die within 24–48 hours. Others survive longer; the skin becomes thickened and wrinkled, and the crust dries and cracks. In the deep portion of the wrinkles, the skin is moist and red. Protracted anorexia results in weight loss. Death usually follows in 3–10 days.

A less severe localized more protracted form of disease that is less often fatal occurs when onset is in older, usually recently weaned pigs. Skin lesions are less severe and often confined to the head and consist of erythema and waxy brown exudate. Affected pigs suffer reduced weight gains relative to peers. In some pigs, lesions may generalize and result in fatality. In others, recovery may require several weeks. Adult swine occasionally have a few brown exudative lesions on the back and flanks, usually unrelated to outbreaks in younger pigs. Other reported outcomes of *S. hyicus* infection include polyarthritis (Hill et al. 1996) and abortion (Duncan and Smith 1992).

Lesions

Gross lesions begin most commonly around eyes, mouth, and ears, in axilla and groin, and in areas of trauma. The affected skin is first erythematous and moist, having a scalded appearance. Lesions spread and coalesce, and exudate quickly accumulates as a greasy brown film, then as bacteria and dirt accumulate, a thick malodorous brown crust forms (Figures 60.1 and 60.2). Below the crusty layer, the skin is erythematous and/or grossly thickened and wrinkled. At necropsy, dehydration and emaciation are evident. There may be linear streaks on renal papillae and accumulation of cellular debris in the ureters and pelvis of kidneys. Lymph nodes draining the skin are swollen and edematous.

Microscopically, subcorneal vesicle and pustules that may extend to include follicular infundibula are the earliest lesions. As lesions progress, there are spongiosis, increased numbers of intraepithelial leukocytes, spongiotic pustules, and accumulation of crust composed of

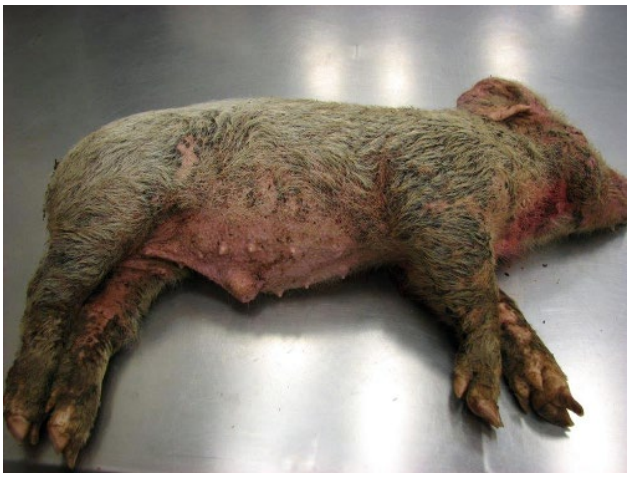


Figure 60.1 Generalized exudative epidermitis in a 3-week-old piglet. *Source:* Courtesy of Dr. Bruce Janke.

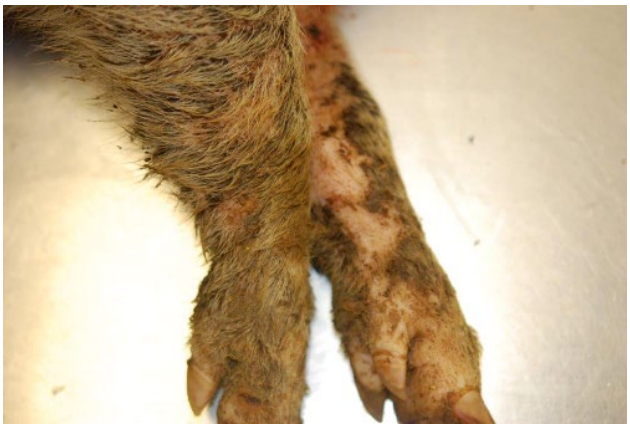


Figure 60.2 Exudative epidermitis in a 3-week-old piglet. Multifocal erythema with inflammatory crusts adherent to the skin and hair. *Source:* Courtesy of Dr. Bruce Janke.

serum, exfoliated stratum corneum, large numbers of neutrophils, necrotic cellular debris, and large numbers of staphylococci. The dermis is expanded by edema, perivascular-to-generalized neutrophilic accumulations, and congested vasculature. As lesions become chronic, orthokeratosis and parakeratosis contribute to a thickened crust, generalized epidermal proliferation results in acanthosis and generalized epidermal thickening, and cellular infiltrates in the dermis transition to predominantly histiocytes, lymphocytes, and plasma cells. In addition to lesions in the skin, suppurative lymphadenitis in subcutaneous lymph nodes and renal lesions are common. Acute renal lesions are likely due to damage by excreted exfoliative toxins of *S. hyicus* on tubular and transitional epithelial cells. Cells lining collecting ducts and the renal pelvis become vacuolated and are later shed, sometimes forming casts that obstruct tubules in the renal pelvis or, in severe cases, the ureters (Blood and Jubb 1957). Purulent pyelonephritis can also develop in pigs with EE that are bacteremic.

Diagnosis

Clinical signs and lesions are characteristic if not pathognomonic for EE in young piglets. Diagnosis of EE in adults or animals with local lesions secondary to trauma is more difficult. Confirmation requires demonstration of typical microscopic lesions and isolation of *S. hyicus* or rarely *S. aureus*, *S. chromogenes*, or *S. sciuri*. Isolation of *S. hyicus* during outbreaks is important for antimicrobial susceptibility testing. Ideal samples for culture are swabs of moist, affected areas of skin taken after removing the overlying crust and enlarged superficial lymph nodes in areas of affected skin.

Staphylococcus hyicus grows well on sheep or bovine blood agar; however, secondary organisms such as *Pseudomonas*, *Proteus*, and other *Staphylococcus* species may also be isolated from EE lesions. Selective media containing potassium thiocyanate (Devriese 1977) or less than 10% NaCl may aid isolation. *S. hyicus* can be identified by using conventional biochemical tests or panel identification systems or by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF). Toxigenic and nontoxigenic strains can be isolated from the same lesion. A method of phage typing *S. hyicus* has been described, which aids in differentiating virulent from avirulent strains (Wegener 1993). An indirect ELISA test for detection of toxins ExhA, ExhB, and ExhC was developed as an alternative to phage typing (Andresen 1999). Additionally identification and toxin profiling with polymerase chain reaction and dot blot hybridization is possible (Andresen and Ahrens 2004; Onuma et al. 2011; Voytenko et al. 2006). However tests for toxin identification are not in widespread use in veterinary diagnostic laboratories.

Other skin diseases may look similar to EE. Swinepox (Chapter 42) has localized lesions and is rarely fatal. Mange (Chapter 65) is pruritic, and mites can be demonstrated in skin scrapings. Ringworm (Chapter 17) has typical expanding superficial lesions in which dermatophytes can be demonstrated by culture or microscopic examination. Pityriasis rosea (Chapter 17) has expanding nongreasy erythematous circular lesions and is self-limiting and nonfatal. Zinc deficiency (Chapter 68) causes parakeratosis that appears as symmetrical dry lesions in 2- to 4 month-old pigs. Finally, dermatosis vegetans (Chapter 17) is a rare inherited disease in Landrace, which also causes a fatal pneumonitis.

Treatment and prevention

Early treatment following onset of disease provides best chance of success, but severely affected animals may not

respond. Antimicrobial drugs are commonly used to treat EE. However, reports indicate that *S. hyicus* is resistant to many antimicrobial agents (Aarestrup and Jensen 2002; Park et al. 2013a; Teranishi et al. 1987; Wegener et al. 1994; Werckenthin et al. 2001). A study comparing antimicrobial susceptibility profiles of toxigenic and nontoxigenic *S. hyicus* isolates concluded that there was not a significant correlation between toxin gene carriage and antimicrobial resistance (Futagawa-Saito et al. 2009). Although regional differences exist, resistance to penicillin, erythromycin, streptomycin, sulfonamides, and tetracycline is a frequent occurrence. In addition, genetic elements conferring methicillin and zinc resistance have been reported in *S. hyicus* (Park et al. 2013b; Slifierz et al. 2014). Therefore antimicrobial susceptibility testing of isolates recovered from EE lesions is recommended to provide evidence of the appropriate treatment. In the absence of drug susceptibility results, ceftiofur, enrofloxacin, and combination of trimethoprim/sulfamethoxazole and lincomycin are best choices. Injection of the antimicrobial is recommended, but it may be given orally in less severe cases. Other treatments include spraying the pigs several times with skin disinfectants such as novobiocin in mineral oil, chlorhexidine, Virkon® (Durvet), or dilute tamed iodine. Fluid and electrolyte replacement is important in severely affected piglets and may be administered orally.

Cross-suckling of gilt piglets with older sows may provide some passive protection. Alternatively autogenous vaccines using strains isolated from the affected herd may be helpful in protracted disease situations. Newly acquired sows or gilts vaccinated prior to farrowing will provide some colostral protection to offspring and help ward off severe forms of EE. Efforts should be made to minimize skin trauma by clipping needle teeth, removing abrasive surfaces, and treating for mange if present. Thorough cleaning and disinfection of facilities should be carried out between farrowings, and sows entering facilities should be washed and disinfected. Control of EE may depend largely on preventing trauma and improving the environment with better ventilation, cleaner and drier pens, controlled humidity, and reduced stocking density.

Staphylococcus aureus

Staphylococcus aureus is the only staphylococcal species apart from *S. hyicus* to be consistently isolated from lesions in pigs. Besides skin infections, *S. aureus* has been associated with septicemia, mastitis, vaginitis, metritis, osteomyelitis, and endocarditis. Although commonly found in the swine facilities and on the skin of healthy pigs, *S. aureus* is not a common pathogen and is not associated with herd outbreaks.

Etiology

Like all staphylococci, *S. aureus* is positive on Gram stain, and it forms white to golden opaque colonies with a double zone of hemolysis on sheep blood agar. The inner zone of complete hemolysis is caused by alpha-hemolysins, while the outer zone of incomplete hemolysis is caused by beta-hemolysins. *S. aureus* grows best under aerobic conditions at 95–98.6°F (35–37°C). It tends to form grape-like clusters of coccoid organisms best observed from solid media. Besides hemolysins, the organism variably produces a number of substances, which may be considered virulence factors. Among these are protein A, teichoic acids, coagulase, staphylokinase, DNase, lipase, hyaluronidase, leukocidin, enterotoxins, and exfoliative toxins.

Public health

Recently there has been a public health concern regarding swine as a reservoir for methicillin-resistant *S. aureus* (MRSA). *S. aureus* in humans is a frequent cause of localized skin infections as well as life-threatening septicemia, pneumonia, endocarditis, and other soft tissue and bone infections (Falcone et al. 2009). MRSA strains emerged in the 1960s and are of great concern given their poor response to treatment and consequent higher mortality rates. Traditionally most MRSA disease has been health-care-associated MRSA (HA-MRSA), being acquired through contact with a health-care setting or health-care personnel (Tiemersma et al. 2004). More recently, MRSA-associated disease has emerged in people in the community apart from health-care-associated risk factors, so-called community-associated MRSA (CA-MRSA) (Vandenesch et al. 2003). Specific genotypes predominate in HC-MRSA and CA-MRSA infections, which assist in distinguishing infection sources (Bal et al. 2016).

Colonization by MRSA has also been reported in livestock including pigs (van Loo et al. 2007), so-called livestock-associated MRSA (LA-MRSA). Studies in Europe, North America, and elsewhere have demonstrated that nasal and skin colonization without clinical infection in pigs is common and caused by the multilocus sequence types ST398, ST9, and ST5 (Armand-Lefevre et al. 2005; Frana et al. 2013; Smith et al. 2009; Smith and Pearson 2011; Wagenaar et al. 2009; Weese et al. 2010). A limited number of reports implicate ST398 and ST9 isolates in a range of human disease conditions similar to other *S. aureus* strains, but these strains may not cause disease as frequently in colonized people as other human MRSA strains (Cuny et al. 2009; Smith and Pearson 2011; van Loo et al. 2007). This difference is likely due to adaption in pigs leading to a reduction in pathogenicity in humans. This is due to a reduced

capacity for adherence to human skin (Uhlemann et al. 2012) and the near absence of human-specific *S. aureus* virulence factors (Hau et al. 2015; Price et al. 2012). While ST398 and ST9 are considered livestock-adapted lineages, the ST5 lineage has historically been a well-adapted HA-MRSA clone and may indicate an increased risk posed to exposed individuals colonized through contact with swine (Bal et al. 2016).

People who spend considerable time in contact with infected pigs are at significantly increased risk for testing positive for LA-MRSA from nasal swabs relative to the general population (Smith et al. 2009; Voss et al. 2005). In one study isolates from pigs and people from the same farm were indistinguishable, suggesting inter-transmission (Khanna et al. 2008). However, the persistence of LA-MRSA in humans is dependent on intensity of animal contact (i.e. contamination of nasal mucus is probably more frequent than true colonization of nasal mucosa), and short-term carriage after animal contact has been described (Frana et al. 2013; Graveland et al. 2011). Transmission from ST398 MRSA-positive farmworkers to family members not in contact with swine has been reported, but spread into the general community seems to be infrequent (Cuny et al. 2009; Graveland et al. 2011). Many unanswered questions remain about LA-MRSA colonization in both humans and swine that need to be addressed prior to implementing a successful bacterial prevention or control strategy on farms or in the community.

Pathogenesis

Damage to skin and mucosal surfaces can predispose to localized *S. aureus*-induced skin lesions similar to EE caused by *S. hyicus*. Some strains of *S. aureus* can produce exfoliative toxins that are similar to those found in *S. hyicus*. *S. aureus* may also invade causing bacteremia that can evolve to life-threatening septicemia in neonates. More often bacteremia leads to abscesses in bones, joints, heart valves, liver, kidney, lymph nodes, and other internal organs. Abscesses may be observed at slaughter in otherwise normal pigs. Ascending infections may lead to mastitis, vaginitis, metritis, and umbilical abscesses.

Clinical signs and lesions

Because *S. aureus* is associated with numerous diseases and other agents can cause similar diseases, clinical signs are not predictive of this organism as the causative agent. Most cases occur in individual animals, and animal-to-animal spread is rare. Neonatal septicemia may be fatal or result in stunted piglets at 7–10 days of age. Umbilical abscesses, polyarthritis, and vegetative endocarditis with cardiac enlargement may also occur, or animals may be found dead with no gross lesions. Chronic infections appear as abscesses of the skin, umbilicus, bones, joints, mammary glands, and internal organs. Osteomyelitis may lead to pathologic fractures particularly in vertebrae. Bone and joint infections in the feet are most often due to extension of skin infections initiated by trauma. Abscesses typically contain creamy white or bloodstained pus and are often surrounded by thick fibrous capsules. Pus may also be in the peritoneal cavity, the pericardial sac, or the lumen of the uterus. A white, non-odorous purulent vaginal discharge occurs with endometritis (Roberson et al. 2007). *S. aureus* may cause sporadic abortions (Kohler and Wille 1980) and has also been rarely isolated from cases of enteritis where enterotoxins may be contributory (Taylor et al. 1982).

Diagnosis and treatment

Diagnosis of *S. aureus* is based on culture from suggestive lesions. Aerobic growth on sheep blood agar of small (1–2 mm diameter), yellow to white opaque colonies with double-zone hemolytic pattern is highly indicative of *S. aureus*. Further identification is accomplished by Gram stain and biochemical testing or MALDI-TOF. *S. aureus* is positive for catalase, coagulase, mannitol, and Voges–Proskauer reagent. These tests should differentiate *S. aureus* from other abscess-causing bacteria such as *Arcanobacterium pyogenes* and streptococci. In the past further identification of *S. aureus* isolates for public health significance was accomplished by phage typing or plasmid profiling. These have largely been replaced by other methods such as multilocus sequence typing (MLST) or protein A gene sequence typing (*spa* typing).

Treatment of individual abscesses can be accomplished by surgical drainage and antimicrobial therapy. Prompt parenteral treatment is recommended to minimize development of extensive and fatal abscess development. Susceptibility testing for appropriate antimicrobial agent is recommended since many isolates are resistant to common drugs such as penicillin, ampicillin, chlortetracycline, oxytetracycline, and spectinomycin. Ceftiofur, enrofloxacin, and trimethoprim/sulfamethoxazole are good choices in the absence of individual isolate testing. Since *S. aureus* infection is an individual animal problem, there is usually no need to treat the whole group. The use of bacterins has been described, but they are not widely available or extensively used. Environmental cleaning of heavily contaminated areas is prudent. *S. aureus* is somewhat resistant to disinfectants especially when protected by organic materials, but it is sensitive to disinfectants, such as phenols, hypochlorites, iodine, and iodophors.

References

Aarestrup FM, Jensen LB. 2002. *Vet Microbiol* 89:83–94.
Ahrens P, Andresen LO. 2004. *J Bacteriol* 186:1833–1837.
Amtsberg G. 1979. *Zentralbl Veterinarmed B* 26:137–152.
Andresen LO. 1998. *FEMS Immunol Med Microbiol* 20:301–310.
Andresen LO. 1999. *Vet Microbiol* 68:285–292.
Andresen LO. 2005. *Vet Rec* 157:376–378.
Andresen LO, Ahrens P. 2004. *J Appl Microbiol* 96:1265–1270.
Andresen LO, Bille-Hansen V, Wegener HC. 1997. *Microb Pathog* 22:113–122.
Andresen LO, Ahrens P, Daugaard L, et al. 2005. *Vet Microbiol* 105:291–300.
Andrews JJ. 1979. *Vet Pathol* 16:432–437.
Armand-Lefevre L, Ruimy R, Andremont A. 2005. *Emerg Infect Dis* 11:711–714.
Baird-Parker AC. 1965. *J Gen Microbiol* 38:363–387.
Bal AM, Coombs GW, Holden MTG, et al. 2016. *J Glob Antimicrob Resist* 6:95–101.
Birgersson A, Jonsson P, Holmberg O. 1992. *Vet Microbiol* 31:181–189.
Blood DC, Jubb KV. 1957. Exudative epidermitis of pigs. *Aust Vet J* 33:126–127.
Chen S, Wang Y, Chen F, et al. 2007. *PLoS One* 2:e147.
Cuny C, Nathaus R, Layer F, et al. 2009. *PLoS One* 4:e6800.
Devriese LA. 1977. *Am J Vet Res* 38:787–792.
Devriese LA, Hajek, V, Oeding P, et al. 1978. *Int J Syst Bacteriol* 28:482–490.
Devriese LA, Vlaminck K, Nuytten J, et al. 1983. *Equine Vet J* 15:263–265.
van Duijkeren EI, Jansen MD, Flemming SC, et al. 2007. *Emerg Infect Dis* 13:1408–1410.
Duncan M, Smith D. 1992. *Can Vet J* 33:75–76.
Falcone M, Serra P, Venditti M. 2009. *Eur J Intern Med* 20:343–347.
Frana TS, Beahm AR, Hanson BM, et al. 2013. *PLoS One* 8:e53738.
Fudaba Y, Nishifuji K, Andresen LO, et al. 2005. *Microb Pathog* 39:171–176.
Futagawa-Saito K, Ba-Thein W, Higuchi T, et al. 2007. *Vet Microbiol* 124:370–374.
Futagawa-Saito K, Ba-Thein W, Fukuyasu T. 2009. *J Vet Med Sci* 71:681–684.
Graveland H, Wagenaar JA, Bergs K, et al. 2011. *PLoS One* 6:e16830.
Hajek V, Devriese LA, Mordarski M, et al. 1986. *System Appl Microbiol* 8:169–173.
Hajsig D, Babic T, Madic J. 1985. *Veterinarski Arh* 55:45–51.
Hau SJ, Sun J, Davies PR, et al. 2015. *PLoS One* 10:e0142832.
Hill BD, Corney BG, Wagner TM. 1996. *Aust Vet J* 73:179–181.
Jarp J. 1991. *Vet Microbiol* 27:151–158.
Kanbar T, Voytenko AV, Alber J, et al. 2008. *J Vet Sci* 9:327–329.
Khanna T, Friendship R, Dewey C, et al. 2008. *Vet Microbiol* 128:298–303.
Kibenge FS, Rood JI, Wilcox GE. 1983. *Vet Microbiol* 8:411–415.
Kim J, Chae C. 2004. *Vet J* 167:104–106.
Kohler B, Wille H. 1980. *Monatsh Veterinarmed* 35:506–510.
Kresse JI, Taylor WD, Stewart WW, et al. 1985. *Vet Microbiol* 10:525–531.
Lammler C. 1991. *J Clin Microbiol* 29:1221–1224.
Lammler C, de Freitas JC, Chhatwal GS, et al. 1985. *Zentralbl Bakteriol Mikrobiol Hyg A* 260:232–237.
Leekitcharoenphon P, Pamp SJ, Andresen LO, et al. 2016. *Vet Microbiol* 185:34–40.
van Loo I, Huijsdens X, Tiemersma E, et al. 2007. *Emerg Infect Dis* 13:1834–1839.
Myllys V. 1995. *J Dairy Res* 62:51–60.
Onuma K, Uoya Y, Koide T, et al. 2011. *Microbiol Immunol* 55:168–173
Park CaKB. 1986. *Korean J Vet Res* 26:251–257.
Park J, Friendship RM, Poljak Z, et al. 2013a. *Can Vet J* Feb;54:139–144.
Park J, Friendship RM, Weese JS, et al. 2013b. *BMC Vet Res* 9:211.
Price LB, Stegger M, Hasman H, et al. 2012. *MBio* 3:e00305-11.
Roberson J, Moll D, Saunders G. 2007. *Vet Rec* 161:821–822.
Rosander A, Guss B, Pringle M. 2011. An IgG-binding protein A homolog in Staphylococcus hyicus. *Vet Microbiol.* 149(1–2):273–276.
Sato H, Kuramoto M, Tanabe T, et al. 1991a. *Vet Microbiol* 28:157–169.
Sato H, Tanabe T, Kuramoto M, et al. 1991b. *Vet Microbiol* 27:263–275.
Sato H, Watanabe T, Higuchi K, et al. 2000. *J Bacteriol* 182:4096–4100.
Shimizu A, Teranishi H, Kawano J, et al. 1987. *Zentralbl Bakteriol Mikrobiol Hyg A* 265:57–61.
Shimizu A, Kloos WE, Berkhoff HA, et al. 1997. *J Vet Med Sci* 59:443–450.
Slifierz MJ, Park J, Friendship RM, et al. 2014. *Can Vet J* 55:489–490.
Smith TC, Pearson N. 2011. *Vector Borne Zoonotic Dis* 11:327–339
Smith TC, Male MJ, Harper AL, et al. 2009. *PLoS One* 4:e4258.
Sompolinsky D. 1953. *Schweiz Arch Tierheilkd* 95:302–309.

Spinola J. 1842. *Die Krankhelten der Schweine*. Berlin: Verlag Hirschwald, pp. 146–148.
Takeuchi S, Kobayashi Y, Morozumi T, et al. 1985. *Nippon Juigaku Zasshi* 47:841–843.
Takeuchi S, Kobayashi Y, Morozumi T. 1987. *Vet Microbiol* 14:47–52.
Tanabe T, Sato H, Kuramoto M, et al. 1993. *Infect Immun* 61:2973–2977.
Tanabe T, Sato H, Watanabe K, et al. 1996. *Vet Microbiol* 48:9–17.
Taylor DJ. 2004. Exudative epidermitis. In Coetzer JAW, Tustin RC, eds. *Infectious Diseases of Livestock*, 2nd ed. Cape Town, South Africa: Oxford University Press.
Taylor SL, Schlunz LR, Beery JT, et al. 1982. *Infect Immun* 36:1263–1266.
Teranishi H, Shimizu A, Kawano J, et al. 1987. *Nippon Juigaku Zasshi* 49:427–432.
Tiemersma EW, Bronzwaer SL, Lyytikäinen O, et al. 2004. *Emerg Infec Dis* 10:1627–1634.
Uhlemann A, Porcella SF, Trivedi S, et al. 2012. *MBio* 3:e00027-12.
USDA 2007. Swine 2006 Part II: Reference of Swine Health and Health Management Practices in the United States. USDA:APHIS:VS, CEAH: Fort Collins, CO.
Vandenesch F, Naimi T, Enright MC, et al. 2003. *Emerg Infect Dis* 9:978–984.
Voss A, Loeffen F, Bakker J, et al. 2005. *Emerg Infect Dis* 11:1965–1966.
Voytenko AV, Kanbar T, Alber J, et al. 2006. *Vet Microbiol* 116:211–216.
Wagenaar JA, Yue H, Pritchard J, et al. 2009. *Vet Micro* 139:405–409.
Watanabe T, Sato H, Hatakeyama Y, et al. 2000. *J Bacteriol* 182:4101–4103.
Wattrang E, McNeilly F, Allan GM, et al. 2002. *Vet Microbiol* 86:281–293.
Weese JA, Reid-Smith R, Rousseau J, et al. 2010. *Can Vet J* 51:749–752.
Wegener HC. 1993. *Res Microbiol* 144:237–244.
Wegener HC, Skov-Jensen EW. 1992. *Epidemiol Infect* 109:433–444.
Wegener HC, Andresen LO, Bille-Hansen V. 1993. *Can J Vet Res* 57:119–125.
Wegener HC, Watts JL, Salmon SA, et al. 1994. *J Clin Microbiol* 32:793–795.
Werckenthin C, Cardoso M, Martel JL, et al. 2001. *Vet Res* 32:341–362.
Whitaker KH, Neu SM, Pace LW. 1990. *J Vet Diagn Invest* 2:244–246.

61

Streptococcosis

Marcelo Gottschalk and Mariela Segura

Relevance

Several streptococcal species reside in tonsils, intestines, and/or genital tract of clinically healthy pigs, and some are potential pathogens. *Streptococcus suis, Streptococcus porcinus*, and *Streptococcus dysgalactiae* subsp. *equisimilis* are generally found in tonsils (Devriese et al. 1994b; Vieira et al. 1998). *Streptococcus hyointestinalis*, *Streptococcus alactolyticus* (*Streptococcus* intestinalis) and *Streptococcus bovis*, and possibly *S. suis* are considered part of the intestinal microflora (Devriese et al. 1994b). *Streptococcus orisuis* and streptococcal mutans-like strains are normal inhabitants of the oral cavities of pigs (Takada and Hirasawa 2007; Takada et al. 2008). Vaginal microflora may include some of the aforementioned species (such as *S. suis*), as well as *Streptococcus hyovaginalis* and *Streptococcus thoraltensis* (Devriese et al. 1997, 1988).

Streptococcus suis is the most important streptococcal swine pathogen worldwide, causing septic diseases mostly in 5- to 10-week-old pigs characterized by septicemia and acute death, meningitis, polyarthritis, polyserositis, and valvular endocarditis. It is also important as a zoonotic agent. Other less common *Streptococcus* spp. isolated from a variety of lesions/disease conditions are discussed, including *S. porcinus* (Lancefield groups E, P, U, and V) and S. dysgalactiae subsp. *equisimilis* (Lancefield groups C, G, and L), among others.

Streptococcus suis

Etiology and prevalence

Streptococcus suis is an encapsulated gram-positive coccus. It was first reported by Jansen and Van Dorssen in the Netherlands (1951) and by Field et al. (1954) in England and was officially recognized as a new species in 1987 (Kilpper-Balz and Schleifer 1987). Since then *S. suis* has been reported globally in both traditional and modern intensive swine operations. Some confusion existed in the original studies regarding early Lancefield groups R, S, and T and their relationship with group D streptococci and the different *S. suis* capsular serotypes. The terminology of Lancefield groups R, S, RS, and T, sometimes employed in papers describing human *S. suis* infections, should not be used to avoid confusion (Gottschalk et al. 2010; Okura et al. 2016).

Nine serotypes, 1–8 and 1/2, were originally described based on antigenicity of capsular polysaccharides (CPS) (Perch et al. 1983). In the following years, a total of 26 additional serotypes, 9–34, were described (Gottschalk et al. 1989, 1991; Higgins et al. 1995). Reference strains originated mostly from diseased pigs, although some originated from clinically healthy pigs, a diseased human, diseases calves, or a diseased lamb. Subsequent phylogenetic analyses of the 16S rRNA and chaperonin 60 (cpn60) genes showed that the reference strains of serotypes 32 and 34 should have been classified as *Streptococcus orisratti* (Hill et al. 2005). More recently, sequence analyses of species-specific genes (*sodA* and *recN*) indicate that the reference strains of serotypes 20, 22, 26, and 33 should also be taxonomically removed from the *S. suis* species (Okura et al. 2016). It has been proposed that serotypes 20, 22, and 26 belong to a new species (*Streptococcus parasuis*) (Okura et al. 2016). However, more extensive studies using a higher number of *S. suis* and *S. suis*-like strains will provide additional insights into the classification of these strains and make the species boundaries clearer (Okura et al. 2016). In fact, all 35 serotypes of *S. suis* (or *S. suis*-like strains) are isolated from diseased pigs (Gottschalk and Segura 2015), and, from the clinical point of view, most laboratories still identify the 35 serotypes.

A majority of *S. suis* strains isolated from diseased pigs belong to serotypes 1–9 (Goyette-Desjardins et al. 2014). Serotype 2 is considered the predominant and most virulent serotype in most Eurasian countries (Berthelot-Hérault et al. 2000; Goyette-Desjardins

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

et al. 2014; Wei et al. 2009; Wisselink et al. 1999). However, serotype 9 is most frequently isolated from diseased pigs in Spain, Germany, and the Netherlands (Goyette-Desjardins et al. 2014; Vela et al. 2005). The situation seems to be different in North America. Although serotypes 2 and 3 are the most prevalent in diseased pigs, serotype 2 has a prevalence typically below 20% in Canada (Gottschalk and Lacouture 2015) and in the United States (Fittipaldi et al. 2009). Strains of lower virulence are present in North America when compared with those from Europe (Fittipaldi et al. 2011). Untypable isolates are recovered mostly from sporadic occurrences of disease (Gottschalk and Lacouture 2015). Some of them are nonencapsulated, hence impossible to serotype using antisera. The use of multiplex PCR for serotyping (Okura et al. 2014) can sometimes identify the serotype of such strains. Some of these strains remain untypable even by PCR due to mutations or even loss of *cps* loci (Zheng et al. 2017). New novel cps *loci*, which would represent new serotypes, have been lately described within strains isolated either from healthy Chinese (Qiu et al. 2016) or diseased Canadian (Zheng et al. 2017) pigs. The use of PCR tests that identify these new novel cps *loci* may significantly reduce the number of untypable isolates.

Genetic diversity exists among *S. suis* isolates within and between serotypes (Princivalli et al. 2009). Though different methods based on DNA have been used for *S. suis* surveillance, they are only effective for short-term epidemiology as they are based on non-characterized genomic differences between isolates. It has been reported that the genetic diversity of *S. suis* is better evidenced by multilocus sequence typing (MLST), mainly for serotype 2 strains. Using this technique, different sequence types (ST) have been identified. Serotype 2 ST1 strains are mostly associated with high virulence and disease in both pigs and humans in Europe, Asia, Africa, and South America (Segura et al. 2017). Serotype 2 ST7, a single locus variant of ST1, is mostly endemic to mainland China (see below). North American serotype 2 strains are mainly ST25 and ST28, with the presence of a low percentage of ST1 strains. The original hypothesis of lower virulence of North American serotype 2 strains (Gottschalk and Segura 2000) was confirmed by experimental infection of animals that demonstrated that ST1 European strains are significantly more virulent than ST28 strains, while ST25 strains are intermediate in virulence (Fittipaldi et al. 2011). Differences in strain virulence belonging to the same ST have also been revealed (Athey et al. 2015).

Public health

Streptococcus suis is an emerging zoonotic agent that has increased in importance in the last 10 years. Serotype 2 is the most common cause of human disease; serotypes 5, 14, and others are also uncommonly reported (Callejo et al. 2016; Goyette-Desjardins et al. 2014; Wertheim et al. 2009).

Meningitis is the most common manifestation evidenced clinically by headache, fever, vomiting, and nervous signs and later by hearing loss. Some patients exhibit signs of sepsis including cutaneous petechiae, ecchymoses, and purpura (Wertheim et al. 2009). Less commonly, endocarditis, arthritis, and endophthalmitis are reported (Doube and Calin 1988; Huang et al. 2005; McLendon et al. 1978; Vilaichone et al. 2002; Voutsadakis 2006). In recent outbreaks in China of unusually high morbidity and mortality, sepsis with a toxic shock-like syndrome is described (Tang et al. 2006; Yu et al. 2006). Case fatality rates vary from less than 3% in most Western countries to 26% in some Asian countries (Gottschalk et al. 2010).

In Western countries where human *S. suis* has been reported, it is rare, usually involving a single person, and is highly occupationally associated in pig farmers, veterinarians, abattoir workers, people who transport pork, meat inspectors, and butchers. Infection is primarily through direct contact of small skin wounds with *S. suis*-infected pigs or contaminated pork, although in some cases no wound is obvious (Gottschalk et al. 2010; Segura et al. 2016). The incubation period with directly infected skin wounds is typically a few hours but may be up to several days (Wertheim et al. 2009). Humans in at-risk occupations also may have asymptomatic *S. suis* colonization of tonsils (Gottschalk et al. 2010; Segura et al. 2016) or nasal mucosa (Bonifait et al. 2014) and may be seropositive (Robertson and Blackmore 1989; Smith et al. 2008). The significance of these findings relative to risk of disease is unknown.

Disease in humans in Southeast Asia can be different. Although sporadic individual cases of disease occur as in the West, albeit more frequently, there have also been two major outbreaks in China in 1998 and 2005 where in each more than 50 people died of toxic shock-like septic disease with relatively low incidence of meningitis (Gottschalk et al. 2010; Tang et al. 2006; Yu et al. 2006). In other Asian countries, such as Vietnam and Thailand, human *S. suis* is among the most frequent causes of bacterial meningitis in adults (Gottschalk et al. 2010). In these countries, *S. suis* is a frequent foodborne disease (Segura et al. 2016) as well as a disease of direct contact with infected swine or contaminated pork. This is likely due to cultural and other factors. There is a high rate of *S. suis* contamination of pork as indicated by a high percentage of positive pork samples in Asian markets (Cheung et al. 2008). The well-documented risk of consuming contaminated undercooked pork is compounded by the common practice of consuming raw pork in Vietnam, Laos, and Thailand (Gottschalk et al. 2010). Since *S. suis* can survive in carcasses at 4°C (39°F) for

6 weeks, contaminated chilled or frozen meat poses risk long after being butchered. In addition to foodborne risks, larger numbers of people in Southeast Asia are at risk by direct contact with *S. suis*-infected pigs compared with the West. In these countries, backyard pig production is common, often including slaughter and processing, and living conditions frequently place pigs and humans in close contact.

Differences between the West and Southeast Asia may also be due to differences in *S. suis* strain virulence or prevalence. It has been proposed that a highly virulent *S. suis* ST7 strain unique to China was responsible for the toxic shock-like outbreaks. However, fatal toxic shock-like cases have occurred in many Western countries where this ST7 strain has never been isolated (Gottschalk et al. 2010; Mancini et al. 2017). Recent information clearly indicates that serotype 2 strains differ in virulence for swine (and probably for humans) according to different geographic regions (see above). Because serotype 2 strains predominate in human cases, it may be that a lower prevalence of virulent serotype 2 in pigs in North America translates to lower transmission to humans and lower prevalence of human disease. In Argentina where virulent European-like serotype 2 ST1 strains are frequently isolated from diseased pigs, a high number of human cases have been reported, a surprising fact for a country with still limited swine production (Callejo et al. 2016).

Finally, a reason for very low prevalence of human *S. suis* in North America and low prevalence in Europe may be partially due to underdiagnosis. *S. suis* is a pathogen very well known by laboratories of human disease in Asia, relatively well known in Europe, and generally poorly known in America. Many diagnostic laboratories working in human diseases in Western countries have misidentified it as enterococci, *Streptococcus pneumoniae, S. bovis*, other streptococci, or even *Listeria* (Gottschalk et al. 2010).

Epidemiology

Natural habitat

The natural habitat of *S. suis* is the upper respiratory tract, particularly the tonsils and nasal cavities, as well as the genital and possibly alimentary tracts of pigs (Devriese et al. 1994b; Hogg et al. 1996). It is easily detected in almost all pigs of any age (MacInnes et al. 2008). Virulent serotype 2 strains can also be carried subclinically by healthy pigs, but in few herds that remain entirely subclinical and in only a low proportion of pigs within these herds (Marois et al. 2007; Monter Flores et al. 1993). In herds with clinical signs, the number of *S. suis* serotype 2 carrier animals is usually higher (Marois et al. 2007). Serotypes 9–34 (as well as untypable strains) are more likely to subclinically colonize the nasal cavities and vagina than to cause disease (Hogg et al. 1996). Individual pigs are usually colonized by more than one *S. suis* serotype (Monter Flores et al. 1993).

Wild boars are known to carry *S. suis*, even to suffer from fatal disease, and may be an important reservoir in some countries (Baums et al. 2007; Risco et al. 2015). In Spain, serotype 9, the most important serotype isolated from clinical disease in domestic pigs, was also shown to be widely present in wild boars (Sánchez del Rey et al. 2014). *S. suis* has also uncommonly been confirmed in cattle (Okwumabua et al. 2017), lambs (Muckle et al. 2014), wild rabbits (Sánchez del Rey et al. 2013), and a dog (Muckle et al. 2010), suggesting that these could serve as unlikely sources for pigs. Additional mammals and birds might also carry *S. suis* (Devriese et al. 1994a), but these isolates need to be confirmed as *S. suis* using current discriminatory methods.

Transmission

Transmission of virulent strains between herds usually occurs by the movement of healthy carrier animals. The introduction of carrier pigs harboring virulent strains (breeding gilts, boars, weaners) into a noninfected recipient herd may result in the subsequent onset of disease in weaners and/or growing pigs. Sows infect their piglets during birthing via contamination from vaginal colonization and probably through the respiratory route (Amass et al. 1997; Cloutier et al. 2003; Robertson et al. 1991; Segura et al. 2016). Although most weaned piglets carry *S. suis* strains, few carry virulent strains capable of inducing the disease (Cloutier et al. 2003; Marois et al. 2007). Even though different serotypes and strains within the same serotype are present in a herd, a single strain usually causes most disease (Cloutier et al. 2003; Marois et al. 2007). However, when predisposing factors are present in a herd that can lower immunity or otherwise lower resistance to *S. suis* invasion (see below), multiple strains of *S. suis* may cause disease in a herd. Horizontal transmission is important, especially during outbreaks, when diseased animals shed higher numbers of bacteria, thereby increasing transmission by direct contact or aerosol (Cloutier et al. 2003). Aerosol transmission without nose-to-nose contact has been confirmed for *S. suis* serotype 2 (Berthelot-Hérault et al. 2001). Another study showed that although prevention of direct contact by spatial separation of pigs reduces the rate at which susceptible pigs become colonized, such intervention measures would not prevent *S. suis* spread in a farm (Dekker et al. 2013).

The importance of environmental contamination, fomites, and insect vectors in transmission of *S. suis* is uncertain. *S. suis* has been isolated from feed troughs of piglets and sows (Robertson et al. 1991), confirming environmental contamination. However, the oral route of infection is not yet been proven, and recent studies suggested that *S. suis* is not able to survive in feed (fine

pellet or crumb feed, with or without formic acid) or in the stomach contents (Warneboldt et al. 2016). *S. suis* may also be transmitted via fomites (Dee and Corey 1993; Robertson et al. 1991) and flies (Enright et al. 1987), although the importance of such vectors has still to be confirmed.

Survival in the environment

The durability of *S. suis* in various environmental conditions has been studied using serotype 2 strains. Viability in water at 39 °F (4 °C) is retained for 1–2 weeks. In experimentally inoculated feces, *S. suis* survives at 32 °F, (0 °C) 48 °F (9 °C), and 72–77 °F (22–25 °C) for 104, 10, and 8 days, respectively. In dust survival at 32 °F (0 °C), 48 °F (9 °C), and 72–77 °F (22-25 °C) is for 54, 25, and 0 days, respectively. Thus, at a summertime or nursery temperature of 72–77 °F (22–25 °C), the organism could survive about 8 days in feces but less than 24 hours in dust (Clifton-Hadley and Enright 1984). Viability is retained in rotting pig carcasses for 6 weeks at 39 °F (4 °C) and for 12 days at 72–77 °F (22–25 °C), proving a potential source for spread by birds, rats, mice, dogs, or other species (Clifton-Hadley and Enright 1984).

Disinfectants commonly used in piggeries can kill *S. suis* in less than 1 minute, even at concentrations less than those recommended by the manufacturers (Clifton-Hadley and Enright 1984; Robertson et al. 1991). Organic matter reduces effectiveness of chemical disinfectants and should be completely removed with thorough washing prior to application. Even though *S. suis* survives in water up to 2 hours at 122 °F (50 °C) but only 10 minutes at 140 °F (60 °C), use of heated pressure washers compared with non-heated is of limited value since water cools rapidly on surfaces, negating potential benefit (Clifton-Hadley and Enright 1984).

Virulence factors

Nearly all studies on virulence factors, pathogenesis, mechanisms of protection, and animal models of disease have used ST1 Eurasian and/or ST7 Chinese serotype 2 strains (Fittipaldi et al. 2012; Segura et al. 2017). Extrapolation of findings to other serotypes or even to other serotype 2 strains such as ST25/ST28 North American strains should be done with caution. Interpretation of the collective results of studies on virulence factors and protection is hampered by variation in experimental designs. There is no generalized agreement among investigators in the *S. suis* field about what defines a strain as virulent, and, on many occasions, strains have been defined as virulent or avirulent solely on the basis of the clinical condition of the animal from which the strain was recovered and/or the site of recovery. For example, not all strains isolated from tonsils of clinically healthy pigs are avirulent, since pigs may be tonsillar carriers of virulent strains without being ill (Segura et al. 2017). Additionally, experimental infection models to evaluate virulence are not readily comparable. There are models in pigs; strains of inbred and outbred mice, rabbits, and zebrafish; and unicellular eukaryotes such as an amoeba (Segura et al. 2017).

The pig remains the most valuable model, but *S. suis* experimental models are not easily standardized. Variations in the type (traditional or minipigs), genetic background, age, health status of the herd of origin, and route of infection further complicate *S. suis* pathogenesis studies in the natural host (Segura et al. 2017). To reproduce disease in a relatively natural way by the respiratory route, intranasal or aerosol routes of inoculation have been used. However, results have not always been positive or reproducible. In most models, prior to *S. suis* exposure, preconditioning the nasal mucosa to favor bacterial invasion is usually needed. These methods include preinfection with other toxin-producing pathogens such as *Bordetella bronchiseptica* (Vecht et al. 1985, 1991) or direct mucosal irritation with acetic acid (Pallarés et al. 2003). Yet these methods may work with very virulent serotype 2 strains, but not with less virulent strains or serotypes (Segura et al. 2016). In fact, there is not a sure way to determine the level of virulence for a given strain of *S. suis*. As a consequence, important discrepancies exist in the literature regarding even the virulence of the same strain (Berthelot-Hérault et al. 2005). Nevertheless, it is clear that differences in virulence exist among serotype 2 strains (Fittipaldi et al. 2011; Gottschalk and Segura 2000).

Difficulties with reliable experimental models of *S. suis* disease have also confounded studies aimed at identification of critical virulence factors. Recent literature is replete with new putative critical virulence factors, confusion, and controversy. For a critical evaluation, readers are invited to consult the studies compiled by Segura et al. (2016, 2017).

The best validated *S. suis* virulence factor is the CPS that plays an important role in bacterial resistance to phagocytosis by leukocytes. The CPS chemical structure of only a few serotypes is known (van Calsteren et al. 2016). The CPS seems to be a necessary but not sufficient virulence factor as most low virulence strains are encapsulated, indicating that additional virulence factors are necessary for full virulence. Unencapsulated strains can also occasionally invade host tissue and cause disease, mainly endocarditis (Lakkitjaroen et al. 2011).

Bacterial cell wall components may be surface exposed even in encapsulated strains, inducing an exaggerated inflammatory response of the host. Among these, peptidoglycan and teichoic and lipoteichoic acid components have been implicated as virulence factors, mainly involved in resistance to killing by phagocytic cells, adherence to host cells, resistance against cationic

antimicrobial peptides, and/or induction of exaggerated inflammation (Fittipaldi et al. 2008a,b). Also, several proteins with a C-terminal cell wall sorting signal including LPXTG or related motifs are thought to be important in virulence when compared to isogenic mutants lacking some of these factors (Segura et al. 2016, 2017).

Products secreted by *S. suis* are also suggested as virulence factors. Of these the hemolysin suilysin (SLY) is the best characterized being toxic to epithelial, endothelial, and phagocytic cells (Gottschalk and Segura 2000; Tenenbaum et al. 2016).

Although the bulk of literature may not confirm some putative virulence factors as significant, some of them may still serve as virulence markers and/or be useful for phenotypic comparison of strains. Examples include the proteins muramidase-released protein (MRP), extracellular factor (EF) (Smith et al. 1997; Vecht et al. 1991), and SLY (Tenenbaum et al. 2016). Although isogenic mutants lacking either MRP or EF proteins or SLY were shown to be as virulent to pigs as the respective parent strains (Allen et al. 2001; Smith et al. 1997), there is a positive association between the presence of these proteins and strain virulence in European and Asiatic countries (Silva et al. 2006; Wei et al. 2009). For example, there is a positive correlation between ST1 virulent strains and the presence of these three factors (Fittipaldi et al. 2011; Callejo et al. 2016). Avirulent *S. suis* serotype 2 strains possessing MRP, EF, and SLY have so far not been described (Fittipaldi et al. 2012). However, the absence of any or all of these proteins is not necessarily associated with a lack of virulence. Most North American and some European and Asian virulent isolates do not produce all these factors (Berthelot-Hérault et al. 2000; Fittipaldi et al. 2009). In North America, ST28 low virulence strains are MRP+, whereas higher virulent ST25 strains are negative for this marker. In both cases these strains are negative for both EF and SLY (Fittipaldi et al. 2011).

Pathogenesis of the infection

Virulent strains of *S. suis* can colonize the tonsils and, to a lesser extent, the respiratory mucosal surfaces in a low number of pigs without producing disease (see above). Colonization is thought to be the requisite first step to subsequent invasion, hematogenous and/or lymphogenous dissemination, and systemic disease (Madsen et al. 2002; Segura et al. 2016). However the exact mechanism(s) of invasion of mucous membranes is (are) unknown. Damage to the mucosal epithelium is thought to play a role, supported by the fact that colonization of the nasal mucosa by *B. bronchiseptica* and its ability to damage the mucosal epithelium by elaboration of dermonecrotic toxin (DNT) predisposes to *S. suis* invasion and septic diseases (Vecht et al. 1985, 1991).

The gastrointestinal tract cannot be excluded as a secondary site of infection in piglets. Studies have shown that *S. suis* is able to translocate from the intestine via the bloodstream to different tissues and cause disease. However, these studies used *S. suis* protected within gastric acid-resistant capsules (Segura et al. 2016). Experimental infection of weaned piglets through the oral route with either virulent serotype 2 or serotype 9 strains did not induce disease (Warneboldt et al. 2016). The conditions leading to sufficient passage of *S. suis* through the stomach are still unclear and might differ in neonatal, suckling, or weaning periods. Further studies on oro-gastrointestinal *S. suis* infections are required (Segura et al. 2016). The intestinal route of infection is, however, very common in humans (Segura et al. 2016).

The pathogenesis of *S. suis* infection is summarized in Figure 61.1. Survival of the organism in the bloodstream may be facilitated by the CPS and cell wall components that efficiently hamper phagocytosis (Fittipaldi et al. 2008a,b, 2012; Smith et al. 1999). The role of host complement in bacterial killing, mediated by opsonophagocytosis, is still controversial (Segura et al. 2016). Bacteria then travel in the bloodstream either free in circulation or attached to the surface of monocytes (Fittipaldi et al. 2012; Gottschalk and Segura 2000). SLY that is produced by some strains seems to contribute to the ability of encapsulated *S. suis* to resist killing by porcine phagocytes in the presence of complete serum lacking specific antibodies (Benga et al. 2008; Tenenbaum et al. 2016).

The mechanism(s) by which *S. suis* is able to cross the blood–brain barrier is only partially understood. Brain microvascular endothelial cells together with choroid plexus epithelial cells constitute the structural basis of the blood–brain barrier. *S. suis* adheres to and invades brain microvascular endothelial cells with or without cytotoxicity (Vanier et al. 2004). It also affects porcine choroid plexus epithelial cell barrier function and integrity through cell death that is predominantly due to necrosis, but apoptosis may also contribute. These and probably other mechanisms facilitate *S. suis* invasion of the CNS (Fittipaldi et al. 2012; Tenenbaum et al. 2016).

Inflammation plays an important role in the pathogenesis of *S. suis*-induced septicemia and meningitis. Bacterial cell wall components are known to induce the production of proinflammatory cytokines by murine, human, and swine cells (Gottschalk and Segura 2000; Fittipaldi et al. 2012). High levels of cytokines are also produced after *in vivo* infections that may lead to sudden death of animals (Domínguez-Punaro et al. 2007). Likewise, and shortly after *S. suis* invasion of the CNS by *S. suis*, there are transcriptional activation of proinflammatory cytokines and chemokines and induction of inflammation that causes CNS clinical signs. Cases in humans and pigs with presence of a shorter incubation

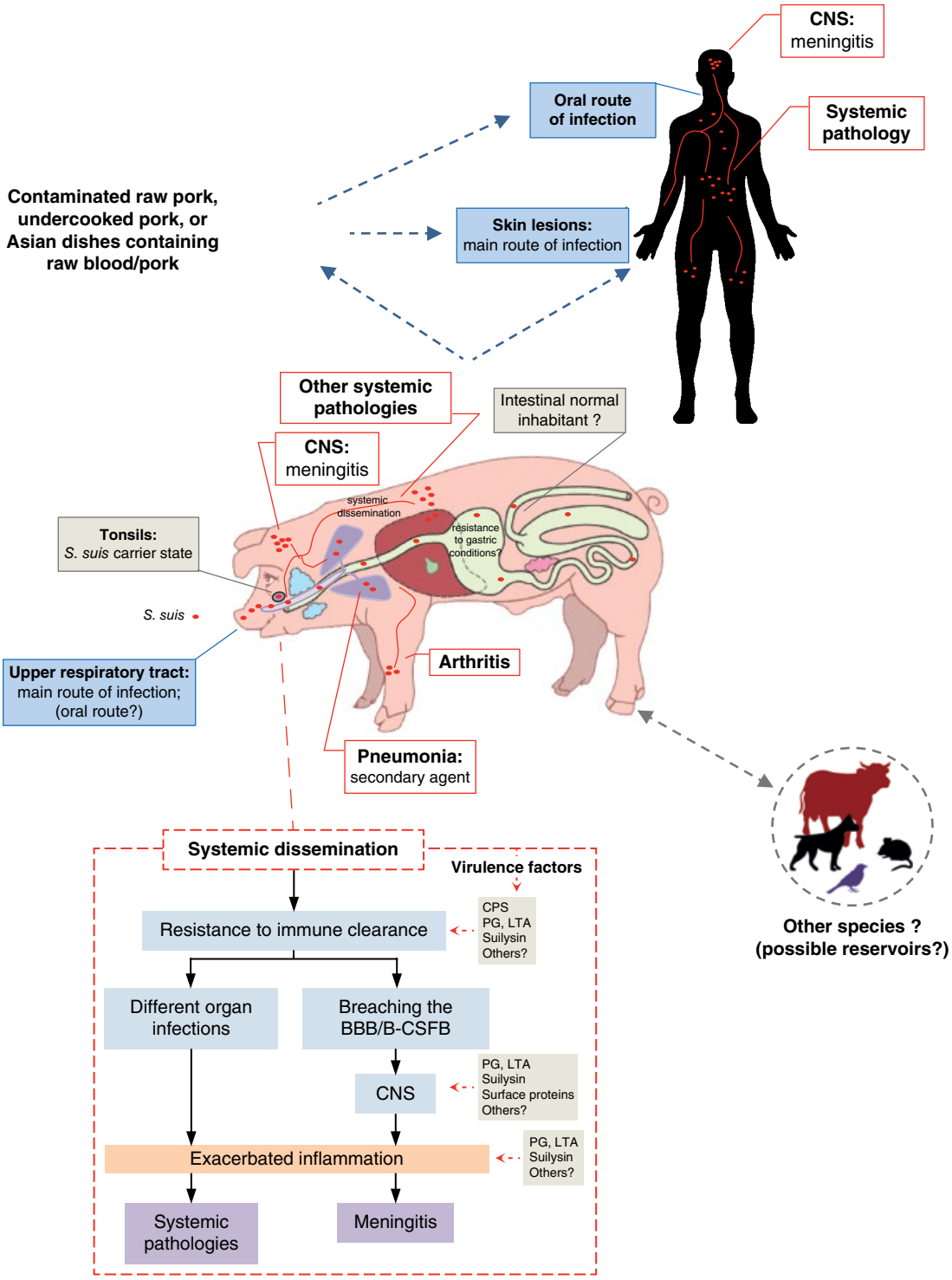


Figure 61.1 Pathogenesis and epidemiological features of *Streptococcus suis*-induced disease. The main route of entry of *S. suis* in pigs is the upper respiratory tract, whereas in humans it enters via skin wounds (handling of infected animals or meat) or the oral route (ingestion of raw or undercooked contaminated pork-derived products). After breaching the mucosal (respiratory, intestinal) or skin barriers, *S. suis* invades different organs and tissues through hematogenous and/or lymphogenous dissemination, leading mainly to meningitis, arthritis, and/or other multiple systemic pathologies (such as septic shock with sudden death, polyserositis, and endocarditis). Access to the central nervous system (CNS) is gained by crossing of the blood–brain barrier (BBB) and/or the blood–cerebrospinal fluid barrier (B-CSFB), leading to severe meningitis. During systemic dissemination, *S. suis* resists the attack of the host innate immune system by avoiding phagocytosis, possibly reducing complement activation, reducing cell activation, and/or inducing the death of leukocytes by means of multiple virulence factors. These include capsular polysaccharide (CPS), suilysin, and/or modifications of chemical properties of the cell wall components peptidoglycan (PG) and lipoteichoic acid (LTA). Several possible mechanisms might be used by *S. suis* to cross the BBB or the B-CSFB, including increased barrier permeability by direct induction of necrosis/apoptosis or indirectly through an intensive inflammatory process leading to bacterial and leukocyte trafficking into the CNS. Direct invasion and translocation of bacteria across these barriers have been reported as well. Finally, induction of inflammatory cytokines and other immune mediators by *S. suis* leads to exacerbated inflammation and is mainly responsible for the clinical signs of disease. Several *S. suis* virulence factors are involved in these last steps of the pathogenesis, including suilysin, PG, LTA, and several surface-anchored proteins. *S. suis* has been isolated from other animal species, including birds, rabbits, mice, dogs, horses, cattle, fallow deer, and wild boars, complicating the global epidemiology of *S. suis*. It has been suggested that some of these animal species might act as reservoirs. *Source:* Segura et al. (2017). Reproduced with permission of Elsevier.

time, more rapid disease progression, and a higher rate of mortality have been also described (Feng et al. 2010; Gottschalk et al. 2010) and may be due, in part, to enhanced induction of inflammation.

Clinical signs and lesions

Even when *S. suis* pig carrier rate is very high, the incidence of the disease varies from period to period and is usually less than 5% (Clifton-Hadley et al. 1986). In the absence of treatment, mortality rates can reach 20% (Cloutier et al. 2003). In most cases, affected animals are generally between 5 and 10 weeks of age, but atypical cases in pigs up to 32 weeks of age and a few hours old have also been described (Cloutier et al. 2003; Lapointe et al. 2002; MacInnes and Desrosiers 1999). The earliest sign is usually a rise in rectal temperature to as high as 108.5 °F (42.5 °C). This may occur initially without any other obvious signs. It is accompanied by a detectable bacteremia or pronounced septicemia that, if untreated, may persist for up to 3 weeks. During this period there are usually a fluctuating fever and variable degrees of poor appetite, depression, and shifting lameness (Clifton-Hadley et al. 1984; MacInnes and Desrosiers 1999).

In peracute cases, pigs may be found dead with no premonitory signs. A proportion of affected pigs typically develop nervous disease as a consequence of meningitis. Early nervous signs include incoordination and adoption of unusual stances, which soon progress to inability to stand, paddling, opisthotonus, convulsions, and nystagmus. The eyes are often staring, with reddening of mucous membranes. Less remarkable clinical signs include malaise and anorexia owing to septicemia and pneumonia as well as lameness caused by arthritis. Less common clinical manifestations of *S. suis* are vegetative valvular endocarditis, rhinitis, abortion (usually due to high temperature during the fever syndrome), and vaginitis (Sanford and Tilker 1982). In North America, *S. suis* is the infectious agent most frequently isolated from cases of vegetative valvular endocarditis in pigs. Affected pigs may die suddenly or show various levels of dyspnea, cyanosis, and wasting. Polyserositis similar to Glässer's disease can sometimes be observed.

Reports from the United Kingdom describe septicemia, meningitis, and polyarthritis, but rarely pneumonia (Heath et al. 1996; MacLennan et al. 1996). In the Netherlands, *S. suis* type 2 is associated with pneumonia in 42% of the cases, followed by meningitis, endocarditis, and polyserositis (Vecht et al. 1985). In North America, early reports indicated that *S. suis* was predominantly isolated from cases of pneumonia (Hogg et al. 1996; Reams et al. 1994; Sanford and Tilker 1982). *S. suis* isolates belonging to serotypes other than 2 have been recovered from cases of bronchopneumonia in Denmark (Perch et al. 1983), the Netherlands (Vecht et al. 1985), Belgium (Hommez et al. 1986), Finland (Sihvonen et al. 1988), Australia (Gogolewski et al. 1990), Canada (Higgins and Gottschalk 1990), and the United States (Reams et al. 1994). In France, *S. suis* was not mainly associated with lung lesions at slaughter (Fablet et al. 2012). The role of *S. suis* as a primary agent of pulmonary lesions, in the absence of other pathogens, is still controversial (Staats et al. 1997). However, *S. suis* is a common pulmonary isolate in porcine respiratory disease complex where it is one of several opportunists contributing to secondary bronchopneumonia (Chapter 21). Clinical manifestations related to meningitis, arthritis, and serositis were induced by intravenous application of a serotype 9 strain in specific-pathogen-free (SPF) piglets (Beineke et al. 2008). In general, there are no differences in lesions observed in infections with strains of different serotypes (Reams et al. 1994).

Significant microscopic lesions are usually limited to the brain, heart, joints, and serosal membranes (Reams et al. 1994). The predominant lesions are neutrophilic meningitis and choroiditis, with hyperemic meningeal blood vessels and fibrinopurulent or suppurative epicarditis (Reams et al. 1994, 1996; Sanford and Tilker 1982). Evidence of encephalitis, edema, and congestion of the brain may be present (Staats et al. 1997). The choroid plexus may have disruption of the plexus brush border, and fibrin and inflammatory cell exudates may be present in the ventricles (Staats et al. 1997). Using immunohistochemistry, bacteria were observed in the cytoplasm of neutrophils and macrophages localized in meningeal lesions (Zheng et al. 2009). Interstitial pneumonia is also observed and is considered a lesion secondary to septicemia (Reams et al. 1994). There is only one report of fibrinous pleuritis and bronchopneumonia in SPF piglets experimentally infected with *S. suis* serotype 2 (Berthelot-Hérault et al. 2001). Microscopic lesions are generally the same for all serotypes (Reams et al. 1994). An exception is in rare cases of fibrinohemorrhagic pneumonia with alveolar septal necrosis where it is suggested that certain strains of *S. suis* may cause vascular lesions (Reams et al. 1995). Less common lesions of hemorrhagic and necrotizing myocarditis, fibrinosuppurative polyserositis, and subacute meningoencephalitis and meningoencephalomyelitis have also been reported by Sanford (1987a,b).

Diagnosis

Diseased pigs

Presumptive diagnosis of *S. suis* infections is generally based on clinical signs, age of animals, and macroscopic lesions, although it is sometimes difficult to differentiate from *Haemophilus parasuis* infections. Confirmation is achieved by the isolation of the infectious agent and observation of typical microscopic lesions in tissues.

Collection of more than one alpha-hemolytic colony from different tissues of the same animal or from different animals in the same herd is recommended, because multiple serotypes and strains of *S. suis* can be involved in a single outbreak, especially under the presence of concomitant diseases such as the porcine reproductive and respiratory syndrome virus (PRRSV) (Higgins and Gottschalk 1990; Reams et al. 1994). Because strains causing disease in herds may change over time (Amass et al. 1997), ongoing surveillance by periodic culture of cerebrospinal fluid (CSF) from pigs with meningitis is recommended. This is especially important to upgrade autogenous vaccines when used as a method of control. Strains considered for inclusion in autogenous vaccines should be isolated from systemic sites such as meninges, spleen, liver, and joints and not from lungs, nasal cavities, or tonsils.

Direct detection of *S. suis* from infected tissues has also been studied (Boye et al. 2000), although its application is limited due to poor differentiation of serotypes. A multiplex nested PCR to detect and differentiate *H. parasuis*, *S. suis*, and *Mycoplasma hyorhinis* in formalin-fixed, paraffin-embedded tissues from pigs with polyserositis has been described (Kang et al. 2012). Detection of some *S. suis* serotypes by PCR (or even the presence of *mrp/ef/sly* positive strains) has also been reported (Wisselink et al. 2002). However, these methods are not used in routine diagnosis in veterinary medicine, although they have been used in humans (Gottschalk et al. 2010; Nguyen et al. 2015). It is important to mention that PCR for direct detection of serotypes 2 and 1 strains will also detect serotypes 1/2 and 14 strains, respectively. Finally, oral fluid samples were recently tested for detection of *S. suis* (Cheong et al. 2017). However, since *S. suis* is normally present in saliva (Dekker et al. 2013), the use of such a sample does not have any practical advantage.

After isolation, biochemical identification of *S. suis* isolates is possible with a minimum of tests when serotyping is available (Higgins and Gottschalk 1990). Devriese et al. (1991) suggested the use of only two tests on pig isolates:amylase positive and Voges–Proskauer (acetoin) negative. However, this simplified identification schema can only be used for isolates recovered from diseased or dead pigs and from sites other than the upper respiratory tract. In addition *S. suis*-like isolates can also be misidentified as *S. suis* using these tests (Okura et al. 2016). More recently evaluation of matrix-assisted laser desorption/ionization time-of-flight mass spectrophotometry (MALDI-TOF MS), as alternative tool for *S. suis* identification, has produced good results (Matajira et al. 2017; Pérez-Sancho et al. 2015).

Serotyping is still an important part of the routine diagnostic procedure. It can be carried out by different techniques, but many laboratories have adopted the coagglutination technique. Some isolates cross-react with more than one typing antiserum, and some strains are autoagglutinating, keeping the percentage of untypable strains around 20% (Gottschalk and Lacouture, 2015). During the last years, PCR tests have been developed that would allow different laboratories to perform complete serotyping without the need of different antisera (Okura et al. 2014). However, as serotypes 2 and 1/2 as well as serotypes 1 and 14 cannot be differentiated by PCR, specific antisera are still needed to differentiate these serotypes. Recently, *in silico* serotype determination from whole genome sequencing short-read data was shown to be useful for complete serotyping with clear differentiation of these serotypes (Athey et al. 2016). It was shown that strains of serotype pairs 1 and 14 and 2 and 1/2 can be differentiated by a missense mutation in the *cpsK* gene (Athey et al. 2016). Multiplex *S. suis* serotyping assay that simultaneously detects 33 serotypes using the Luminex xTAG universal array technology has also been developed (Bai et al. 2015).

Genetic tools may be useful in distinguishing isolates of *S. suis*, in determining the origin of infection in a given herd, in monitoring the kinetics of an outbreak, or in selecting strain(s) for inclusion in a vaccine. Restriction fragment length polymorphisms, random amplified polymorphic DNA, ribotyping, pulsed-field gel electrophoresis, and multiple-locus variable number tandem repeat analysis have been used (Martinez et al. 2002; Tian et al. 2004). More recently, 876 genes were defined as the minimum core genome (MCG) of the *S. suis* species. Different single nucleotide polymorphisms (SNPs) were located in the MCG genes and were referred to as MCG SNPs that can be used to classify and compare isolates within different MCG. This typing system facilitates the application of genome data to the surveillance of *S. suis* strains in a given region. The MCG groups may also be used to define *S. suis* subpopulations with the potential to cause severe clinical infections and large–scale outbreaks (Zheng et al. 2014).

Genomic fingerprinting allows the identification of isolates involved in outbreaks within a herd (Cloutier et al. 2003). Atypically, several distinct strains of *S. suis* serotype 2 may be isolated from systemic sites in the same or different animals (Marois et al. 2007). In herds affected with serotypes other than 2, such as serotype 1/2, clinical manifestations of the disease are more likely to be the result of inherent herd factors than the virulence of a specific isolate (Martinez et al. 2002). The great genetic heterogeneity of *S. suis* strains, the isolation of different strains within the same herd, and the predominance or not of particular strains in clinical disease are evidence that infection by *S. suis* is a dynamic process and reinforce the idea that its epidemiology is very complex (Vela et al. 2003). Isolates of *S. suis* serotype 2 from different geographical origins may be genotypically and

phenotypically different (Rehm et al. 2007). However, some strains share genotypic and phenotypic characteristics. For example, the use of MLST has shown that most serotype 2 strains involved in swine diseases from Eurasia are ST1, MRP$^+$, EF$^+$, and SLY$^+$, whereas those from North America are ST25 or ST28, MRP^v, EF$^-$, and SLY$^-$.

The advent of whole genome sequencing has contributed to the identification of further diversity within the *S. suis* species, as evidenced by more than 1000 published genomes of various serotypes and STs, yet a vast majority are incomplete annotations with only a few being circularized closed genome sequences. Nevertheless, genome comparisons between different strains and serotypes have identified major variations in gene content (Segura et al. 2017). Extensive genomic comparisons involving a sample of >300 strains of different serotypes and genetic backgrounds, although of limited geographic diversity (United Kingdom and Vietnam only), identified genetic differences between systemic, respiratory, and carriage *S. suis* isolates and a generalized tendency that disease-causing isolates have significantly smaller genomes than nonclinical isolates (Weinert et al. 2015). However, it is apparent that systemic isolates of these more virulent strains with reduced genomes are also carried in the nasopharynx niche (Weinert et al. 2015).

Surveillance in clinically healthy pigs

Detection of *S. suis* from tonsils or nasal cavities has no practical utility in the diagnosis of *S. suis* disease. *S. suis* is a normal inhabitant of the upper respiratory tract and is present in almost all herds, and mixtures of avirulent and virulent strains of different serotypes may reside in the same pig or herd (MacInnes et al. 2008). These sites are highly contaminated, and traditional bacterial isolation presents a low sensitivity (Gottschalk et al. 1999). The presence of a specific *S. suis* serotype in high numbers in nasal cavities and/or tonsils may reflect active transmission of the infection rather than a carrier state (Cloutier et al. 2003; Marois et al. 2007). The prevalence of a specific serotype may also be underestimated in nasal swabs or tonsils due to the lack of sensitivity of the isolation method. Selective isolation using immunomagnetic beads coated with serotype 2-specific antibodies or use of PCR significantly increases the detection rate from tonsils when compared with the standard isolation technique (Cloutier et al. 2003; Gottschalk et al. 1999; Marois et al. 2007). However, and as mentioned above, the PCR for serotype 2 and 1 strains based on detection of the CPS gene will also detect serotype 1/2 and 14 strains, respectively (Marois et al. 2004; Wisselink et al. 2002). PCR tests for direct detection of *ef* or *mrp* positive strains from tonsils have also been described (Swildens et al. 2005; Wisselink et al. 1999); however, this technique can only be used where virulent strains are positive for these factors. In addition, since some serotypes can also harbor one of these genes, a positive PCR does not definitively mean that virulent strains positive for both factors are present. Since many *S. suis*-like strains are also normal inhabitants of the upper respiratory tract, untypable isolates recovered from tonsils or nasal cavities should be confirmed as *S. suis* by genetic methods (Okwumabua et al. 2003). Biochemical identification alone, either traditional or by the use of rapid multitests, may be misleading (Okura et al. 2016). Up to 50% of isolates recovered from tonsils of healthy pigs and biochemically identified as being *S. suis* may be negative using a species-specific PCR (unpublished observations). On the other hand, many *S. suis* isolates, mainly serotypes 9–34 as well as untypable strains, may give atypical biochemical profiles that will clearly underestimate the prevalence of real *S. suis* (Gottschalk et al. 1991; Okura et al. 2016).

Serologic tests for the detection of antibodies against *S. suis* have been evaluated (del Campo Sepúlveda et al. 1996; Kataoka et al. 1996). Recently, an ELISA test has been used to measure antibodies against *S. suis* in different tests (Wallgren et al. 2016). However, these tests are generally not useful since many cross-reactions may be detected with different strains of *S. suis* or other streptococci. In addition, *S. suis* does not induce high antibody titers, even after an experimental infection (Calzas et al. 2015). A herd/strain-specific ELISA test using either whole bacteria or a protein extract of the predominant virulent *S. suis* strain from the herd may allow the evaluation of maternal antibody levels to determine the best time for vaccination and to monitor antibody levels after either natural infection or vaccination (Cloutier et al. 2003; Lapointe et al. 2002). Practically, this approach has little utility as a routine test owing to the marked diversity of strains infecting herds and the impracticality of developing herd/strain-specific serology tests. A whole-cell ELISA test has also been reported (but not validated) to measure exposure to *S. suis* in humans (Smith et al. 2008).

Treatment

The choice of the best antibacterial agent against *S. suis* infections should be based on susceptibility of the organism, the type of infection, and the mode of administration. Determination of minimal inhibitory concentrations (MICs) in isolates from Europe showed that emergence of resistance among *S. suis* derived from swine clinical cases appeared to be limited. Resistance to ampicillin, ceftiofur, enrofloxacin, florfenicol, penicillin, and trimethoprim/sulfamethoxazole was low, whereas that to tetracycline was constantly very high (El Garch et al. 2016; van Hout et al. 2016). However, strains isolated from tonsils of healthy animals or from the environment of slaughterhouses are usually multiresistant (Soares et al. 2015; Zhang et al. 2015). In North America, a large

number of isolates were shown to be moderately susceptible to penicillin, but the sensitivity rate to amoxicillin and ampicillin was around 90% (Dee and Corey 1993). Even if MIC values for penicillin may gradually increase with time, pigs infected with strains showing higher MICs may still respond well to treatment with this antibiotic (Callens et al. 2013; Varela et al. 2013). Serotype–specific patterns of susceptibility to antimicrobials have been described and may differ between regions (Marie et al. 2002; Vela et al. 2005).

MacInnes and Desrosiers (1999) suggested that ampicillin, ceftiofur, gentamicin, tiamulin, and a combination of trimethoprim and a sulfonamide are the most useful antibacterial products for parenteral treatment. However, the choice of antimicrobials used for the treatment of diseased animals should preferably be based on knowledge of the local pattern of resistance (Dee and Corey 1993). Early recognition of nervous signs including holding back of ears, squinting, and dog-sitting followed by immediate parenteral treatment with an appropriate antibiotic with or without an anti-inflammatory agent maximizes pig survival (Amass et al. 1997). During an outbreak, pigs should be monitored 2–3 times daily, and new cases treated. In segregated early weaned pigs with acute *S. suis* meningitis, excellent results were obtained with injection of penicillin and dexamethasone (Varela et al. 2013). In outbreaks where response to parenteral treatment of acute cases is poor, it is advisable to treat all the pigs in a pen when one is affected or found dead (MacInnes and Desrosiers 1999). Treatment can also be administrated via the drinking water or in medicated feed. However, treatment needs to be started very quickly and be continued for at least 5 days (Denicourt and Le Coz 2000). Amoxicillin is frequently used since it rapidly achieves high plasma levels and diffuses well into the extracellular space (Denicourt and Le Coz 2000). In other studies, use of ampicillin and penicillin G did not significantly reduce disease in animals exposed to a coinfection with *S. suis* and PRRSV (Halbur et al. 2000; Schmitt et al. 2001). They found that treatment with ceftiofur was the only regimen that significantly reduced mortality, severity of gross lung lesions, and recovery of *S. suis* from tissues at necropsy. Another study reported that the pharmacokinetics of ceftiofur hydrochloride in pigs coinfected with PRRSV and *S. suis* was significantly different compared with that of clinically healthy pigs, indicating that the health status of an affected animal may influence the availability of this antimicrobial (Day et al. 2015).

Prevention

Reduction of predisposing factors

Streptococcus suis emerged as an important pathogen with intensification of the swine industry. In addition to virulence of involved strains, other factors impact development of disease including immune status of the herd, mixing of infected and naïve uninfected pigs, concurrent infections and immunosuppression, quality of the environment, and other management factors. Control of these factors helps prevent disease in herds harboring virulent strains.

Overcrowding, poor ventilation, excessive temperature fluctuations, and mixing of pigs with an age spread of more than 2 weeks seem to be the most important stress factors involved in the development of *S. suis* infection in susceptible pigs (Dee and Corey 1993). Management practices such as all-in/all-out pig flow can help reduce the incidence of the disease. Dividing large buildings into smaller rooms can help minimize temperature fluctuations and the age spread between pigs. Cleaning each room between groups of pigs reduces buildup of microorganisms and improves health status, average daily gain, and feed conversion (Dee and Corey 1993).

Infection with some viruses is known to render pigs more susceptible to *S. suis* disease or potentiate lesions in pigs. Control of such viruses helps minimize the impact of *S. suis*. Acute infections with virulent North American PRRSV significantly increase susceptibility to *S. suis* disease (Thanawongnuwech et al. 2000). Likewise, piglets infected with PRRSV acquired *in utero* are more susceptible to infection and disease following challenge by *S. suis* serotype 2 (Feng et al. 2001). Concurrent infection with pseudorabies (Aujeszky's) and influenza virus may enhance clinical disease caused by *S. suis* (Iglesias et al. 1992; Lin et al. 2015).

Antimicrobial preventive medication

A wide range of antibiotics are used in pig husbandry for targeted prophylactic and metaphylactic use (Seitz et al. 2016). Strategies for prophylactic antimicrobial medication for *S. suis* should reflect consideration of bioavailability, route of administration (feed or water), competition (feed and water availability), and serum concentration needed to kill *S. suis* (Amass et al. 1997). Penicillin and amoxicillin are frequently used in most countries where metaphylactic use is still allowed (Seitz et al. 2016). Byra et al. (2011) reported that potassium penicillin G administered in drinking water is effective in reducing mortality associated with *S. suis* infection.

Immunization

As the complexity of *S. suis* epidemiology in swine increases (multiple serotypes, multiple strains within serotypes with high phenotypical diversity), field reports describing difficulty in disease control and management, especially "vaccine failure", are common. In fact, *S. suis* is a clear example of a bacterial disease extremely difficult to be controlled through vaccination. For a complete and recent review, see Segura (2015).

Vaccines used in the field to prevent *S. suis* disease are either autogenous or (a very few) commercial bacterins, and results have been inconsistent (Halbur et al. 2000; Reams et al. 1996; Segura 2015). Possible reasons for failure of autogenous vaccines include degradation of protective antigens caused by heat or formalin processing, inadequate bacterial antigen concentrations, the adjuvant included in the formulation, low production of antibodies, and/or production of antibodies to antigens not associated with protection (del Campo Sepúlveda et al. 1996; Segura 2015). Field studies also reported failure of a commercial serotype 2 bacterin to protect against nursery mortality (reviewed by Segura [2015]). In a few cases, good protection against challenge with serotype 2 could be obtained with bacterins (Baums et al. 2009). Wisselink et al. (2001) showed that a bacterin with a water-in-oil emulsion adjuvant produced better results than with an aluminum hydroxide-based adjuvant. It is clear that protection (if any) is serotype dependent and commercial vaccines should not be used without clear information about the serotype/strain involved in the clinical cases. Even within the same serotype, protection obtained with a commercial bacterin may be difficult to predict due to high phenotypic variation within strains of the same serotype, based on available data for serotype 2 (Segura 2015). Efficacy of autogenous *S. suis* bacterins is uncertain as there is little published data. It is difficult to assess observations made in the field, because there are many uncontrolled factors in commercial herds that can influence the results. Certainly, any attempt to assess the efficacy of an autogenous *S. suis* vaccine in the field must include equal numbers of non-vaccinated controls (Segura 2015).

The nature of antibodies that are needed to obtain protection is unclear. Antigenicity of CPS and the need for anti-capsular antibodies in protection are still controversial. Pigs experimentally or naturally infected with *S. suis* type 2 only produced low levels of antibodies against the CPS (Calzas et al. 2015). Wisselink et al. (2002) showed that antibodies against the CPS and other bacterial components are essential for full protection against homologous challenge. Indeed, a recent study showed very good protection using a CPS-glycoconjugate vaccine based on purified CPS of *S. suis* serotype 2 conjugated to a carrier protein (Goyette-Desjardins et al. 2016). Since the structure and antigenicity of the CPS is the same for all strains within a serotype, this vaccine would be protective against any serotype 2 strain. However, a previous study showed that the serotype-specific protection obtained with a bacterin was due to the presence of opsonic antibodies directed to antigens different from the CPS (Baums et al. 2009).

Most vaccination-challenge studies have been carried out with piglets. Because *S. suis* disease is most often at 6–10 weeks of age and the first of 2 doses of bacterin need be given at approximately 3–4 weeks of age, interference by maternal antibodies must be considered. Lapointe et al. (2002) found better response to vaccination in 2–4-week-old pigs with lower levels of maternal antibodies to the serotype 1/2 bacterin strain as compared with cohorts with higher maternal titers.

Vaccination of sows and gilts has also been sometimes described as effective (Swildens et al. 2007). Sow vaccination is less costly and labor intensive, thus representing an economical alternative to piglet vaccination. Yet available results indicate that sow vaccination with bacterins as a means to protect their piglets is a matter of controversy. Vaccinated sows with a formalin-killed *S. suis* serotype 2 bacterin five and three weeks before parturition responded poorly or not at all to vaccination and resulted in low maternal immunity transferred to the litters (Blouin et al. 1994). In a field trial, vaccination of sows with a commercial serotype 2 bacterin did not consistently reduce nursery mortality due to *S. suis* (Torremorrell et al. 1997). Similarly, administration of a *S. suis* serotype 14 bacterin to sows four and one week pre-farrowing failed to confer protection against mortality and only partially protected against morbidity in their progeny after homologous challenge (Amass et al. 2000). In contrast, a field study with a *S. suis* serotype 2 autogenous bacterin showed that opsonizing antibodies were increased in the serum and colostrum of sows vaccinated five and three weeks pre-farrowing, specific antibodies were detected in their litters, and pigs were protected up to 6 weeks of age (Baums et al. 2010). A recent study showed that 5-week-old piglets from vaccinated sows were protected against an experimental infection with virulent strains of *S. suis* (Hsueh et al. 2017). These data suggest that protective passive maternal immunity for *S. suis* serotype 2 can be achieved by bacterin vaccination of sows. However, without subsequent active vaccination of the piglets to lengthen protection, this strategy might lead to highly susceptible growers (Baums et al. 2010). As in the case of piglet vaccination, scientific studies (with control groups) that include production and evaluation of antibodies in vaccinated sows, duration of maternal antibodies, and functional evaluation of such antibodies must still be done to confirm the usefulness of sow vaccination with bacterins (Segura 2015).

In herds infected with multiple strains or serotypes of *S. suis*, multivalent vaccines or vaccines that provide a strong degree of cross-immunity are needed to provide adequate control of infection. Bacterins do not seem to fulfill this requisite (Segura 2015). Subunit vaccines based on proteins conserved among serotypes may be more useful in providing practicable heterologous

protection. Although a large amount of information regarding the use of subunit candidates has been published in recent years, data are still experimental, and no commercial vaccine is yet available (Segura 2015). With a few exceptions, proposed *S. suis* candidates concern serotype 2 only, and strictly homologous protection was evaluated. Only one protein candidate, surface antigen one (Sao), has been shown to induce cross-serotype protection (Hsueh et al. 2017). Confusing data using different animal models, different strains, and vaccine formulated with adjuvants inducing deleterious side effect have been published (Segura 2015). More scientific studies confirmed by different research groups must be performed in order to identify good vaccine candidates (Segura 2015).

Of these protein vaccine candidates, MRP, EF, and SLY have been frequently proposed as protective (Wisselink et al. 2001). However, consensus of studies is that high levels of antibodies against these proteins do not provide protection and their use as subunit vaccines remains controversial (Segura 2015). In addition, since these proteins are not widely present among virulent serotype 2 strains, their usefulness as cross-protective subunit vaccines is limited, unless combined with other more universal immunogenic proteins (Segura 2015).

Based on the observation that convalescent animals are protected against reinfection, low doses of virulent *S. suis* were administered as a live vaccine and were shown to induce good protection against challenge, although the low dose virulent vaccine induced some pathology (Schmitt et al. 2001). Many studies have shown that infection of mice with live avirulent *S. suis* mutants confers homologous and, in some cases, heterologous protection (Gottschalk and Segura 2000; Segura 2015). Only a few live bacterial candidates have been tested in pigs with mixed results (Segura 2015). Side effects such as hyperthermia, extra connective tissue reactions, lameness, convulsions, and others have been reported with some of the experimental live vaccines when used in pigs (Segura 2015). Further attenuation would thus be necessary for routine use in pigs. The challenge in creating a live vaccine is to obtain a fully attenuated strain that induces protective immunity and is also safe. In addition, since *S. suis* is also a zoonotic agent, safety of a live vaccine is a major public health concern. The possibility that an attenuated strain in pigs is not completely attenuated in humans cannot be completely excluded. The risks of introducing a live vaccine strain into a commercial herd have not been well established and require further studies (Segura 2015).

Because virulent systemic strains of *S. suis* rarely colonize the upper respiratory tract of sows and gilts and few piglets are colonized with these strains by weaning, induction of nasal colonization in young pigs with the herd's systemic strain was suggested as a means of disease prevention (Oliveira et al. 2001). Results showed that inoculation of 5-day-old piglets with the herd's systemic strain of *S. suis* tended to be more effective in reducing the morbidity and mortality than the colonization of piglets by nose-to-nose contact with inoculated sows (Oliveira et al. 2001). However, once again, the use of a virulent strain with an important zoonotic potential should be carefully evaluated.

Eradication

Attempts to eradicate *S. suis* infection have focused only on serotype 2. Medicated early weaning (MEW) is ineffective since *S. suis* is a very early colonizer. Cesarean section can be used to derive pigs free of *S. suis* from infected dams. According to Clifton-Hadley et al. (1986), only depopulation and restocking with "clean" pigs will ensure eradication of the infection, and in most herds this is cannot be justified economically. The problem is in determining whether a pig or herd is "clean." There is no diagnostic test able to detect with high confidence the presence of virulent strains in carrier pigs. The definition of a "clean" herd would then need to be a herd with a history free of endemic disease due to *S. suis*. Still, the presence of carrier animals harboring virulent strains cannot be ruled out. In the presence of predisposing factors (see above), these strains may induce clinical disease. Even if accomplished, strict biosecurity measures are needed that include eliminating rodents, and perhaps flies, in order to prevent reinfection (Amass et al. 1997). Mills (1996) described procedures used to establish a purebred minimal-disease herd from gilts that were carriers of a virulent strain of *S. suis* type 2. Amass et al. (1996) did not recommend such an approach, but instead recommend optimization of management and environment of pigs coupled with strategic medication of clinically ill animals for control and prevention of mortality caused by *S. suis*. It has been suggested that *S. suis* may be purportedly eliminated from tonsils of sows through vaccination with a bacterin combined with medication, resulting in progeny free of pathogenic *S. suis* (Swildens et al. 2007). However, this approach has not been successful in the field (unpublished data). Byra et al. (2011) showed that potassium penicillin G administered in drinking water was effective in reducing mortality associated with *S. suis* infection as well as tonsillar carriage of *S. suis*.

Given the cost of eliminating *S. suis*, the risk of failure, the difficulty in maintaining a free herd, and the lack of tools to monitor herd status, it would appear reasonable to direct resources toward control measures rather than eradication.

Infections caused by beta-hemolytic streptococci

Streptococcus porcinus

The name *Streptococcus porcinus* was proposed in 1984 (Collins et al. 1984) to represent streptococci of serological groups E, P, U, and V that formed a single DNA–DNA homology group. *S. porcinus* has a unique phenotypic profile in addition to serologic differences that can be used to help identify the species.

Streptococcus porcinus can be isolated from tonsils, pharynx, and nasal cavities of clinically healthy pigs. In fact, it is one of the most common bacterial species recovered from tonsils at slaughterhouses (O'Sullivan et al. 2011). It is also occasionally found in the vaginal mucus of sows and in the semen and prepuce of boars. It is considered to be more of a secondary invader than a primary pathogen in conditions such as pneumonia, enteritis, encephalitis, and arthritis (Wessman 1986). *S. porcinus* group E has been associated in the United States with a contagious entity in growing pigs known as streptococcal lymphadenitis, jowl abscesses, or cervical abscesses. Transmission is by contact, drinking water, or ingestion of food contaminated by abscess discharge or infected feces. The organisms enter through the mucosa of the pharyngeal or tonsillar surfaces and are carried to the lymph nodes primarily of the head and neck region where abscesses are formed (Wessman 1986). Losses due to this disease in the United States were important in the 1960s, but the incidence and importance has since dramatically declined. The disease is not recognized as an important economic entity in other countries, where the bacterium represents only a few percent of the microorganisms isolated from abscesses in swine (Wessman 1986). A report of an outbreak from Spain mentioned that 80% of 50 feeder pigs had mandibular and retropharyngeal purulent lymphadenitis (Real et al. 1992). Antibiotic treatment is not usually successful in abscessed swine or in elimination of carriers. Resistance to tetracycline has been reported (Lämmler and Bahr 1996). Vaccination is possible but has not been widely used since the condition is not widespread.

Streptococcus porcinus groups P, U, and V were isolated by Hommez et al. (1991) from pig lungs, genital organs, and brains. However, no histological lesions could be associated with their presence. *S. porcinus* groups P and V were associated with abortions in pigs (Lämmler and Bahr 1996; Plagemann 1988). Katsumi et al. (1998) reported that from a total of 170 beta-hemolytic streptococci isolated from lesions in slaughtered pigs, 22.4% were identified as *S. porcinus*. Of those, 3.0% belonged to group E, 3.0% to group P, and 8.2% to group U, and 8.2% were ungroupable.

Finally, many strains of *S. porcinus* were supposedly recovered from the human female genitourinary tract and might be considered an emerging human pathogen (Pereira et al. 2013). However, it was later reported that these strains belong to a new species, *Streptococcus pseudoporcinus* (Bekal et al. 2006). Since *S. pseudoporcinus* can also be isolated from dairy cows, subspecies denominations have been proposed:*S. pseudoporcinus* subsp. *hominis* subsp. nov. for the human isolates and *S. pseudoporcinus* subsp. *lactis* subsp. nov. for the dairy isolates (Shewmaker et al. 2012). More recently, a confirmed *S. porcinus* was recovered from a wound in an abattoir worker (Shewmaker et al. 2012).

Streptococcus dysgalactiae

Vieira et al. (1998), based on multilocus enzyme electrophoresis typing and genomic DNA relatedness, proposed that alpha- and nonhemolytic streptococci of Lancefield group C are designated as S. dysgalactiae subsp. *dysgalactiae*, while beta-hemolytic streptococci belonging to Lancefield groups C, G, or L are designated as S. dysgalactiae subsp. *equisimilis*. In swine, members of the S. dysgalactiae subsp. *equisimilis* species are all beta-hemolytic streptococci. Although members of the normal flora, they are considered the most important beta-hemolytic streptococci involved in lesions in pigs (Hommez et al. 1991). S. dysgalactiae subsp. *equisimilis* can be reliably identified using MALDI-TOF MS (Matajira et al. 2017).

Streptococcus dysgalactiae subsp. *equisimilis* is common in nasal and throat secretions, tonsils, and vaginal and preputial secretions. In fact, vaginal secretions and milk from post-parturient sows are the most likely sources of infection for the piglets (Woods and Ross 1977). Streptococci enter the bloodstream via skin wounds, the navel, and tonsils. A bacteremia or septicemia occurs, and the organisms settle in one or more tissues, giving rise most commonly to arthritis, endocarditis, or meningitis. Infection is usually first seen in pigs between 1 and 3 weeks of age. Joint swelling and lameness are the most obvious and persistent clinical signs. Elevated temperatures, lassitude, roughened hair coat, and inappetence may also be noted. Early lesions consist of periarticular edema, swollen hyperemic synovial membranes, and turbid synovial fluid. Necrosis of articular cartilage may be observed 15–30 days after onset and may become more severe. Fibrosis and multifocal abscessation of periarticular tissues and hypertrophy of synovial villi also occur. Hill et al. (1996) reported that in lame pigs up to 12 weeks of age, the most common causative agent was S. dysgalactiae subsp. *equisimilis* and most of the pigs culled for arthritis were under 6 weeks of age (Hill et al. 1996). Hommez et al. (1991) mentioned that S. dysgalactiae

subsp. *equisimilis* is frequently isolated from pigs with septicemia, arthritis, or valvular endocarditis. Katsumi et al. (1998) reported that during a 7-year period, 77.6% of beta-hemolytic streptococci isolated from slaughtered pigs in Japan belonged to the S. dysgalactiae species. More recently, severe disseminated suppurative meningoencephalomyelitis due to S. dysgalactiae subsp. *equisimilis* has been described (Kasuya et al. 2014). It has also been isolated from several tuberculosis-like lesions in condemned pigs at the slaughterhouse in Spain (Cardoso-Toset et al. 2015).

Beta-hemolytic streptococci are sensitive to beta-lactam antibiotics. High MIC values to tetracyclines, danofloxacin, spectinomycin, tiamulin, and clindamycin have also been reported (Moreno et al. 2016). Long-acting antibacterial agents may be beneficial, and treatment should be given before the inflammatory process is well advanced. Insufficient consumption of colostrum or milk or inadequate levels of antibodies, especially in gilts, may predispose to disease (Windsor 1978). Since baby pigs are virtually assured of being exposed to S. dysgalactiae, effective preventive measures should be followed. Adequate intake of colostrum may ensure that the piglets receive protective antibodies (Zoric et al. 2004). Traumatic injuries to the feet and legs should be minimized by reducing the abrasiveness of the floor surface in the nursing area (Zoric et al. 2009). There are no recent reports about vaccination against groups C or L streptococci. Autogenous bacterins have been used, and a reduction in incidence of arthritis has been reported when sows were vaccinated before farrowing (Woods and Ross 1977).

Other streptococci

Streptococcus equi subsp. *zooepidemicus* (*Streptococcus zooepidemicus*) is classified in Lancefield's group C and causes respiratory disease in a variety of mammals. In Europe and the United States, *S. zooepidemicus* is the primary cause of equine respiratory tract infections in foals and infertility in mares. However in China, *S. zooepidemicus* is the mainly reported in swine. In 1975, an outbreak of *S. zooepidemicus* in swine occurred in western China, resulting in substantial economic losses (Feng and Hu 1977). From the 1990s to the present, sporadic cases and regional epidemics are still impacting the pig industry in China (Mao et al. 2008). A combined vaccine with porcine circovirus type 2 and *S. zooepidemicus* was developed in that country (Lin et al. 2014). *Streptococcus epidemicus* has also been isolated from several tuberculosis-like lesions in condemned pigs at the slaughterhouse in Spain (Cardoso-Toset et al. 2015).

Bacterial streptococcal strains were isolated from the lungs and kidney of two pigs with lesions associated with pneumonia and septicemia, respectively. The two isolates were recovered from animals on different farms located in difference provinces of Spain and in different years. The isolates were classified as a new species, *Streptococcus plurextorum* sp. nov. (Vela et al. 2009). Another new streptococcal species, *Streptococcus porci* sp. nov. has been associated with pneumonia and pericarditis (Vela et al. 2010). So far, there are no data about the habitat and/or the virulence properties of these two species.

References

Allen AG, Bolitho S, Lindsay H, et al. 2001. *Infect Immun* 69:2732–2735.

Amass S, Wu C, Clark LK. 1996. *J Vet Diagn Invest* 8:64–67.

Amass S, San Miguel P, Clark L. 1997. *J Clin Microbiol* 35:1595–1959.

Amass S, Stevenson G, Vyverberg B, et al. 2000. *Swine Health Prod* 8:217–219.

Athey TB, Auger JP, Teatero S, et al. 2015. *PLoS One* 10:e0137760.

Athey TB, Teatero S, Lacouture S, et al. 2016. *BMC Microbiol* 16:162.

Bai X, Liu Z, Ji S, et al. 2015. *J Microbiol Methods* 117:95–99.

Baums C, Verkühlen G, Rehm T, et al. 2007. *Appl Environ Microbiol* 73:711–717.

Baums C, Kock C, Beineke A, et al. 2009. *Clin Vaccine Immunol* 16:200–208.

Baums CG, Brüggemann C, Kock C, et al. 2010. *Clin Vaccine Immunol* 17:1589–1597.

Beineke A, Bennecke K, Neis C, et al. 2008. *Vet Microbiol* 128:423–430.

Bekal S, Gaudreau C, Laurence R, et al. 2006. *J Clin Microbiol* 44:2584–2586.

Benga L, Fulde M, Neis C, et al. 2008. *Vet Microbiol* 132:211–219.

Berthelot-Hérault F, Morvan H, Kéribin AM, et al. 2000. *Vet Res* 31:473–479.

Berthelot-Herault F, Gottschalk M, Labbe A, et al. 2001. *Vet Microbiol* 82:69–80.

Berthelot-Hérault F, Gottschalk M, et al. 2005. *Can J Vet Res* 69:236–240.

Blouin C, Higgins R, Gottschalk M, et al. 1994. *Can J Vet Res* 58:49–54

Bonifait L, Veillette M, Létourneau V, et al. 2014. *Appl Environ Microbiol* 80:3296–3304.

Boye M, Feenstra A, Tegtmeier C, et al. 2000. *J Vet Diagn Invest* 12:224–232.
Byra P, Cox W, Gottschalk M, et al. 2011. *Can Vet J* 52:272–276.
Callejo R, Zheng H, Du P, et al. 2016. *J Med Microbiol Case Rep* 3 doi: 10.1099.
Callens BF, Haesebrouck F, Maes D, et al. 2013. *Microb Drug Resist* 19:146–151.
van Calsteren MR, Goyette-Desjardins G, Gagnon F, et al. 2016. *J Biol Chem* 291:8387–8398.
Calzas C, Lemire P, Auray G, et al. 2015. *Infect Immun* 83:441–453.
del Campo Sepulveda E, Altman E, Kobisch M, et al. 1996. *Vet Microbiol* 52:113–125.
Cardoso-Toset F, Gómez-Laguna J, Amarilla SP, et al. 2015. *PLoS One* 9:e0139130.
Cheong Y, Oh C, Lee K, et al. 2017. *J Vet Sci*. 18:283–289.
Cheung P, Lo K, Cheung T, et al. 2008. *Int J Food Microbiol* 127:316–320.
Clifton-Hadley F, Enright M. 1984. *Vet Rec* 114:585–587.
Clifton-Hadley F, Alexander T, Enright M, et al. 1984. *Vet Rec* 115:562–564.
Clifton-Hadley F, Alexander T, Enright M. 1986. *Proc Pig Vet Soc* 14:27–34.
Cloutier G, D'Allaire S, Martinez G, et al. 2003. *Vet Microbiol* 97:135–151.
Collins M, Farrow J, Katic V, et al. 1984. *Syst Appl Microbiol* 5:402–413.
Day DN, Sparks JW, Karriker LA, et al. 2015. *J Vet Pharmacol Ther* 38:475–481.
Dee S, Corey MM 1993. *Swine Health Prod* 1:17–20.
Dekker N, Bouma A, Daemen I, et al. 2013. *PLoS One* 8:e61339.
Denicourt M, Le Coz P. 2000. In Martineau GP, ed. *Streptococcus suis* 2000 Update: Nine Strategic and Practical Steps to Quickly Understand *Streptococcus suis* Infection and Disease. Nice, France: Virbac.
Devriese L, Kilpper-Balz A, Schleifer K. 1988. *Int J Syst Bacteriol* 38:440–441.
Devriese L, Ceyssens K, Hommez J, et al. 1991. *Vet Microbiol* 26:141–150.
Devriese L, Haesebrouck F, De Herdt P, et al. 1994a. *Avian Pathol* 23:721–724.
Devriese L, Hommez J, Pot B, et al. 1994b. *J Appl Bacteriol* 77:31–36.
Devriese L, Pot B, Vandamme P, et al. 1997. *Int J Syst Bacteriol* 47:1073–1077.
Domínguez-Punaro M, Segura M, Plante M, et al. 2007. *J Immunol* 179:1842–1854.
Doube A, Calin A. 1988. *Ann Reheum Dis* 47:598–599.
El Garch F, de Jong A, Simjee S, et al. 2016. *Vet Microbiol* 194:11–22.
Enright M, Alexander T, Clifton-Hadley FA. 1987. *Vet Rec* 121:132–133.
Fablet C, Marois C, Dorenlor V, et al. 2012. *Res Vet Sci* 93:627–630.
Feng Z, Hu J. 1977. *Anim Husbandry Vet Med Lett* 2:7–12.
Feng W, Laster S, Tompkins M, et al. 2001. *J Virol* 75:4889–4895.
Feng Y, Zhang H, Ma Y, et al. 2010. *Trends Microbiol* 18:124–131.
Field H, Buntain D, Done J, 1954. *Vet Rec* 66:453–455.
Fittipaldi N, Sekizaki T, Takamatsu D, et al. 2008a. *Mol Microbiol* 70:1120–1135.
Fittipaldi N, Sekizaki T, Takamatsu D, et al. 2008b. *Infect Immun* 76:3587–3594.
Fittipaldi N, Fuller T, Teel J, et al. 2009. *Vet Microbiol* 139:310–317.
Fittipaldi N, Xu J, Lacouture S, et al. 2011. *Emerg Infect Dis* 17:2239–2244.
Fittipaldi N, Segura M, Grenier D, et al. 2012. *Future Microbiol* 7:259–279.
Gogolewski R, Cook R, O'Connell C. 1990. *Aust Vet J* 67:202–204.
Gottschalk M, Lacouture S. 2015. *Can Vet J* 56:1093–1094.
Gottschalk M, Segura M. 2000. *Vet Microbiol* 76:259–272.
Gottschalk M, Higgins R, Jacques M, et al. 1989. *J Clin Microbiol* 27:2633–2635.
Gottschalk M, Higgins R, Jacques M, et al. 1991. *J Clin Microbiol* 29:2590–2594.
Gottschalk M, Lacouture S, Odierno L. 1999. *J Clin Microbiol* 37:2877–2881.
Gottschalk M, Xu J, Calzas C, et al. 2010. *Future Microbiol* 5:371–391.
Goyette-Desjardins G, Auger JP, Xu J, et al. 2014. *Emerg Microbes Infect* 3:e45.
Goyette-Desjardins G, Calzas C, Shiao TC, et al. 2016. *Infect Immun* 84:2059–2075.
Halbur P, Thanawongnuwech R, Brown G, et al. 2000. *J Clin Microbiol* 38:1156–1160.
Heath P, Hunt B, Duff J, et al. 1996. *Vet Rec* 139:450–451.
Higgins R, Gottschalk M. 1990. *J Vet Diagn Invest* 2:249–252.
Higgins R, Gottschalk M, Boudreau M, et al. 1995. *J Vet Diagn Invest* 7:405–406.
Hill B, Corney B, Wagner T. 1996. *Aust Vet J* 73:179–181.
Hill J, Gottschalk M, Brousseau R, et al. 2005. *Vet Microbiol* 25:107:63–69.
Hogg A, Amass S, Hoffman L, et al. 1996. In Proceedings, American Association of Swine Practitioners, pp. 79–81.
Hommez J, Devriese L, Henrichsen J, et al. 1986. *Vet Microbiol* 11:349–355.
Hommez J, Devriese L, Castryck F, et al. 1991. *J Vet Med B* 38:441–444.
van Hout J, Heuvelink A, Gonggrijp M. 2016. *Vet Microbiol* 194:5–10.
Hsueh KJ, Cheng LT, Lee JW, et al. 2017. *BMC Vet Res* 13:15.
Huang YT, Yeng LJ, Ho SW, et al. 2005. *J Microbiol Immunol Infect* 38:306–313.

Iglesias J, Trujano M, Xu J. 1992. *Am J Vet Res* 53:364–367.
Jansen E, Van Dorssen C. 1951. *Tijdschr Diergeneeskd* 76:815–832.
Kang I, Kim D, Han K, et al. 2012. *Can J Vet Res* 76:195–200.
Kasuya K, Yoshida E, Harada R, et al. 2014. *J Vet Med Sci* 76:715–718.
Kataoka Y, Yamashita T, Sunaga S, et al. 1996. *Vet Med Sci* 58:369–372.
Katsumi M, Kataoka Y, Takahashi T, et al. 1998. *J Vet Med* 60:129–131.
Kilpper-Balz R, Schleifer K. 1987. *Int J Syst Bacteriol* 37:160–162.
Lakkitjaroen N, Takamatsu D, Okura M, et al. 2011. *J Med Microbiol* 60:1669–1676.
Lämmler C, Bahr K. 1996. *Med Sci Res* 24:177–178.
Lapointe L, D'Allaire S, Lebrun A, et al. 2002. *Can J Vet Res* 66:8–14.
Lin HX, Ma Z, Yang XQ, et al. 2014. *Vet Microbiol* 171:198–205.
Lin X, Huang C, Shi J, et al. 2015. *PLoS One* 10:e0124086.
MacInnes J, Desrosiers R. 1999. *Can J Vet Res* 63:83–89.
MacInnes J, Gottschalk M, Lone A, et al. 2008. *Can J Vet Res* 72:242–248.
MacLennan M, Foster G, Dick K, et al. 1996. *Vet Rec* 139:423–424.
Madsen L, Svensmark B, Elvestad K, et al. 2002. *J Comp Pathol* 126:57:65.
Mancini F, Adamo F, Creti R, et al. 2017. *J Infect Chemother* 22:774–776.
Mao Y, Fan H, Lu C. 2008. *FEMS Microbiol Lett* 286:103–109.
Marie J, Morvan H, Berthelot-Herault F, et al. 2002. *J Antimicrob Chemother* 50:201–209.
Marois C, Bougeard S, Gottschalk M, et al. 2004. *J Clin Microbiol* 42:3169–3175.
Marois C, Le Devendec L, Gottschalk M, et al. 2007. *Can J Vet Res* 71:14–22.
Martinez G, Harel J, Gottschalk M. 2002. *Can J Vet Res* 66:240–248.
Matajira CE, Moreno LZ, Gomes VT, et al. 2017. *J Vet Diagn Invest* 29:245–249.
McLendon BR, Bron AJ, Mitchell CJ. 1978. *Br J Ophthalmol* 62:729–731.
Mills G. 1996. *Irish Vet J* 49:674–677.
Monter Flores J, Higgins R, D'Allaire S, et al. 1993. *Can Vet J* 34:170–171.
Moreno LZ, da Costa BL, Matajira CE, et al. 2016. *Diagn Microbiol Infect Dis* 86:178–180.
Muckle A, Giles J, Lund L, et al. 2010. *Can Vet J* 51:773–774.
Muckle A, López A, Gottschalk M, et al. 2014. *Can Vet J* 55:946–949.
Nguyen BH, Phan DH, Nguyen HX, et al. 2015. *J Infect Dev Ctries* 9:624–630.
O'Sullivan T, Friendship R, Blackwell T, et al. 2011. *Can J Vet Res* 75:106–111.
Okura M, Lachance C, Osaki M, et al. 2014. *J Clin Microbiol* 52:1714–1719.
Okura M, Osaki M, Nomoto R, et al. 2016. *Pathogens* 5(pii):E45 doi: https://doi.org/10.3390/pathogens5030045.
Okwumabua O, O'Connor M, Shull E. 2003. *FEMS Microbiol Lett* 218:79–84.
Okwumabua O, Peterson H, Hsu HM, et al. 2017. *J Vet Diagn Invest* 29:160–168.
Oliveira S, Batista L, Torremorell M, et al. 2001. *Can J Vet Res* 65:161–167.
Pallarés F, Halbur P, Schmitt C, et al. 2003. *Can J Vet Res* 67:225–228.
Perch B, Pedersen, K, Henrichsen J. 1983. *J Clin Microbiol* 17:993–996.
Pereira N, Powell AM, Nyirjesy P, et al. 2013. *J Low Genit Tract Dis* 17:e18–21.
Pérez-Sancho M, Vela AI, García-Seco T, et al. 2015. *Front Public Health* 3:202.
Plagemann O. 1988. *Zentralbl Veterinarmed B* 35:770–772.
Princivalli M, Palmieri C, Magi G, et al. 2009. *Euro Surveill* 14(33) pii:19310.
Qiu X, Bai X, Lan R, et al. 2016. *Appl Environ Microbiol* 82:7102–7112.
Real F, Ferrer O, Rodriguez J. 1992. *Vet Rec* 131:151–152.
Reams R, Glickman L, Harrington D, et al. 1994. *J Vet Diagn Invest* 6:326–334.
Reams R, Harrington D, Glickman L, et al 1995. *J Vet Diagn Invest* 7:406–408.
Reams R, Harrington D, Glickman L, et al. 1996. *J Vet Diagn Invest* 8:119–121.
Rehm T, Baums C, Strommenger B, et al. 2007. *J Med Microbiol* 56:102–109.
Risco D, Fernández-Llario P, Cuesta JM, et al. 2015. *J Zoo Wildl Med* 46:370–373.
Robertson I, Blackmore DK. 1989. *Epidemiol Infect* 103:157–164.
Robertson I, Blackmore D, Hampson D, et al. 1991. *Epidemiol Infect* 107:119–126.
Sánchez del Rey V, Fernández-Garayzábal JF, Briones V, et al. 2013. *Vet Microbiol* 165:483–486.
Sánchez del Rey V, Fernández-Garayzábal JF, Mentaberre G, et al. 2014. *Vet J* 200:464–467.
Sanford SE. 1987a. *Can J Vet Res* 51:481–485.
Sanford SE. 1987b. *Can J Vet Res* 51:486–489.
Sanford SE, Tilker A, 1982. *J Am Med Vet Assoc* 181:673–676.
Schmitt C, Halbur P, Roth J, et al. 2001. *Vet Microbiol* 78:29–37.
Segura M. 2015. *Expert Rev Vaccines* 14:1587–1608.
Segura M, Calzas C, Grenier D, et al. 2016. *FEBS Lett* 590:3772–3799.

Segura M, Fittipaldi N, Calzas C, et al. 2017. *Trends Microbiol* 25:585–599.
Seitz M, Valentin-Weigand P, Willenborg J. 2016. *Curr Top Microbiol Immunol* 398:103–121.
Shewmaker PL, Steigerwalt AG, Whitney AM, et al. 2012. *J Clin Microbiol* 50:3591–3597.
Sihvonen L, Kurl D, Henrichsen J. 1988. *Acta Vet Scand* 29:9–13.
Silva L, Baums C, Wisselink H, et al. 2006. *Vet. Microbiol* 115:117–127.
Smith H, Wisselink H, Stockhofe-Zurwieden N, et al. 1997. *Adv Exp Med Biol* 418:651–655.
Smith H, Damman M, van der Velde J, et al. 1999. *Infect Immun* 67:1750–1756.
Smith T, Capuano A, Boese B, et al. 2008. *Emerg Infect Dis* 14:1925–1927.
Soares TC, Gottschalk M, Lacouture S, et al. 2015. *Can J Vet Res* 79:279–284.
Staats J, Feder I, Okwumabua O, et al. 1997. *Vet Res Commun* 21:381–407.
Swildens B, Wisselink H, Engel B, et al. 2005. *Vet Microbiol* 109:223–228.
Swildens B, Nielen M, Wisselink H, et al. 2007. *Vet Rec* 160:619–621.
Takada K, Hirasawa M. 2007. *Int J Syst Evol Microbiol* 57:1272–1275.
Takada K, Igarashi M, Yamaguchi Y, et al. 2008. *Microbiol Immunol* 52:64–68.
Tang J, Wang C, Yang W, et al. 2006. *PLoS Med* 3:e151.
Tenenbaum T, Seitz M, Schroten H, et al. 2016. *Future Microbiol* 11:941–954.
Thanawongnuwech R, Brown G, Halbur P, et al. 2000. *Vet Pathol* 37:143–152.
Tian Y, Aarestrup F, Lu CP. 2004. *Vet Microbiol* 103:55–62.
Torremorrell M. Pijoan C, Trigo E. 1997. *Swine Health Prod* 5:139–143.
Vanier G, Segura M, Friedl P, et al. 2004. *Infect Immun* 72:1441–1449.
Varela NP, Gadbois P, Thibault C, et al. 2013. *Anim Health Res Rev* 14:68–77.
Vecht U, van Leengoed L, Verheijen E. 1985. *Vet Q* 7:315–321.
Vecht U, Wisselink H, Jellema M, et al. 1991. *Infect Immun* 59:3156–3162.
Vela A, Goyache J, Tarradas C, et al. 2003. *J Clin Microbiol* 41:2498–2502.
Vela A, Moreno M, Cebolla J, et al. 2005. *Vet Microbiol* 105:143–147.
Vela A, Casamayor A, Sánchez Del Rey V, et al. 2009. *Int J Syst Evol Microbiol.* 59:504–508.
Vela A, Perez M, Zamora L, et al. 2010. *Int J Syst Evol Microbiol* 60:104–108.
Vieira V, Teixeira L, Zahner V, et al. 1998. *Int J Syst Bacteriol* 48:1231–1243.
Vilaichone RK, Vilaichone W, Nunthapisud P, et al. 2002. *J Med Assoc Thai* 85(Supl 1):S109–S117.
Voutsadakis IA. 2006. *Clin Colorectal Cancer* 6:226–228.
Wallgren P, Nörregård E, Molander B, et al. 2016. *Acta Vet Scand* 58:71
Warneboldt F, Sander SJ, Beineke A, et al. 2016. *Pathogens* 5(3).
Wei Z, Li R, Zhang A, et al. 2009. *Vet Microbiol* 137:196–201.
Weinert LA, Chaudhuri RR, Wang J, et al. 2015. *Nat Commun* 6:6740.
Wertheim H, Nghia H, Taylor W, et al. 2009. *Clin Infect Dis* 48:617–625.
Wessman G. 1986. *Vet Microbiol* 12:297–328.
Windsor R. 1978. *Vet Annu* 18:134–143.
Wisselink H, Reek F, Vecht U, et al. 1999. *Vet Microbiol* 67:143–157.
Wisselink H, Vecht U, Stockhofe-Zurwieden N, et al. 2001. *Vet Rec* 148:473–477.
Wisselink H, Joosten J, Smith H. 2002. *J Clin Microbiol* 40:2922–2929.
Woods R, Ross, R.F. 1977. *Am J Vet Res* 38:33–36.
Yu H, Jing H, Chen Z, et al. 2006. *Emerg Infect Dis* 12:914–920.
Zhang C, Zhang Z, Song L, et al. 2015. *Biomed Res Int* 2015:284–303.
Zheng P, Zhao YX, Zhang AD, et al. 2009. *Vet Pathol* 46:531–535.
Zheng H, Ji S, Lan R, et al. 2014. *J Clin Microbiol* 52:3568–3572.
Zheng H, Qiu H, Roy D, et al. 2017. *Vet Res* 48:10 doi: https://doi.org/10.1186/s13567-017-0417-6
Zoric M, Sjölund M, Persson M, et al. 2004. *Infect Dis Vet Public Health* 51:278–284.
Zoric M, Nilsson E, Lundeheim N, et al. 2009. *Acta Vet Scand.* 23:51:23.

62

Swine Dysentery and Brachyspiral Colitis

David J. Hampson and Eric R. Burrough

Overview

This chapter reviews a group of diseases of swine resulting from infection with anaerobic intestinal spirochetes of the genus *Brachyspira*. These include swine dysentery (SD), a severe mucohemorrhagic colitis (dysentery) caused by infection with strongly hemolytic *Brachyspira* species, as well as milder forms of brachyspiral colitis caused by weakly hemolytic *Brachyspira* species. Among the latter is a specific disease called porcine intestinal spirochetosis or porcine colonic spirochetosis (PIS/PCS) resulting from infection with *Brachyspira pilosicoli*.

Summary of recent developments

Emergence of new species of *Brachyspira* and of atypical strains of *Brachyspira hyodysenteriae* has confounded diagnosis of SD and interpretation, particularly when *B. hyodysenteriae* is detected in herds without apparent SD. Besides *B. hyodysenteriae*, the classical agent of SD, two other strongly beta-hemolytic species (*Brachyspira hampsonii* and *Brachyspira suanatina)* are also agents of SD in some parts of the world. Additionally, isolates of *B. hyodysenteriae* are increasingly cultured from herds without apparent SD. Some of these isolates exhibit typical strong hemolysis on blood agar, while others are atypically weakly hemolytic. Lack of apparent SD in these herds suggests the existence of weakly virulent or avirulent strains of *B. hyodysenteriae*. Alternatively, herd-specific cofactors such as alteration of colonic microbiota by diet or other cofactors needed for expression of SD by virulent *B. hyodysenteriae* may be lacking. This means that a definitive diagnosis of SD requires the confirmation of a strongly beta-hemolytic *Brachyspira* spp. in the colonic mucosa or feces of pigs with typical dysentery and/or lesions. It also means that the detection of *B. hyodysenteriae* in herds without apparent SD does not definitively prove that these strains have the potential to cause SD in these herds. Encouragingly, an expanding array of molecular diagnostic techniques is becoming available to support disease diagnosis and investigation.

Of particular concern is the worldwide emergence of pathogenic *Brachyspira* species and strains with reduced susceptibility to commonly used antimicrobials. Efforts to identify alternatives to antimicrobials to help control these infections are ongoing.

Overview of *brachyspira* species

The genus *Brachyspira* contains nine officially recognized and several unofficial species. These gram-negative anaerobic bacteria are genetically distinct from other spirochetes and have adapted to occupy specialized niches in the large intestines of various birds and mammals. Seven *Brachyspira* species colonize swine: the three most common pathogenic species are *B. hyodysenteriae* and *B. hampsonii*, both agents of SD, and *B. pilosicoli*, the cause of PIS (or PCS). Of the other potentially pathogenic species, *B. suanatina* is an agent of SD that has only been reported in Scandinavia (Mushtaq et al. 2015), and some strains of *Brachyspira murdochii* and *Brachyspira intermedia* may occasionally cause colitis in swine. The final species colonizing swine is *Brachyspira innocens*, a nonpathogenic commensal. Relationships between the recognized species, based on their 16S rRNA gene sequences, are shown in Figure 62.1.

All the *Brachyspira* species resemble short slender snakes with several loose coils (Figure 62.2). Those that colonize swine are 5–11 μm in length and 0.2–0.4 μm in width. *Brachyspira* have two sets of periplasmic flagella that wind around the cell body beneath the outer envelope, with each set anchored at opposite ends of the spirochete cell. These flagella confer a corkscrew-like motility that helps them to penetrate and move through the viscous digesta and mucus overlying the colonic epithelium.

Brachyspira spp. DNA has a low guanine and cytosine ratio, from 24.6 to 28%. Genome sizes are from

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

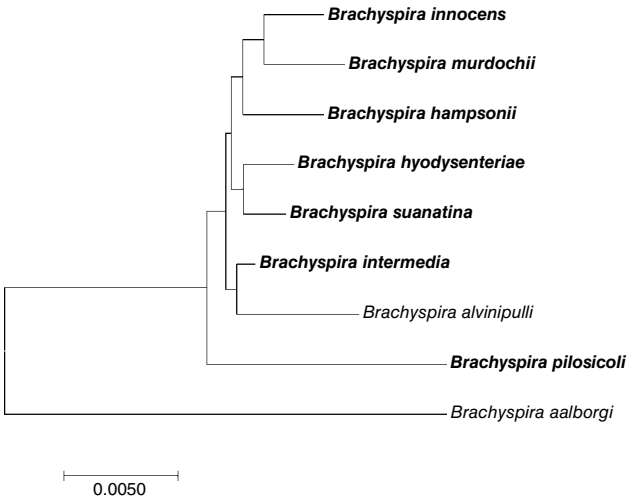


Figure 62.1 Relationships between the nine officially named *Brachyspira* species based on the 16S rRNA gene sequences of the type strains. The seven species that are known to colonize pigs are marked in bold. *Source:* Courtesy of Drs. David Hampson and Tom La.

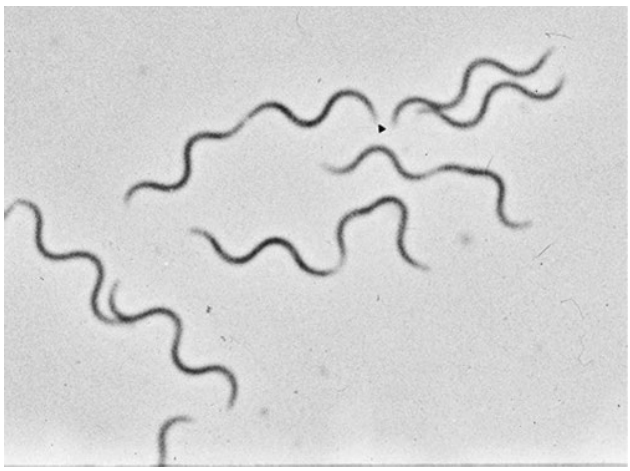


Figure 62.2 Cells of *Brachyspira hyodysenteriae* as viewed under a phase-contrast microscope.

approximately 2.5 to 3.2 million base pairs (Mbp), each with >2300 protein coding sequences. Most species share close similarities in their 16S rRNA gene sequences, implying that separation of species occurred relatively recently. *B. hyodysenteriae* strains contain a approximately 36 kb plasmid (Bellgard et al. 2009; Black et al. 2015), a 3.2 kb plasmid is present in *B. intermedia* strain PWS/A^T (Håfström et al. 2011), and a putative plasmid sequence and a bacteriophage sequence occur in *B. suanatina* strain AN4859/03 (Mushtaq et al. 2015). *B. hyodysenteriae* contains a prophage-like gene transfer agent (GTA) that randomly packages approximately 7.5 kb fragments of host DNA and transfers it to other *B. hyodysenteriae* strains (Humphrey et al. 1997; Matson et al. 2005). Other *Brachyspira* species harbor similar GTA sequences (Motro et al. 2009; Stanton et al. 2003), although it is not known whether they are functional. The GTAs could contribute to the extensive gene rearrangements that occur within and between *Brachyspira* species (Mappley et al. 2012; Zuerner et al. 2004).

The *Brachyspira* species are anaerobic but can tolerate brief oxygen exposure. They grow slowly *in vitro* and can easily be overgrown by other anaerobic members of the enteric microbiota unless selective isolation medium is used. Agar should be made selective with the addition of 400 µg/mL spectinomycin and 25 µg/mL each of colistin and vancomycin to make CVS medium (Jenkinson and Wingar 1981). The more selective BJ medium (Kunkle and Kinyon 1988) containing lower concentrations of the three antimicrobials, but with 25 µg/mL spiramycin and 12.5 µg/mL rifampin added, can also be used for agents of SD, but is not recommended for *B. pilosicoli* as it may inhibit growth. Typically *Brachyspira* form a low flat film of growth after 3–5 days at 98.6–108 °F (37–42 °C), without forming colonies. A zone of beta-hemolysis surrounds growth on plates containing 5% defibrinated ovine or bovine blood. Hemolysis is strong for *B. hyodysenteriae, B. hampsonii*, and *B. suanatina* and weak for the other *Brachyspira* species. Atypical weakly hemolytic isolates of *B. hyodysenteriae* do occur. Strongly hemolytic isolates typically display a characteristic enhanced hemolysis, or ring phenomenon, around defects or slits in the agar (Figure 62.3).

Useful features that may help to distinguish between *Brachyspira* species include their strength of beta-hemolysis, their ability to produce indole, and their enzymatic profile in the commercial API-ZYM kit (Fellström et al. 1997). Some comparative features of the porcine spirochetes are presented in Table 62.1. None can be completely relied upon for identification as strains with atypical phenotypes occasionally are found (Thomson

Figure 62.3 Blood agar plate showing zones of weak beta-hemolysis (left) and strong beta-hemolysis with ring phenomenon (right). *Source:* Courtesy of Joann Kinyon.

Table 62.1 Differentiation of seven *Brachyspira* species that colonize swine using strength of hemolysis on Trypticase soy blood agar, biochemical reactions, and utilization of sugars.

Species	Hemolysis	Indole	Hippurate	API-ZYM[a]
B. hyodysenteriae	Strong[b]	+[c]	–	1
B. hampsonii	Strong	–	–	3
B. suanatina	Strong	+[d]	–	5
B. intermedia	Weak	+	–	1
B. innocens	Weak	–	–	2
B. murdochii	Weak	–	–	3
B. pilosicoli	Weak	–[c]	+[e]	4

[a] Reactions in the commercial API-ZYM test:

1) Alpha-glucosidase positive; alpha-galactosidase negative.
2) Alpha-glucosidase positive or negative; alpha-galactosidase positive.
3) Alpha-glucosidase negative; alpha-galactosidase negative.
4) Variable reactions, including positive reactions for both enzymes; beta-glucosidase negative.
5) Alpha-galactosidase negative; beta-glucosidase positive.

[b] Weakly hemolytic strains of *B. hyodysenteriae* have been recorded.
[c] Indole-negative strains of *B. hyodysenteriae* and indole-positive strains of *B. pilosicoli* have been recorded.
[d] Weakly positive indole.
[e] Hippurate-negative strains of *B. pilosicoli* have been recorded.
+ positive reaction; – negative reaction.

et al. 2001). For example, atypical strains of *B. hyodysenteriae* have been described that are indole negative (Fellström et al. 1999), while others may be weakly hemolytic (La et al. 2016a; Mahu et al. 2016).

Swine dysentery

Relevance

SD was first recognized in the 1920s, but the spirochetal etiology was not determined until the early 1970s (Glock and Harris 1972; Taylor and Alexander 1971). The causal agent was originally named *Treponema hyodysenteriae* (Harris et al. 1972), but this species and the weakly beta-hemolytic *Treponema innocens* (Kinyon and Harris 1979) were later transferred to the genus *Serpula* and then to *Serpulina* (Stanton 1992) and are now classified in the genus *Brachyspira* (Ochiai et al. 1997). It is now known that *B. hyodysenteriae* is not the sole etiologic agent of SD. Strongly beta-hemolytic *B. hampsonii* and *B. suanatina* can also induce SD in susceptible pigs. SD is therefore redefined as a disease of pigs with characteristic mucohemorrhagic diarrhea (dysentery) and colitis from which any strongly beta-hemolytic *Brachyspira* spp. is recovered by culture of tissues or feces (Burrough 2017).

In infected herds SD causes considerable financial loss due to mortality, slow growth rates, poor feed conversion, and costs of treatment. Costs also arise from the need to implement preventative measures in herds that do not have SD and from the disruption to the supply and movement of pigs when the disease is introduced into seedstock herds. The disease can also represent a welfare issue unless it is controlled.

Etiology

Of the three known agents of SD, *B. hyodysenteriae* remains the most commonly recognized and reported species worldwide and is also the best characterized. *B. hyodysenteriae* is a discrete species within the genus *Brachyspira*, and analysis of the population structure using multilocus enzyme electrophoresis (MLEE), multilocus sequence typing (MLST), and multiple-locus variable number tandem repeat analysis (MLVA) has shown that the species is diverse, contains numerous genetically distinct strains, and is a nonrecombinant clonal species (Hidalgo et al. 2010a; La et al. 2009b; Lee et al. 1993a; Mirajkar and Gebhart 2014; Osorio et al. 2012; Råsbäck et al. 2007b; Rugna et al. 2015). A recent MLST investigation of *B. hampsonii* revealed a similar population structure (Mirajkar et al. 2015), with the species being divided into three "clades," now known as genomovars (Mirajkar et al. 2016). The existence of numerous distinct strains of *B. hyodysenteriae* can also be demonstrated using techniques such as pulsed-field gel electrophoresis (PFGE) (Atyeo et al. 1999a) and/or random amplified polymorphic DNA analysis (Hidalgo et al. 2010b). Before the advent of molecular methodology, *B. hyodysenteriae* isolates were differentiated into serogroups and serovars, defined on the basis of the antigenic properties of lipooligosaccharides (LOS) extracted from their cell envelopes (Hampson et al. 1997). It is now rare for serotyping to be undertaken, and commercial antisera are not available.

Molecular analysis of isolates has shown that new variants of *B. hyodysenteriae* may emerge on farms (Atyeo et al. 1999a; Hidalgo et al. 2010b). Other than random mutational and recombination events, GTAs may contribute to this strain "microevolution" through transduction of new sequences from other *Brachyspira* species or strains. Newly emerged strains could have altered phenotypic properties, including altered antimicrobial susceptibility, colonization potential, or virulence. Many virulence factors are identified for *B. hyodysenteriae*.

The hemolytic activity of *Brachyspira* spp. is likely an essential virulence factor. The strength of hemolysis has been suggested as a sensitive indicator of potential to induce SD in pigs (Burrough et al. 2012). Three genes (*tlyA*, *tlyB*, and *tlyC*) encoding putative hemolysins of *B. hyodysenteriae* were originally identified based on their ability to induce a hemolytic phenotype in *Escherichia coli* (ter Huurne et al. 1994). The *tly* genes may be regulatory elements rather than encoding hemolysins;

nevertheless, inactivation of *tlyA* has been shown to reduce both the hemolytic activity and the virulence of *B. hyodysenteriae* (Hyatt et al. 1994). Another gene (*hlyA*) encodes an 8.93 kDa acyl carrier protein with hemolytic activity (Hsu et al. 2001). Four other genes predicted to be involved in the hemolytic phenotype include those encoding hemolysin III, hemolysin activation protein, hemolysin III channel protein, and hemolysin (Black et al. 2015; La et al. 2016a). Atypical weakly hemolytic isolates of *B. hyodysenteriae* have been recovered from herds where SD did not appear to be clinically present, although strongly hemolytic isolates also have been recovered from other herds without disease (Hampson et al. 1992, 2016; La et al. 2016a; Lysons et al. 1982; Mahu et al. 2016; Thomson et al. 2001). These weakly hemolytic isolates may be of reduced virulence or be fully avirulent, but to date their potential to cause disease has not been fully explored under standard experimental conditions.

Brachyspira hyodysenteriae has many genes involved in motility and chemotaxis (Bellgard et al. 2009) and exhibits both chemotaxis and viscotaxis toward mucin (Milner and Sellwood 1994; Naresh and Hampson 2010). This affinity for mucin allows *B. hyodysenteriae* to associate with the gut mucosa (Kennedy et al. 1988). Disruptions introduced to *B. hyodysenteriae* flagellar genes (*flaA* and *flaB*) have resulted in reduced motility and colonization ability (Kennedy et al. 1997; Rosey et al. 1996). Colonization of the colonic mucosa may also be enhanced by the NADH oxidase activity of *B. hyodysenteriae* by protecting from oxygen toxicity. Consistent with this, strains with an inactivated NADH oxidase (*nox*) gene show a reduced ability to colonize swine and cause disease (Stanton et al. 1999).

The *B. hyodysenteriae* 36 kb plasmid has also been associated with virulence (La et al. 2011). Application of a DNA microarray to compare the gene content of strains reported to have differences in virulence identified a block of six genes on the plasmid that appeared to be correlated with virulence (La et al. 2014). The function of these genes is unclear, but they may enhance colonization capacity and hence increase the likelihood that a particular strain will colonize to levels where it can induce disease (La et al. 2014). In an analysis of 332 genes encoding predicted virulence factors in *B. hyodysenteriae* isolates from German herds, the only differences between isolates from herds without disease compared with those with disease were in the block of six plasmid genes and in genes encoding some outer membrane proteins, genes associated with iron metabolism, and those associated with hemolysis in the case of weakly hemolytic isolates (La et al. 2016a).

A number of outer membrane proteins and lipoproteins of *B. hyodysenteriae* have been described (Hampson et al. 2006). A 39 kDa variable surface protein (Bhp39) encoded by linked gene copies may be involved in immune avoidance due to differential gene expression (McCaman et al. 2003; Witchell et al. 2006). LOS, a semi-rough form of lipopolysaccharide found in the outer envelope of *B. hyodysenteriae* (Halter and Joens 1988), may produce localized toxic effects that disrupt the colonic epithelial barrier (Greer and Wannemuehler 1989; Nibbelink et al. 1997; Nuessen et al. 1983).

Compared with *B. hyodysenteriae*, much less is known about potential virulence determinants in *B. hampsonii* and *B. suanatina*, but future comparative analysis of the three species may reveal both common and novel virulence mechanisms.

Public health

None of the agents of SD are known to infect human beings.

Epidemiology

The incidence of SD varies in different countries and regions and changes with time. SD caused by *B. hyodysenteriae* remains a relatively common and important endemic problem in many countries. An overall decline in SD incidence occurred in the United States in the 1990s following establishment of new high health status herds in nontraditional swine-rearing states, and with introduction of larger units, multisite production and early weaning systems, but a recent reemergence of disease has occurred both in the United States and in Canada (Burrough 2017). Routine medication with carbadox may also have suppressed the disease, as the incidence has increased again in areas of the world where carbadox has been withdrawn from use (Hampson et al. 2015).

P280/1, the first recorded strain of what is now known as *B. hampsonii* (genomovar 3), was isolated in the 1980s from a pig in the United Kingdom (Atyeo et al. 1999b). Around 2007, *B. hampsonii* strains of genomovars 1 and 2 appeared and rapidly became widespread in pigs in the United States and Canada (Chander et al. 2012; Rubin et al. 2013b). More recently *B. hampsonii* genomovar 1 was isolated from pigs in Belgium and Germany (Mahu et al. 2014; Rohde et al. 2014), while a survey of pig herds in Australia failed to identify either *B. hampsonii* or *B. suanatina* (La et al. 2016b). To date *B. suanatina* has only been isolated in Sweden and Denmark and was first identified in the early 2000s (Råsbäck et al. 2007a).

Agents of SD naturally infect pigs (including feral pigs) and some species of birds (rheas, chickens, ducks, and geese). On infected farms, different agents of SD have been isolated from mice, rats, dogs, and waterfowl. Waterfowl appear to be a significant reservoir and potential means of introduction and spread for *B. hampsonii*, as this agent has been recovered from migratory waterfowl samples in North America and Europe

(Martínez-Lobo et al. 2013; Rubin et al. 2013a), and an avian-origin isolate of *B. hampsonii* induced SD when experimentally inoculated into pigs (Aller-Morán et al. 2016a). Similarly, *B. suanatina* has been recovered from waterfowl in Sweden and Denmark, and an avian-origin isolate induced clinical signs of SD following experimental inoculation in pigs (Råsbäck et al. 2007a).

On endemically infected swine farms, transmission mainly occurs by ingestion of feces containing spirochetes. This is especially likely in single-site, farrow-to-finish herds with continuous flow and poor biosecurity. Agents of SD may be spread by contact with clothing or footwear of animal caretakers that are contaminated with pig feces. Transmission between pens may occur in housing systems where there are open channels between pens. Lagoon water containing effluent can be a source of infection (Glock et al. 1975) and should not be recycled. Feral pigs, other animals including rats and mice, and waterfowl that access farms or their water supplies are potential reservoirs that may transmit infection (Joens and Kinyon 1982; Phillips et al. 2009).

New outbreaks of SD typically occur following introduction of asymptomatic carrier pigs that are not quarantined and/or treated prophylactically. Outbreaks also occur in herds following transmission via rodents or birds from adjacent infected farms, by introduction through contaminated feed or animal trucks, or by visitors who have had contact with infected pigs. When investigating risk factors for SD, Robertson et al. (1992) found that allowing visitors onto farms and the presence of rodents were both associated with disease. In contrast, heightened biosecurity, such as the provision of boots and protective clothing for visitors, the presence of security fencing, on-site feed mixing, and obtaining replacement breeders from a consistent source, were protective. *B. hyodysenteriae* is shed in the feces for variable periods of time, and recovered asymptomatic pigs may transmit infection to susceptible pigs for at least 70 days (Songer and Harris 1978).

Brachyspira hyodysenteriae is relatively resistant in moist feces. It survives in feces diluted in water for 48 days at temperatures from 32 to 50 °F (0–10 °C), for 7 days at 77 °F (25 °C), and less than 24 hours at 98.6 °F (37 °C) (Chia and Taylor 1978). In another study it survived for 10 days in soil at 50 °F (10 °C), for 78 days in soil in the presence of 10% pig feces, and for 112 days in pure pig feces (Boye et al. 2001). Drying of dysenteric feces rapidly kills *B. hyodysenteriae* (Chia and Taylor 1978). Phenolics and sodium hypochlorite are the most effective disinfectants.

Pathogenesis

The pathogenesis of SD is incompletely understood and has been recently reviewed (Burrough 2017). Individual animal susceptibility to development of SD varies and is, in part, due to differences in the colonic microbiota. Gnotobiotic pigs are variably colonized by *B. hyodysenteriae* but do not develop SD, while gnotobiotes inoculated with intestinal scrapings or content from pigs with dysentery readily develop disease (Meyer et al. 1974a,b). These findings suggest that infection with other microorganisms along with an agent of SD is required for disease expression. Anaerobes such as *Bacteroides vulgatus* and *Fusobacterium necrophorum* were shown to successfully fulfill this requirement (Harris et al. 1978).

Following ingestion, *B. hyodysenteriae* survives the acidic environment of the stomach and eventually reaches the large intestine where they colonize and produce disease. Experimentally, an inoculum of 10^5–10^{10} colony-forming units (cfu) may produce SD (Kinyon et al. 1977). Proliferation and colonic mucosal colonization are aided by the ability of *B. hyodysenteriae* to utilize available substrate, penetrate and move through viscous mucus down a chemotactic gradient into the crypts, and avoid potential oxygen toxicity at the surface of the colonic mucosa. Clinical signs and lesions start to develop as bacterial cell numbers reach $10^6/cm^2$ of mucosa (Hughes et al. 1977; Whipp et al. 1979). Spirochetes typically appear in the feces 1–4 days before onset of SD (Costa et al. 2014; Kinyon et al. 1977; Rubin et al. 2013b), concurrent with a shift in colonic bacteria from predominantly gram-positive to mainly gram-negative species (Robinson et al. 1984); however, preclinical shedding of up to 8 days has been reported (Wilberts et al. 2014a).

Brachyspira hyodysenteriae strains vary in their virulence (Achacha et al. 1996), but the basis is poorly understood. The presence of spirochetes close to epithelial cells in the lumen and crypts of the cecum and colon stimulates an outpouring of mucus (Wilcock and Olander 1979a,b). They attach to epithelial cells in the crypt, but the significance of this is unclear since attachment to animal cell cultures does not cause cellular damage or invasion (Bowden et al. 1989; Knoop et al. 1979).

The mechanisms of tissue destruction in SD are not fully understood. There is a loss of sulfated mucins in the colonic mucosa of pigs with acute SD relative to controls, and the mucus layer becomes disorganized and lacks the striations apparent in age-matched control pigs (Quintana-Hayashi et al. 2015; Wilberts et al. 2014a). Additionally, mucin production increases dramatically in pigs with SD due to the *de novo* production of mucin 5AC and increased production of mucin 2 (Quintana-Hayashi et al. 2015). Increased mucus affords increased binding sites for spirochetes, and alteration of the barrier structure may allow improved access to the underlying epithelium. The hemolysin(s) and LOS may then cause local damage, resulting in epithelial sloughing. Subsequent mucosal and submucosal invasion by secondary bacteria and the protozoan *Balantidium coli* may contribute to lesion formation.

Diarrhea appears to result from failure of epithelial transport of sodium and chloride ions from lumen to blood resulting in colonic malabsorption, and not from the activity of prostaglandins released from the inflamed mucosa or spirochetal enterotoxins, or from direct tissue damage induced by spirochetal cell components (Argenzio et al. 1980; Schmall et al. 1983; Whipp et al. 1978). Occasional peracute deaths may arise from endotoxin release.

Under experimental conditions diet has been shown to have a major influence on SD expression. Colonization by *B. hyodysenteriae* can be inhibited by feeding diets that are either highly digestible (Pluske et al. 1996) or rich in inulin (Hansen et al. 2010, 2011; Thomsen et al. 2007), while disease expression is exacerbated in pigs fed diets with increased insoluble fiber (Wilberts et al. 2014b). Diet-related protective mechanisms likely include causing changes in the physicochemical environment of the large intestine, changes in the colonic microbiota with increases in species that directly inhibit spirochetes (Klose et al. 2010; Leser et al. 2000; Mølbak et al. 2007), or a reduction in other anaerobic bacteria that facilitate *B. hyodysenteriae* colonization and augment inflammation and lesion formation (Joens et al. 1981; Whipp et al. 1979).

Clinical signs

SD occurs mainly in grower and finisher pigs and less frequently in weaner pigs. It may begin a few weeks after animals are moved from the nursery, coinciding with a dietary change and removal of antimicrobials used to control respiratory and enteric diseases. Older suckling piglets from gilts that have not been exposed to *B. hyodysenteriae* and piglets in newly infected herds are occasionally affected.

The first evidence of SD is usually soft, yellow to gray feces. Partial anorexia and increased rectal temperature of 104–105 °F (40–40.5 °C) may occur; however, a febrile response is not a consistent feature or predictive of spirochete shedding (Jacobson et al. 2007). A few hours to days after onset of clinical disease, large amounts of mucus and often flecks of blood are found in the feces. This progresses to watery feces containing blood, mucus, and shreds of white mucofibrinous exudate (dysentery) with concurrent staining of the perineum. Most pigs recover over several weeks, but their growth rate remains depressed. Prolonged diarrhea leads to dehydration, and the animals become weak and emaciated.

The incubation period for SD is variable, ranging from 4 days to 3 months, but disease usually occurs within 10–14 days in naturally exposed pigs. Disease usually spreads gradually, with new animals becoming affected daily. The course varies between individual animals. Occasional pigs are peracutely affected and die within a few hours to days.

In outbreaks of SD, morbidity in weaner pigs may approach 90%, and mortality may be 30% if effective treatment is delayed. Experimentally, mortality in untreated pigs may exceed 50% (Kinyon et al. 1977). In chronically affected herds, particularly if they are being medicated, the disease may not be clinically evident.

In experimentally induced SD, the occurrence and severity of disease is dependent upon the degree of stress on the pig, the quantity of infectious inoculum, the growth phase of the culture (with active log-phase inoculum being most infectious), the diet, the group size, and pig weight (Jacobson et al. 2004).

On endemically infected swine farms, clinical signs often recur cyclically at 3- to 4-week intervals in individual pigs and in large groups. Reappearance may occur after removal of antimicrobials from the water or feed or from various stressors, including moving to new pens, mixing with different animals, weighing, a change in feed, overcrowding, and/or extreme changes in ambient temperature.

Lesions

Lesions are limited to all or part of the cecum through the descending colon with spiral colon being most consistently affected in acute SD (Wilberts et al. 2014a). Distribution of lesions tends to be more diffuse in the later stages of the disease.

Typical lesions in acute SD include hyperemia and edema of the large intestinal wall, mesentery, and mesenteric lymph nodes. The large intestinal mucosa is usually edematous with loss of the typical rugose appearance and is covered by mucus, fibrin, and flecks of blood (Figure 62.4). Contents are soft to watery and contain exudate.

As disease progresses, large intestinal mural edema decreases and mucosal lesions become more severe. Exudation of fibrin increases, forming thick pseudomembranes composed of fibrin, mucus, and blood. As lesions become chronic, mucosal exudates contain less mucus and blood and are typically composed of a thin layer of fibrinonecrotic exudate resembling lesions of enteric salmonellosis (see Chapter 59). Lesions can also be observed in the colons of clinically healthy pigs as segmental reddened areas of the mucosa usually covered with mucus, but with normal contents.

Microscopic lesions are limited to the cecum, colon, and rectum. In the acute phase of disease, spirochetes are most numerous in the lumen and within superficial crypts (Figure 62.5), but may also be observed attached to luminal epithelium within disrupted epithelial and goblet cells, and in the superficial lamina propria, often in higher numbers surrounding blood vessels. The mucosa and submucosa are thickened due to vascular congestion, serofibrinous effusion, and infiltrating

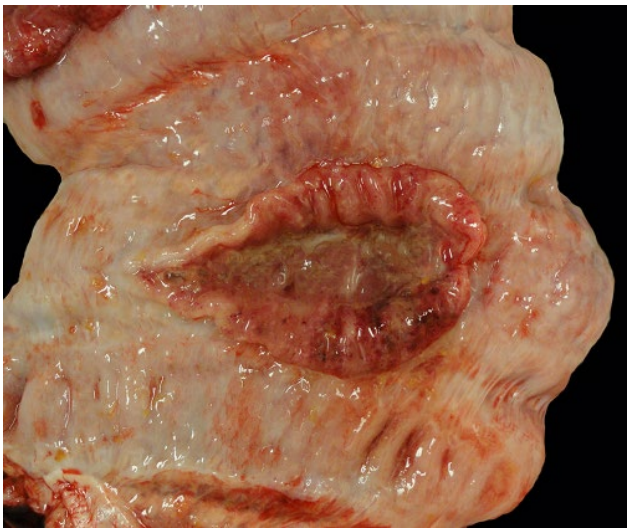


Figure 62.4 Spiral colon from a pig 10 days after intragastric inoculation with *Brachyspira hampsonii*. There is serosal hyperemia and mesocolonic edema. A segment has been opened to reveal mucosal thickening, hemorrhage, and watery contents containing mucus, fibrin, and blood. *Source:* Courtesy of Dr. Eric Burrough.

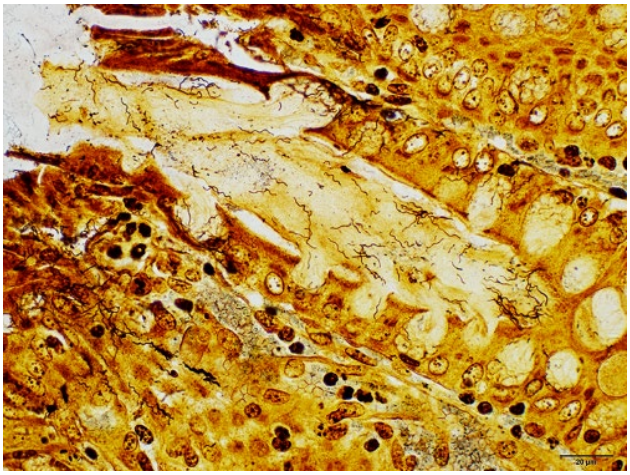


Figure 62.5 *Brachyspira hyodysenteriae* within a colonic crypt and adjacent epithelium of a pig with swine dysentery (Warthin–Starry; bar = 20 µm). *Source:* Courtesy of Dr. Eric Burrough.

leukocytes, predominantly neutrophils. Erosion of luminal epithelium results from loss of cohesion, necrosis, and shedding of enterocytes. Superficial lamina propria subjacent to erosions may have neutrophilic cuffing of blood vessels, hemorrhages, and invasion by luminal bacteria and protozoa. Expulsion of mucus from goblet cells fills colonic crypts 3 days after onset of clinical disease, and hyperplasia of goblet cells occurs after 5 days (Jacobson et al. 2007).

Later changes include accumulation of fibrin, mucus, and cellular debris in mucosal crypts and on the luminal surface of the large intestine. Superficial necrosis of the mucosa may be extensive, but deep ulceration is not typical. Numbers of neutrophils, lymphocytes, and plasma cells increase in the lamina propria. Chronic mucosal lesions usually include less hyperemia and edema, more extensive superficial necrosis, and extensive superficial mucosal fibrinonecrotic exudate.

Diagnosis

A presumptive diagnosis of SD can be made by the demonstration of large numbers of typical serpentine spirochetes using nonspecific stains or dark-field microscopy in fecal or mucosal smears or in tissue sections from pigs with typical dysentery and/or lesions. A definitive diagnosis of SD requires the demonstration of a strongly beta-hemolytic *Brachyspira* spp. in the colonic mucosa or feces of pigs with typical dysentery and/or lesions (Burrough 2017). Traditionally confirmation of strongly beta-hemolytic *Brachyspira* spp. has been done by selective anaerobic culture and analysis of phenotypic properties of the isolated organisms. In the United Kingdom an indirect fluorescent antibody test has been used to detect *B. hyodysenteriae* in smears (Hunter and Saunders 1977), although false-positive reactions can occur. Monoclonal antibodies (MAbs) should improve specificity (Lee and Hampson 1996), but when one was bound to magnetic beads to extract *B. hyodysenteriae* from feces, this did not increase the detection sensitivity (Corona-Barrera et al. 2004b). *B. hyodysenteriae* and *B. hampsonii* can be rapidly identified and localized within lesions in histologic sections or detected in feces or mucosal smears using oligonucleotide probes for fluorescent *in situ* hybridization (FISH) (Boye et al. 1998; Burrough et al. 2013; Wilberts et al. 2015). PCR can also be used for rapid direct detection in feces or mucosal smears. However, culture remains a more sensitive assay and is preferred since it allows for assessment of hemolysis.

Samples for testing are best collected from several acutely affected untreated animals, as they have large numbers (10^8–10^9/g) of *Brachyspira* in their colonic mucosa and feces. Colon and feces are ideal samples. Oral fluids are a complementary sample type allowing pen-level detection of agents of SD, particularly when pigs have active disease (Warneke et al. 2013). Samples should be kept moist and refrigerated during transit. Asymptomatic pigs are not optimal as they may only periodically shed the organism at detectable levels (>10^3 cells/mL contents) in their feces. However, *B. hyodysenteriae* has been identified in pigs from apparently healthy herds (Hampson et al. 2016; La et al. 2016b) if sufficient numbers of samples are tested. If disease is mild or subclinical, large numbers of samples should be tested. Pooling batches of five rectal swabs for testing increases the rate of detection, since the slight reduction in sensitivity by dilution is more than offset by the advantage of testing five times the number of animals (Fellström et al. 2001).

Optimal culture media and conditions are as described earlier. On blood agar, agents of SD produce zones of strong beta-hemolysis around a film of growth in which colonies are hard to distinguish. Spirochetes can be observed in resuspended samples using phase-contrast or dark-field microscopy. Plates without hemolysis should be re-incubated and regularly checked for up to 10 days. False-negative results can occur due to inappropriate handling or storage of samples, such as exposure to extreme temperatures, drying, or transport delays. Mixed spirochete species can be present on a plate, so individual isolates must be cloned to purity before phenotypic characterization (Table 62.1) or other means of speciation.

PCR amplification of specific sequences is widely used for speciation. The most common targets used for identifying *B. hyodysenteriae* are portions of the *nox* gene (Atyeo et al. 1999b), the 23S rRNA gene (Leser et al. 1997), or the *tlyA* gene (Fellström et al. 2001). Recently, however, it has been reported that some avian strains of *B. hampsonii* may cross-react in these *B. hyodysenteriae* PCRs and PCRs for other *Brachyspira* species (Aller-Morán et al. 2016b). *B. hampsonii* is usually identified using a *nox*-based PCR (Rubin et al. 2013b), while PCRs based on the *tlyA* and *rpoC* genes were recently described for *B. hampsonii* and *B. suanatina*, respectively (La et al. 2016b). Modifications to the basic PCRs have included a commonly used duplex reaction for identifying *B. hyodysenteriae* and *B. pilosicoli* (La et al. 2003), several multiplexed real-time PCRs for *Brachyspira* species (Song and Hampson 2009; Willems and Reiner 2010), and recently a high resolution melting assay for *B. hampsonii* (Scherrer et al. 2016). Other PCR-based methodology that allows identification of a range of *Brachyspira* species involves amplification of portions of specific genes (usually *nox* or 16S rRNA), followed either by sequencing and phylogenetic analysis of the products against know sequences (Atyeo et al. 1999b) or by restriction enzyme digestion of the products to give species-specific banding patterns after gel electrophoresis (RFLP analysis). Targets for the latter include the genes for 16S rRNA (Stanton et al. 1997), 23S rRNA (Barcellos et al. 2000), and the *nox* gene (Rohde et al. 2002; Rohde and Habighorst-Blome 2012).

PCR is usually conducted on growth from the primary isolation plate, although various PCR tests have been described for direct detection of pathogenic spirochetes in clinical samples. Isolation followed by PCR can take 3–5 days, but this method has the advantage that the isolates are available for phenotypic evaluation, antimicrobial sensitivity testing, and/or strain typing.

As an alternative to PCR, isolates in primary culture can be subjected to MALDI-TOF MS, another rapid methodology for *Brachyspira* speciation that can discriminate all known agents of SD (Calderaro et al. 2013; Warneke et al. 2014).

Although serologic tests have been developed for identifying herds with SD (La and Hampson 2001), very few laboratories use them. Generally, these tests have not been based on species-specific antigens, and so they have had low specificity and/or sensitivity. An enzyme-linked immunosorbent assay (ELISA) using LOS as plate-coating antigen has proved helpful for identifying infected herds, but not for detecting individual pigs with SD (Joens et al. 1982). LOS-based ELISA systems require knowledge of the serotypes of organisms present in the herds to be tested so that the appropriate LOS can be used as plate-coating antigen. Serological ELISAs using recombinant *B. hyodysenteriae* surface proteins Bhlp29.7 and H114 as plate-coating antigen have been described (Hampson et al. 2016; La et al. 2009a; Song et al. 2015). The ELISA using H114 correctly detected apparently healthy herds that subsequently were confirmed to contain pigs colonized with *B. hyodysenteriae* (Hampson et al. 2016). Besides serum, ELISAs run on meat juices collected at slaughter may be useful for detecting herds infected with *B. hyodysenteriae* (Song et al. 2012).

The range of diagnostic tests available for *Brachyspira* detection has expanded considerably in recent years. Newer molecular assays that improve the specificity of an etiologic diagnosis lack the sensitivity of selective anaerobic culture. Diagnostic approaches for SD should be sufficiently broad to detect all potentially pathogenic spirochetes and also have sufficient sensitivity to detect small numbers of spirochetes typically shed from asymptomatic carriers. Selective anaerobic culture, which has the capacity to detect all known agents of SD and provides a hemolytic phenotype, should continue to be an integral part of *Brachyspira* detection, diagnosis, and surveillance for SD.

A number of enteric diseases may be confused with SD, and SD often occurs concurrently with other enteric infections (Møller et al. 1998). Proliferative enteropathy (PE) caused by *Lawsonia intracellularis* (Chapter 58) may clinically resemble SD, but SD does not affect the small intestine. Salmonellosis (Chapter 59) can have similar clinical signs and lesions; however, with salmonellosis, there may be hemorrhage or necrosis in parenchymatous organs and lymph nodes and mucosal lesions in the small intestine. Deep ulcerative enteric lesions also are much more typical of salmonellosis. Trichuriasis (Chapter 67) may usually be differentiated from SD on the basis of the presence of numerous *Trichuris suis* in the large intestine; however, prepatent trichuriasis can mimic SD and requires microscopic examination of the large intestine and/or culture to differentiate. Gastric ulcers and other hemorrhagic conditions may result in blood in the feces, but this tends to be "tarry" due to digestion of the blood, and the mucosa of the large intestines lacks lesions. PIS/PCS represents the most difficult differential diagnosis, as it can closely resemble mild cases of SD.

Immunity

Changes occur in antibody levels and in cell-mediated reactivity in pigs with SD, but their importance is unclear. Serum IgG levels to *B. hyodysenteriae* correlate with duration of clinical signs, while IgA levels in the colon reflect recent exposure (Rees et al. 1989b). Neither is strongly correlated with protection from SD (Joens et al. 1982; Rees et al. 1989b).

Pigs recovered from SD may be protected against subsequent challenge with *B. hyodysenteriae* for up to 17 weeks (Joens et al. 1979; Olson 1974), although some animals (7–43%) remain susceptible (Jenkins 1978; Joens et al. 1979; Rees et al. 1989a) and approximately 10% may only become protected after two bouts of disease (Rees et al. 1989a). Immunity is partially serotype specific and directed against LOS antigens (Joens et al. 1983).

Complement components in conjunction with immune serum may be involved in clearance of *B. hyodysenteriae* from the colon (Joens et al. 1985). Cell-mediated immunity also may be involved in protection as inhibition of peripheral blood leukocyte migration, a delayed hypersensitivity response, and a T-cell proliferative response to *B. hyodysenteriae* antigens are found in convalescent pigs (Jenkins et al. 1982). CD8αα cell proliferation has been recorded in pigs recovered from SD (Waters et al. 2000a). By comparing circulating leukocytes and lymphocyte subpopulations in pigs, Jonasson et al. (2004) deduced that γδ T cells and CD8+ cells may be associated with susceptibility to infection, while monocytes and CD4+ CD8+ T cells appear to be the major responding lymphocytes. Increased numbers of neutrophils, γδ T cells, and specific antibodies have been found during the recovery phase (Jonasson et al. 2006).

Following experimental infection or vaccination with a bacterin vaccine, there is some protection against serotypes of *B. hyodysenteriae* other than those to which the animals have been exposed (Kennedy et al. 1992; Parizek et al. 1985). It is not known whether infection with one *Brachyspira* species confers any protection against infection with another species.

Prevention and control

Methods to treat pigs or control outbreaks

Only a few effective antimicrobials remain available for treatment of SD, and resistance to important drugs such as the pleuromutilins is increasing. These drugs should only be used for specific therapy of clinical disease and for eradication programs. It is important to use either agar or broth dilution methods to determine the minimum inhibitory concentration (MIC) of the antimicrobials. For comparative studies, broth dilution testing may be conducted using VetMIC Brachy panels (National Veterinary Institute, Uppsala, Sweden), incorporating the *B. hyodysenteriae* type strain B78^T as a control (Karlsson et al. 2003).

Severely affected animals may require intramuscular antimicrobials for at least 3 days; however, in most cases water medication for 5–7 days is effective. If this is not possible, then in-feed medication for 7–10 days can be used, although affected animals may have a low feed intake. Pigs should have free access to drinking water. Oral glucose–electrolyte solutions can be given to severely affected pigs. Treatment of acute SD may be followed by in-feed medication at reduced levels for 2–4 weeks to prevent reinfection.

The drugs most commonly used to treat SD are the pleuromutilins tiamulin and valnemulin, as well as tylosin and lincomycin. Their dosage rates and potential side effects are listed in Table 62.2. Based on pharmacokinetic properties and *in vitro* susceptibility data, pleuromutilins appear to be the most suitable antimicrobials. In pigs experimentally infected with susceptible isolates of either *B. hyodysenteriae* or *B. hampsonii*, water medication with tiamulin resolved clinical disease within 24 hours and eliminated viable spirochete shedding within 72 hours (Wilberts et al. 2014c). Unfortunately, decreased susceptibility to tiamulin among *B. hyodysenteriae* isolates has been reported in several countries, and a recent investigation of Italian isolates of *B. hyodysenteriae* suggests transnational spread of resistant strains throughout Europe (Rugna et al. 2015). Tiamulin usage selects for clones of *B. hyodysenteriae* with decreased tiamulin susceptibility (Karlsson et al. 2004). To reduce the risk of emerging resistance to pleuromutilins, other drugs should be used if MIC determinations or herd records indicate their efficacy. Resistance to both tylosin and lincomycin frequently occurs in *Brachyspira* species (Hommez et al. 1998; Karlsson et al. 2003). Macrolide and lincosamide resistance may be caused by point mutations in the 23S rRNA gene and/or ribosomal protein genes, with tylosin resistance developing within 2 weeks *in vitro* (Hillen et al. 2014; Karlsson et al. 1999). Multidrug-resistant strains of *B. hyodysenteriae* are also increasing (Duinhof et al. 2008; Šperling et al. 2011).

Tylvalosin is a modification of an older drug (acetylisovaleryltylosin) that may also be useful for treatment and control of SD when used through the water and followed by in-feed medication. Other antimicrobials including bacitracin, spiramycin, gentamicin, dimetridazole, ronidazole, virginiamycin, olaquindox, and carbadox have been used for treatment and prevention of SD. Unfortunately, resistance to several of these has been reported, and the availability of others is now greatly reduced internationally. Many of these drugs have low MICs against *B. hyodysenteriae*, but their pharmacokinetic properties result in low concentrations in the gastrointestinal tract that makes them only suitable as a prophylactic (de Graaf et al. 1988). Both carbadox and

Table 62.2 Dosage level, duration of administration, and side effects for four drugs commonly used for the treatment of swine dysentery.

Drug	Dosage and duration	Side effects
Tiamulin	10 mg/kg bw; im for 1–3 days 8 mg/kg bw; po for 5–7 days in drinking water In-feed medication with 100–180 ppm for 10–14 days, followed by 30–40 ppm for 2–4 weeks	Rare: erythema. Local reactions at the injection site. Lethal side effects may occur in combination with ionophores
Valnemulin	In-feed medication 3–4 mg/kg bw for 1–4 weeks	Adverse effects including lethargy, depression, erythema, edema, pyrexia, ataxia, anorexia, and deaths have been reported. Lethal side effects may occur in combination with ionophores
Tylosin	10 mg/kg bw; im twice daily for 3 days followed by appropriate in-feed or water medication 5–10 mg/kg bw; po in drinking water for 5–7 days Followed by in-feed medication with 100 g/ton feed for 3 weeks, followed by in-feed medication 40 g/ton feed	Diarrhea, pruritus, erythema, rectal edema, and prolapse have been reported
Lincomycin	8 mg/kg bw; po in drinking water for at least 5 days but not to exceed 10 days In-feed medication 100 g/ton feed for 3 weeks or until signs of disease disappear, followed by 40 g/ton	Rare

Note: The information in this table is an abbreviated summary of product labeling. For information regarding withdrawal times (which vary extensively between countries), review national regulations and product labels.
bw, body weight; im, intramuscular; po, per os (oral).

metronidazole may induce expression of the *B. hyodysenteriae* GTA, which in turn may increase transfer of resistance genes between strains (Stanton et al. 2008). Ionophore growth promoters such as salinomycin and monensin may prevent losses; however, toxicity may occur if they are used with pleuromutilins or drugs that interfere with hepatic metabolism. Various other additives and ingredients have been examined for *in vitro* efficacy against *Brachyspira* spp. (Kutasi et al. 2016: Vande Maele et al. 2016), but few *in vivo* studies have been reported.

All-in/all-out management with cleaning and disinfection between batches reduces the risk of reinfection of medicated pigs and limits spread of infection. Ideally affected batches of pigs should be moved to clean buildings after medication to break the infection cycle. Careful disposal of infected bedding, the use of boot scrubbers and disinfectant footbaths, cleaning and disinfection of equipment used in infected areas, and changing of protective clothing are all vital measures. As outbreaks of SD are often associated with stressful conditions such as pig handling, crowding, transportation, severe weather conditions, or dietary changes, it is important to minimize these as much as possible in herds at risk. Attention should also be paid to the form and composition of the diet as SD expression is heavily influenced by dietary factors.

Mice and rats can serve as potential reservoirs of agents of SD in pig herds, so implementation of effective rodent control is essential. Unfortunately, it is virtually impossible to prevent mechanical transmission of infectious material via birds, waterfowl, and other potential wildlife vectors in outdoor facilities.

Bacterin vaccines for *B. hyodysenteriae* are available commercially in some countries and may provide a degree of protection (Diego et al. 1995). Unfortunately, they tend to be LOS serogroup specific, which then requires the use of autogenous or multivalent preparations. They are relatively difficult and costly to produce on a large scale because of the fastidious growth requirements of *B. hyodysenteriae*. One publication reported that immunization with a *B. hyodysenteriae* bacterin actually exacerbated dysentery (Olson et al. 1994). A proteinase-digested bacterin may offer better protection than conventional bacterins (Waters et al. 2000b).

Apparently naturally avirulent or low virulence strains of *B. hyodysenteriae* have been used experimentally as vaccines (Hudson et al. 1976), sometimes in combination with bacterins (Lysons et al. 1986). Modified live strains have been produced by inducing mutations in genes affecting motility (Rosey et al. 1996), hemolysis (Hyatt et al. 1994), and protection from oxygen toxicity (Stanton et al. 1999); however, such strains may have a reduced ability to colonize pigs, and they have produced only limited protection.

Although the use of a recombinant *B. hyodysenteriae* 38 kDa flagellar protein as a vaccine failed to prevent colonization in experimentally infected pigs (Gabe et al. 1995), recombinant Bhlp29.7 outer membrane lipoprotein did provide a 50% reduction in incidence of disease (La et al. 2004). A combination of four other recombinant

surface proteins identified using a "reverse vaccinology" strategy gave similar levels of protection under experimental conditions (Song et al. 2009), demonstrating the general validity of this approach. The emergence of *B. hampsonii* and *B. suanatina* further complicates the vaccination issue for SD as commercial vaccines would likely need to protect against at least two of these agents depending on the geographic market.

Methods to prevent introduction or avoid outbreaks of SD

Closed herds or those in closed pyramids should remain free of SD if they are geographically isolated and use precautions to prevent contamination. Introduction of new stock is the greatest risk, so a reliable history of the source herd is essential. Purchased animals should be quarantined for at least 3 weeks and treated to eliminate agents of SD. Infectious materials also may be carried into a herd by fomites such as workers' boots, farm implements, feed or animal trucks, and wild birds or animals; thus measures must be implemented to prevent these.

Methods to eliminate agents of SD from herds

The cost for eliminating SD may be recouped in 6–12 months through improved production and reduced drug usage (Windsor and Simmons 1981; Wood and Lysons 1988). Depending on the herd structure, production system, and economic considerations, SD eradication can be accomplished in several ways. These include intensive medication of all pigs for a short period, use of medicated early weaning and multisite production, or an ongoing program of emptying and disinfection of each herd unit in a cycle and introducing medicated animals to cleaned and disinfected units. Considerable effort is needed to plan and organize the eradication and to achieve the full understanding and cooperation of all personnel involved. Generally, eradication becomes more difficult as herd size increases and as the company operation becomes larger and more complex. Wood and Lysons (1988) suggested that the chances of an eradication program succeeding were around 80–90% in carefully selected herds. A combination of total depopulation, cleanup, disinfection, and repopulation with SD-free stock is sometimes the only method available to eliminate *B. hyodysenteriae*; however, it is generally the most expensive method and should only be considered if financially feasible (Polson et al. 1992; Wood and Lysons 1988). Projected cost and probability of success should influence the choice of method.

Attempts to eliminate SD without total depopulation should only be undertaken if an effective antimicrobial agent against agents of SD isolated from the unit is available. It is also important to establish a source of replacement breeding stock that is free from SD or to isolate and medicate all replacement stock before allowing them to enter the herd.

General guidelines for an SD eradication program include the following. Diagnosis should be confirmed by laboratory testing, and several strongly beta-hemolytic isolates should be tested for their MIC to available antibiotics. Isolates also should be typed by methods such as *nox* gene sequencing to determine if more than one strain is present. Herds with a continuous production system should try to change to batch production before the eradication. The program should be performed during a warm season when environmental survival of agents of SD is diminished. The number of animals in the herd should be reduced, and ideally all weaner, grower, and finisher pigs should be removed. Stock replacement should cease during the program. A rodent and insect control program should be implemented, and measures taken to stop wild birds from entering buildings and to protect water supplies from contamination with waterfowl feces. Dogs and cats should not be allowed within the herd area. Environmental contamination of all areas where pigs are housed, as well as the watering and feeding equipment, should be removed by high-pressure washing with hot water and disinfection. A disinfection program using glutaraldehyde and calcium hydroxide has been described and was effective in eliminating *B. hampsonii* from a commercial farrow-to-wean system (Burrough and Sexton 2013). Slats should be lifted, and slurry tanks emptied, cleaned, and disinfected. In outdoor production units, shelters should be cleaned, disinfected, and relocated to fresh sites. Remaining organic material should be burned, and the ground plowed and reseeded or left empty for several months. Sows, gilts, and boars should be medicated via drinking water or feed for at least 14 days and then moved into cleaned and disinfected buildings that have been empty for at least 2 weeks. Piglets born during the medication period should be weaned, treated parenterally with the same antimicrobial, and finished off-site. Piglets born after the sows have finished their medication can be weaned and reared on-site.

Brachyspiral colitis

Relevance

Brachyspiral colitis is an umbrella term used to describe diarrhea and colitis in pigs infected with one or more pathogenic *Brachyspira* spp. and reflects an increased understanding of the diversity of species within this genus and the role that different species and strains may play in inducing inflammation in the large intestine (Hampson 2014). This term is most applicable when discussing weakly beta-hemolytic *Brachyspira* spp., and

practitioners should approach these agents with the understanding that pathogenicity is often dependent upon a combination of host, microbial, and dietary factors.

The best characterized disease in pigs associated with weakly beta-hemolytic *Brachyspira* spp. is PIS/PCS. Taylor et al. (1980) first described PIS/PCS in a study in which pigs that were orally challenged with the weakly beta-hemolytic intestinal spirochete strain P43/6/78^{T} developed mucoid diarrhea containing flecks of blood and had lesions of colitis. This strain is now the type strain of the species *B. pilosicoli* and has previously been referred to as *Anguillina coli* (Lee et al. 1993b); *Serpulina coli* (Duhamel et al. 1993), a group IV weakly hemolytic intestinal spirochete (Fellström and Gunnarsson 1995); and *Serpulina pilosicoli* (Trott et al. 1996a). Pigs with PIS/PCS exhibit variable loss of condition, which leads to increased time to reach market weight and disrupts efficient production flow (Duhamel 1998).

Occasionally other weakly hemolytic *Brachyspira* species have been implicated in causing colitis and chronic diarrhea in pigs. Some strains of *B. intermedia* have been suspected to have pathogenic potential in pigs: this is not altogether surprising given that *B. intermedia* is a recognized enteric pathogen in adult poultry (Hampson and McLaren 1999). *B. murdochii* is the species most frequently reported to be associated with lesions of mild colitis in pigs (Komarek et al. 2009; Weissenböck et al. 2005), and experimental infection of pigs with a *B. murdochii* strain confirmed that it was mildly pathogenic (Jensen et al. 2010). Infections or colonization with one or more of the nonpathogenic or mildly pathogenic *Brachyspira* species is quite common in domestic pigs (Osorio et al. 2013): arguably their greatest significance is that these spirochetes may complicate diagnosis of SD and/or PIS/PCS – both of which are much more economically important diseases and require implementation of rigorous control measures. Accordingly, the remainder of this section is dedicated to PIS/PCS.

Etiology of PIS/PCS

Studies using MLEE, MLST, and MLVA have demonstrated that *B. pilosicoli* forms a distinct species, that strains show extensive diversity, and that the population is recombinant (Lee and Hampson 1994; Neo et al. 2013a,b; Trott et al. 1998). PFGE also has been commonly used for typing strains of *B. pilosicoli* (Atyeo et al. 1996; Fossi et al. 2003). Extensive genomic rearrangements between strains have been observed, and strains vary in the size of their genomes (Mappley et al. 2012; Zuerner et al. 2004).

The *B. pilosicoli* strain 95/1000 has a approximately 2.59 Mbp circular chromosome, genes for a GTA, and an integrated prophage, but does not possess plasmids (Wanchanthuek et al. 2010). The *B. pilosicoli* outer envelope contains LOS, and this is serologically heterogeneous among different strains (Lee and Hampson 1999). *B. pilosicoli* lacks the *rfbBADC* gene cluster found on the *B. hyodysenteriae* plasmid, and hence the species are predicted to have a different LOS structure. A number of outer membrane proteins and lipoproteins of *B. pilosicoli* have been described (Trott et al. 2001). More work is needed to define their potential role in disease, including whether they may be involved in attachment and/or in generating protective immunity.

Brachyspira pilosicoli is cultured under the same anaerobic conditions as *B. hyodysenteriae*. After 3–5 days on Trypticase soy blood agar, *B. pilosicoli* forms a thin spreading surface haze surrounded by a zone of weak beta-hemolysis. Slicing the agar prior to inoculation can improve the recovery of *B. pilosicoli*, but a zone of enhanced hemolysis is not usually seen. Once isolated, the spirochete grows readily in various anaerobic liquid media, as described for *B. hyodysenteriae*.

Public health

Brachyspira pilosicoli colonizes human beings who are usually either immunocompromised or live in developing communities where hygiene is poor and fecal contamination of water supplies may occur (Margawani et al. 2004). Infection may be associated with chronic diarrhea and/or failure to thrive. Strains of *B. pilosicoli* from humans can cause disease when inoculated into pigs and chickens (Duhamel et al. 1995; Muniappa et al. 1997; Trott et al. 1995, 1996b). The potential for transmission of *B. pilosicoli* from animals to humans exists, although the risk of healthy pig industry workers developing disease from contact with pigs is slight.

Epidemiology

PIS/PCS has been reported in most pig-producing countries. Increased recognition of the condition has resulted from improved diagnostic methods, the withdrawal of routine antimicrobial growth promoters, and the fact that other major intestinal diseases are now better controlled in many countries.

Investigations in different regions have found that a variable but often high proportion of farms having pigs with persistent diarrhea problems are infected with *B. pilosicoli*, whereas little or no infection occurs in farms without diarrhea.

A wide range of species may be naturally infected with *B. pilosicoli*, and typical clinical signs and lesions have been recorded in all of them (Duhamel 2001). Isolates from pigs, dogs, birds, and humans can be closely related genetically (Lee and Hampson 1994; Trott et al. 1998).

Transmission occurs by the fecal–oral route, and infection may be introduced into naïve herds by carrier pigs.

B. pilosicoli can persist in the environment, and the disease can recur between batches of pigs if the premises are not adequately cleaned and disinfected. Feral animals and birds may be a source of infection. On one pig farm *B. pilosicoli* was detected in chickens, effluent pond water, and wild ducks on the effluent pond (Oxberry and Hampson 2003). An isolate from the pond belonged to the same genetic type as one from a pig, a finding consistent with a previous observation, suggesting that feral water birds may contaminate water supplies and so represent a potential source of *B. pilosicoli* infection for pigs (Oxberry et al. 1998). Rodents appear unlikely to serve as an important long-term biologic reservoir of *B. pilosicoli*.

The on-farm epidemiology of *B. pilosicoli* can be highly variable (Oxberry and Hampson 2003). Sometimes the incidence is low and largely confined to one age group, while in other herds it may be widespread and associated with numerous different strains. The presence of multiple *B. pilosicoli* strains within certain farms might explain why PIS/PCS commonly recurs in convalescent animals or in those treated with antimicrobials. In such cases reinfection may be with a different strain, possibly having different antigenic determinants, antimicrobial susceptibilities, or potential to colonize and cause disease.

In Finland, most farms had their own distinct genotypes of *B. pilosicoli*, while it was rare to find the same type on different farms (Fossi et al. 2003). The within-farm genotypes appeared fairly stable, as the same genotypes were found on three farms that were reexamined after 3 years.

Brachyspira pilosicoli is shed in the feces, and this may be intermittent and continue over weeks in some pigs. It is relatively resistant in the environment, surviving in lake water at 39 °F (4 °C) for 66 days (Oxberry et al. 1998) and remaining viable at 50 °F (10 °C) for 119 days in soil and for 210 days in soil with 10% pig feces and in feces (Boye et al. 2001). Although it is susceptible to many common disinfectants, the efficacy of some of them is reduced by the presence of organic matter (Corona-Barrera et al. 2004a).

Pathogenesis

Cells of *B. pilosicoli* are motile, and strains vary in their attraction to mucin (Naresh and Hampson 2010). Once in the large intestine, the spirochete is able to penetrate the mucus overlying the colonic mucosa. In the initial stage of infection, *B. pilosicoli* cells adhere in large numbers to the luminal surface of cecal and colonic epithelial cells. Attachment occurs to mature apical enterocytes between crypt units, but not to immature cells deeper in the crypts (Trott et al. 1996b).

Despite the availability of genome sequences, few *B. pilosicoli* virulence factors have been identified. Cellular attachment by *B. pilosicoli* strains has been achieved *in vitro* using intestinal epithelial cell lines, but putative adhesins or host cell receptors have not been identified (Muniappa et al. 1998; Naresh et al. 2009). Colonized monolayers demonstrated a time-dependent series of changes including accumulation of actin at the cell junctions, a loss of tight junction integrity, and evidence of apoptosis – although the mechanisms causing these changes were not identified. Interestingly, addition of the stress hormone norepinephrine to *in vitro* cultures enhances *B. pilosicoli* growth, attraction to mucin, and attachment to Caco-2 cells (Naresh and Hampson 2011). This may cause pigs to become more susceptible to infection with *B. pilosicoli* (and potentially other pathogenic *Brachyspira* spp.) during periods of stress.

As with SD, *B. pilosicoli* colonization and/or disease expression can be influenced by diet. An analysis of risk factors on farms revealed that reduced prevalence results from using home-mixed and/or non-pelleted diets (Stege et al. 2001). Adding carboxymethylcellulose to an experimental pig diet increased the viscosity of the intestinal contents and enhanced colonization with *B. pilosicoli* (Hopwood et al. 2002). High levels of soluble non-starch polysaccharide ("soluble fiber") in grains like barley and rye also may increase viscosity and therefore enhance *B. pilosicoli* colonization. Consistent with this, pigs fed diets based on cooked white rice (highly digestible and low in soluble fiber) have shown reduced colonization with *B. pilosicoli* compared with pigs fed conventional diets (Hampson et al. 2000; Lindecrona et al. 2004). Feeding a pelleted diet rather than meal increased the risk of colonization, but fermented liquid feed or lactic acid had no influence on colonization (Lindecrona et al. 2004).

Clinical signs

PIS/PCS most commonly occurs soon after weaning or in recently mixed growers placed on a new diet, but it can occur in finishers and occasionally in pregnant sows and recently introduced breeding stock. PIS/PCS may affect groups of pigs in a unit or be present in pigs of mixed ages. Various manifestations may be seen in weaners, growers, and finishers on the same farm. Not all infected animals develop diarrhea; however, subclinical infections still may depress growth rates.

The first clinical signs are hollowing of the flanks and the passage of loose, sometimes sticky feces. Fecal consistency changes to that of wet cement or porridge and may glisten. These may be the only signs in finishers, but weaners and growers usually develop watery to mucoid diarrhea that is green or brown and occasionally contains thick tags of mucus and sometimes flecks of blood. Diarrhea is usually self-limiting and lasts 2–14 days, although some animals may relapse.

Affected pigs appear unthrifty, have fecal staining of the perineum, have a tucked-up appearance, and sometimes are febrile but usually continue to eat. Pigs that develop loose feces may show significant loss of condition, decreased feed conversion, and delays in reaching market weight (Thomson et al. 1997, 1998).

Brachyspira pilosicoli may be shed in the feces within 2–7 days of experimental inoculation, although the incubation period may extend to 20 days. Between 17 and 100% of pigs may become infected, with 17–67% developing diarrhea and 8–100% having colitis (Duhamel 2001). Mortality is rare in the field.

Pigs with PIS/PCS may have concurrent sickness that exacerbates disease, particularly intestinal diseases such as SD, salmonellosis, PE, or infection with porcine circovirus type 2 (PCV2) (Duhamel et al. 1995; Girard et al. 1995; Møller et al. 1998; Stege et al. 2000; Thomson et al. 1998, 2001).

Lesions

Gross lesions of PIS/PCS are limited to the cecum and colon and may be subtle, particularly in the early stages. Soon after the onset of clinical signs, the cecum and colon may be flaccid and fluid filled with an edematous serosal surface and enlarged mesenteric and colonic lymph nodes. The large intestinal contents are usually abundant, watery, green or yellow, and frothy (Figure 62.6). There may be mild mucosal congestion and hyperemia with variable catarrhal exudate and/or multifocal erosion or necrosis. In later stages, the mucosa is thickened, and petechiae or ecchymoses may be observed on the surface. Hemorrhages, erosions, or necrotic foci may occur, and there may be small tags of adherent fibrin, necrotic material, and digesta that appear as conical scales on the mucosa.

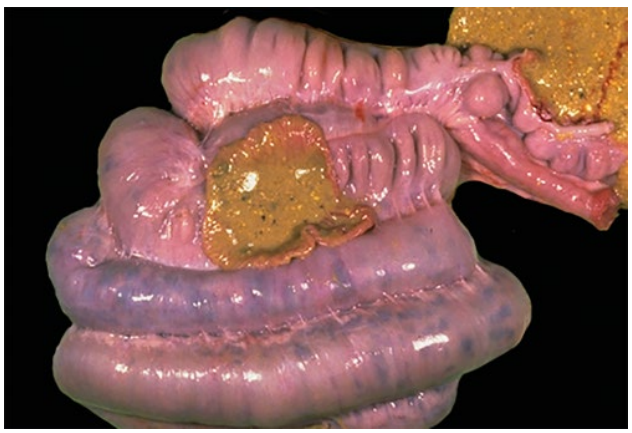


Figure 62.6 Cecum and spiral colon from a pig 21 days after intragastric inoculation with *Brachyspira pilosicoli*. Note abundant mucoid to watery contents in the cecum (top) and colon (center). *Source:* Courtesy of Dr. Gerald E. Duhamel.

Microscopic lesions are generally confined to the mucosa and submucosa but may extend into the muscularis. The mucosa is usually thickened, edematous, and occasionally hyperemic and characterized by dilated, elongated crypts filled with mucus, cellular debris, and degenerate inflammatory cells. The presence of *B. pilosicoli* within crypts and the lamina propria may be associated with neutrophilic exocytosis (crypt abscesses) and a mixed infiltrate of neutrophils and lymphocytes in the lamina propria. In chronic infections, the lamina propria is usually infiltrated with large numbers of monocytes, lymphocytes, and plasma cells (Duhamel 2001). The crypt cell mitotic rate can be increased, and immature, cuboidal, or squamous epithelium may be present on the surface of the mucosa between crypt units. Columnar epithelium lining the surface of the colon may be covered by a fringe of spirochetes attached end-on to form a characteristic "false brush border" (Girard et al. 1995; Taylor et al. 1980) (Figure 62.7). Although this attachment is pathognomonic for PIS/PCS, it is not always found (Thomson et al. 1997). Occasionally *Balantidium coli* may be seen on the surface of the affected colon (Taylor et al. 1980; Trott et al. 1996b).

Brachyspira pilosicoli cells may be observed inside dilated colonic crypts (Trott et al. 1996b), invading through tight junctions between colonic enterocytes, within goblet cells (Thomson et al. 1997), and within the lamina propria (Duhamel 2001). Invasion has been observed both concurrent with and independent of attachment of spirochetes to the epithelium. In humans, *B. pilosicoli* has been isolated from the bloodstream of individuals with severe clinical disease or impaired immunity, and evidence of systemic spread has been

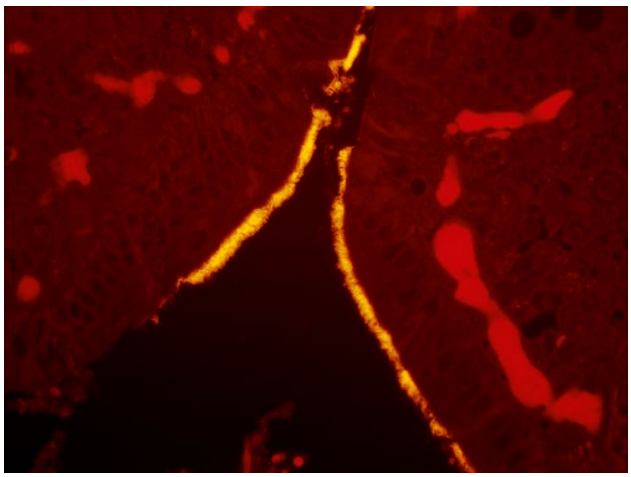


Figure 62.7 Fluorescent *in situ* hybridization using a probe specific for *Brachyspira* spp. on a section of colon from a pig with PIS/PCS. Note the dense fringe of fluorescent spirochetes attached by one cell end to the surface of the colonic epithelium to form a "false brush border". *Source:* Courtesy of Dr. Eric Burrough.

observed in experimentally infected chickens (Mappley et al. 2013; Trott et al. 1997). Systemic spread or spirochetemia has not been recorded in pigs, but they cannot be excluded.

Epithelial damage followed by local invasion and subsequent colitis combine to cause an increase in the water content of the cecal and colonic contents, together with excess mucus production. Erosion of the epithelium leads to replacement by immature cells and reduction of the surface area of the colon for absorption of water, electrolytes, and volatile fatty acids. This then leads to decreased feed conversion efficiency and diminished weight gain (Duhamel 1998; Thomson et al. 1997).

Diagnosis

A definitive diagnosis of PIS/PCS requires the confirmation of *B. pilosicoli* in pigs with typical clinical disease and lesions. *B. pilosicoli* can be confirmed in tissue sections using immunohistochemistry with specific antibodies or FISH with specific oligonucleotide probes (Boye et al. 1998; Jensen et al. 2000) where it is observed attached to the surface of colonic enterocytes (Figure 62.8), within dilated intestinal crypts, and occasionally within the lamina propria. *B. pilosicoli* can also be confirmed by culture or PCR of affected colonic mucosa or feces.

However, clinically normal pigs may shed *B. pilosicoli*, and confirmation in feces does not alone confirm PIS/PCS. The significance of positive fecal culture of *B. pilosicoli* needs to be interpreted in the context of a complete diagnostic investigation (Thomson et al. 1998). Fecal samples for culture and/or PCR should be obtained from a cross section of affected pigs and, when possible, correlated with lesions.

CVS medium is preferred for isolating *B. pilosicoli* as BJ medium may inhibit *B. pilosicoli* growth and is not recommended (Duhamel and Joens 1994; Trott et al. 1996c). Pure cultures of spirochetes can be differentiated by MALDI-TOF MS or by using the biochemical tests outlined in Table 62.1, specifically strength of beta-hemolysis, hippurate hydrolysis, and lack of beta-glucosidase activity (Fellström and Gunnarsson 1995; Trott et al. 1996c).

Reported PCR tests for *B. pilosicoli* target the genes for 16S rRNA (Fellström et al. 1997), 23S rRNA (Leser et al. 1997), or the *nox* gene (Atyeo et al. 1999b). Duplex and multiplex PCRs, sometimes also detecting other enteric pathogens, and real-time PCR assays that allow spirochete quantitation also have been described (see section on SD).

As with *B. hyodysenteriae*, RFLP analysis can be used to identify *B. pilosicoli* isolates. Indirect fluorescent antibody tests using MAbs raised against specific outer membrane proteins of *B. pilosicoli* have potential for diagnostic use on feces (Lee and Hampson 1995), but a MAb-based immunomagnetic separation of *B. pilosicoli* from feces did not improve the sensitivity of detection compared with culture/PCR (Corona-Barrera et al. 2004b).

Clinical signs of PIS/PCS can be very similar to those of PE (Chapter 58), with which it may occur. Salmonellosis (Chapter 59), post weaning colibacillosis (Chapter 52), SD and yersiniosis (Chapter 64), PCV2 enteritis (Chapter 30), trichuriasis (Chapter 67), and "nonspecific colitis," a diet-responsive colitis (Chapter 15; Smith and Nelson 1987), all should be considered in the differential diagnosis of porcine colitis.

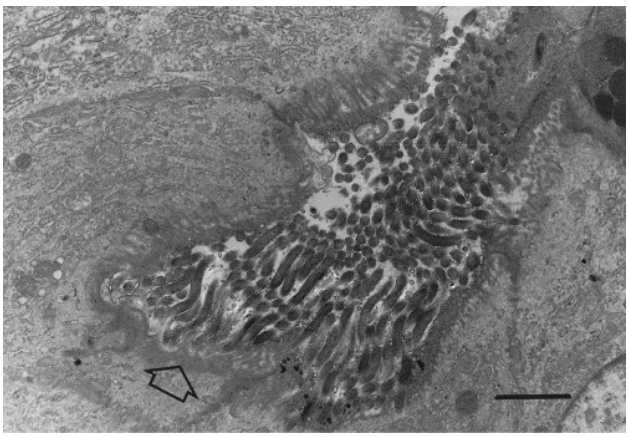


Figure 62.8 Transmission electron photomicrograph of the colonic epithelium from a pig with PIS/PCS. Note the large numbers of spirochetes attached by one cell end to the apical membrane of enterocytes causing effacement of the microvilli and disruption of terminal web microfilaments (arrow). Bar = 2 µm. *Source:* Courtesy of Drs. Darren Trott and David Hampson.

Immunity

The host immune mechanisms directed against *B. pilosicoli* are poorly understood. Agglutinating serum antibodies have been recorded in pigs recovered from experimental infection (Taylor et al. 1980), while no significant antibody levels against whole cell preparations of *B. pilosicoli* were found after 18 days in experimentally infected pigs with mild colitis (Hampson et al. 2000). In other experiments, pigs developed low levels of serum IgG against *B. pilosicoli* whole cell extracts and membrane preparations 2–7 weeks after challenge (Zhang et al. 1999; Zhang and Duhamel 2002). Little is known about protective immunity in PIS/PCS. The existence of long-term colonization suggests that the spirochete may be able to evade immune mechanisms. Cross-protection is unlikely as *B. pilosicoli* strains show considerable variability in their LOS (Lee and Hampson 1999). Nothing is known about maternal immunity, although natural infection has not been recorded in unweaned piglets.

Prevention and control

Methods to treat pigs or control outbreaks

Treatment and control of PIS/PCS are largely modeled on procedures developed for SD, although modifications can be made because of the milder economic impact of PIS/PCS. Antimicrobial therapy can be used to reduce *B. pilosicoli* infection and maintain productivity while improving welfare. It may also be required to prevent sudden increases in morbidity due to recent introduction of naïve pigs, change of diet, or other stressors. Affected pigs should be treated by water or feed medication at similar levels and durations as recommended for SD. Parenteral treatment may be necessary for severely ill pigs. Although information on the *in vitro* antimicrobial susceptibility of *B. pilosicoli* is limited, a number of antimicrobials that are effective against *B. hyodysenteriae*, including tiamulin, valnemulin, carbadox, dimetridazole, and to a lesser extent lincomycin, have been shown to have low MIC values when tested against porcine *B. pilosicoli* isolates (Fossi et al. 2000; Hommez et al. 1998; Kinyon et al. 2002; Trott et al. 1996c). Fewer isolates have been found to be susceptible to tylosin, and resistance to several antimicrobials, including tiamulin, has been recorded (Pringle et al. 2012). Olaquindox may be a useful prophylactic as it has MIC values <1.0 μg/mL against *B. pilosicoli*, and the spirochete could not be isolated from herds previously receiving 100 ppm olaquindox in-feed (Fellström et al. 1996).

Management strategies that limit access of pigs to contaminated environments can reduce the impact of PIS/PCS. Replacing continuous-flow systems with all-in/all-out systems reduces the risk of infection (Stege et al. 2001). Modification to the diet composition and/or physical form or adding zinc oxide in the feed at 3 kg/ton may be helpful (Love 1996).

Methods to avoid outbreaks of PIS/PCS

It can be difficult to avoid the introduction of *B. pilosicoli* into herds because of the presence of reservoir hosts such as wild birds, although similar strategies to those for SD can be followed. Unfortunately, no effective vaccines are available for *B. pilosicoli*. An autogenous bacterin induced systemic antibody titers, but the pigs still became colonized and developed diarrhea after challenge (Hampson et al. 2000).

Methods to eliminate *B. pilosicoli* from herds

The methods described for the elimination of SD also may be effective for PIS/PCS, but the economic impact generally does not warrant such costly procedures. Fossi et al. (2001) reported eradicating *B. pilosicoli* from a 60-sow herd by tiamulin treatment followed by relocation of the breeding herd, thorough cleaning and disinfection of the premises, and then returning the adult animals to the original location. This protocol would be more difficult to follow in larger herds, and the existence of reservoir hosts presents an ongoing threat of reintroduction.

References

Achacha M, Messier S, Mittal KR. 1996. *Can J Vet Res* 60:45–49.

Aller-Morán LM, Martínez-Lobo FJ, Rubio P, et al. 2016a. *Vet J* 214:10–13.

Aller-Morán LM, Martínez-Lobo FJ, Rubio P, et al. 2016b. *J Vet Diagn Invest* 28:755–759.

Argenzio RA, Whipp SC, Glock RD. 1980. *J Infect Dis* 142:676–684.

Atyeo RF, Oxberry SL, Hampson DJ. 1996. *FEMS Microbiol Lett* 141:77–81.

Atyeo RF, Oxberry SL, Hampson DJ. 1999a. *Epidemiol Infect* 123:133–138.

Atyeo RF, Stanton TB, Jensen NS, et al. 1999b. *Vet Microbiol* 67:49–62.

Barcellos DE, de Uzeda M, Ikuta N, et al. 2000. *Vet Microbiol* 75:189–198.

Bellgard MI, Wanchanthuek P, La T, et al. 2009. *PLoS One* 4(3):e4641.

Black ML, Moolhuijzen PM, Barrero R, et al. 2015. *PLoS One* 10(6):e0131050.

Bowden CA, Joens LA, Kelley LM. 1989. *Am J Vet Res* 50:1481–1485.

Boye M, Jensen TK, Moller K, et al. 1998. *Mol Cell Probes* 12:323–330.

Boye M, Baloda SB, Leser TD, et al. 2001. *Vet Microbiol* 81:33–40.

Burrough ER. 2017. *Vet Pathol* 54:22–31.

Burrough ER, Sexton C. 2013. In Proceedings of the 44th Annual Meeting on American Association of Swine Veterinarians, pp. 551–556.

Burrough ER, Strait EL, Kinyon JM, et al. 2012. *J Vet Diagn Invest* 24:1025–1034.

Burrough ER, Wilberts BL, Bower LP, et al. 2013. *J Vet Diagn Invest* 25:407–412.

Calderaro A, Piccolo G, Montecchini S, et al. 2013. *J Proteomics* 78:273–280.

Chander Y, Primus A, Oliveira S, et al. 2012. *J Vet Diagn Invest* 24:903–910.

Chia SP, Taylor DJ. 1978. *Vet Rec* 103:68–70.

Corona-Barrera E, Smith DG, Murray B, et al. 2004a. *Vet Rec* 154:473–474.

Corona-Barrera E, Smith DGE, La T, et al. 2004b. *J Med Microbiol* 53:301–307.

Costa MO, Hill JE, Fernando C, et al. 2014. *BMC Vet Res* 10:129.

Diego R, Lanza I, Carvajal A, et al. 1995. *Vaccine* 13:663–667.

Duhamel GE. 1998. *Large Anim Pract* 19:14–22.

Duhamel GE. 2001. *Anim Health Res Rev* 2:3–17.

Duhamel GE, Joens LA. 1994. *Laboratory Procedures for Diagnosis of Swine Dysentery*. USA: American Association of Veterinary Laboratory Diagnosticians, Inc..
Duhamel GE, Mathiesen MR, Schafer RW, et al. 1993. In Conference of Research Workers in Animal Disease (Abstract), p. 14.
Duhamel GE, Muniappa N, Gardner I, et al. 1995. *Pig J* 35:101–110.
Duinhof TF, Dierikx CM, Koene MG, et al. 2008. *Tijdschr Diergeneeskd* 133:604–608.
Fellström C, Gunnarsson A. 1995. *Res Vet Sci* 59:1–4.
Fellström C, Pettersson B, Johansson K, et al. 1996. *Am J Vet Res* 57:807–811.
Fellström C, Pettersson B, Thompson J, et al. 1997. *J Clin Microbiol* 35:462–467.
Fellström C, Karlsson M, Pettersson B, et al. 1999. *Vet Microbiol* 70:225–238.
Fellström C, Zimmerman U, Aspan A, et al. 2001. *Anim Health Res Rev* 2:37–43.
Fossi M, Saranpaa T, Rautiainen E. 2000. *Acta Vet Scand* 41:355–358.
Fossi M, Heinonen M, Pohjanvirta T, et al. 2001. *Anim Health Res Rev* 2:53–57.
Fossi M, Pohjanvirta T, Pelkonen S. 2003. *Epidemiol Infect* 131:967–973.
Gabe JD, Chang R-J, Sloiany R, et al. 1995. *Infect Immun* 63:142–148.
Girard C, Lemarchand T, Higgins R. 1995. *Can Vet J* 36:291–294.
Glock RD, Harris DL. 1972. *Vet Med Small Anim Clin* 67:65–68.
Glock RD, Vanderloo KJ, Kinyon JM. 1975. *J Am Vet Med Assoc* 166:277–278.
de Graaf GJ, Jager LP, Baars AJ, et al. 1988. *Vet Q* 10:34–41.
Greer JM, Wannemuehler MJ. 1989. *Microbiol Pathol* 7:279–288.
Håfström T, Jansson DS, Segerman B. 2011. *BMC Genomics* 12:395.
Halter MR, Joens LA. 1988. *Infect Immun* 56:3152–3156.
Hampson DJ. 2014. In Proceedings of the 23rd International Pig Veterinary Society Congress, pp. 40–46.
Hampson DJ, McLaren AJ. 1999. *Avian Pathol* 28:113–117.
Hampson DJ, Cutler R, Lee BJ. 1992. *Vet Rec* 131:318–319.
Hampson DJ, Atyeo RF, Combs BG. 1997. In Hampson DJ, Stanton TB, eds. *Intestinal Spirochaetes in Domestic Animals and Humans*. England: CAB International, pp. 175–209.
Hampson DJ, Robertson ID, La T, et al. 2000. *Vet Microbiol* 73:75–84.
Hampson DJ, La T, Adler B, et al. 2006. *Microbiology* 152:1–2.
Hampson DJ, La T, Phillips ND. 2015. *Porc Health Manag* 1:8.
Hampson DJ, La T, Phillips ND, et al. 2016. *Vet Microbiol* 191:15–19.
Hansen CF, Phillips ND, La T, et al. 2010. *J Anim Sci* 48:2859–2865.
Hansen CF, Hernández A, Mansfield J, et al. 2011. *Br J Nutr* 106:1506–1513.
Harris DL, Glock RD, Christensen CR, et al. 1972. *Vet Med Small Anim Clin* 67:61–64.
Harris DL, Alexander TJ, Whipp SC, et al. 1978. *J Am Vet Med Assoc* 172:468–471.
Hidalgo Á, Carvajal A, La T, et al. 2010a. *J Clin Microbiol* 48:2859–2865.
Hidalgo Á, Carvajal A, Pringle M, et al. 2010b. *Epidemiol Infect* 138:76–85.
Hillen S, Willems H, Herbst W, et al. 2014. *Vet Microbiol* 172:223–229.
Hommez J, Devriese LA, Castryck F, et al. 1998. *Vlaams Diergen Tijdschrift* 67:32–35.
Hopwood DE, Pethick DW, Hampson DJ. 2002. *Br J Nutr* 88:523–532.
Hsu T, Hutto DL, Minion FC, et al. 2001. *Infect Immun* 69:706–711.
Hudson MJ, Alexander TJ, Lysons RJ, et al. 1976. *Res Vet Sci* 21:366–367.
Hughes R, Olander HJ, Kanitz DL, et al. 1977. *Vet Pathol* 14:490–507.
Humphrey SB, Stanton TB, Jensen NS, et al. 1997. *J Bacteriol* 179:323–329.
Hunter D, Saunders CN. 1977. *Vet Rec*, 101:303–304.
ter Huurne AAHM, Muir S, Van Houten M, et al. 1994. *Microbiol Pathol* 16:269–282.
Hyatt DR, ter Huurne AAHM, Van der Zeist BAM, et al. 1994. *Infect Immun* 62:2244–2248.
Jacobson M, Fellström C, Lindberg R, et al. 2004. *J Clin Microbiol* 53:273–280.
Jacobson M, Lindberg R, Jonasson R, et al. 2007. *Res Vet Sci* 82:287–298.
Jenkins EM. 1978. *Vet Med Small Anim Clin* 73:931–936.
Jenkins EM, Mohammad A, Klesius PH. 1982. In Proceedings of the 6th Pig Veterinary Society Congress, p. 41.
Jenkinson SR, Wingar CR. 1981. *Vet Rec* 109:384–385.
Jensen TK, Møller K, Boye M, et al. 2000. *Vet Pathol* 37:22–32.
Jensen TK, Christensen AS, Boye M. 2010. *Vet Pathol* 47:334–338.
Joens LA, Kinyon JM. 1982. *J Clin Microbiol* 15:994–997.
Joens LA, Harris DL, Baum DH. 1979. *Am J Vet Res* 40:1352–1354.
Joens LA, Glock RD, Whipp SC, et al. 1981. *Vet Microbiol* 6:69–77.
Joens LA, Nord NA, Kinyon JM, et al. 1982. *J Clin Microbiol* 15:249–252.
Joens LA, Whipp SC, Glock RD, et al. 1983. *Infect Immun* 39:460–462.

Joens LA, Deyoung DW, Glock RD, et al. 1985. *Am J Vet Res* 46:2369–2371.
Jonasson R, Johannisson A, Jacobson M, et al. 2004. *J Med Microbiol* 53:267–272.
Jonasson R, Andersson M, Råsbäck T, et al. 2006. *J Med Microbiol* 55:845–855.
Karlsson M, Fellström C, Heldtander MU, et al. 1999. *FEMS Microbiol Lett* 172:255–260.
Karlsson M, Fellström C, Gunnarsson A, et al. 2003. *J Clin Microbiol* 41:2596–2604.
Karlsson M, Aspan A, Landen A, et al. 2004. *J Med Microbiol* 53:281–285.
Kennedy MJ, Rosnick DK, Ulrich RG, et al. 1988. *J Gen Microbiol* 134:1565–1567.
Kennedy MJ, Rosnick DK, Ulrich RG, et al. 1992. In Proceedings of the 12th International Pig Veterinary Society Congress, p. 273.
Kennedy MJ, Rosey EL, Yancey Jr RJ. 1997. *FEMS Microbiol Lett* 153:119–128.
Kinyon JM, Harris DL. 1979. *Int J Syst Bacteriol* 29:102–109.
Kinyon JM, Harris DL, Glock RD. 1977. *Infect Immun* 15:638–646.
Kinyon JM, Murphy D, Stryker C, et al. 2002. In Proceedings of the 17th International Pig Veterinary Society Congress, p. 50.
Klose V, Bruckbeck R, Henikl S, et al. 2010. *J Appl Microbiol* 108:1271–1280.
Knoop FC, Schrank GD, Ferraro FM. 1979. *Can J Microbiol* 25:399–405.
Komarek V, Maderner A, Spergser J, et al. 2009. *Vet Microbiol* 134:311–317.
Kunkle RA, Kinyon JM. 1988. *J Clin Microbiol* 26:2357–2360.
Kutasi J, Jakob L, Jurkovich V, et al. 2016. *Acta Microbiol Immunol Hung* 8:1–7.
La T, Hampson DJ. 2001. *Anim Health Res Rev* 2:45–52.
La T, Phillips ND, Hampson DJ. 2003. *J Clin Microbiol* 41:3372–3375.
La T, Phillips ND, Reichel MP, et al. 2004. *Vet Microbiol* 102:97–109.
La T, Phillips ND, Hampson DJ. 2009a. *Vet Microbiol* 133:98–104.
La T, Phillips ND, Harland BL, et al. 2009b. *Vet Microbiol* 138:330–338.
La T, Phillips ND, Wanchanthuek P, et al. 2011. *Vet Microbiol* 153:150–155.
La T, Phillips ND, Thomson JR, et al. 2014. *Vet Res* 45:131.
La T, Rohde J, Phillips ND, et al. 2016a. *PLoS One* 11(8):e0160362.
La T, Phillips ND, Hampson DJ. 2016b. *PLoS One* 11(12):e0167424.
Lee JI, Hampson DJ. 1994. *J Med Microbiol* 40:365–371.
Lee BJ, Hampson DJ. 1995. *FEMS Microbiol Lett* 131:179–184.
Lee BJ, Hampson DJ. 1996. *FEMS Microbiol Lett* 136:193–197.
Lee BJ, Hampson DJ. 1999. *J Med Microbiol* 48:411–415.
Lee JI, Hampson DJ, Combs BG, et al. 1993a. *Vet Microbiol* 34:35–46.
Lee JI, Hampson DJ, Lymbery AJ, et al. 1993b. *Vet Microbiol* 34:273–285.
Leser TD, Møller K, Jensen TK, et al. 1997. *Mol Cell Probes* 11:363–372.
Leser TD, Lindecrona RH, Jensen TK, et al. 2000. *Appl Environ Microbiol* 66:3290–3296.
Lindecrona RH, Jensen TK, Møller K. 2004. *Vet Rec* 154:264–267.
Love R. 1996. *Aust Assoc Pig Vet News* 20:6.
Lysons RJ, Lemcke RM, Bew J, et al. 1982. In Proceedings of the 7th International Pig Veterinary Society Congress, p. 40.
Lysons RJ, Burrows MR, Debney TG, et al. 1986. In Proceedings of the 9th International Pig Veterinary Society Congress, p. 180.
Mahu M, de Jong E, De Pauw N, et al. 2014. *Vet Rec* 174:47.
Mahu M, De Pauw N, Vande Maele L, et al. 2016. *Vet Res* 47:66.
Mappley LJ, Black ML, AbuOun M, et al. 2012. *BMC Genomics* 13:454.
Mappley LJ, Tchórzewska MA, Nunez A, et al. 2013. *J Med Microbiol* 62:297–302.
Margawani KR, Robertson ID, Brooke JC, et al. 2004. *J Med Microbiol* 53:325–332.
Martínez-Lobo FJ, Hidalgo Á, García M, et al. 2013. *PLoS One* 8:e82626.
Matson EG, Thompson MG, Humphrey SB, et al. 2005. *J Bacteriol* 187:5885–5892.
McCaman MT, Auer K, Foley W, et al. 2003. *Microbes Infect* 5:1–6.
Meyer RC, Simon J, Byerly CS. 1974a. *Vet Pathol* 11:515–526.
Meyer RC, Simon J, Byerly CS. 1974b. *Vet Pathol* 11:527–534.
Milner JA, Sellwood R. 1994. *Infect Immun* 62:4095–4099.
Mirajkar NS, Gebhart CJ. 2014. *PLoS One* 9:e107176.
Mirajkar NS, Bekele AZ, Chander YY, et al. 2015. *J Clin Microbiol* 53:2908–2918.
Mirajkar NS, Phillips ND, La T, et al. 2016. *J Clin Microbiol* 54:2942–2949.
Mølbak L, Thomsen LE, Jensen TK, et al. 2007. *J Appl Microbiol* 103:1853–1867.
Møller K, Jensen TK, Jorsal SE, et al. 1998. *Vet Microbiol* 62:59–72.
Motro Y, La, T, Bellgard MI, et al. 2009. *Vet Microbiol* 134:340–345.
Muniappa N, Mathiesen MR, Duhamel GE. 1997. *J Vet Diagn Invest* 9:165–171.

Muniappa N, Ramanathan MR, Tarara MP, et al. 1998. *J Spiro Tick-borne Dis* 5:44–53.
Mushtaq M, Zubair S, Råsbäck T, et al. 2015. *BMC Microbiol* 15:208.
Naresh R, Hampson DJ. 2010. *Microbiology* 156:191–197.
Naresh R, Hampson DJ. 2011. *Microbiology* 157:543–547.
Naresh R, Song Y, Hampson DJ. 2009. *PLoS One* 4(12):e8352.
Neo E, La T, Phillips ND, et al. 2013a. *Gut Pathogens* 5:24.
Neo E, La T, Phillips ND, et al. 2013b. *Vet Microbiol* 163:299–304.
Nibbelink SK, Sacco RE, Wannemuehler MJ. 1997. *Microb Pathog* 23:181–187.
Nuessen ME, Joens LA, Glock RD. 1983. *J Immunol* 131:997–999.
Ochiai S, Adachi Y, Mori K. 1997. *Microbiol Immunol* 41:445–452.
Olson LD. 1974. *Can J Comp Med* 28:7–13.
Olson LD, Dayalu KI, Schlink GT. 1994. *Am J Vet Res* 55:67–71.
Osorio J, Carvajal A, Naharro G, et al. 2012. *PLoS One* 7(6):e39082.
Osorio J, Carvajal A, Naharro G, et al. 2013. *Res Vet Sci* 95:861–869.
Oxberry SL, Hampson DJ. 2003. *Vet Microbiol* 93:109–120.
Oxberry SL, Trott DJ, Hampson DJ. 1998. *Epidemiol Infect* 121:219–225.
Parizek R, Stewart R, Brown K, et al. 1985. *Vet Med* 80:80–86.
Phillips ND, La T, Adams PJ, et al. 2009. *Vet Microbiol* 134:294–299.
Pluske JR, Siba PM, Pethick DW, et al. 1996. *J Nutr* 126:2920–2933.
Polson DD, Marsh WE, Harris DL. 1992. In Proceedings of the 12th International Pig Veterinary Society Congress, p. 510.
Pringle M, Landén A, Unnerstad HE, et al. 2012. *Acta Vet Scand* 54:54.
Quintana-Hayashi MP, Mahu M, De Pauw N, et al. 2015. *Infect Immun* 83:1610–1609.
Råsbäck T, Jansson DS, Johansson KE, et al. 2007a. *Environ Microbiol* 9:983–991.
Råsbäck T, Johansson K-E, Jansson DS, et al. 2007b. *Microbiology* 153:4074–4087.
Rees AS, Lysons RJ, Stokes CR, et al. 1989a. *Res Vet Sci* 47:263–269.
Rees AS, Lysons RJ, Stokes CR, et al. 1989b. *Vet Immunol Immunopathol* 47:263–269.
Robertson ID, Mhoma JRL, Hampson DJ. 1992. *Aust Vet J* 69:92–93.
Robinson IM, Whipp SC, Bucklin JA, et al. 1984. *Appl Environ Microbiol* 48:964–969.
Rohde J, Habighorst-Blome K. 2012. *Vet Microbiol* 158:211–215.
Rohde J, Rothkamp A, Gerlach GF. 2002. *J Clin Microbiol* 40:2598–2600.
Rohde J, Habighorst-Blome K, Seehusen F. 2014. *Vet Microbiol* 168:432–435.
Rosey EL, Kennedy MJ, Yancey RJ. 1996. *Infect Immun* 64:4154–4162.
Rubin JE, Harms NJ, Fernando C, et al. 2013a. *Microb Ecol* 66:813–822.
Rubin JE, Costa MO, Hill JE, et al. 2013b. *PLoS One* 8:e57146.
Rugna G, Bonilauri P, Carra E, et al. 2015. *Vet J* 203:115–119.
Scherrer S, Borgström A, Frei D, et al. 2016. *Vet Microbiol* 193:17–21.
Schmall MS, Argenzio RA, Whipp SC. 1983. *Am J Vet Res* 44:1309–1316.
Smith WJ, Nelson EP. 1987. *Vet Rec* 121:334.
Song Y, Hampson DJ. 2009. *Vet Microbiol* 137:129–136.
Song Y, La T, Phillips ND, et al. 2009. *Vet Microbiol* 137:111–119
Song Y, Frey B, Hampson DJ. 2012. *BMC Vet Res* 8:6.
Song Y, La T, Phillips ND, et al. 2015. *Vet J* 206:365–370.
Songer JG, Harris DL. 1978. *Am J Vet Res* 39:913–916.
Šperling D, Smola J, Čížek A. 2011. *Vet Rec* 168:215.
Stanton TB. 1992. *Int J Syst Bacteriol* 42:189–190.
Stanton TB, Fournie-Amazouz E, Postic D, et al. 1997. *Int J Syst Bacteriol* 47:1007–1012.
Stanton TB, Rosey EL, Kennedy MJ, et al. 1999. *Appl Environ Microbiol* 65:5028–5034.
Stanton TB, Thompson MG, Humphrey SB, et al. 2003. *FEMS Microbiol Lett* 224:225–229.
Stanton TB, Humphrey SB, Sharma VK, et al. 2008. *Appl Environ Microbiol* 74:2950–2956.
Stege H, Jensen TK, Møller K, et al. 2000. *Prev Vet Med* 46:279–292.
Stege H, Jensen TK, Møller K, et al. 2001. *Prev Vet Med* 50:153–164.
Taylor DJ, Alexander TJL. 1971. *Br Vet J* 127:58–61.
Taylor DJ, Simmons JR, Laird HM. 1980. *Vet Rec* 106:326–332.
Thomsen LE, Knudsen KE, Jensen TK, et al. 2007. *Vet Microbiol* 119:152–163.
Thomson JR, Smith WJ, Murray BP, et al. 1997. *Infect Immun* 65:3693–700.
Thomson JR, Smith WJ, Murray BP. 1998. *Vet Rec* 142:235–239.
Thomson JR, Smith WJ, Murray BP, et al. 2001. *Anim Health Res Rev* 2:31–36.
Trott DJ, McLaren AJ, Hampson DJ. 1995. *Infect Immun* 63:3705–3710.
Trott DJ, Stanton TB, Jensen NS, et al. 1996a. *Int J Syst Bacteriol* 46:206–215.
Trott DJ, Huxtable CR, Hampson DJ. 1996b. *Infect Immun* 64:4648–4654.

Trott DJ, Stanton TB, Jensen NS, et al. 1996c. *FEMS Microbiol Lett* 142:209–214.
Trott DJ, Jensen NS, Saint Girons I, et al. 1997. *J Clin Microbiol* 35:482–485.
Trott DJ, Mikosza ASJ, Combs BG, et al. 1998. *Int J Syst Bacteriol* 48:659–668.
Trott DJ, Alt DP, Zuerner RL, et al. 2001. *Anim Health Res Rev* 2:19–30.
Vande Maele L, Heyndrickx M, Maes D, et al. 2016. *J Vet Med Sci* 78:325–328.
Wanchanthuek P, Bellgard MI, La T, et al. 2010. *PLoS One* 5(7):e11455.
Warneke H, Kinyon J, Burrough E, et al. 2013. In Proceedings of the 6th International Conference on Colonic Spirochaetal Infections in Animals and Humans, p. 43.
Warneke HL, Kinyon JM, Bower LP, et al. 2014. *J Vet Diagn Invest* 26:635–639.
Waters WR, Hontecillas R, Sacco RE, et al. 2000a. *Immunology* 101:333–341.
Waters WR, Pesch BA, Hontecillas R, et al. 2000b. *Vaccine* 18:711–719.
Weissenböck H, Maderner A, Herzog AM, et al. 2005. *Vet Microbiol* 111:67–75.
Whipp SC, Harris DL, Kinyon JM, et al. 1978. *Am J Vet Res* 39:1293–1296.
Whipp SC, Robinson IM, Harris DL, et al. 1979. *Infect Immun* 26:1042–1047.
Wilberts BL, Arruda PH, Kinyon JM, et al. 2014a. *Vet Pathol* 51:1096–1108.
Wilberts BL, Arruda PH, Kinyon JM, et al. 2014b. *PLoS One* 9:e114741.
Wilberts BL, Arruda PH, Warneke HL, et al. 2014c. *Res Vet Sci* 97:341–347.
Wilberts BL, Warneke HL, Bower LP, et al. 2015. *J Vet Diagn Invest* 27:41–46.
Wilcock BD, Olander HJ. 1979a. *Vet Pathol* 16:450–465.
Wilcock BD, Olander HJ. 1979b. *Vet Pathol* 16:567–573.
Willems H, Reiner G. 2010. *Berl Munch Tierarztl Wochenschr* 123:205–209.
Windsor EN, Simmons JR. 1981. *Vet Rec* 122:482–484.
Witchell TD, Coutts SA, Bulach DM, et al. 2006. *Infect Immun* 74:3271–3276.
Wood EN, Lysons RJ. 1988. *Vet Rec* 121:277–279.
Zhang P, Duhamel GE. 2002. In Proceedings of the 17th International Pig Veterinary Society Congress, p. 188.
Zhang P, Witters NA, Duhamel GE. 1999. *Adv Exp Med Biol* 473:191–197.
Zuerner RL, Stanton TB, Minion FC, et al. 2004. *Anaerobe* 10:229–237.

63

Tuberculosis

Suelee Robbe-Austerman and Charles O. Thoen

Relevance

Tuberculosis (TB) continues to cause significant economic losses to swine producers throughout the world. TB due to *Mycobacterium tuberculosis* (human), *Mycobacterium bovis* (cattle), *Mycobacterium caprae* (goat), and other members of the closely related *Mycobacterium tuberculosis* complex (MTBC) has not been a concern in commercial swine production in developed countries for many years. However tuberculous lesions continue to be reported in the cervical and mesenteric lymph nodes of swine during meat inspection, primarily due to the ubiquitous *Mycobacterium avium* complex (MAC) of organisms. Gross and microscopic lesions are similar for all tubercular mycobacteria in swine and cannot be used to distinguish between them. While TB caused by MTBC is rarely found in commercial swine production in developed countries, feral swine and wild boar are widely identified as reservoirs and spillover hosts in various countries throughout the world including Argentina, France, Portugal, New Zealand, Spain, South Korea, South Africa, and the United States (Molokai, Hawaii) (Pesciaroli et al. 2014). In regions where MTBC are endemic in feral swine, these organisms must be considered along with MAC as causes of TB in domestic swine (Bailey et al. 2013). In countries without control programs for various members of the MTBC, endemic TB in humans, cattle, goats, or others can pose a serious risk for swine. Understanding the causative agent when investigating cases or an outbreak of TB in swine is critical, as prevention and control approaches to disease caused by MTBC or MAC organisms are significantly different.

Regardless of the causative agent, the processing of tuberculous swine carcasses is costly and results in significant economic losses. Regulations of the meat and poultry inspection program of the USDA require that unaffected portions of swine carcasses with tuberculous lesions in more than one primary site, such as cervical and mesenteric lymph nodes, be cooked at 170 °F (76.7 °C) for 30 minutes before being approved for human food (*Code of Federal Regulations*, Title 9, Sec. 311.2). The value of a cooked carcass is only about 20–25% of the value of a carcass not cooked. In processing plants where cooking facilities are not available (most high-volume modern plants), the carcass is condemned, and there is no salvage value.

There has been no direct campaign in the United States to eradicate TB in swine. However the percentage of swine with suspect tuberculous lesions at slaughter peaked in 1922 and has progressively reduced since (Table 63.1). In the United States, two major factors have reduced the number of tuberculous lesions at slaughter. The first was the campaign to eradicate bovine tuberculosis (bTB), which was started in 1917, and had successfully eradicated bTB in most areas of the United States by 1960 (Olmstead and Rhode 2004). The second major factor was the modernization of swine production. The introduction of farrowing barns and nursery facilities, which limited piglet exposure to potentially contaminated dirt and organic bedding, parallels the continued reduction of condemnation rates from the 1960s to current times. Despite the dramatic reduction in condemnation rates throughout the years, sporadic outbreaks can cause significant economic hardship to individual swine operations.

Etiology

In humans and cattle, the term tuberculosis is exclusively limited to disease caused by MTBC organisms. However, in birds and swine, TB can also be caused by MAC and occasionally other *Mycobacterium* species. The *Mycobacterium* genus contains a large number of species, 177 currently named (Euzeby 1997). The vast majority of these species are soil- and water-dwelling organisms that may act as opportunistic pathogens

Diseases of Swine, Tenth Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson.

Table 63.1 Prevalence of tuberculosis in swine in the United States as determined by inspection in abattoirs under federal supervision.

Year	Number slaughtered	Percent tuberculosis[a]	Percent condemned[b]
1912	34,966,378	4.69	0.12
1917	40,210,847	9.89	0.19
1922	34,416,439	16.38	0.20
1927	42,650,443	13.54	0.14
1932	45,852,422	11.38	0.08
1937	36,226,309	9.48	0.08
1942	50,133,871	7.96	0.026
1947	47,073,370	8.50	0.023
1952	63,823,263	4.40	0.015
1956	66,781,940	4.76	0.010
1962	67,109,539	2.25	0.008
1968	72,325,507	1.35	0.005
1972	83,126,396	0.85	0.007
1978	71,805,911	0.75	0.006
1983	79,992,743	0.41	0.003
1989	82,110,688	0.67	0.002
1995	94,490,329	0.21	0.003
2004[c]	102,707,038	0.0364	0.001
2008[c]	115,949,655	0.0178	0.0018
2016	117,833,370	0.0068	0.0005

Sources: Data compiled from USDA (1922, 1973, 1979, 1984, 1990, 1996); Feldman (1963).
[a] Includes all carcasses with evidence of tuberculosis, varying in extent from only small foci in cervical lymph nodes to generalized involvement.
[b] Includes carcasses with evidence of generalized tuberculosis.
[c] FSIS Electronic Animal Disposition Reporting System (eADRS), USDA.

under certain conditions; among these is *Mycobacterium avium* subsp. *hominissuis* (MAH), a member of the MAC. For these environmental exposure is the main mode of infection; transmission from animal to animal is generally not considered significant. Only a very few mycobacteria are specialized host-adapted pathogens where animal-to-animal transmission is the primary mode of infection for immunocompetent individuals. These include all members of the MTBC, *M. avium* subspecies *avium* (MAA) in birds, *M. avium* subspecies *paratuberculosis* (MAP) in ruminants, and *Mycobacterium leprae* in humans. There are exceptions, but documenting these has been difficult due to the slow-growing nature of mycobacteria and the difficultly in accurate subspecies characterization and genotyping. Only recently has it been possible with the advent of whole genome sequencing (WGS) and careful epidemiological investigation to document human-to-human transmission of an environmental mycobacteria, *Mycobacterium abscessus* (Bryant et al. 2016).

A review of slaughter surveillance records at the US National Veterinary Services Laboratories (NVSL) over the last 8 years (2009–2016) indicates that of the 289 swine granulomas submitted, 123 were diagnosed by histology as mycobacteriosis compatible and of the ones that were cultured, 70 (97%) were identified as MAC and 2(3%) were identified as *Mycobacterium intermedium*. This is consistent to what historically has been reported in the United States (Thoen et al. 1975).

Prior to the recent routine characterization of isolates by molecular methods, *M. avium* and closely related isolates were identified by a combination of phenotypic characteristics and biochemical tests and then, if needed, further characterized by serovars. These methods were cumbersome and time consuming. Today identification is routinely completed to the species and subspecies level using rapid and accurate methods that include hybridization probes, high performance liquid chromatography (HPLC), matrix-assisted laser desorption/ionization time-of-flight mass spectrophotometry (MALDI-TOF MS), and polymerase chain reaction (PCR) (Lin et al. 2015; Turenne et al. 2007). The characterization of DNA has replaced serology for subspecies identification, and attempts to compare the two approaches indicate poor correlation between genotypes and serovars (Frothingham and Wilson 1993). Many molecular methods

have been used to identify mycobacterial subspecies and to genotype MAC including multiplex PCR targeting insertion elements, PCR targeting large sequence polymorphisms, and partial sequencing of *rpoB* and *hsp65 genes* (Higgins et al. 2011; Semret et al. 2006; Shin et al. 2010; Turenne et al. 2006). Higher resolution genotyping methods include restriction fragment length polymorphism (RFLP), variable number tandem repeats (VNTR), and multispacer sequence typing (MST) (Cayrou et al. 2010; Komijn et al. 1999; Thibault et al. 2007). WGS has become the gold standard genotyping method, far surpassing the resolution of previous technologies since the introduction of benchtop next-generation sequencing instruments in 2011 (Köser et al. 2012).

The primary reasons for subspecies characterization and genotyping of MAC isolates recovered from swine are to help determine the source of infection, to link cases or outbreaks to a common strain, or to understand the evolutionary history of MAC. Based on comparison of sequences of 10 genes in 56 strains of genetically diverse MAC, it was proposed that MAA, *Mycobacterium avium* subsp. *sylvaticum* (MAS), and MAP each represent a recently evolved clone of genetically similar host-adapted pathogens and that they evolved from the genetically diverse group of MAH organisms that are distributed in a wide variety of environmental habitats (Turenne et al. 2008). Since then, WGS data strongly supports and extends these findings. Figure 63.1 shows an unrooted phylogenetic tree based on whole genome sequences of 104 publically available MAC isolates representing genetic and geographic diversity of the four subspecies within MAC. Branch lengths represent the genetic distance and variation of each of the subspecies. It is clear that isolates belonging to each of MAA, MAS, and MAP form tiny clusters of highly related organisms on the tree. When considered in light of the host species and geographic origin of each isolate, this strongly suggests that MAA, MAS, and MAP are specialized pathogens that have recently evolved and that are fairly constrained to their host species. In contrast all of the remaining unnamed scattered branches on the tree represent a genetically diverse group of isolates classified as MAH. This suggests that MAH is a very large genetically diverse group of organisms without host species or geographic constraints on any of the MAH branches. Isolates from pigs, humans, ruminants, and birds with broad geographic dispersion are found throughout the branches. This is not surprising since MAH are known to be widely distributed in the environment (Falkinham III 2016; Kirschner et al. 1992; Turennne et al. 2007). Most data suggests that MAS is a phenotypic variant of MAA (Turenne et al. 2007); consequently further discussions in this chapter will include both as MAA. In summary, current data suggest that MAA and MAP are specialized pathogens in birds and ruminants, respectively,

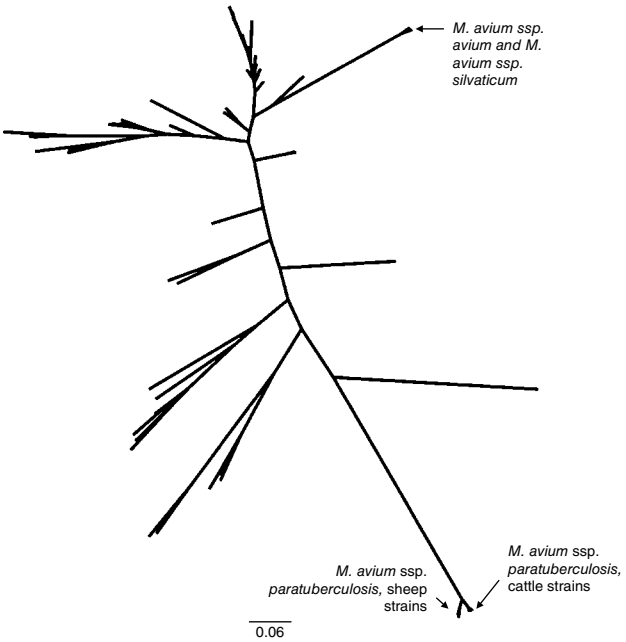


Figure 63.1 Phylogenetic tree of 104 geographically diverse *Mycobacterium avium* complex (MAC) isolates using kSNP. *Mycobacterium avium* ssp. *avium* (MAA), *M. avium* ssp. *silvaticum* (MAS), and *M. avium* ssp. *paratuberculosis* (MAP) are identified on the tree. All other branches are classified as *M. avium* ssp. *hominissuis* (MAH). These data support the current evidence that MAH is an environmental organism with a great deal of genetic diversity. On the other hand, MAP and MAA appear to be genetically bottlenecked, a pattern seen in other obligate pathogens with clear evidence of animal-to-animal transmission.

and are not widespread in the environment. In contrast, the etiological agent primarily responsible for swine TB, MAH, is ubiquitous in the environment, and outbreaks or increases in prevalence of swine TB are likely due to greater exposure to environments and/or environmental conditions where MAH thrive.

Public health

The MAC, especially MAH, cause disseminated disease or pneumonia in AIDS patients, less frequently in organ transplantees, and in other immunocompromised individuals (Abad and Razonable 2016). The incidence of MAC in humans has declined and is directly correlated with effective HIV treatment (Collins et al. 2016). While sporadic infections do occur and can be very serious, MAC are commonly identified as contaminants or incidental findings in the culture laboratory, and consequently MAC diagnosis may be overestimated (Sultani et al. 2016). Risk factors for infection in humans include immune compromise, chronic pulmonary disease, and advanced age. Chlorination of water delivery systems provides a preferential environment for proliferation of

MAC and other nontuberculous mycobacteria in biofilms (Falkinham III 2016). To date there is no data that implicates exposure to infected swine or consumption of pork as an increased risk for human infection.

Epidemiology

The epidemiology of TB in swine is dependent on the distribution of the offending mycobacterial species. Because MTBC and MAA are primarily host-adapted animal pathogens, the prevalence and geographic distribution of swine TB caused by these organisms directly correlate with the geographic distribution, prevalence, and infection rate of the mycobacterial animal host species. Unlike host-adapted tubercular mycobacteria, MAH is an ubiquitous environmental organism that is distributed throughout the world, infecting and establishing disease opportunistically when swine and other animals are susceptible either by an overwhelming dose or by a suppressed immune system.

The primary sources of information on the prevalence and geographic distribution of TB in pigs are the data obtained from meat inspection records. In the United States during 1922, 16.38% of all swine slaughtered under federal supervision had tuberculous lesions, and in 0.2% the disease was so extensive that the entire carcass was condemned (Table 63.1). Since 1922 there has been a gradual decline. The prevalence by 2008 and 2016 had decreased to 0.0178 and 0.0068%, respectively, with only 0.0018 and 0.0005% having evidence of generalized TB. The eradication of *M. bovis* is not likely the only or even primary cause of the reduction in tuberculous lesions found at slaughter. *M. bovis* has not been detected in domestic pigs during slaughter surveillance in the United States for at least 27 years (data is not available for prior years), yet the prevalence of TB in swine has continued to decrease during the same time period. This significant reduction in MAC-associated TB directly correlates with the modernization of swine production where pigs in environmentally controlled housing have limited exposure to soil, standing water, and organic bedding. Indeed, numerous reports have shown that when production systems include sawdust, wood shavings, straw, and/or peat, the incidence of TB caused by MAC increases (Álvarez et al. 2011; Matlova et al. 2004, 2005).

Sporadic outbreaks of MAC-associated TB have been reported on farms that have not been associated with organic bedding or feeding peat; however, published data describing these outbreaks is limited. One extensive cross-sectional study in Okinawa, Japan, was not able to identify specific risk factors associated with size, density, location of farm, or all-in/all-out practices (Hibiya et al. 2010). This highlights the challenges faced by veterinarians when investigating these outbreaks. Identifying risk factors or pinpointing likely sources can be difficult with retrospective studies or investigations because of the chronic nature of TB and the lack of clinical disease. The time from exposure to detection at slaughter may be several weeks or even months.

Sources of infection

Mycobacterium tuberculosis Complex (MTBC)

Pigs are highly susceptible to *M. bovis and M. tuberculosis* (Francis 1958), as well as other MTBC. *M. bovis* is not a frequent cause of TB in swine in localities where the disease in cattle is controlled by a campaign of eradication. Where *M. bovis* is endemic in cattle, the infection may be transmitted to swine by the feeding of unpasteurized milk and dairy by-products. Oral fluids and feces of tuberculous cattle or wildlife may contain viable *M. bovis*, which provides an obvious hazard where swine and cattle are maintained in a common pen or field. The practice of feeding swine the offal from abattoirs or uncooked garbage is unwise, because such material may contain tuberculous material from beef carcasses. Fichandler and Osborne (1966) described an epidemic of TB in a herd of swine in Connecticut that was fed improperly cooked offal from tuberculous cattle. Other MTBC organisms also infect swine. *M. caprae*, which is closely related to *M. bovis* and distributed throughout Europe and Asia, readily infects swine (Sabrina et al. 2011). *Mycobacterium microti*, endemic in wild rodents in the United Kingdom and Europe, has rarely been identified in swine at slaughter (Taylor et al. 2006).

Mycobacterium avium subsp. *avium* (MAA)

Experimental evidence suggests that MAA and MAH are similarly pathogenic to swine (Agdestein et al. 2012). Avian TB caused by MAA in commercial poultry was historically considered a significant source of swine TB. Due to the change in production systems of both poultry and swine, MAA incidence in poultry is lower, and exposure of infected poultry and litter to swine is less likely (González et al. 2002). Wild birds have an estimated MAA prevalence of 0.3–1.7% (Friend 1999) and are also a risk factor for MAA in swine (Alfredsen and Skjerve 1993). Currently MAA is an infrequent isolate recovered from swine slaughter surveillance cases in the United States. When MAA is confirmed as the cause of swine TB, control of potential domestic and/or wild bird tissue and/or fecal sources is indicated since it is a host-adapted pathogen and long-term establishment in the environment would not be expected.

Mycobacterium avium subsp. *hominissuis* (MAH)

The vast majority of isolates recovered from swine slaughter surveillance cases in the United States are MAH, suggesting that environmental sources rather

than birds are most common. However the role of birds in MAH epidemiology is poorly understood. Birds can be infected with MAH, and the prevalence of infection is unknown. It is also unknown whether direct transmission of MAH from birds to pigs occurs with any significance. Further confounding the potential association of MAH between birds and pigs is the impact of avian feces and debris, as this material may enhance the proliferation of MAH in the environment.

Because MAH is ubiquitous and likely exists within the vast majority of swine facilities with little to no TB prevalence, the cause of MAH outbreaks is likely due to the quantity (dose), timing of exposure, or both. Organic beddings such as wood shavings and peat are well-documented risk factors for TB in swine, and there is good evidence they provide an ideal environment for mycobacterial proliferation (Pakarinen et al. 2007). The distribution of environmental mycobacteria is far from random. They are more prevalent in environments that are damp, warm, low in pH, low in dissolved oxygen, high in soluble zinc, high in humic acid, and high in fulvic acid (Kirschner et al. 1992). Generally MAH proliferates in areas where there is active biodegradation of organic matter rather than in sterile soils. Biofilm production by MAH is also well documented (Johansen et al. 2009). This characteristic protects the organism from desiccation, disinfectants, and mechanical removal from pipes, tanks, and other colonized surfaces. Survival and proliferation in potable water systems is typical of MAH and is enhanced by the use of chorine and other water disinfectant treatments due to the removal of competing organisms (Falkinham III 2016). While swine TB caused by MAH due to contaminated drinking water has not been confirmed in the literature, it should be considered as a possibility in outbreaks in modern swine facilities.

Age of MAH exposure appears to impact the severity of lesions at slaughter. Piglets experimentally infected as early as 4 weeks of age can have significant lesions at slaughter. In one of the larger experimental infection studies, pigs infected at 8 weeks of age (slaughtered 17 weeks post infection) had the most severe lesions at slaughter followed by pigs at infected at 4 weeks of age and slaughtered 18.5 weeks later. Pigs infected later in life, 12, 16, and 20 weeks of age, and slaughtered 12, 10.5, and 6 weeks later, respectively, had fewer lesions (Acland and Whitlock 1986).

The data supporting significant pig-to-pig transmission of MAH is somewhat controversial. In natural outbreaks where higher resolution genotyping such as RFLP was conducted, the diversity in genotypes within outbreaks did not support clonal transmission of a single genotype (Matlova et al. 2004). Furthermore multiple strains of MAH can readily be found within individual pigs, suggesting environmental exposure (Wellenberg et al. 2010). Finally the highly diverse pattern of MAH displayed in the WGS phylogenetic tree (Figure 63.1) does not support clustering associated with long-term pig-to-pig transmission. Findings have been mixed in experimental transmission studies. Acland and Whitlock (1986) reported limited transmission evidenced by few microscopic lesions and no gross lesions between contacts and experimentally infected pigs. However, in other studies, contact pigs housed with orally inoculated pigs developed gross as well as microscopic lesions (Ray et al. 1972; Thoen et al. 1976b), confirming significant lateral transmission under the conditions of the experiments. There is good evidence that pigs infected with MAH shed the organism in their feces (Agdestein et al. 2014), thus augmenting environmental contamination. In Norway maintaining sows and slaughter pigs in the same pen was a risk factor for tuberculous lesions at slaughter (Alfredsen and Skjerve 1993), suggesting a role for lateral transmission from chronically infected sows. It is unknown whether the amount of MAH shed by infected pigs is high enough to reach a clinically significant threshold without environmental replication and whether the amount shed is sufficient to increase the amount already present through environmental replication to a threshold dose needed for disease. The current data, although far from settled, suggests that pigs play a limited role in the transmission of MAH.

Other mycobacteria infecting swine

Various species of mycobacteria other than MAC and MTBC have been isolated from swine and other animals. Swine appear to acquire mycobacteria that are representative of what is contained within their environment. For example, in South Texas, 98 feral pigs were examined and cultured; no MTBC were found, but 65/98 were culture positive for non-MTBC mycobacteria, and of the 15 identified, none were MAA, one was MAH, and 14 were miscellaneous other environmental mycobacteria (Campbell et al. 2011; Higgins et al. 2011). It is common to culture mycobacteria from morphologically normal lymph nodes of pigs; consequently isolating mycobacteria without supportive microscopic lesions of TB may or may not be significant. Finally there are several reports of culture of MAP from pig tissue, although it does not appear to be common and is most often the result of being co-located with cattle (Miranda et al. 2011).

Pathogenesis

The development of disease in swine depends on the ability of the tubercle bacillus to multiply within tissues of the host and to induce a host response. Although acid-fast bacilli initially encounter granulocytes and humoral components, activated mononuclear macrophages are

considered to be more important in protection of the host against mycobacteria (Olsen et al. 2010).

The capacity of MAC to produce progressive disease may be related to certain complex lipids present in the cell wall, such as the glycopeptidolipids (previously referred to as C-mycosides) localized in the exterior portion of the cell envelope (Rastogi and Barrow 1994). However, it appears that the effect of these components alone or together on phagolysosome fusion cannot fully account for virulence. Available information suggests that a combination of toxic lipids and factors released by virulent tubercle bacilli may cause disruption of the phagosome, interfere with phagolysosome formation, alter the release of hydrolytic enzymes from the attached lysosomes, and/or inactivate the lysosomal enzymes released into the cytoplasmic vacuole (Thoen et al. 2006). Some MAC strains are susceptible to bactericidal mechanisms of macrophages; however, the importance of reactive nitrogen intermediates and oxygen radicals in macrophages of animals exposed to virulent tubercle bacilli remains to be elucidated (Thoen et al. 2009). A detailed review of TB granuloma formation and its importance in the disease process has recently been published (Ramakrishnan 2012). Granulomas begin to appear after the adaptive immune system is stimulated, typically 2–3 weeks post inoculation. This is consistent with other work showing sensitized lymphocytes and detectable mycobacterial antibodies starting at 14–28 days post exposure to *M. avium* or *M. bovis* (Muscoplat et al. 1975; Thoen et al. 1979).

Clinical signs

TB in swine is usually subclinical; consequently, swine producers usually become aware of it when contacted by the slaughter plant with news of unusually high condemnation rates. Generally, tuberculous lesions are limited to small foci in a few lymph nodes associated with the digestive tract. Nonspecific clinical signs are sometimes observed in pigs with generalized TB, but they are not sufficient to suggest TB. Abortions and wasting due to MAH have been reported in rare cases of generalized disease (Eisenberg et al. 2012; Wellenberg et al. 2010).

Lesions

As seen in abattoirs in developed countries, tuberculous lesions in swine are usually limited to lymph nodes of the cervical and mesenteric regions. The lesions vary in appearance from small, yellowish white, caseous foci, a few millimeters in diameter to diffuse enlargement of the entire node (Figure 63.2). The disease may be localized in one group of nodes or may involve a number of lymph nodes along the digestive tract. The frequent occurrence of MAC in lesions limited to the cervical lymph nodes in naturally infected swine indicates that infection usually occurs by ingestion. In a study of lesion distribution in 81 pigs with tuberculous lesions at slaughter, 25 (31%) had lesions in lymph nodes in 1 of 3 sites (head, mesenteric, or inguinal), 53 (65%) in 2 of these sites, and 3 (4%) in all 3 sites (Matlova et al. 2005).

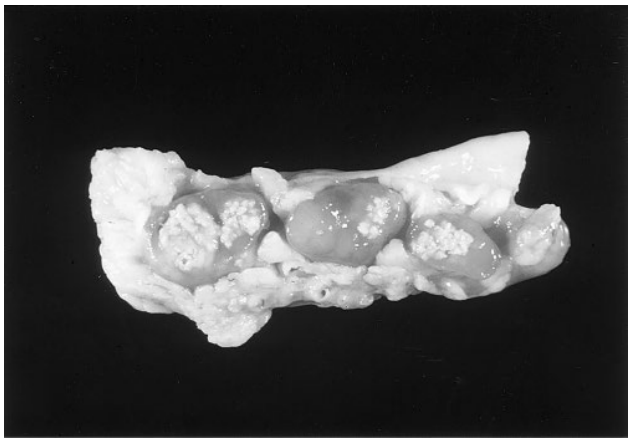


Figure 63.2 Tuberculous lesions in a mesenteric lymph node of a pig at slaughter.

Disseminated disease is relatively uncommon in swine, but is more likely with *M. bovis* (MTBC) than with MAH or MAA (MAC) (Francis 1958) and can still be observed in countries where MTBC or MAA are endemic in their host species. Feldman (1938) in an abattoir study of naturally occurring TB in swine showed that 30% of *M. bovis* cases were disseminated compared with only 0.0003% of *M. avium* cases. Distribution of gross lesions in disseminated disease is similar for both *M. bovis* and MAH or MAA. Lymph nodes of the head, neck, and mesentery are the most commonly affected, followed by liver, spleen, lung, other lymph nodes, bones, and notably the uterus and mammary glands. Infected mammary glands are the source of organisms that are shed in bovine milk of tuberculous cows and must be considered a possible source, in addition to fecal shedding, of transmission from chronically MTBC- or MAC-infected sows.

While gross differences between tuberculous lesions caused by MAC and MTBC are not specific enough to allow definitive differentiation, some features are generally more characteristic of each. MTBC typically cause discrete well-formed encapsulated tubercles, while MAC form focal, predominantly histiocytic infiltrates with occasional necrosis that blend at the margins with surrounding tissue architecture. In an infection with MAC, the lymph nodes may be enlarged and firm with no discrete purulent foci, or there may be one or more foci of caseous necrosis with indistinct borders. Mineralization is seldom demonstrable, except in very chronic lesions.

The cut surface of the lesion has a homogeneous or slightly nodular appearance with a few caseous foci. Relatively large areas of caseation may be present and occasionally will involve the entire lymph node. Although there may be diffuse fibrosis, there is typically little encapsulation of necrotic foci. As a result, the focal lesions due to MAC are not easily enucleated. In contrast, focal lesions due to MTBC tend to be discrete, caseous, mineralized, and well encapsulated and are relatively easy to separate from the surrounding tissue. These distinctions are by no means absolute, and there are many variations in the gross appearance of tuberculous lesions in lymph nodes of swine.

Microscopic lesions in MAC infections in lymph nodes and in other tissues include multifocal-to-coalescing infiltration by epithelioid and multinucleate Langhans and foreign body-type macrophages. There may be some necrosis and mineralization, especially in advanced lesions, but mineralization is not usually prominent. Fibrosis is variably observed, but encapsulation of lesions is uncommon. Acid-fast stains reveal very few organisms in the cytoplasm of macrophages. Similar changes are observed in sows and slaughter pigs (Thoen et al. 1976a). Lesions in MTBC infection have similar distribution and character, but tend toward earlier and more caseous necrosis, more distinct granuloma formation rimmed by epithelioid macrophages, fewer multinucleate cells, more mineralization, and fibrous encapsulation of granulomas (Figure 63.3; Karlson and Thoen 1971). However, consistent histopathological differentiation between lesions caused by MTBC and MAC is not possible. Furthermore, coinfections of MAC and MTBC have been reported (Barandiaran et al. 2015).

Of note is *Rhodococcus equi* that can cause granulomas in submaxillary lymph nodes of slaughter-age swine that are grossly indistinguishable from MAC or MTBC. Komijn et al. (2007) found that of granulomas detected predominantly in submaxillary lymph nodes of 0.75% of slaughter pigs in the Netherlands, *R. equi* and not MAC could be cultured from 45%. *R. equi*, like MAC, can also be routinely cultured from lymph nodes without lesions. An abattoir study from Poland reported that 26.6% of 395 submandibular lymph nodes without lesions were culture positive for *R. equi* (Witkowski et al. 2016).

Diagnosis

Antemortem testing

A clinical diagnosis of TB in swine is usually not possible since most cases will be asymptomatic or present with only nonspecific malaise. Animals with disseminated disease may have granulomas on the liver and other organs that can be visualized with ultrasound. There are several options for antemortem diagnostics available, either focused on the humoral (serology) or cell-mediated immune response.

Within the last few decades, several ELISAs for MAC in swine have been evaluated, and some have recently been commercialized (although not sold in the United States) for use in serum and in meat juice. Examples include PrioCHECK™ *M. avium* Antibody ELISA Kit (Thermo Fisher Scientific, Inc., Waltham, MA) and ID Screen™ *Mycobacterium avium* (IDvet, Grabels, France). A lateral flow assay has also been evaluated for MTBC in the wild boar (Boadella et al. 2011). The available data estimating the sensitivity and specificity of these assays are limited, but they appear to perform similarly in pigs as they do in other species known to have a fairly strong humoral response to mycobacterial infections. Specificities range from 92 to 95% with sensitivities from

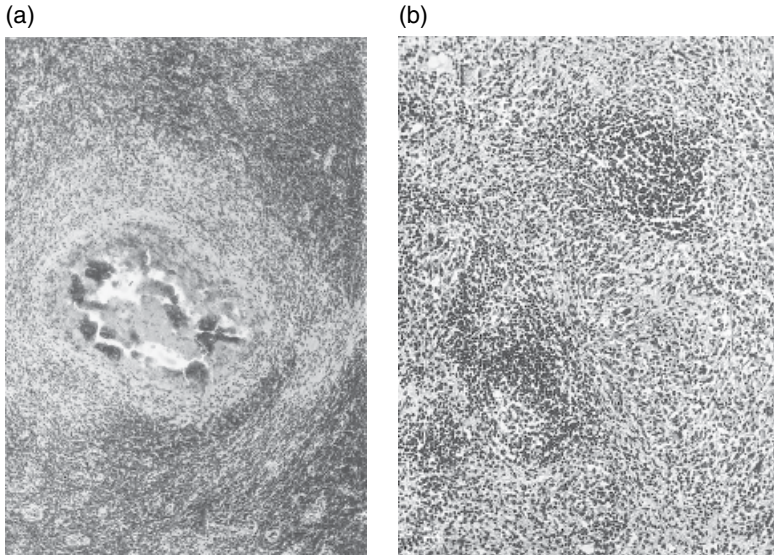


Figure 63.3 Tuberculous changes in cervical lymph nodes in swine. (a) Mammalian tubercle bacillus infection. Peripheral fibrosis, necrosis, and calcification are typical of lesions due to bovine or human types of tubercle bacilli (H&E ×40). (b) *Mycobacterium avium* infection. Diffuse cellular infiltration with little necrosis (H&E ×95).

50 to 70%, sufficient for use on a herd or population basis but problematic in low prevalence populations and insufficient for use in individuals (Wisselink et al. 2010).

Classical assays exploiting the cell-mediated immune system have also been evaluated including the intradermal (skin) test and the gamma interferon ELISA, but in most places only the skin test is readily available (Faldyna et al. 2012; Stepanova et al. 2011). There is very little recent work published on the skin test, but the test has been used for many years. Because it is a required test for export to several countries, most agriculture ministries make purified protein derivatives (PPDs) from the various *Mycobacterium* species important in that country/region available for testing, along with instructions for use and interpretation. For the United States, PPDs are available from NVSL, Ames, IA. In pigs, 0.1 mL is injected intradermally in the skin on the back of the ear, and the test is read 48 hours later. A positive test is indicated by focal swelling and redness and occasionally even hemorrhage and/or ulceration. The skin test is recommended for use on a herd, not individual, basis as false-positive and false-negative tests may occur (Payeur and Songer 1992).

Post mortem testing

Gross lesions of granulomatous lymphadenitis or disseminated granulomas are sufficient for TB to be included as a possible cause, but are not sufficiently specific for a definitive diagnosis. *R. equi* and other bacteria can cause granulomatous lymphadenitis grossly and microscopically indistinguishable from TB, and *Trueperella pyogenes* is a common cause of disseminated encapsulated abscesses in swine that can easily be confused with generalized TB. Characteristic gross and microscopic lesions with demonstration of acid-fast organisms allow a strong presumptive diagnosis of TB. An unequivocal diagnosis requires confirmation of the *Mycobacterium* spp. in tissues with lesions by culture and identification or by direct PCR (Miller et al. 1999).

Sampling for an outbreak investigation

WGS of isolated MAC gives veterinarians unprecedented resolution to identify and recognize potential sources of an outbreak; consequently it is worth discussing how to properly sample animals and the environment for mycobacterial culture. First, it is important to characterize the outbreak occurring in the animals, so tissues with lesions from at least 3–5 animals should be cultured. Sampling and combining multiple lesions into one pooled culture for each animal will help to economically identify coinfections of different strains within the animal. The pens should be investigated for areas of decomposing; damp organic matter and samples of 25 g each should be collected and frozen or refrigerated and shipped on ice to a testing laboratory within 4 days. If feed is a suspected source, 25 g samples of feed from multiple areas of the lot should be collected. Finally two 1 L samples of each water source should be collected, as well as sampling any area of chronic moisture where biofilms may have formed. This may include areas in the pens with evident algae/mold or areas with algae/mold in a water-holding tank, water line, or other component of the water delivery system. Sampling the biofilm is critical. Where there is algae/mold, a clean brush can be used to scrub the area in order to loosen the scale and algae, then collect that material and either freeze or refrigerate, and ship within 4 days. Submit all samples to a laboratory specializing in mycobacterial culture.

Prevention and control

The known epidemiology of the detected *Mycobacterium* spp. will direct efforts at prevention and control. For host-adapted *Mycobacterium* spp. (MTBC and MAA), implementation or increasing stringency of biosecurity measures aimed at preventing contact with the host (including bird-proofing buildings, feed, and water sources), host feces (bedding, contaminated lagoon surface water, etc.), or their by-products used as feed ingredients (meat and bone meal, fat, etc.) should successfully control the disease. For TB caused by environmental mycobacteria such as MAH, the solutions can be much more challenging. Wood shavings, sawdust, and peat should be avoided for use as bedding. While straw is reported to be associated with tuberculous lesions, it is not mentioned in the literature as often, so it may be a superior choice if bedding material is required. Standing water that supports the decomposition of organic matter creating "swampy areas" should be cleaned up or fenced off. Water delivery systems should be evaluated for evident biofilms, and the biofilms removed, followed by disinfection (see below). Timing of exposure matters, and for market hogs, the older the pigs are past 8 weeks when significantly exposed, the less likely and severe the lesions. Therefore, reducing risk factors for younger pigs is more impactful than for older pigs in minimizing carcass condemnations at slaughter. In most outbreaks, attention to these factors eliminates or greatly reduces slaughter condemnations. However, in some confined herds with few apparent risk factors, it may be difficult to consistently reduce condemnations. In these herds it may be useful to skin-test sows and cull positives, suggesting that recontamination of the environment by chronically infected sows may sometimes be significant in initiating new infections/outbreaks.

Appropriate tuberculocidal disinfectants should be employed. All organic matter should be physically removed, and appropriate contact times as indicated on the product labels should be followed. The most useful products for premises use are phenol based, such as One-Stroke Environ™, Tek-Trol™, and Pheno-Tek II™. These products tend to maintain effectiveness in the presence of limited organic matter. Biofilm production by MAH can provide robust protection against most disinfectants, so mechanical removal of any evident biofilm is critical prior to disinfection. Even with the best disinfectants, only a reduction in numbers of bacteria will be achieved. Consequently disinfecting without fundamentally changing the environment to prevent the regrowth of MAH is bound to fail.

The swine industries in developed countries have achieved great success in controlling and preventing TB. With the more recent realization that sporadic outbreaks of carcass condemnations are the result of MAH, an environmental mycobacterium, unique challenges to control are evident. Gaps in knowledge certainly exist, and understanding risk factors associated with changes in productions systems as the industry evolves will require sound epidemiology and applied research. This research will be best employed by a partnership between veterinarians and producers who encounter and identify prevalence changes and researchers capable of identifying species and strains of tubercular mycobacteria. As consumer preferences continue to impact types of production systems, environmental risks may change, and veterinarians and producers may need to balance carcass condemnation rates with evolving housing and husbandry practices.

References

Abad CL, Razonable RR. 2016. *J Clin Tuber Other Mycobact Dis* 4:1–8.

Acland HM, Whitlock RH. 1986. *J Comp Pathol* 96:247–266.

Agdestein A, Johansen TB, Kolbjørnsen O, et al. 2012. *BMC Vet Res* 8:11.

Agdestein A, Olsen I, Jørgensen A, et al. 2014. *Vet Res* 45:46.

Alfredsen S, Skjerve E. 1993. *Prev Vet Med* 15:253–259.

ÁLvarez J, Castellanos E, Romero B, et al. 2011. *Epidemiol Infect* 139:143–148.

Bailey SS, Crawshaw TR, Smith NH, et al. 2013. *Vet J* 198:391–397.

Barandiaran S, Pérez AM, Gioffré AK, et al. 2015. *Epidemiol Infect* 143:966–974.

Boadella M, Lyashchenko K, Greenwald R, et al. 2011. *J Vet Diagn Invest* 23:77–83.

Bryant JM, Grogono DM, Rodriguez-Rincon D, et al. 2016. *Science* 354:751–757.

Campbell TA, Long DB, Bazan LR, et al. 2011. *J Wildl Dis* 47:974–978.

Cayrou C, Turenne C, Behr MA, et al. 2010. *Microbiology* 156:687–694.

Collins L, Clement M, Stout J. 2016. *Open Forum Infect Dis* 3(suppl 1):579–579.

Eisenberg T, Volmer R, Eskens U, et al. 2012. *Vet Microbiol* 159:69–76.

Euzeby JP. 1997. *Int J Syst Bacteriol* 47:590–592.

Faldyna M, Gopfert E, Kudlackova H, et al. 2012. *J Vet Diagn Invest* 24:376–378.

Falkinham III JO. 2016. *Clin Microbiol News Lett* 38:1–7.

Feldman WH. 1938. *J Am Vet Med Assoc* 92:681.

Feldman WH. 1963. Tuberculosis. In TG Hull, ed. *Diseases Transmitted from Animals to Man*, 5th ed. Springfield, IL: Charles C Thomas.

Fichandler PD, Osborne AD. 1966. *J Am Vet Med Assoc* 148:167–169.

Francis J. 1958. *Tuberculosis in Animals and Man: A Study in Comparative Pathology*. London: Cassell, p. 177.

Friend M. 1999. Tuberculosis. In Friend M, Franson JC, eds. *Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds*. Reston: USGS, pp. 93–98.

Frothingham R, Wilson KH. 1993. *J Bacteriol* 175:2818–2825.

González M, Rodriguez-Bertos A, Gimeno I, et al. 2002. *Avian Dis* 46:1055–1061.

Hibiya K, Kazumi Y, Nishiuchi Y, et al. 2010. *Comp Immunol Microbiol Infect Dis* 33:401–421.

Higgins J, Camp P, Farrell D, et al. 2011. *BMC Vet Res* 7:77.

Johansen TB, Agdestein A, Olsen I, et al. 2009. *BMC Microbiol* 9:159.

Karlson AG, Thoen CO. 1971. *Am J Vet Res* 32:1257–1261.

Kirschner RA Jr, Parker BC, Falkinham III JO. 1992. *Am Rev Respir Dis* 145(2 Pt 1):271–275.

Komijn RE, de Haas PE, Schneider MM, et al. 1999. *J Clin Microbiol* 37:1254–1259.

Komijn RE, Wisselink HJ, Rijsman VM, et al. 2007. *Vet Microbiol* 120:352–357.

Köser CU, Ellington MJ, Cartwright EJP, et al. 2012. *PLoS Pathog* 8:e1002824.

Lin CS, Su CC, Hsieh SC, et al. 2015. *J Microbiol Immunol Infect* 48:205–212.

Matlova L, Dvorska L, Palecek K, et al. 2004. *Vet Microbiol* 102:227–236.

Matlova L, Dvorska L, Ayele WY, et al. 2005. *J Clin Microbiol* 43:1261–1268.

Miller JM, Jenny AL, Ellingson JL. 1999. *J Vet Diagn Invest* 11:436–440.

Miranda C, Matos M, Pires I, et al. 2011. *Food Res Int* 44:3276–3277.
Muscoplat CC, Thoen CO, Chen AW, et al. 1975. *Am J Vet Res* 36:1167–1171.
Olmstead AL, Rhode PW. 2004. *J Econ Hist* 64:734–772.
Olsen I, Barletta RG, Thoen CO. 2010. Mycobacterium. In Gyles CL, Prescott JF, Songer G, et al., eds. *Pathogenesis of Bacterial Infections in Animals*. Hoboken, NJ: Wiley-Blackwell, pp. 113–132.
Pakarinen J, Nieminen T, Tirkkonen T, et al. 2007. *Vet Microbiol* 120:105–112.
Payeur JB, Songer JG. 1992. Mycobacteriosis (tuberculosis) in swine. In Cole JR, Hall RF, Peters J, et al., eds. *Pork Industry Handbook*. East Lansing, MI: Michigan State University Cooperative Extension Service.
Pesciaroli M, Alvarez J, Boniotti MB, et al. 2014. *Res Vet Sci* 97(suppl):S78–S85.
Ramakrishnan L, 2012. *Nat Rev Immunol* 12:352–366.
Rastogi N, Barrow WW. 1994. *Res Microbiol* 145:243–252.
Ray JA, Mallmann VH, Mallmann WL, et al. 1972. *Am J Vet Res* 33:1333–1345.
Sabrina R, Javier B, Beatriz R, et al. 2011. *Emerg Infec Dis* 17:532.
Semret M, Turenne CY, de Haas P, et al. 2006. *J Clin Microbiol* 44:881–887.
Shin SJ, Lee BS, Koh WJ, et al. 2010. *J Clin Microbiol* 48:4057–4062.
Stepanova H, Pavlova B, Stromerova N, et al. 2011. *Vet Immunol Immunopathol* 142:107–112.
Sultani F, Barnes K, Jepson A, et al. 2016. *Eur Resp J* 48(suppl):60.
Taylor C, Jahans K, Palmer S, et al. 2006. *Vet Rec* 159:59–60.
Thibault VC, Grayon M, Boschiroli ML, et al. 2007. *J Clin Microbiol* 45:2404–2410.
Thoen CO, Jarnagin JL, Richards WD. 1975. *Am J Vet Res* 36:1383–1386.
Thoen CO, Himes EM, Weaver DE, et al. 1976a. *Am J Vet Res* 37:775–778.
Thoen CO, Johnson DW, Himes EM, et al. 1976b. *Am J Vet Res* 37:177–181.
Thoen CO, Armbrust AL, Hopkins MP. 1979. *Am J Vet Res* 40:1096–1099.
Thoen CO, Barletta RG, Thoen CO, et al. 2006. Pathogenesis of *Mycobacterium bovis*. In Thoen CO, Steele JH, Gilsdorf MJ, eds. *Mycobacterium bovis Infection in Animals and Humans*. Hoboken, NJ: Wiley-Blackwell Publishing, pp. 18–33.
Thoen CO, Lobue PA, Enarson DA, et al. 2009. *Vet Ital* 45:135–181.
Turenne CY, Semret M, Cousins DV, et al. 2006. *J Clin Microbiol* 44:433–440.
Turenne CY, Wallace R, Behr MA. 2007. *Clin Microbiol Rev* 20:205–209.
Turenne CY, Collins DM, Alexander DC, et al. 2008. *J Bacteriol* 190:2479–2487.
USDA. 1922. *Yearbook of Agriculture*. Washington, DC: Washington Government Printing Office.
USDA. 1973. Statistical Summary. Federal Meat and Poultry Inspection for Calendar Year 1972. MPI-1.
USDA. 1979. Statistical Summary. Federal Meat and Poultry Inspection for Calendar Year 1978. MPI-1.
USDA. 1984. Statistical Summary. Federal Meat and Poultry Inspection for Calendar Year 1983. MPI-1.
USDA. 1990. Statistical Summary. Federal Meat and Poultry Inspection for Calendar Year 1989. MPI-1.
USDA. 1996. Statistical Summary. Federal Meat and Poultry Inspection for Calendar Year 1995.
Wellenberg GJ, de Haas PEW, van Ingen J, et al. 2010. *Vet Rec* 167:451–454.
Wisselink HJ, van Solt-Smits CB, Oorburg D, et al. 2010. *Vet Microbiol* 142:401–407.
Witkowski L, Rzewuska M, Takai S, et al. 2016. *BMC Microbiol* 16:98.

64

Miscellaneous Bacterial Infections

André Broes, David J. Taylor, and Guy-Pierre Martineau

Actinobaculum (Eubacterium) suis

Relevance, etiology, and public health

Actinobaculum suis causes cystitis and pyelonephritis in individual or small groups of sows and is carried by boars. It is not a public health concern. *A. suis* was earlier classified as *Corynebacterium suis*, *Actinomyces suis*, and *Eubacterium suis* (Lawson et al. 1997; Soltys and Spratling 1957; Yassin et al. 2015).

A. suis is a gram-positive pleomorphic rod, 2–3 µm long and 0.3–0.5 µm wide, and it occurs as "Chinese letters" and palisades in smears of infected tissues. It is nonmotile, does not form spores, and grows on blood agar under anaerobic conditions. Colonies are 2–3 mm and nonhemolytic after 2 days; then they flatten and develop a characteristic dry, gray, opaque surface with a crenated edge attaining a size of 4–5 mm in 5–6 days. Growth is enhanced by the addition of urea to media with a final concentration of 1.2% (w/v). The organism produces urease.

Epidemiology and pathogenesis

A. suis-associated urinary tract disease in sows has been reported in North and South America, Europe, Asia, and Australia. The pig is the main host of *A. suis* and most male pigs, aged 6 months or more, harbor *A. suis* in the preputial diverticulum. The organism may be found on the floors of pens occupied by male pigs, and uninfected males are readily infected when they are housed with carriers (Jones and Dagnall 1984). It is rarely isolated from the vagina of healthy females. Carr and Walton (1990) isolated *A. suis* from footwear of handlers working with boars.

Urinary tract infection is by the ascending route. Larsen et al. (1986) demonstrated that some strains of *A. suis* are heavily fimbriated and adhere to glycoconjugate receptors on epithelial cells of the porcine bladder. Infection of the ureters and kidneys follows infection of the bladder.

Most cases occur in females 1–3 weeks after mating with an infected boar. Water restriction and the presence of crystalluria may predispose to infection (Wendt and Sobestiansky 1995). Cases may also occur at other times of the reproductive cycle, by recent infection of the urinary tract or by recrudescence of previously existing disease.

Clinical signs, lesions, and diagnosis

Clinical signs may develop 2–3 weeks after service by an infected boar or may be delayed until farrowing. Affected sows or gilts may die suddenly or be found ill, depressed, or thirsty with hunched backs. Hematuria is the main sign in the acute phase; later affected animals are uremic, pass bloodstained, purulent urine with or without a vulval discharge, and exhibit weight loss. Clinically affected sows frequently die from renal failure. Mild cases may occur in which inappetence and vulval discharge are the only obvious signs.

Lesions are limited to the urinary tract where there is ascending inflammation terminating in pyelonephritis. The mucosa of the urethra, bladder, and ureters is inflamed, coated with catarrhal or fibrinopurulent exudate; hemorrhagic; or necrotic. Affected kidneys often have irregular yellow areas of degeneration in the parenchyma that are visible on the surface. The renal pelvis may be dilated and contain mucoid fluid in which flakes of necrotic debris and altered blood are present. The medullary pyramids may contain dark foci of necrosis. The ureters can be dilated and filled with reddish purulent urine.

Diagnosis is based on clinical signs and bacterial culture of urine or urinary tract. The presence of hematuria 2–3 weeks after service by a boar suggests *A. suis* cystitis and pyelonephritis, rather than cystitis caused by *Escherichia coli* (Chapter 52). Postmortem examination

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

reveals aforementioned lesions. *A. suis* is easily seen in Gram-stained films, often with other bacteria, notably streptococci. Anaerobic incubation for 4 days is essential for isolation from urine or affected tissue. A selective medium for the isolation of *A. suis* has been described (Dagnall and Jones 1982). Recently Amigo et al. (2012) have described a gel-based PCR, which appeared to be far more sensitive than traditional culture. Using this assay they detected the microorganism in 8.9% (17/192) and 82.2% (37/45) sow urine and boar preputial swabs, respectively, compared with 0 and 31% (14/45) by culture.

Prevention and control

A. suis is sensitive *in vitro* to several antibiotics including penicillin and tetracyclines (Biksi et al. 2003). Antibiotics are frequently effective, but relapses occur, and early slaughter of affected animals is recommended. Prolonged treatment for 20 days with ampicillin given at 20 mg/kg has been suggested (Wendt and Sobestiansky 1995). Chronically affected animals in poor bodily condition and those that have not responded to treatment should be euthanized. Use of artificial insemination may help prevent disease.

References

Amigo CR, Sena de Gobbi DD, Gomes VT, et al. 2012. *ScientificWorldJournal* 2012:572732.

Biksi I, Major A, Fodor L, et al. 2003. *Acta Vet Hung* 51:53–59.

Carr J, Walton JR. 1990. Investigation of the pathogenic properties of Eubacterium (Corynebacterium) suis. In Proc 11th Int Congr Pig Vet Soc.

Dagnall GJR, Jones JET. 1982. *Res Vet Sci* 32:389–390.

Jones JET, Dagnall GJR. 1984. *J Hyg (Camb)* 93:381–388.

Larsen JL, Hogh P, Hovind-Hougen K. 1986. *Acta Vet Scand* 27:520–530.

Lawson PA, Falsen E, Akervall E, et al. 1997. *Int J Syst Bacteriol* 47:899–903.

Soltys MA, Spratling FR. 1957. *Vet Rec* 69:500.

Wendt M, Sobestiansky J. 1995. *Dtsch Tierarztl Wochenschr* 102:21–22.

Yassin AF, Spröer C, Pukall R, et al. 2015. *Int J Syst Evol Microbiol* 65:615–624.

Actinomyces hyovaginalis

Actinomyces hyovaginalis was first isolated from purulent vaginal discharge, organs of aborted fetuses, and a variety of body sites of pigs (Collins et al. 1993; Hommez et al. 1991) and later from sheep (Collins et al. 1993; Foster et al. 2012) and a goat (Schumacher et al. 2009). Two biovars, type II and III, are described based on phenotypic and biochemical differences (Storms et al. 2002). Type II strains, known as "vaginal" and thought to be vaginal flora of pigs, have been isolated in mixed culture from vaginal discharges and infrequently in pure culture from aborted pig fetuses. Type III strains, known as "general," have been cultured from a variety of porcine tissues including nasal cavity, tonsil, lung, kidney, uterus, joint, liver, and urinary bladder (Hommez et al. 1991; Storms et al. 2002). Baele et al. (2001) reported type III strains among the most common isolates from tonsils of pre- and post weaned pigs, suggesting it as a commensal, and Aalbaek et al. (2003) reported type III strains in pure or mixed culture from unique pyemic lung lesions in market pigs at slaughter, suggesting hematogenous distribution of an infection that they proposed as beginning as an inhaled opportunist in the lung.

Based on limited data, it seems that type II strains are infrequent causes of sporadic abortion and that type III strains cause disseminated pyemic lesions in a variety of organs with lung lesions being the best characterized. Due to the difficulty in isolation and identification of *A. hyovaginalis*, associated disease is likely underreported.

Actinomyces species are facultatively anaerobic, gram-positive, pleomorphic, non-acid-fast, non-sporing, non-motile, slow-growing bacteria. They are part of the skin and mucosal flora of human beings and animals. Identification of *Actinomyces* species by conventional phenotypic and biochemical methods is difficult and unreliable, due to slow growth as well as poor reproducibility and lack of discriminatory power of biochemical tests. Sequencing of the 16S ribosomal RNA (rRNA) definitively identifies these microorganisms (Hall et al. 1999).

Lesions are best characterized in aborted fetuses (Hogg et al. 2012) and in lungs (Aalbaek et al. 2003; Liljegren et al. 2003). In fetuses from a litter aborted 3 weeks prior to term, inconsistent gross lesions included minimal fibrinous exudate in the thoracic cavities and on the placental surface. Microscopic lesions were also inconsistent and included subacute necrotizing and suppurative placentitis and moderate, subacute suppurative bronchopneumonia. Gram-positive pleomorphic bacteria were observed in suppurative exudate in the placenta and lung. Gross lesions in lungs of slaughter pigs were randomly distributed irregularly round white-to-cream foci that contained coagulated necrotic material. Microscopically, foci were composed centrally by coagulated necrotic lung tissue with scattered indistinct basophilic bacteria surrounded by a

thin zone of aggregated neutrophils and then a zone of macrophages and peripherally by a zone of fibrosis.

Diagnosis of *A. hyovaginalis* requires culture and typical microscopic lesions. Culture must be anaerobic with fastidious anaerobic blood agar, and identification requires extensive biochemical testing or sequencing of the 16S rRNA gene. Culture from vaginal discharge should not be considered diagnostic for reproductive failure since *A. hyovaginalis* appears to be common vaginal flora.

References

Aalbaek B, Christensen H, Bisgaard M, et al. 2003. *J Comp Pathol* 129:70–77.

Baele M, Chiers K, Devriese LA, et al. 2001. *J Appl Mircobiol* 91:997–1003.

Collins MD, Stubbs S, Hommez J, et al. 1993. *Int J Syst Bacteriol* 43:471–473.

Foster G, Wragg P, Koylass MS, et al. 2012. *Vet Microbiol* 157:471–475.

Hall V, O'Neill GL, Magee JT, et al. 1999. *J Clin Microbiol* 37:2255–2261.

Hogg RA, Wessels ME, Koylass MS, et al. 2012. *Vet Rec* 170:127.

Hommez J, Devriese LA, Miry C, et al. 1991. *Zentralbl Veterinarmed B* 38:575–580.

Liljegren CH, Aalbaek B, Nielsen OL, et al. 2003. *APMIS* 111:531–538.

Schumacher VL, Hinckley L, Gilbert K, et al. 2009. *J Vet Diagn Invest* 21:380–384.

Storms V, Hommez J, Devriese LA, et al. 2002. *Vet Microbiol* 84:93–102.

Bacillus anthracis: Anthrax

Relevance, etiology, and public health

Anthrax has been recently reviewed (Mogridge et al. 2010; World Health Organization 2008). Anthrax is rare in swine. Swine, along with rats and dogs, are resistant to anthrax and often survive infection. Outbreaks in swine are usually limited with low morbidity and mortality. There are three clinical forms in swine – pharyngeal (cervical), intestinal, or septicemic – each resembling more common swine diseases that confound clinical recognition and proper diagnosis. The primary importance of anthrax and its recognition and diagnosis in swine is twofold. First, proper disposal of carcasses and decontamination of premises are essential to prevent spread of anthrax spores. Second, anthrax is zoonotic, and infected swine and their pork products are a hazard to humans.

Anthrax is caused by *Bacillus anthracis*, a large encapsulated gram-positive, aerobic, spore-forming, nonmotile rod. *B. anthracis* grows well on common laboratory media. On blood agar plates, colonies can usually be detected within 12 hours. After 24 hours at 37°C, the colonies are nonhemolytic and have a "ground-glass" appearance with irregular, wavy borders that give them the "Medusa head" characteristic. *B. anthracis* may be distinguished from other members of the genus by biochemical tests and PCR techniques.

B. anthracis exists in two forms, the multiplicative vegetative form found in tissues or the environmentally resistant spore form found in the environment. The cycle of infection involves exposure of the host to environmental spores, their uptake and germination to vegetative forms within the host, and then localized or generalized multiplication resulting in tissue damage and often death. Following death, the shedding of vegetative forms to the environment through exuding body fluids, putrefaction, or the opening of the carcass through the agency of a scavenger or human prosector results in sporulation. Spores can remain viable under appropriate environmental conditions for 50 or more years.

Human exposure occurs when animals that have died from the disease are examined postmortem without appropriate precautions or during carcass disposal. Infection through open skin wounds causes focal cellulitis and lymphadenitis in humans that is often self-limiting. More serious is inhalation of spores that leads to flu-like symptoms and progresses to often fatal sepsis and toxemia. This is more likely when infected body fluids or tissues have been exposed to air and sporulation has occurred, sporulation requiring only 4–10 hours. Ingestion of vegetative forms or *B. anthracis* in undercooked contaminated pork can lead to anorexia, bloody diarrhea, and abdominal pain that can progress to often fatal septicemia and toxemia.

Epidemiology and pathogenesis

Anthrax primarily affects herbivores, occurs worldwide, and is endemic in many warmer climates including most countries of Africa and Asia, in many countries in southern Europe, and in certain locales in North and South America (World Health Organization 2008). Where endemic, outbreaks in livestock (including swine housed outdoors) and in feral animals usually occur when rains have ended a period of drought. In confined swine outbreaks are sporadic and rare, not seasonal, and are usually point sources most likely due to contamination

of feed by large numbers of spores (Ferguson 1986). This occurs from feeding contaminated animal protein products, forages containing animals killed by anthrax or contaminated vegetation originating from an endemic area. Direct pig-to-pig transmission rarely occurs. Biting flies and mosquitoes may transmit the disease (Turell and Knudson 1987).

Exposure to *B. anthracis* in swine is usually by the ingestion of spores. Spores of *B. anthracis* are noninvasive and need to transit the mucosal epithelial lining of the gastrointestinal tract to germinate. Follicle-associated epithelium of the tonsillar crypts in the pharyngeal form and that of lymphoid patches in the intestinal form facilitate uptake of spores and delivery to the underlying lamina propria. Their germination is rapid, requiring only 10–15 minutes for conversion to vegetative forms. Two major and several more minor virulence factors are operative in evading host defenses, creating tissue damage, and resulting in death in fatal anthrax. The anionic polypeptide capsule is required for virulence and greatly reduces phagocytosis by macrophages and neutrophils. Anthrax toxin binds to and damages many cell types. It impairs phagocytosis in macrophages, reduces oxidative response in neutrophils, acts as a chemokine for neutrophils, and is cytotoxic for macrophages and other types of cells. In highly susceptible herbivores and occasionally pigs, multiplication of *B. anthracis* begins locally, extends to regional lymph nodes, and rapidly becomes systemic, leading to terminal shock and death (septicemic form). In most cases of porcine anthrax, multiplication and tissue damage is limited to the site of invasion and regional lymph nodes (i.e. the pharyngeal/cervical region or the ileum and proximal colon where lymphoid patches are most numerous).

Clinical signs and lesions

The incubation period in swine ranges from 1 to 8 days. The pharyngeal form is characterized by cervical edema, lethargy, inappetence, vomiting, and pyrexia to 107 °F (41.7 °C). Animals may recover spontaneously or die due to suffocation. Pigs with intestinal anthrax exhibit any combination of lethargy, anorexia, vomiting, ataxia, loose sometimes bloody feces, constipation, and pyrexia up to 107.4 °C (41.9 °C) (Brennan 1953; Redmond et al. 1997). Death follows in severe cases, but less severe cases are more commonly followed by recovery (Brennan 1953; Redmond et al. 1997). The septicemic form usually manifests as sudden death or as lethargy, tremor, and pyrexia followed shortly by death. Young pigs may develop septicemia more frequently than older swine (Ferguson 1986).

Necropsy of anthrax cases is discouraged to reduce environmental contamination by spores and limit human exposure. However, since anthrax may not be suspected based only on clinical presentation, the gross lesions of the disease are important. Large pigs that have died may have a bloody discharge from the nose, mouth, and/or anus (Edgington 1990), and small ones can be pale and dehydrated. In the pharyngeal form the cervical tissues have a gelatinous consistency and are separated by straw-colored, pink, or red hemorrhagic fluid. The tonsils are usually covered with fibrinous exudate and can be necrotic. The pharyngeal mucosa is frequently inflamed and swollen. Mandibular and pharyngeal lymph nodes are enlarged to several times their normal size, and the cut surfaces are hemorrhagic or yellow and necrotic. In the intestinal form there is segmental necrotizing enteritis most commonly affecting the ileum and spinal colon. Affected segments have mucosal hemorrhage and adherent fibrinonecrotic exudate, there is mural thickening, and fibrin can adhere to the serosa. The peritoneal cavity can contain copious pinkish fluid that clots when exposed to air. Mesenteric lymph nodes are typically enlarged, and hemorrhagic or necrotic and edema of the mesentery is common. In the septicemic form, there are variable amounts of serosanguinous peritoneal fluid, serosal and renal cortical petechia, splenic enlargement, and generalized lymphadenomegaly.

Diagnosis

Diagnosis of anthrax in swine is made difficult by its rare occurrence, lack of unique clinical signs, and similarity of various forms to other more common swine diseases. Cervical swelling can also be caused by necrotoxic clostridial infections (Chapter 51) and cervical lymphadenitis caused by *S. porcinus* (Chapter 61). Enteritis and/or typhlocolitis with pyrexia can also be caused by *Escherichia coli* (Chapter 52), *Salmonella* spp. (Chapter 59), *Brachyspira* spp. (Chapter 62), classical swine fever virus (Chapter 39), and African swine fever virus (Chapter 25). Sudden death caused by septicemia can also be caused by *A. suis* (Chapter 48), *E. coli* (Chapter 52), *E. rhusiopathiae* (Chapter 53), *H. parasuis* (Chapter 54), *S. choleraesuis* (Chapter 59), and *S. suis* (Chapter 61). Exudation of bloody fluid from all body orifices in the septicemic form, from the nose and mouth in the cervical form, and from the anus in the intestinal form should raise suspicion in fatal cases.

The officially recommended methods for the diagnosis of anthrax are in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (World Organization for Animal Health (OIE) 2016). If anthrax is suspected based on clinical presentation, the recommended method of confirmation is the M'Fadyean smear, which is a microscopic examination for encapsulated bacilli in a smear of blood from a dead animal stained with polychrome methylene blue stain. Necropsy examination is not recommended due to exposure of vegetative forms

to oxygen and formation of spores. Gross lesions are as described above. Culture should not be attempted unless appropriate safety precautions are available. Personnel handling the organism should be vaccinated. *B. anthracis* is readily cultured, and typical nonhemolytic colonies can be identified by their biochemical characteristics or by PCR (Fasanella et al. 2003; Hutson et al. 1993). The organism can also be directly detected from clinical samples by PCR (Drago et al. 2002). PCR kits are commercially available.

Prevention and control

Pigs with clinical anthrax can recover completely after penicillin treatment (Ferguson 1986). Oxytetracycline is effective against *B. anthracis* and may be used parenterally (Edgington 1990). Infection may persist for up to 21 days after infection in a population (Redmond et al. 1997); this factor must be considered before animals are sent for human consumption. Immunity develops in recovered animals, and serum antibody to the toxin can be detected. Vaccines can stimulate protective immunity. Kaufmann et al. (1973) used the Sterne strain anthrax vaccine, an avirulent spore vaccine, in swine with success.

The World Health Organization (2008) provides details of control measures. Qualified medical personnel can give persons exposed to the infection prophylactic antimicrobials such as penicillin and tetracyclines, and clinical disease should be immediately treated. Vaccination can protect humans against longer-term exposure. Control of the spread of anthrax differs significantly from control of most other animal diseases because it depends on preventing access to viable spores of *B. anthracis* in soil, manures, or contaminated feeds. Prevention of environmental contamination by the long-lived spores is essential. Preferred disinfectants are 5% freshly prepared sodium hydroxide or 10% formaldehyde (Edgington 1990). Few spores are formed in the unopened carcasses of animals dead of anthrax. The orifices and any cuts in a carcass should be covered with disinfectant-soaked cotton wool to prevent sporulation and spread of infection. Carcasses, bedding, and other combustible articles should be destroyed by incineration, preferably on the spot, or by deep burial in at least 4 ft (1.25 m) of soil with the carcass covered with lime. Disinfectants should be used prior to clearing out infected premises, and then exposed surfaces as well as equipment and tools should be scrubbed or pressure washed with the disinfectant.

References

Brennan ADJ. 1953. *Vet Rec* 65:255–258.

Drago L, Lombardi A, Vecchi ED, et al. 2002. *J Clin Microbiol* 40:4399.

Edgington AB. 1990. *Vet Rec* 127:321–324.

Fasanella A, Losito S, Adone R, et al. 2003. *J Clin Microbiol* 41:896–899.

Ferguson LC. 1986. Anthrax. In Leman AD, Straw B, Glock RD, et al., eds., *Diseases of Swine*, 6th ed. Ames, IA: Iowa State University Press, pp. 622–627.

Hutson RA, Duggleby CJ, Lowe JR, et al. 1993. *J Appl Bacteriol* 75:463–472.

Kaufmann AF, Fox MD,Kolb RC. 1973. *J Am Vet Med Assoc* 163:442.

Mogridge J, Shadomy S, Turnbull P. 2010. *Bacillus anthracis*. In Gyles CL, Prescott JF, Songer JG, et al., eds. *Pathogenesis of Bacterial Infections in Animals*. Hoboken, NJ: Wiley-Blackwell, pp. 91–112.

Redmond C, Hall GA, Turnbull PCB, et al. 1997. *Vet Rec* 141:244–247.

Turell MJ, Knudson GB. 1987. *Infect Immun* 55:1859–1861.

World Health Organization. 2008. Turnbull P, ed. *Anthrax in Humans and Animals*, 4th ed. Geneva, Switzerland: WHO Press, World Health Organization.

World Organization for Animal Health (OIE). 2016. Anthrax. Chapter 2:1:1. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Paris: OIE, pp. 87–97.

Burkholderia pseudomallei: Melioidosis

Relevance, etiology, and public health

Melioidosis is a chronic bacterial infection of swine by *Burkholderia pseudomallei* seen in tropical and subtropical regions. Humans can be infected. Both pigs and people may contract infection from animals by consumption of contaminated meat, from water contaminated by animal or human feces, and from other environmental sources. *B. pseudomallei* is now considered a candidate agent for bioterrorists.

B. pseudomallei, formerly known as *Pseudomonas pseudomallei*, is a short, gram-negative rod that does not form spores. It produces rough (wrinkled) or mucoid colonies on a wide variety of laboratory media at 37 °C and grows on MacConkey agar to give colorless colonies.

Melioidosis in humans can be fatal, presenting as septicemia, pneumonia, or chronic suppurative lesions of skin, lymph nodes, or bone. Mortality in humans is

20–50% with or without treatment. It is rarely transmissible from person to person and is usually acquired by the ingestion of contaminated food and water. This contamination can be purely environmental or result from contamination of water and food with infected animal feces. Ingestion of improperly cooked meat from infected animals may also result in human infection.

Epidemiology and pathogenesis

B. pseudomallei is present in water and soil in tropical and subtropical areas and may infect pigs when water supplies are contaminated or when they consume infected animal or plant matter. It has been reported from Australia (Millan et al. 2007), Malaysia (Omar et al. 1962), and the Caribbean.

Phenolic (2% Lysol), chlorine (0.1–0.5%), and oxidizing disinfectants (1% Virkon®, 3% hydrogen peroxide) and formaldehyde (4%) are effective against *B. pseudomallei*.

Clinical signs, lesions, and diagnosis

Infection is often clinically inapparent, but a raised rectal temperature of 40–42 °C (104–108 °F) may develop for up to 4 days. Unsteady gait, lameness or weakness, slight nasal discharge, and subcutaneous swellings of the limbs may be seen. Deaths may occur but are rare in adults in which abortions and uterine discharges have been recorded (Laws and Hall 1964; Millan et al. 2007; Omar et al. 1962; Rogers and Andersen 1970).

Lesions are found in slaughter pigs in which clinical signs have not been seen and in pigs that have died from the disease. They consist of large abscesses filled with creamy or caseous yellow-green pus in the lungs, liver, spleen, kidney, and mesenteric and subcutaneous lymph nodes.

Melioidosis should be suspected in tropical environments on clinical grounds when prolonged raised rectal temperatures and unsteady gait are associated with subcutaneous swellings of the limbs. More frequently, presumptive diagnosis is based on the typical creamy abscesses found at slaughter (Ketterer et al. 1986). Diagnosis is confirmed by culture. A selective medium is used for environmental samples (Peacock et al. 2005). The identity of suspect colonies can be confirmed biochemically (Hemarajata et al. 2016). A hypersensitivity test resembling a tuberculin test (the melioidin test) and serum agglutination and complement fixation tests have been used to confirm diagnosis in the live pig. Antibody has been demonstrated within 7 days of experimental infection (Najdenski et al. 2004).

Prevention and control

B. pseudomallei is resistant *in vitro* to aminoglycosides but susceptible to some cephalosporins and to amoxicillin: clavulanic acid. The disease can be prevented by use of clean or chlorinated water supplies and preventing access to contaminated soil. As the disease is of public health importance, infected carcasses should be disposed of safely. Rigorous criteria for meat inspection of slaughter pigs will help to safeguard public health.

References

Hemarajata P, Baghdadi JD, Hoffman R, et al. 2016. *J Clin Microbiol* 54:2866–2873.

Ketterer PJ, Webster WR, Shield J, et al. 1986. *Aust Vet J* 63:146–149.

Laws L, Hall WTK. 1964. *Aust Vet J* 40:309–314.

Millan JM, Mayo M, Janmaat A, et al. 2007. *Vet J* 174:200–202.

Najdenski H, Kussowski V, Vesschnova A. 2004. *J Vet Med B* 51:225–230.

Omar AR, Cheah KK, Mahendranathan T. 1962. *Br Vet J* 118:421–429.

Peacock SJ, Chieng G, Cheng AG, et al. 2005. *J Clin Microbiol* 43:5359–5361.

Rogers RJ, Andersen DJ. 1970. *Aust Vet J* 46:292.

Campylobacter spp.

Relevance and etiology

A number of *Campylobacter* species are known to be residents of the gastrointestinal tract in swine including *Campylobacter coli* (Doyle 1944), *Campylobacter jejuni* (Svedhem and Kaijser 1981), *Campylobacter sputorum* subsp. *mucosalis* (Lawson et al. 1981), *Campylobacter hyointestinalis* (Gebhart et al. 1985), *Campylobacter lari* (Young et al. 2000), and *Campylobacter lanienae* (Sasaki et al. 2003). Historically, *C. coli* was erroneously thought to cause swine dysentery (Chapter 62), and both *C. sputorum* subsp. *mucosalis* and *C. hyointestinalis* were erroneously thought to cause porcine proliferative enteropathy (Chapter 58). The primary importance of campylobacters in swine is as one of many sources for infection of humans. *C. jejuni* and *C. coli* are among the

main causes of bacterial gastroenteritis in humans in all industrialized countries (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) 2015; Huang et al. 2016). Although mild diarrheal disease can be produced in neonatal pigs by inoculation with *C. coli* (Olubunmi and Taylor 1982) or *C. jejuni* (Boosinger and Power 1988; Vitovec et al. 1989), campylobacters are not known to produce any generally recognized enteric or other diseases in swine.

Campylobacters are gram-negative curved rods or short spiral organisms from 0.5 to 8 µm in length and 0.3 µm in diameter with a single polar flagellum (Penner 1988). They do not form spores, but some *C. jejuni* have a polysaccharide capsule probably involved in virulence (Bacon et al. 2001). They are microaerobic and grow most readily on media containing blood or other sources of iron. Most commercial media for isolation also incorporate selective antimicrobials such as cefoperazone and amphotericin B (Corry et al. 1995). Growth occurs readily at 37 °C, but they are often cultivated at higher temperatures of 40–42 °C. Colonies generally appear after 48 hours of incubation. *C. coli* forms sprawling watery colonies on blood agar after 48 hours, and *C. jejuni* form slightly smaller colonies 2–3 mm in diameter. They can be identified presumptively to species using biochemical tests. However identification to species level is hindered by variations in methodology and the subjective interpretation of biochemical results and isolates with atypical phenotypes (Linton et al. 1997).

Public health

C. jejuni and C. coli are among the most common causes of food-borne bacterial enteric disease in humans (EFSA and ECDC 2015; Huang et al. 2016; Silva et al. 2011). *C. jejuni*, *C. coli*, and *C. hyointestinalis* of swine origin can cause disease in humans (EFSA and ECDC 2015; Gorkiewicz et al. 2002). Typical symptoms include vomiting, diarrhea, fever and headache. The disease is normally self-limiting. Rarely serious complications may result including septicemia or autoimmune neuropathies such as Guillain–Barré and Miller-Fisher syndromes. Of recent concern is the emergence of antimicrobial resistant strains in affected humans (EFSA and ECDC 2015).

Livestock carrying *Campylobacter* pose an important risk for human infection from contamination of carcasses at slaughter, of milk, and water contaminated by livestock wastes and slurries. Poultry is the main foodborne source of human campylobacteriosis, but contact with infected swine, consumption of contaminated pork products, or consumption of water contaminated by swine effluent may lead to human infection (EFSA and ECDC 2015; Horrocks et al. 2009; Kapperud et al. 1992).

Epidemiology

Campylobacter species are found in swine worldwide. *C. coli* is most commonly found in the pig but also occurs in a wide range of hosts including cattle and poultry (Alter et al. 2005; Horrocks et al. 2009; Jensen et al. 2006; Oporto and Hurtado 2011). *C. jejuni* is less frequently isolated from swine but is present in a wide range of mammalian and avian species where it may cause enteritis (Giacomelli et al. 2015; Horrocks et al. 2009). It is also commonly carried in the tract of a wide range of birds and mammals, including food-producing animals and pets, without causing clinical signs (Keller et al. 2007; Moore et al. 2005; Petersen et al. 2001). *C. hyointestinalis* and *C. mucosalis* are also found in other mammalian species (Giacomelli et al. 2015; Oporto and Hurtado 2011).

Infection takes place by the oral route and is usually direct from one infected pig to another. Organisms are carried principally on the ileal and large intestinal mucosa and shed in the feces. As the organism can survive in feces and contaminated water, indirect transmission may occur. Birds, rodents, and insects may contaminate feed and water and introduce infection to a herd (Alter et al. 2005). Piglets become infected from maternal feces (Leblanc-Maridor et al. 2011b; Soultos and Madden 2007), through contaminated water, or by horizontal transmission between animals or via farmers, domestic animals, or other environmental sources (Alter et al. 2005; Hume et al. 2002; Quintana-Hayashi and Thakur 2012). Maternal immunity does not prevent colonization. Infected animals remain carriers for long periods and pass 10^3–10^4 organisms/g feces for months (Leblanc-Maridor et al. 2008; Weijtens et al. 1999).

Enteric disease in pigs

Enteric disease has been reproduced in naïve neonatal pigs with *C. coli* (Olubunmi and Taylor 1982) or *C. jejuni* (Babakhani et al. 1993; Bibiana et al. 2009; Boosinger and Power 1988; Vitovec et al. 1989). The neonatal pig is an established model for human *C. jejuni* enterocolitis; however, campylobacters are not known to produce any generally recognized enteric or other diseases in any age of pigs.

Similar disease and lesions are described in neonatal pigs inoculated with *C. coli* and *C. jejuni*. Piglets exhibit mucoid to watery and occasionally bloody diarrhea. Gross lesions of mucosal hyperemia, edema, multifocal hemorrhage, and occasional accumulation of mucus are observed most consistently in the cecum and colon and occasionally in the small intestine. Microscopic lesions are limited to the cecum and colon and include multifocal erosions of the luminal epithelium, sparse aggregates

of neutrophils in the lamina propria adjacent to erosions, and edema of the lamina propria and submucosa.

Confirmation of enteric campylobacteriosis in neonatal pigs would require demonstration of typical clinical signs and lesions, confirmation of *C. jejuni* or *C. coli*, and elimination of other more common causes of neonatal porcine diarrhea. These include colibacillosis (Chapter 52), clostridiosis (Chapter 51), rotaviruses (Chapter 43), coronaviruses (Chapter 31), or PRRSV (Chapter 41). *C. jejuni* or *C. coli* can be confirmed in affected large intestinal mucosa by culture (see above) or by PCR (Denis et al. 1999; Jensen et al. 2005; Leblanc-Maridor et al. 2011a).

Prevention and control

Campylobacters are susceptible to most farm disinfectants. It is possible to maintain pig units founded from primary hysterectomy-derived stock free from infection by strict isolation. Nevertheless, Kolstoe et al.(2015) underlined the difficulty of controlling campylobacters as even high levels of biosecurity in Norwegian specific-pathogen-free (SPF) herds did not seem to be sufficient to keep *Campylobacter* spp. out of 6 of the 10 herds tested. Supplementation of the diet of piglets with high levels of zinc oxide can reduce the *C. coli* load, potentially leading to a lower contamination risk for meat during slaughter (Bratz et al. 2013).

References

Alter T, Gaull F, Kasimir S, et al. 2005. *Vet Microbiol* 108:251–261.

Babakhani FK, Bradley GA, Joens LA. 1993. *Infect Immun* 61:3466–3475.

Bacon DJ, Szymanski CM, Burr DH, et al. 2001. *Mol Microbiol* 40:769–777.

Bibiana FL, Adriance SM, Joens LA. 2009. *Foodborne Pathog Dis* 6:377–385.

Boosinger TR, Power TA. 1988. *Am J Vet Res* 49:456–458.

Bratz K, Gölz G, Riedel C, et al. 2013. *J Appl Microbiol* 115:1194–1202.

Corry JE, Post DE, Colin P, et al. 1995. *Int J Food Microbiol* 26:43–76.

Denis M, Soumet C, Rivoal K, et al. 1999. *Lett Appl Microbiol* 29:406–410.

Doyle LP. 1944. *Am J Vet Res* 5:3–5.

European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC). 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. *EFSA J* 13(12):4329.

Gebhart CJ, Edmonds P, Ward GE, et al. 1985. *J Clin Microbiol* 21:715–720.

Giacomelli M, Follador N, Coppola LM, et al. 2015. *Vet J* 204:333–337.

Gorkiewicz G, Feierl G, Zechner R, et al. 2002. *J Clin Microbiol* 40:2601–2605.

Horrocks SM, Anderson RC, Nisbet DJ, et al. 2009. *Anaerobe* 15:18–25.

Huang JY, Henao OL, Griffin PM. 2016. *MMWR Morb Mortal Wkly Rep* 65:368–371.

Hume ME, Droleskey RE, Sheffield CL, et al. 2002. *Curr Microbiol* 45:128–132.

Jensen AN, Andersen MT, Dalsgaard A, et al. 2005. *J Appl Microbiol* 99:292–300.

Jensen AN, Dalsgaard A, Baggesen DL, et al. 2006. *Vet Microbiol* 116:96–105.

Kapperud G, Skjerve E, Bean NH, et al. 1992. *J Clin Microbiol* 30:3117–3121.

Keller J, Wieland B, Wittwer M, et al. 2007. *Zoonoses Public Health* 54:2–7.

Kolstoe EM, Iverfsen T, Ostensvik O, et al. 2015. *Zoonoses Public Health* 62:125–130.

Lawson GHK, Leaver JL, Pettigrew GW, et al. 1981. *Int J Syst Bacteriol* 31:385–391.

Leblanc-Maridor M, Denis M, Lalande F, et al. 2008. *Vet Microbiol* 131:309–317.

Leblanc-Maridor M, Denis M, Chidaine B, et al. 2011a. *Vet Microbiol* 154:171–179.

Leblanc-Maridor M, Garénaux A, Beaudeau F, et al. 2011b. *J Microbiol Methods* 85:53–61.

Linton D, Lawson AJ, Owen RJ, et al. 1997. *J Clin Microbiol* 35:2568–2572.

Moore JE, Corcoran D, Dooley JS, et al. 2005. *Vet Res* 36:351–382.

Olubunmi PA, Taylor DJ. 1982. *Vet Rec* 111:197–202.

Oporto B, Hurtado A. 2011. *Foodborne Pathog Dis* 8(7):807–813.

Penner JL. 1988. The genus Campylobacter: a decade of progress. *Clin Microbiol Rev* 1:157–172.

Petersen L, Nielsen EM, Engberg J, et al. 2001. *Appl Environ Microbiol* 67:3115–3121.

Quintana-Hayashi MP, Thakur S. 2012. *Appl Environ Microbiol* 78:2698–2705.

Sasaki Y, Fujisawa T, Ogikubo K, et al. 2003. *J Vet Med Sci* 65:129–131.

Silva J, Leite D, Fernandes M, et al. 2011. *Front Microbiol* 2:200.

Soultos N, Madden RH. 2007. *J Appl Microbiol* 102:916–920.

Svedhem A, Kaijser B. 1981. *J Infect* 3:37–40.

Vitovec J, Koudela B, Stěrba J, et al. 1989. *Zentralbl Bakteriol* 271:91–103.

Weijtens MJBM, Reinders RD, Urlings HAP, et al. 1999. *J Appl Microbiol* 86:63–70.

Young CR, Harvey R, Anderson R, et al. 2000. *Res Vet Sci* 68:75–78.

Chlamydia

Relevance, etiology, and public health

Chlamydiaceae have been detected in various avian and mammalian species including the pig in a variety of conditions including conjunctivitis, rhinitis, pneumonia, enteritis, arthritis, and various reproductive disorders. However the importance of *Chlamydiaceae* as pig pathogens remains controversial because they are frequently detected in mixed infections with other pathogens, a limited number of inoculation studies have been done in swine, and confirmatory testing has not been readily available.

The nomenclature of the *Chlamydiaceae* is confusing. In 1999, it was proposed to reassign the single genus *Chlamydia* into two genera, *Chlamydia* and *Chlamydophila*. However this proposal was never officially approved, and it was recently proposed to reunite the *Chlamydiaceae* into a single genus, *Chlamydia* (Gunn and Lofstedt 2016). Four species of *Chlamydia* are found in swine *i.e. Chlamydia suis* (previously *Chlamydia trachomatis*-like), *Chlamydia abortus (previously* ruminant *Chlamydia psittaci* serovar 1), *Chlamydia pecorum*, and *Chlamydia psittaci*. Evidence for chlamydia-induced disease in pigs is most compelling for *C. suis* as a potential cause of pneumonia and neonatal diarrhea and for *C. abortus* as a cause of early embryonic death (i.e. irregular returns to estrus).

Chlamydia are gram-negative obligate intracellular bacteria that primarily multiply in mucosal epithelial cells lining the respiratory, urogenital, and gastrointestinal tracts. Elementary bodies are the infectious extracellular forms that are environmentally resistant, inactive particles 0.2–0.3 µm (200–300 nm) in diameter. Once in contact with mucosal epithelial cells, they are internalized, reorganize into reticulate bodies, and divide by binary fission within phagocytic vesicles. Condensation of progeny reticulate bodies to elementary bodies followed by cell lysis completes the life cycle. Elementary bodies can survive for considerable lengths of time in the environment where they are resistant to drying.

Epidemiology and public health

Chlamydial infections have been reported in pigs worldwide. Seroprevalence in various countries has been reported to be as high as 40–99% in fattening pigs, sows, and boars (Schautteet and Varompay 2011). *Chlamydiaceae* have been demonstrated in various pig samples including conjunctiva, lung, intestines, aborted fetal tissues, genital tract, and semen.

Common infection sources, infection routes, possible vectors, and infection kinetics have not been extensively examined. Direct transmission from infected swine is likely the greatest risk factor, including vertical *in utero* transmission. *C. suis*, *C. abortus, C. pecorum*, and *C. psittaci* have been demonstrated in boar semen (Kauffold et al. 2006; Teankum et al. 2006) and *C. suis* in extended boar semen (Hamonic et al. 2016), suggesting the potential of venereal transmission through natural or artificial service. In addition proximity to other animals potentially infected with each chlamydial species should be considered a risk factor. These include ruminants, horses, rabbits, guinea pigs, and mice for *C. abortus*, ruminants for *C. pecorum*, and birds for *C. psittaci*. Keeping poultry and pigs on the same site has been confirmed as a risk factor for *C. psittaci* infection in pigs (Eggemann et al. 2000; Vanrompay et al. 2004). *Chlamydiaceae* survive drying and can persist in dust in an infected environment. Flies or dust may be involved in the transmission of chlamydial conjunctivitis.

Until recently, *C. suis* was thought to only infect pigs. However it has been detected in conjunctival, pharyngeal, and rectal samples from clinically normal humans (De Puysseleyr et al. 2014a, 2015). To date, human clinical disease has not been associated with *C. suis*. Of concern due to the close genetic relationship between *C. suis* and *C. trachomatis* is the potential for tetracycline-resistant strains of *C. suis* to transfer resistance to *C. trachomatis* in coinfected humans (De Puysseleyr et al. 2014b).

C. abortus is among the leading cause of abortion in small ruminants but can also cause abortion in pigs, horses, rabbits, guinea pigs, and mice. It is a well-known zoonotic agent able to induce abortion in humans. Transmission to humans from infected fetuses and placenta from aborted ruminants is well established. Transmission of *C. abortus* from pigs to humans has not yet been confirmed, but it remains a potential risk for pregnant women handling aborted porcine fetuses or placenta.

C. pecorum is not known to infect humans and poses no zoonotic risk.

C. psittaci can cause mild flu-like symptoms, fever, myalgia, diarrhea, encephalitis, and/or pneumonia in humans. Most cases of human psittacosis are zoonotic from exposure to birds or aerosolized bird dander and/or desiccated bird feces. However, a recent report of pneumonia in veterinary students exposed to *C. psittaci*-infected equine fetal membranes confirms the potential of mammalian-to-human zoonosis (Polkinghorne and Greub 2017). To date, transmission of *C. psittaci* from pigs to humans has not been reported.

Pathogenesis, clinical signs, and lesions

Elementary bodies enter by the respiratory, oral, or genital routes and multiply in epithelial cells or are taken up by macrophages and distributed to lymph nodes. Infection may be local at the portal of entry and remain inapparent or latent; may cause local disease such as pneumonia, enteritis, or disturbances of reproduction; or may become generalized and produce systemic disease. A majority of chlamydial infections in swine are likely inapparent.

Chlamydia suis

C. suis has been identified in pigs affected with conjunctivitis, rhinitis, pneumonia, enteritis, and various reproductive disorders, and it is the best characterized pathogenic chlamydial species in swine. A greater degree of genetic diversity exists among strains of *C. suis* than among other chlamydial species (Bush and Everett 2001; Everett et al. 1999), suggesting variation in virulence among strains. Oral inoculation studies in neonatal gnotobiotic pigs produced acute watery diarrhea due to replication predominantly in small intestinal apical villus enterocytes resulting in severe villous atrophy and lymphangitis (Guscetti et al. 2009; Rogers and Andersen 1996). In contrast, oral inoculation of 21-day-old colostrum-fed pigs resulted in villous atrophy in the ileum and intestinal lymphangitis, but no diarrhea (Rogers and Andersen 2000). Studies utilizing intranasal and intratracheal or aerosol inoculation with *C. suis* of neonatal gnotobiotic or 6-week-old conventional pigs produced pyrexia, dyspnea, and coughing due to necrotizing bronchointerstitial pneumonia (Rogers et al. 1996; Sachse et al. 2004). All lobes of lungs in the neonatal gnotobiotic pigs were grossly consolidated. Immunohistochemistry in the gnotobiotic pigs revealed chlamydial antigen in nasal and bronchial epithelial cells, alveolar pneumocytes, and alveolar macrophages. Deposition of *C. suis* in the conjunctival sac of neonatal gnotobiotic pigs resulted in pleocellular conjunctivitis with intraepithelial chlamydial antigen, but gross lesions of conjunctivitis were not observed (Rogers and Andersen 1999).

Chlamydia abortus

C. abortus (ruminant *C. psittaci* serovar 1) is a major cause of abortion in sheep, goats, and cattle. In the pig *C. abortus* has been primarily associated with abortion, weak neonates, and irregular returns to estrus (Camenisch et al. 2004; Eggemann et al. 2000; Hoelzle et al. 2000; Thoma et al. 1997). A single small reproductive inoculation study in swine has been reported. Four sows at 42 days of gestation were inoculated with an ovine isolate of *C. abortus* resulting in detection of *C. abortus* antigen in placenta but not in abortion (Vazquez-Cisneros et al. 1994). In another study (Camenisch et al. 2004), cervical swabs were obtained 1–3 days following irregular return to estrus from 65 sows on 24 different farms, and an additional 128 swabs from sows on 14 farms without a history of irregular returns were also collected. *C. suis* was detected in 10.8% of sows with irregular returns, but none of the sows with normal reproductive history.

Chlamydia pecorum

C. pecorum has been identified in ruminants, koalas, and pigs. In these species it has been associated with various infections such as conjunctivitis and reproductive problems. Confirmatory inoculation studies in swine are not reported.

Chlamydia psittaci

C. psittaci is the agent of psittacosis in birds. In pigs, *C. psittaci* has been isolated from the genital tract and the lung of sows without apparent disease (Busch et al. 2000; Vanrompay et al. 2004). Oral inoculation of 3-day-old gnotobiotic pigs with avian-origin *C. psittaci* resulted in minimal replication in small intestinal enterocytes, mild villous atrophy, and softening of stools, but not diarrhea (Guscetti et al. 2000).

Diagnosis

Presumptive diagnosis is difficult because clinical signs of chlamydial infection are not distinctive and may include conjunctivitis, pneumonia, neonatal enteritis, and reproductive failure. Differential diagnosis should include common causes of the observed clinical signs and gross lesions, and *Chlamydia* spp. as well as other potential causal agents should be confirmed or excluded by testing.

Most veterinary diagnostic laboratories do not routinely test for *Chlamydiaceae* in pigs. Handling chlamydias is dangerous, and severe human infections can result. Appropriate safety precautions should be observed. *Chlamydia* may be detected in smears and in histologic specimens after staining by Giemsa's method or Koster's stain.

Commercial *Chlamydia spp*. antigen ELISAs may be used to detect antigen in swabs or extracts of tissue (Guscetti et al. 2009). Most of them detect the chlamydial family-specific LPS antigen and therefore detect all chlamydial species. Immunofluorescence or immunohistochemical staining of histological sections can be also used for confirmation (Chasey et al. 1981). Commercial *Chlamydiaceae* family-specific mouse monoclonal antibodies are available.

Chlamydia can be grown in embryonated chicken eggs and in cell cultures such as McCoy, HeLa, Vero, or CaCo cells. However pig strains do not grow well on the established cell lines (Schiller et al. 2004), and the zoonotic potential of many *Chlamydia* spp. makes cultivation as a routine diagnostic method impractical.

PCR technology is currently preferred for confirmation as it allows sensitive and specific identification of the *Chlamydia* spp. present (De Puysseleyr et al. 2014b; Li et al. 2011; Pantchev et al. 2010). Current PCR tests target the *omp*A, the 16S–23S rRNA, or the *inc*A genes. Targeting the 16S–23S genes increases sensitivity as multiple copies of these genes are usually present in the organism. A microarray hybridization assay for the identification of chlamydial species has also been developed. The test proved to allow unambiguous identification of chlamydial species (Hoffmann et al. 2015; Sachse et al. 2012). Sequencing of PCR products allows comparison with sequences of reference strains and strain identification for epidemiological studies.

Chlamydial antibodies may be detected using serological assays such as the complement fixation test (CFT), the microimmunofluorescence test (MIF), or ELISAs (Mohamad et al. 2010). Two types of ELISAs have been used based either on a recombinant major outer membrane protein (OMP) or the LPS (Hoelzle et al. 2004). The presence of antibody does not confirm disease, only exposure. Moreover the available serological assays cannot determine the *Chlamydia* species involved.

Prevention and control

Regular cleaning and disinfection of premises is probably worthwhile as *Chlamydiaceae* can survive up to 30 days in feces. *Chlamydiaceae* are susceptible to a wide range of disinfectants including oxidizing, phenolic, and chlorine-based products, quaternary ammonium compounds, and some detergents.

Active infections can be treated with antimicrobials. Tetracyclines are the drug of choice. However strains of *C. suis* resistant to tetracyclines have been reported (Donati et al. 2016; Schautteet et al. 2013). Macrolides could be an alternative when such strains are involved.

No chlamydial vaccines are commercially available for the pig although an immune response has been demonstrated to *C. suis* in the genital tract of sows (De Clercq et al. 2014). The efficacy of ruminant *C. abortus* vaccines in prevention of *C. abortus*-induced reproductive failure in swine is unknown.

References

Bush RM, Everett KD. 2001. Molecular evolution of the Chlamydiaceae. *Int J Syst Evol Microbiol.* 51(Pt 1):203–220.

Busch M, Thoma R, Schiller I, et al. 2000. *J Vet Med B Infect Dis Vet Public Health* 47:471–480.

Camenisch U, Lu ZH, Vaughan L, et al. 2004. *Vet Rec* 155:593–596.

Chasey D, Davies P, Dawson M. 1981. *Br Vet J* 137:634–638.

De Clercq E, Devriendt B, Yin L, et al. 2014. *Vet Res* 45:95.

De Puysseleyr K, De Puysseleyr L, Dhondt H, et al. 2014a. *BMC Infect Dis* 14:560.

De Puysseleyr K, De Puysseleyr L, Geldhof J, et al. 2014b. *PLoS One* 9:e96704.

De Puysseleyr L, De Puysseleyr K, Braeckman L, et al. 2015. *Transbound Emerg Dis* 64:826–833.

Donati M, Balboni A, Laroucau K, et al. 2016. *PLoS One* 25:11:e0149914.

Eggemann G, Wendt M, Hoelzle LE, et al. 2000. *Dtsch Tierarztl Wochenschr* 107:3–10.

Everett KDE, Bush RM, Andersen AA. 1999. *Int J Syst Bacteriol* 49:415–440.

Gunn A, Lofstedt R. 2016. *Vet Rec* 179:193–194.

Guscetti F, Hoop R, Schiller I, et al. 2000. *J Vet Med B Infect Dis Vet Public Health* 47:561–572.

Guscetti F, Schiller I, Sydler T, et al. 2009. *Vet Microbiol* 135:157–168.

Hamonic G, Pasternak JA, Käser T, et al. 2016. *Theriogenology* 86:949–956.

Hoelzle LE, Hoelzle K, Wittenbrink MM. 2004. *Vet Microbiol* 103:85–90.

Hoelzle LE, Steinhausen G, Wittenbrink MM. 2000. *Epidemiol Infect* 125:427–439.

Hoffmann K, Schott F, Donati M, et al. 2015. *PLoS One*. 10:e0143576.

Kauffold J, Melzer F, Henning K, et al. 2006. *Theriogenology*. 65:1750–1758.

Li Y, Wang Y, Nie F, et al. 2011. *J Vet Diagn Invest* 23(4):673–681.

Mohamad KY, Rekiki A, Berri M, et al. 2010. *Vet Microbiol* 143:424–428.

Pantchev A, Sting R, Bauerfeind R, et al. 2010. *Comp Immunol Microbiol Infect Dis* 33:473–484.

Polkinghorne A, Greub G. 2017. *Clin Microbiol Infect* 23:693–694.

Rogers DG, Anderson AA. 1996. *J Vet Diagn Invest* 8:433–440.

Rogers DG, Andersen AA. 1999. *J Vet Diagn Invest* 11:341–344.

Rogers DG, Andersen AA. 2000. *J Vet Diagn Invest* 12:233–239.

Rogers DG, Anderson AA, Hunsaker BD. 1996. *J Vet Diagn Invest* 8:45–55.

Sachse K, Grossmann E, Berndt A, et al. 2004. *Comp Immunol Microbiol Infect Dis* 27:7–23.

Sachse K, Kuehlewind S, Ruettger A, et al. 2012. *Vet Microbiol* 157:476–480.

Schautteet K, De Clercq E, Miry C, et al. 2013. *J Med Microbiol* 62:331–334.

Schautteet K, Vanrompay D. 2011. *Vet Res* 42:29.

Schiller I, Schifferli A, Gysling P, et al. 2004. *Vet J* 168:74–80.

Teankum K, Pospischil A, Janett F, et al. 2006. *Vet Microbiol* 116:149–157.

Thoma R, Guscetti F, Schiller I, et al. 1997. *Vet Pathol* 34:467–469.

Vanrompay D, Geens T, Desplanques A, et al. 2004. *Vet Microbiol* 99:59–66.

Vazquez-Cisneros C, Wilsmore AJ, Bollo E. 1994.Experimental infections of pregnant sows with ovine Chlamydia psittaci strains. *Vet Microbiol.* 42:383–387.

Enterococci

Enterococci are normal inhabitants of the intestinal flora, but some strains have the capacity to colonize the mucosal surface of the small intestine extensively. Some enterococcal species that show typical adhesion to the apical surface of the enterocytes of the small intestine of young animals have been described as associated with diarrhea in different animal species (Vancanneyt et al. 2001). Cases have been reported in piglets between 2 and 20 days of age (Cheon and Chae 1996; Drolet et al. 1990).

Taxonomic studies have shown that most of these enterococci are members of the *E. faecium* species group, mainly *E. durans*, *E. villorum/E. porcinus*, and *E. hirae* (De Graef et al. 2003; Jonach et al. 2014; Vancanneyt et al. 2001). Enteroadherent *enterococci* are involved in cases of the New Neonatal Porcine Diarrhoea Syndrome (NNPDS) reported in the Scandinavian countries (Hermann-Bank et al. 2015; Jonach et al. 2014; Larsson et al. 2014).

The pathogenesis of enteric disease associated with adherent enterococci is unclear. Adherence involves fibrillar projections (Tzipori et al. 1984), and diarrhea is not associated with enterotoxin production or substantial mucosal injury (Cheon and Chae 1996). Because of the natural resistance of enterococci to some antibacterial agents, antimicrobial susceptibility testing is advised before treatment. Sanitation between farrowings is presumed to be helpful in prevention. Due to the lack of knowledge about the clinical and epidemiological aspects of this infection, other preventive measures are difficult to recommend.

References

Cheon D, Chae C. 1996. *J Vet Diagn Invest* 8:123–124.

De Graef EM, Devriese LA, Vancanneyt M, et al. 2003. *Int J Syst Evol Microbiol* 53:1069–1074.

Drolet R, Higgins R, Jacques M. 1990. *Méd Vét Québec* 20:114–115.

Hermann-Bank ML, Skovgaard K, Stockmarr A, et al. 2015. *BMC Vet Res* 11:139.

Jonach B, Boye M, Stockmarr A, et al. 2014. *BMC Vet Res* 10:68.

Larsson J, Lindberg R, Aspán A, et al. 2014. *J Comp Pathol* 151:137–147.

Tzipori S, Hayes J, Sims L, et al. 1984. *J Infect Dis* 150:589–593.

Vancanneyt M, Snauwaert C, Cleenwerck I, et al. 2001. *Int J Syst Evol Microbiol* 51:393–400.

Klebsiella pneumoniae

Relevance and etiology

Since 2011 several outdoor breeding herds in the United Kingdom (mostly in East Anglia) were diagnosed with septicemia in preweaning piglets due to infection with *Klebsiella pneumoniae* subsp. *pneumoniae* (Animal and Plant Health Agency (APHA) 2015). In 2016 the disease was also reported in preweaning piglets in indoor units in several Australian states (Victoria, Queensland, and New South Wales) as well as in Quebec (Canada) and the United States. Prior to these reports of outbreaks, it was more commonly diagnosed causing sporadic disease in individual pigs such as septicemia in piglets and mastitis in sows.

K. pneumoniae subsp. *pneumoniae* belongs to the *Enterobacteriaceae* family. They are gram-negative, rod-shaped, and nonmotile bacteria. They are facultatively anaerobic, produce lysine decarboxylase (LDC) but not ornithine decarboxylase (ODC), and are generally positive in the Voges–Proskauer (VP) test. Colonies are non-hemolytic and usually mucoid.

Klebsiella pneumoniae is a commensal of the porcine digestive tract and is present in the environment in both soil and water sources. It is frequently found in sawdust, which is an ideal environment for survival and multiplication.

K. pneumoniae is a cause of various human infections including pneumonia, meningitis, urinary tract infections, and neonatal septicemia, but it is not considered a zoonotic agent.

Clinical signs, lesions, and diagnosis

Klebsiella septicemia infrequently occurs in pre-weaned 1- to 4-week-old piglets. Piglets are found dead or recumbent and cyanotic. In the UK and Australian outbreaks, the cases occurred during summer months (APHA 2015; New South Wales (NSW) Department of Primary Industries and Local Land Services 2016). Overall mortality is variable but usually low (<5%), and within-litter mortality is also variable and usually low with 1–2 piglets dying per litter. However cases with mortality as high as 100% have been reported in Australia. The duration of herd disease varies from a few weeks to as long as 12 weeks. Although *K. pneumoniae* can be associated with mastitis, sows in the same farrowing group as affected piglets are not usually affected.

Lesions are nonspecific and similar to those observed in septicemia due to other bacteria such as *Streptococcus suis* (Chapter 61), *Actinobacillus suis* (Chapter 48), *E. rhusiopathiae* (Chapter 53), or *Escherichia coli* (Chapter 52). Postmortem findings include purple discoloration of skin on the ventrum and extremities and the presence of fibrin strands in the abdominal cavity, serosal petechiae, pleural effusion, and congested lymph nodes. *K. pneumoniae* is easily isolated in abundant pure culture from various tissues using standard aerobic culture on blood agar.

Prevention and control

The reasons for the emergence of outbreaks of *K. pneumoniae* septicemia in piglets are unknown. Molecular characterization of some UK isolates has shown that all are sequence type 25 (ST25) with a unique combination of a 4.3 kb plasmid and rmpA virulence gene (Bidewell et al. 2013). Four recently characterized Quebec isolates also have the same ST25. All of the isolates have an innate resistance to beta-lactams, and most have shown *in vitro* sensitivity to other antimicrobials tested. However occasional isolates with resistance to multiple antimicrobials have been detected.

In UK outdoor units, interventions included antimicrobial treatment of remaining piglets in affected litters, introduction of creep feed prior to weaning, or treatment of neonatal piglets with a combination of antimicrobial and iron. Some of these interventions resulted in cessation or reduction in mortality. However, in the absence of untreated control groups, their efficacy cannot be assessed since the disease was sometimes self-limiting on farms without interventions.

References

Animal and Plant Health Agency (APHA). 2015. New developments in *Klebsiella pneumoniae* septicaemia outbreaks. In GB Emerging Threats: Pig Diseases: July to September 2015. 19:6–7.

Bidewell C, Williamson S, Rogers J, et al. 2013. *Klebsiella pneumoniae* subsp. *pneumoniae* sequence type 25: re-emergence as a cause of septicaemia in piglets in 2012. In Proc European Porcine Health and Management/Pig Veterinary Society Symposium 5:57.

New South Wales (NSW) Department of Primary Industries and Local Land Services. 2016. *Klebsiella* outbreaks causing septicaemia in sucking pigs. In Animal Health Surveillance 1:6.

Listeria monocytogenes

Relevance, etiology, and public health

Listeria monocytogenes is commonly carried in the tonsils and intestines of swine and is a rare cause of septicemia in piglets, nervous signs in all age groups, and abortion in sows. More importantly the organism is an uncommon cause of serious foodborne disease in humans, and its carriage by slaughter swine is of concern to the food industry.

L. monocytogenes is a gram-positive bacillus 1.2 µm by 0.5 µm and does not form spores. The organism grows at temperatures as low as minus 1 °C and up to 45 °C and can multiply in the presence of nutrients in refrigerated conditions or at room temperature. It is aerobic/aerotolerant and produces 1 mm, grayish, and opaque colonies with a narrow zone of β-hemolysis on blood agar. It is capable of growth on a number of media, and the use of chromogenic media and molecular methods (PCR) to differentiate *L. monocytogenes* from other species (*L. innocua*) is of particular importance when detection is required (Raorane-Abhay et al. 2015; Yukako et al. 2016).

L. monocytogenes is a cause of septicemia, abortion, and nervous signs in humans. Pregnant women, newborn infants, the elderly, and the immunosuppressed are most at risk. Although the organism is present in the environment, food, particularly meat products, is an important source (Beloeil et al. 2003; Boscher et al. 2012; Ristori et al. 2014). Thevenot et al. (2006) have reviewed the contamination of pork and pork products with *L. monocytogenes*. Contamination increases from farm to raw meat because of cross-contamination and multiplication of the organism (Larivière-Gauthier et al. 2014). Up to 30% of raw minced pork meat may be contaminated (Ristori et al. 2014).

Epidemiology and pathogenesis

L. monocytogenes occurs worldwide and is carried by most food animal species, and clinical disease occasionally develops. Carriage of *L. monocytogenes* is common in pigs in the intestine and tonsils. Studies in various countries suggest that about 10% of slaughter pigs are carriers. Carriage rate seems to increase when liquid feed or silage is fed. Exposure in pigs is principally by ingestion. Shedding is in the feces and in the products of

abortion. The organism can be detected in manure for at least 55 days (Grewal et al. 2007) and is susceptible to most approved disinfectants. *L. monocytogenes* is destroyed by pasteurization, but food recontamination can occur after heating.

The organism has two modes of entry. In nervous disease it may travel up nerves to the brain by retrograde movement, and in bacteremia/septicemia, it enters through the tonsil or gut. Invasion of privileged sites such as the brain, joints, and uterus follows bacteremia/septicemia. Clinical signs are caused by the invasion, intracellular multiplication, and production of listeriolysin. Neonatal and pregnant animals are most likely to be clinically affected.

Clinical signs, lesions, and diagnosis

Subclinical infection is common; clinical listeriosis is uncommon. Septicemia in suckling piglets is often evidenced by sudden death or pyrexia, lethargy, and hyperemia or purple discoloration of skin on extremities. Nervous disease is characterized by tremors, lateral nystagmus, lateral recumbency, and paddling. Abortion, stillbirths, and the birth of weak piglets may all occur in affected sows.

Lesions in piglets may include hepatomegaly ± splenomegaly with large numbers of small foci of necrosis, moist lungs that fail to collapse, variable hydrothorax, and enlarged moist lymph nodes (Lopez and Bildfell 1989). Consistent microscopic lesions include multifocal hepatic and splenic necrosis with large numbers of intralesional small rod-shaped bacteria and fibrin thrombi in microvasculature. In pigs with nervous disease, gross lesions are often not observed; microscopic lesions include suppurative meningitis, perivascular cuffing, and microabscess formation in the brain. In reproductive failure, hepatic and splenic necrosis as described in neonates is sometimes observed in stillborn and weak-born pigs.

Clinical signs and lesions are not specific to listeriosis in any of its various forms. Other causes of each clinical syndrome must be included in differentials. Of particular concern is pseudorabies virus that can cause rapid death of neonates and produce multifocal hepatic and splenic necrosis similar to listeriosis. Confirmation of listeriosis requires typical clinical signs and lesions as well as detection of *L. monocytogenes*. The organism is present in clinical specimens in high numbers and produces typical hemolytic colonies on blood agar. For contaminated specimens, tonsillar material, cecal contents, feces, environmental samples, and foods, enrichment in the cold at 4 °C overnight in a simple enrichment medium or one supplemented with antimicrobials followed by subculture onto a chromogenic selective medium gives good results.

PCR using primers based on the *prs and prfA* gene sequences have been used for qualitative detection and confirmation and *prf*A for quantitative demonstration of the organism using real-time PCR (Yukako et al. 2016).

Prevention and control

The organism is sensitive to a number of antimicrobials including penicillins and aminoglycosides, and affected pigs may recover if treated early in the course of disease. Paralyzed pigs should be humanely euthanized. Composting of manures at more than 55 °C or use of aerobic composting can reduce numbers of *Listeria* in wastes (Grewal et al. 2007).

References

Beloeil PA, Chauvin C, Toquin MT, et al. 2003. *Vet Res* 34:737–748.

Boscher E, Houard E, Denis M. 2012. *J Food Prot* 75:889–895.

Grewal S, Sreevatsan S, Michel FC. 2007. Persistence of Listeria and Salmonella during swine manure treatment. *Compost Sci Util* 15:53–62.

Larivière-Gauthier G, Letellier A, Kérouanton A, et al. 2014. *J Food Prot* 77:2121–2128.

Lopez A, Bildfell R. 1989. *Can Vet J* 30:828–829.

Raorane-Abhay V, Swapnil P, Doijad SP, et al. 2015. *Int J Curr Microbiol Appl Sci* 4:788–798.

Ristori CA, Rowlands RE, Martins CG, et al. 2014. *Foodborne Pathog Dis* 11:969–973.

Thevenot D, Dernburg A, Vernozy-Rozand C. 2006. *J Appl Microbiol* 101:7–17.

Yukako S, Ida M, Nishino Y, et al. 2016. *J Vet Med Sci* 78(3):477–479.

Rhodococcus equi

Relevance, etiology, and public health

Rhodococcus equi causes granulomatous lymphadenitis affecting the lymph nodes of the head and neck of the pig. The lesions can be confused at slaughter with those of tuberculosis (Chapter 63), and it is important for this reason rather than as a cause of clinical disease. *R. equi* is also a cause of infection and mortality in immunocompromised humans. Swine are not known to be a risk factor for human infection.

R. equi (Goodfellow 1987) was previously named *Corynebacterium equi*. This gram-positive coccobacillus is non-sporulating, but possesses a capsule. The cell wall contains mycolic acid, and there is an abundant acidic polysaccharide capsule, which is the basis for serotyping schemes (Nakazawa et al. 1983). *R. equi* produces pinkish colonies on solid media. Colonies are slow growing, requiring 48 hours to reach a size of 2–4 mm. The typical colony is irregularly rounded, buff pink, smooth, and mucoid. *R. equi* is biochemically unreactive.

R. equi is of importance to public health as a cause of granulomatous lesions in the cervical lymph nodes of pigs. These must be distinguished from those of tuberculosis caused by the *Mycobacterium avium* complex (MAC) and other *mycobacteria* and are therefore of concern in meat inspection. *R. equi* can infect humans and is becoming more common as a cause of necrotizing pneumonia and chronic illness in immunocompromised humans, particularly in those suffering from HIV infections (Weinstock and Brown 2002). Infection results in high mortality, averaging 25%. Human isolates are similar to some pig strains. However, pig-to-human or pork-to-human transmission is not documented.

Epidemiology and pathogenesis

Little is known of the epidemiology of *R. equi* infection in swine, but *R. equi* has been reported worldwide and infects swine, cattle, deer, horses, sheep, goats, wild birds, and humans (Woolcock et al. 1979). *R. equi* infection is likely to be acquired from the environment by ingestion in swine housed on pasture or in yards contaminated with *R. equi* (Barton and Hughes 1984). The bacterium is readily isolated from the feces of such pigs. Komijn et al. (2007) examined 15,900 cervical lymph nodes for granulomatous lesions in the Netherlands and found them in 0.75% pigs. *R. equi* was isolated from 44% of these. It is present in dust and even in cobwebs of farm buildings in areas where it occurs and is relatively resistant to chemical disinfectants.

Infection of pigs is usually by the oral route. The way in which *R. equi* causes granulomatous lymphadenitis of the head and neck in swine is not clear.

Clinical signs, lesions, and diagnosis

R. equi infection in swine is normally subclinical and has rarely been associated with clinical disease. The lesions of granulomatous lymphadenitis are detected only at slaughter. Affected submandibular and cervical nodes are enlarged and contain multiple yellow-tan foci that are often in a subcapsular location. Caseation and calcification of these foci sometimes occurs.

Diagnosis is postmortem. Gross and microscopic lesions are as described. Microbiologic identification of *R. equi* and elimination of mycobacterial infection are necessary to confirm diagnosis. Selective media (Makrai et al. 2005; Woolcock et al. 1979) improve the results of culture as the creamy or pink domed colonies of *R. equi* require 48 hours to reach a size of 2–4 mm. A quantitative PCR targeting the chromosomal gene choE and the virulence plasmid gene vapA can also be used (Rodríguez-Lázaro et al. 2006).

Prevention and control

R. equi-induced disease is not sufficiently important to necessitate antemortem diagnosis and treatment in swine or dedicated preventive measures.

References

Barton MD, Hughes KL. 1984. *Vet Microbiol* 9:65–76.

Goodfellow M. 1987. *Vet Microbiol* 14:205–209.

Komijn RE, Wisselink HJ, Rijsman VM, et al. 2007. *Vet Microbiol* 120:352–357.

Makrai L, Fodor L, Vendeg I, et al. 2005. *Acta Vet Hung* 53:275–285.

Nakazawa M, Kubo M, Sugimoto C, et al. 1983. *Microbiol Immunol* 27:837–846.

Rodríguez-Lázaro D, Lewis DA, Ocampo-Sosa AA, et al. 2006. *Appl Environ Microbiol* 72: 4256–4263.

Weinstock DM, Brown AE. 2002. *Rhodococcus equi*: an emerging pathogen. *Clin Infect Dis* 34:1379–1385.

Woolcock JB, Farmer AMT, Mutimer MD. 1979. *J Clin Microbiol* 9:640–642.

Treponema pedis: Cutaneous Spirochetosis

Relevance, etiology, and public health

Treponema pedis, a spirochete previously isolated from digital dermatitis in cattle (Evans et al. 2009), has now been isolated from ear necrosis, decubital shoulder ulcers, and other chronic skin lesions in pigs (Karlsson et al. 2014; Pringle et al. 2009; Svartström et al. 2013). Other bacteria such as *Staphylococcus hyicus* and streptococci have been isolated from lesions of ear necrosis in the past, and *T. pedis* is likely not the sole or initiating agent. The syndrome of ear necrosis in swine has not yet been reproduced experimentally. *T. pedis* has no known public health significance.

T. pedis is characterized by its morphology, cultural characteristics, 16S rRNA gene sequences and flaB2 gene sequences. It is approximately 0.25 µm in diameter and 4–6 µm in length with a 3 : 6 : 3 flagellar pattern, does not form spores, is anaerobic, and produces esterases, lipases, and trypsin-like proteases. Cultivation is in strictly anaerobic conditions at 37 °C on fastidious anaerobe agar (FAA) with 10% added horse blood. Colonies become obvious in 4–5 days.

Epidemiology and pathogenesis

Chronic skin lesions including ear necrosis, decubital shoulder ulcers, and spirochetal granuloma have been reported in pigs from most parts of the world. Pigs appear to be the only species affected by these manifestations, but *T. pedis* is also found in digital dermatitis in cattle and sheep. Transmission in pigs may be through skin and ear biting as Pringle et al. (2009) found the organism in the gingiva of affected pigs. Persistence in the swine environment is not known, but infection persists in cattle slurry for some days.

The infection appears to be localized in the skin in infected lesions, possibly caused by biting or abrasion in the first instance. The production of esterases, lipases, and proteases may aid the development of the lesion. Initial infection and penetration into the tissue may be limited by the anaerobic nature of the organism.

Clinical signs, lesions, and diagnosis

Ear necrosis (see also Chapter 17) is a condition in which small inflamed areas on the margins of the ears develop after weaning, spread, intensify, and ultimately cause loss of the ear in severe cases. The lesions begin as small areas of damage on the edge of the ear and near its junction with the head, become scabby, extend, and become necrotic (Pringle et al. 2009). They can lead to loss of the entire pinna. On resolution, they heal, leaving a scar. Similar lesions may occur on the flanks and upper parts of the hind limb, and Svartström et al. (2013) describe a shoulder ulcer from which they have obtained the organism. Morbidity is usually low, and affected pigs do not perform significantly differently from unaffected controls (Busch et al. 2010). An extensive literature associates this condition and its severity with behavioral changes attributed to housing and management (Smulders et al. 2008).

The lesion is a chronic ulcerative and pustular dermatitis. A thick crust of fibrin exudate and inflammatory cells covers its edges with vasculitis with or without thrombosis in the underlying blood vessels. Bacteria can be demonstrated in the superficial layers, and spirochetes can be seen in silver-stained sections deeper in the tissue. Healed lesions may be seen as scars on older animals.

Ear necrosis and localized necrotic lesions elsewhere on the body are suggestive of infection by *T. pedis*, but *S. hyicus* (Chapter 61) may be present in some lesions. Pig pox lesions must be ruled out (Chapter 30) but are typically localized and rarely lose their scabs. Bite wounds are much more difficult to differentiate, and behavior must be watched to confirm the relevant importance of simple biting in the present condition. The progressive nature of the gross lesions suggests spirochetal involvement, and the organisms can be demonstrated in the inflamed tissue. A preliminary identification can be made microscopically in tissue sections utilizing silver stains.

Isolation is by inoculation of fastidious agar broth enriched with fetal calf (25%) and rabbit (10%) serum with added rifampicin and enrofloxacin in anaerobic conditions. Growth can be purified by the inoculation of 0.22 µm pore size Millipore filters on FAA followed by streaking to produce the pinpoint grayish slightly hemolytic colonies (Pringle et al. 2009). The organisms can be also be detected by using a PCR for treponemes (Pringle et al. 2009).

Prevention and control

Treatment of individual pigs is possible using parenteral antimicrobials but is rarely carried out, as the condition does not warrant it. Topical lincomycin–spectinomycin combinations have been used in cattle to treat *T. pedis*. Local skin disinfection may also be of value. Improvement of environmental conditions and particularly the provision of manipulable materials may improve the environmental/social reasons for flank and ear biting. Formaldehyde and glutaraldehyde can reduce infection in the environment.

References

Busch ME, Jensen IM, Korsgaard J. 2010. The development and consequences of ear necrosis in a weaner herd and two growing finishing herds. In Proc Int Pig Vet Soc, p. 45.

Evans NJ, Brown JM, Demirkan I, et al. 2009. *Int J Syst Evol Microbiol* 59:987–991.

Karlsson F, Klitgaard K, Kåre Jensen T. 2014. *Vet Microbiol* 171:122–131.

Pringle M, Backhans A, Otman F, et al. 2009. *Vet Microbiol* 139:279–283.

Smulders D, Hautekiet V, Verbeke G, et al. 2008. *Anim Welfare* 17:61–69.

Svartström O, Karlsson F, Fellström C, et al. 2013. *Vet Microbiol.* 166:617–623.

Trueperella abortisuis

Trueperella abortisuis, previously *Arcanobacterium abortisuis*, was first isolated from the placenta and tissues of an 87-day-gestation aborted litter of pigs having lesions and intralesional bacteria in Japan (Azuma et al. 2009; Murakami et al. 2011; Yassin et al. 2011). Additional isolates have been obtained from placenta after abortion, such as vaginal discharges, rectal swabs from piglets, and boar sperm (Hijazin et al. 2012b; Metzner et al. 2013; Ulbegi-Mohyla et al. 2011). Although this organism caused a single case of sporadic abortion, its significance as a reproductive pathogen of swine is unknown.

T. abortisuis is a gram-positive, short diphtheroid-shaped organism. It grows under microaerobic or anaerobic conditions and produces a narrow zone of complete hemolysis on sheep blood agar. Colonies demonstrate CAMP-like activity with alpha-hemolytic *Staphylococcus aureus*. It is most easily identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Hijazin et al. 2012a; Ulbegi-Mohyla et al. 2011) or by amplification and sequencing of 16S rRNA gene sequence and gene *sodA* encoding superoxide dismutase (Metzner et al. 2013).

T. abortisuis is usually isolated in mixed culture with other bacterial species, making the importance of this novel species unclear. In the index case, 87-day-gestation fetuses were aborted with gross lesions of subcutaneous edema, congested lung, and increased fluids in the body cavities typical of *in utero* autolysis. Microscopic lesions were consistent among three fetuses and included suppurative bronchopneumonia and necrosuppurative placentitis, each with large numbers of intralesional gram-positive filamentous and/or rod-shaped bacteria. Primary antisera produced using cultured *T. abortisuis* was used to confirm the intralesional bacterial identity by immunohistochemistry.

Diagnosis of *T. abortisuis* abortion requires demonstration of lesions in fetuses and/or placenta with morphologically consistent intralesional bacteria and culture of *T. abortisuis*. Demonstration of *T. abortisuis* in vaginal discharges or in placenta absent other lesions should not be considered confirmatory given the potential confounder of fecal contamination.

References

Azuma R, Murakami S, Ogawa A, et al. 2009. Erratum in: *Int J Syst Evol Microbiol* 59:3184.

Hijazin M, Hassan AA, Alber J, et al. 2012a. *Vet Microbiol* 157:243–245.

Hijazin M, Ulbegi-Mohyla H, Alber J, et al. 2012b. *Berl Munch Tierarztl Wochenschr* 125:32–37.

Metzner M, Erhard M, Sammra O, et al. 2013. *Berl Munch Tierarztl Wochenschr* 126: 423–426.

Murakami S, Ogawa A, Azuma R, et al. 2011. *J Vet Med Sci* 73:797–799.

Ulbegi-Mohyla H, Hassan AA, Hijazin M, et al. 2011. *Vet Microbiol* 148:431–433.

Yassin AF, Hupfer H, Siering C, et al. 2011. *Int J Syst Evol Microbiol* 61:1265–1274.

Trueperella pyogenes

Relevance, etiology, and public health

Trueperella pyogenes (formerly *Arcanobacterium pyogenes, Actinomyces pyogenes*, and *Corynebacterium pyogenes*) is a common cause of suppurative lesions in pigs and ruminants throughout the world. Clinical disease can result from the destruction of tissues. As a consequence of *T. pyogenes* infection, carcasses at slaughter may contain unsightly abscesses filled with creamy pus, resulting in economic losses from trimming or condemnation. Rare cases of human infection with *T. pyogenes* have been reported (Levy et al. 2009; Plamondon et al. 2007). Patients often had a history of close contact with domestic animals.

T. pyogenes is a small, non-sporing, gram-positive pleomorphic rod. Growth is enhanced by the addition of serum or blood. *T. pyogenes* is aerobic or facultatively anaerobic and grows best at 37 °C. Colonies are small, taking 48 hours to achieve a diameter of 1 mm. They form narrow zones of complete hemolysis after 24 hours on blood agar. *T. pyogenes* is proteolytic; glucose is fermented, but fermentation of other carbohydrate reactions is variable. Identification is presently most easily carried out using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Hijazin et al. 2012).

Epidemiology and pathogenesis

Infections with *T. pyogenes* are found worldwide in ruminants, pigs, and horses (Ribeiro et al. 2015). It is part of the skin and mucosal flora of the host species and can be isolated in discharges from the upper respiratory tract, udder, vulva, and feces in infected animals. Transmission

may be direct or indirect via infected fomites. The organism can survive freezing and drying when protected in organic matter such as discharge. *T. pyogenes* is susceptible to a range of disinfectants.

Infection is opportunistic resulting from the invasion of skin or mucous membranes by resident *T. pyogenes* (Jarosz et al. 2014). Adhesion to tissues is aided by neuraminidases, fimbriae, and collagen binding protein (CBPA), and tissue damage results from production of the pyolysin (Billington et al. 1997; Jost and Billington 2005). The organism can multiply locally on surfaces such as inflamed bronchiolar epithelium, in the vaginal and uterine mucosa, and in the urinary tract. It causes ascending or hematogenous infections in the mammary gland and spreads by bacteremia to colonize existing minor lesions in joints, lungs, vertebral bodies, and parenchymatous organs. Their location and extent cause the clinical signs.

Clinical signs, lesions, and diagnosis

The clinical signs are quite variable, since *T. pyogenes* is responsible for a range of pathological lesions. Randomly distributed pulmonary abscesses occur due to septic emboli from *T. pyogenes*-infected skin lesions including ear or tail necrosis. Endocarditis, bronchopneumonia, and adhesive peritonitis may be fatal and be associated with fever. Suppurative osteomyelitis generally affects the vertebral bodies, leading to pathological fractures, vertebral collapse, and compression of the spinal cord. Lameness results from polyarthritis or from cellulitis and periarthritis. Purulent secretion from the teat is seen in mastitis, and abscesses are prominent in involuted glands. Creamy discharge may be seen on the vulva in endometritis, and the urine may be flecked with pus in cystitis and pyelonephritis. Subcutaneous or intramuscular abscesses are often clinically inapparent and are discovered only at postmortem or slaughter. Mortality occurs when damage to an organ becomes life threatening as in pyelonephritis in sows. Frequently abscesses do not cause clinical signs other than loss of condition.

T. pyogenes causes creamy, greenish mucoid pus on inflamed mucous surfaces and appears as flecks of pus in the urine in cases of cystitis and as ropes of pus in the kidney pelvis and ureters in pyelonephritis and in the uterus in metritis. The most noticeable and pathognomonic lesions are abscesses, which may arise in almost every tissue in the body. Such abscesses vary from a few millimeters to several centimeters in size, usually have a thick fibrous capsule, and contain yellow-green pus of variable consistency. They may be in joints, over cracked ribs, in contaminated injection sites, and in parenchymatous organs. Mastitis may be confined to one gland or may involve several glands.

T. pyogenes infection is the major cause of purulent abscessation and purulent discharges of all types in pigs, and its presence should be strongly suspected in such cases. Other pyogenic bacteria such as staphylococci (Chapter 60) may also cause abscessation, endocarditis, mastitis, endometritis, and pyelonephritis, whereas *E. coli* (Chapter 52) and *Actinobaculum suis* (see above) must also be considered. Confirmation of *T. pyogenes* requires the demonstration of the organism in typical lesions by laboratory culture or by real-time qPCR (Aghamiri et al. 2014). Pinhead beta-hemolytic colonies on blood agar are suggestive of *T. pyogenes*, and identification can be confirmed using standard bacteriological procedures or more easily by MALDI-TOF MS (Hijazin et al. 2012).

Prevention and control

T. pyogenes is sensitive to a wide range of antimicrobial agents including penicillin, tetracycline, and erythromycin. Some strains have been shown to be resistant to sulfonamides and trimethoprim. Antimicrobial treatments only are poorly effective due to difficulty achieving therapeutic levels in abscesses. Abscesses may be removed surgically where they can be identified in affected individuals.

Effective vaccines are not available. Prevention requires management to reduce or prevent conditions that predispose to the development of *T. pyogenes* infections. The implementation of needle-free injection systems in market hog production has been reported to increase the occurrence of injection site abscesses in pork carcasses (Gerlach et al. 2012).

References

Aghamiri SM, Haghkhah M, Ahmadi MR, et al. 2014. *Reprod Domest Anim* 49:233–238.

Billington SJ, Jost BH, Cuevas WA, et al. 1997. *J Bacteriol* 179:6100–6106.

Gerlach BM, Houser TA, Hollis LC, et al. 2012. *Meat Sci* 92:805–807.

Hijazin M, Hassan AA, Alber J, et al. 2012. *Vet Microbiol* 157:243–245.

Hijazin M, Ulbegi-Mohyla H, Alber J, et al. 2012. *Berl Munch Tierarztl Wochenschr* 125:32–37.

Jarosz ŁS, Gradzki Z, Kalinowski M. 2014. *Pol J Vet Sci* 17:395–404.

Jost BH, Billington SJ. 2005 *Antonie Van Leeuwenhoek* 88:87–102.
Levy CE, Pedro RJ, Von Nowakonski A, et al. 2009. *Emerg Infect Dis* 15:1131–1132.
Plamondon M, Martinez G, Raynal L, et al. 2007. *Eur J Clin Microbiol Infect Dis* 26:663–666.
Ribeiro MG, Risseti RM, Bolaños CA, et al. 2015. *Vet Q* 35:82–87.

Yersinia spp.

Relevance, etiology, and public health

The primary importance of *Yersinia enterocolitica* and, to a lesser extent, *Yersinia pseudotuberculosis* in swine is as a source for foodborne infection of humans that handle or consume contaminated pork products. Swine are the primary carrier of *Y. enterocolitica* and source for human yersiniosis. Most infections by *Y. enterocolitica* and *Y. pseudotuberculosis* in swine are asymptomatic, and their importance in enteric disease in commercial swine production is relatively minor. Of additional significance in swine, antibody to *Y. enterocolitica* serotype O:9 cross-reacts in serological tests for *Brucella* spp., causing false-positive serologic tests for swine brucellosis.

Yersinia belongs to the *Enterobacteriaceae. S*pecies isolated from pigs include *Y. pseudotuberculosis, Y. pseudotuberculosis* subsp. *pestis* (the plague bacillus), *Y. enterocolitica, Y. intermedia, Y. frederiksenii*, and *Y. kristensenii*. *Y. enterocolitica* and *Y. pseudotuberculosis* are most important in swine. Yersinias are gram-negative, non-sporulating coccobacilli or short rods. Capsules, attachment antigens, and enterotoxins have been described. Individual species can be divided into biotypes and serotypes and possess plasmids and virulence factors. *Y. enterocolitica* and *Y. pseudotuberculosis* grow at temperatures between 4 and 37°C and can therefore multiply in refrigerated products. They are aerobic or facultatively anaerobic. They grow on routine media upon which they appear as small 1–2 mm colonies within 24–48 hours.

Yersiniosis has been the third to fourth most frequent zoonosis in the European Union (EU) from 2004 to 2011 (Laukkanen-Ninios et al. 2014). Most cases are foodborne, associated with the preparation or consumption of undercooked pork. The relative prevalence as a foodborne illness is much lower in the United States where it ranked eighth in 2011 (Scallon et al. 2011) and is over-represented as a regional and seasonal disease associated with the preparation or consumption of chitterlings or "chitlins," a dish prepared from swine intestines (Drummond et al. 2012). The vast majority of human yersiniosis is caused by *Y. enterocolitica*, with *Y. pseudotuberculosis* accounting for less than 1%. Approximately 75% of human yersiniosis is attributed to the preparation or consumption of contaminated raw or undercooked pork (Laukkanen-Ninios et al. 2014; Van Damme et al. 2015). Predictably, the same serotypes and genotypes predominate in swine and human infections (Fredricksson-Ahomaa et al. 2007; Laukkanen-Ninios et al. 2014).

Most human yersiniosis manifests as self-limiting enterocolitis in children 5 years of age or younger that exhibit pyrexia, vomiting, and severe often bloody diarrhea. Adults may experience similar signs, but more commonly acute ileitis and purulent mesenteric lymphadenitis result in acute abdominal pain along with vomiting and diarrhea mimicking acute appendicitis (Bancerz-Kisiel and Szweda 2015; Drummond et al. 2012).

Epidemiology, clinical signs, and lesions

Y. enterocolitica can be isolated from a wide variety of mammals and birds, with the pig being the primary reservoir (Bancerz-Kisiel and Szweda 2015; Drummond et al. 2012). It can also be detected in the environment, including ponds and lakes (Greenwood et al. 1990). In contrast, *Y. pseudotuberculosis* is most commonly found in rodents and uncommonly in pigs. *Y. enterocolitica* is reported in swine worldwide. Limited prevalence data exists, but studies have indicated that 32–53% of US farms (Bhaduri et al. 2005; Wesley et al. 2008), 48–80% of Canadian farms (Farzan et al. 2010; Pilon et al. 2000), 69% of English farms (Ortiz Martínez et al. 2010), 25–50% of German farms (Gürtler et al. 2005; von Altrock et al. 2006; Wehebrink et al. 2008), 80–100% of Belgian, Italian, and Spanish farms (Martínez et al. 2011), and 36–80% of pigs on Finnish farms (Asplund et al. 1990; Laukkanen et al. 2009) are infected. Factors known to increase risk for farm infection are purchase of swine from outside sources, feeding of animal protein products, and use of surface or shallow-well water sources (Virtanen et al. 2014; Wesley et al. 2008). Factors that increase carriage rate on farms include solid unbedded flooring (Vilar et al. 2013), antibiotic-free rearing (Funk et al. 2013), and continuous-flow management practices.

Y. enterocolitica infects swine orally, persists in tonsils for long periods of time, and is shed in feces as soon as 5 days and for up to 10 weeks following initial infection (Fukushima et al. 1983, 1984; Nielsen et al. 1996). Grower and finishing pigs are most often infected with rates of infection much lower in breeding animals and piglets.

Enteropathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* do not generally cause clinical disease in pigs on commercial swine farms (Laukkanen-Ninios et al. 2014). Watery diarrhea containing bits of fibrin and enterocolitis

have been reproduced in colostrum-deprived neonatal pigs by inoculation with *Y. enterocolitica* (Robins-Browne et al. 1985; Schiemann 1988; Shu et al. 1997), confirming potential pathogenicity in swine and serving as a model of human yersiniosis. However, *Y. enterocolitica* infection in neonates in a commercial setting is uncommon, and associated diarrheal disease is not generally recognized. Inoculation of 11- to 24-week-old pigs with *Y. enterocolitica* resulted in colonization and fecal shedding, but no clinical disease was observed (Fukushima et al. 1984). Similarly, colonization by *Y. enterocolitica* in a commercial setting is most common in 2- to 5-month-old pigs, but clinical disease is not generally observed (Fukushima et al. 1983; Nesbakken et al. 2006; Vilar et al. 2013; Virtanen et al. 2012). Neef and Lysons (1994) orally inoculated 10-week-old pigs with *Y. pseudotuberculosis* and produced watery diarrhea in 25% and gross and/or microscopic lesions of enterocolitis in 50%. This suggests the potential for clinically significant disease in a commercial setting and is consistent with the findings of Thomson et al. (1998) where *Y. pseudotuberculosis* was the sole isolate from pigs on 4 of 85 farms where enterocolitis and diarrheal disease were confirmed in 15–50 kg (33–110 lb) pigs. However, this report is atypical, and pigs shedding *Y. pseudotuberculosis* on commercial pig farms are generally without clinical disease.

Lesions reported in *Y. enterocolitica*-inoculated neonatal colostrum-deprived pigs and in *Y. pseudotuberculosis*-inoculated conventional pigs are the same. Grossly, lesions were confined to the ileum, cecum, and colon where there were scattered erosions in the mucosa. Microscopic lesions were unique and observed consistently in the ileum, cecum, and colon and inconsistently in the jejunum. Multifocal microabscesses that contained matted colonies of bacteria were observed in the lamina propria that later expanded and resulted in multifocal necrosis and erosion of the superficial mucosa. Additionally, randomly scattered septic microabscesses were observed in the liver of neonatal pigs in only one study (Shu et al. 1997).

Diagnosis, prevention, and control

Diagnosis of porcine enteric yersiniosis requires clinical signs of diarrhea, typical microscopic lesions, and confirmation of infection by culture or PCR. Positive culture in diarrheic pigs is insufficient since subclinical shedding of *Yersinia* species is relatively common and there are a large number of other causes of diarrhea in swine. Gross lesions of erosive ileitis are nonspecific since they are also observed in cases of enteric salmonellosis (Chapter 59) that is a much common disease in commercial swine.

Y. enterocolitica and *Y. pseudotuberculosis* can readily be isolated at 37°°C on blood and MacConkey agar from tissues with lesions. Most isolation methods for *Yersinia* use cold enrichment techniques in which tissues or samples under investigation are enriched at 4°°C, followed by subculture onto a selective medium. Food microbiologists use a range of methods including various selective media and immunomagnetic enrichment (Arrausi-Subiza et al. 2014; Rasmussen et al. 1995; Van Damme et al. 2015).

Absent clinical disease, Yersinia infections in swine are rarely treated, prevented, or otherwise controlled. Isolates are often sensitive to oxytetracycline, neomycin, sulfonamides, and spectinomycin. Presently there are insufficient data on how to reduce the prevalence of *Yersinia* spp. on infected farms. Control of foodborne disease is best accomplished in the slaughter plant to reduce fecal contamination of carcasses and in home with appropriate sanitation during food preparation and cooking pork and pork products to appropriate core temperatures (Laukkanen-Ninios et al. 2014).

References

Arrausi-Subiza M, Ibabe JC, Atxaerandio R, et al. 2014. *BMC Vet Res* 10:192.

Asplund K, Tuovinen V, Veijalainen P, et al. 1990. *Acta Vet Scand* 31:39–43.

Bancerz-Kisiel A, Szweda W. 2015. *Ann Agric Environ Med* 22:397–402.

Bhaduri S, Wesley IV, Bush EJ. 2005. *Appl Environ Microbiol* 71:7117–7121.

Drummond N, Murphy BP, Ringwood T, et al. 2012. *Foodborne Pathog Dis* 9:179–189.

Farzan A, Friendship RM, Cook A, et al. 2010. *Zoonoses Public Health* 57:388–396.

Fredricksson-Ahomaa M, Stolle A, Korkeala H. 2007 *FEMS Immunol Microbiol* 47:315–329.

Funk JA, ABley MJ, Bowman AS, et al. 2013. *Foodborne Pathog Dis* 10:514–519.

Fukushima H, Nakamura R, Ito Y, et al. 1983. *Vet Microbiol* 8:469–483.

Fukushima H, Nakamura R, Ito Y, et al. 1984. *Vet Microbiol* 9:375–389.

Greenwood MH, Hooper WL, Rodhouse JC. 1990. *Epidemiol Infect* 104:351–360.

Gürtler M, Alter T, Kasimir S, et al. 2005. *J Food Prot* 68:850–854.

Laukkanen R, Ortiz Martínez P, Siekkinen K, et al. 2009. *Foodborne Pathog Dis* 6:681–688.

Laukkanen-Ninios R, Freddriksson-Ahomaa M, Korkeala H. 2014. *Compr Rev Food Sci Food Saf* 13:1165–1191.

Martínez PO, Fredriksson-Ahomaa M, Pallotti A, et al. 2011. *Foodborne Pathog Dis* 8:445–450.
Neef NA, Lysons RJ. 1994. *Vet Rec* 135:58–63.
Nesbakken T, Iversen T, Eckner K, et al. 2006. *Int J Food Microbiol* 111:99–104.
Nielsen B, Heisel C, Wingstrand A. 1996. *Vet Microbiol* 48:293–303.
Ortiz Martínez P, Mylona S, Drake I, et al. 2010. *Int J Food Microbiol* 139:64–69.
Pilon J, Higgins R, Quessy S. 2000. *Can Vet J* 41:383–387.
Rasmussen HN, Rasmussen OF, Christensen H, et al. 1995. *J Appl Bacteriol* 78:563–568.
Robins-Browne RM, Tzipori S, Gonis G, et al. 1985. *J Med Microbiol* 19:297–308.
Scallon E, Hoekstra RM, Angulo FJ, et al. 2011. *Emerg Infect Dis* 17:7–15.
Schiemann DA. 1988. *Can J Vet Res* 52:325–330.
Shu D, Simpson HV, Xu RJ, et al. 1997. *N Z Vet J* 43:50–56.
Thomson JR, Smith WJ, Murray BP. 1998. *Vet Rec* 142:235–239.
Van Damme I, Berkvens D, Vanantwerpen G, et al. 2015. *Int J Food Microbiol* 204:33–40.
Vilar MJ, Virtanen S, Heinonen M, et al. 2013. *Foodborne Pathog Dis* 10:595–602.
Virtanen S, Nikunen S, Korkeala H. 2014. *J Food Prot* 77:116–121.
Virtanen S, Salonen L, Laukkanen-Ninios R, et al. 2012. *Appl Environ Microbiol* 78:3000–3003.
Von Altrock A, Louis AL, Rosler U, et al. 2006. *Berl Munch Tierarztl Wochenschr* 119:391–399.
Wehebrink T, Kemper N, Grosse Beilage E, et al. 2008. *Berl Munch Tierarztl Wochenschr* 121:27–32.
Wesley IV, Bhaduri S, Bush E. 2008. *J Food Prot* 71:1162–1168.

Section V

Parasitic Diseases

65

External Parasites

Matthew T. Brewer and John H. Greve

The importance of external parasites to pork production varies greatly among geographic regions because of differences in climate and husbandry systems. Free-roaming pastured swine are exposed to more attacks by arthropods than confined swine. Confinement rearing of swine results in a large number of host animals in close association, thereby allowing for the buildup of large populations of ectoparasites. Flies, fleas, lice, mites, and ticks are all external parasites that can produce a range of clinical signs in swine, with rubbing and skin lesions being the most common. In addition, external parasites can be vectors of microbial pathogens. More difficult to appreciate are economic losses due to reduced growth rate, reduced feed efficiency, and loss of carcass value at slaughter. Indeed, skin blemishes from insect bites present at slaughter may lead to unnecessary trimming or even condemnation. In addition, products improperly used to treat ectoparasitism may produce residues in the tissues, causing contamination of the pork. Some external parasites are vectors of microbial pathogens. Addressing ectoparasite infestation involves a combination of husbandry practices along with treatment of pigs and the environment.

Sarcoptic mange

The most important ectoparasite of swine worldwide is scabies (sarcoptic mange, sarcoptosis). Herds infested with scabies suffer reduced growth rates, reduced feed efficiency, and decreased fertility in breeding sows (Kessler et al. 2003). The real economic importance tends to be underestimated, because pork producers may not recognize the presence of scabies in their herds when it occurs. Two clinical forms of scabies are recognized: a chronic hyperkeratotic form most commonly seen in sows and a pruritic hypersensitive form most commonly seen in growing pigs. Scabies historically has a high prevalence in swine herds (40–90% in some locales), with prevalence within infested herds varying from about 20 to 95%. Estimates of herd prevalence are difficult because mites burrow and low numbers of mites may not be detected by skin scraping and modern production systems and seedstock suppliers have been successful in elimination of scabies.

Etiology and life cycle

Scabies is caused by a burrowing arachnid mite that is thought to be a single species with host-adapted variants; therefore scabies of pigs is caused by *Sarcoptes scabiei* var. suis. The mite of swine scabies is host specific; hence sources of mites are other swine as reservoir hosts other than swine have not been implicated.

The mite has a globular body approximately 0.5 mm in length, barely visible to the naked eye and more easily seen when viewed against a dark background. When observed with low-power magnification, the mite has four pairs of short, stumpy legs, some of which bear a long, unjointed pedicle that terminates in a sucker-like pulvillus. These pedicles occur on legs I and II in the female mite and legs I, II, and IV in the male. The legs that do not have pedicles bear long bristles. The third and fourth pair of legs does not extend beyond the body margin. A distinguishing feature of *Sarcoptes* is that the anus is terminal, at the very posterior margin of the organism (Figure 65.1).

Sarcoptes scabiei are permanent parasites of the skin. After mating on the surface, the females make tunnels into the upper two-thirds of the epidermis, laying a string of 40–50 eggs behind them as they progress forward. Burrowing is by extra-oral digestion of the stratum corneum, stratum granulosum, and stratum spinosum. Burrows do not typically extend deeper than the stratum spinosum. In about 30 days the females die in the burrows. The larvae hatch in 3–5 days and molt into nymphs, and the nymphs molt into adults, all within the burrows. The adults reach the surface by means of side

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

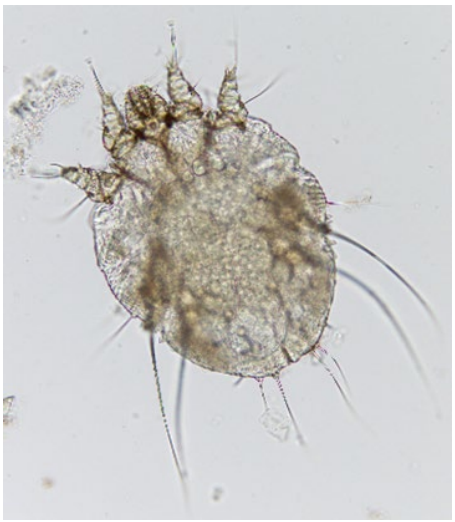


Figure 65.1 *Sarcoptes scabiei*, adult. Note that the third and fourth pairs of legs do not protrude beyond the body margin and the terminal anus, which is at the very posterior of the margin of the mite.

tunnels, and mating occurs in surface pockets, starting the cycle over. The time from egg to fertilized female takes 10–25 days, all of which is spent on the host pig.

Epidemiology

The main reservoir of scabies mites in a herd is sows, which transmit the mites by physical contact. Boars are less involved with the herd's daily activities because of the increasing use of artificial insemination, but they can serve as another source of infestation. The usual infestation in sows and boars is characterized by hyperkeratotic lesions on the inner (scaphal) surface of the ears. A few adult swine also may have mites and hyperkeratotic lesions elsewhere. Susceptible pigs become infested while suckling infested sows or huddling with infested pigs. Transmission from pig to pig is fairly slow. The mite stage responsible for transmission is considered to be newly fertilized females, which are on the skin surface instead of in the tunnels. Swine management practices that facilitate the spread of mites are group housing of sows, continuous-flow systems for growing pigs, and larger group sizes of growing pigs. The prevalence and severity of scabies tends to increase during cool months and decrease during warm months (Davies et al. 1991).

Environmental contamination is not very important in transmission of scabies mites; however pigs may become infested when placed in pens immediately after infested pigs have been removed (Smith 1986). Although mites have been kept alive for 3 weeks under optimum laboratory conditions, under field conditions, survival off of the host is poor, usually no more than 3 days. Viability is reduced by desiccation, sunlight, heat, and extreme cold.

Within a group of pigs, there seems to be a smaller population of pigs that harbor large numbers of mites but do not develop a severe form of hypersensitive mange. The larger population of pigs harbor few mites but develop a marked hypersensitivity reaction (Davies et al. 1996a, b). In the latter population the number of mites declines over time as the level of hypersensitivity increases (Cargill and Dobson 1979; Davis and Moon 1990). Continuing exposure over time from pen mates maintains the allergic reaction and clinical signs in the hypersensitive pigs.

Clinical signs and pathogenesis

Pruritus is the most consistent clinical sign of scabies. Generalized pruritus occurs from 2 to 11 weeks after infestation. The onset of pruritus and the intensity of rubbing will depend on the number of mites in the initial exposure and the level of ongoing exposure. After infestation, pigs develop encrusted lesions that are rich in mites, especially in the inner (scaphal) surface of the ears. These plaque-like lesions may coalesce to cover up to 70% of the surface of the pinnae, but they will regress with time. The development of pruritus may result in connective tissue proliferation and hyperkeratinization, leading to alopecia and/or abrasions, especially over the flanks.

Sarcoptes mites secrete histiocytic enzymes that dissolve live skin cells that are subsequently ingested by the parasite. These enzymes, along with mite feces and eggs, are capable of inducing hypersensitivity reactions. Focal erythematous skin papules associated with hypersensitivity occur in most animals as encrustations subside. The papules occur primarily on the rump, flank, and abdomen. Histologically, papules contain large numbers of eosinophils, mast cells, and lymphocytes. At this stage, few mites can be demonstrated on skin scrapings. Desensitization has not been demonstrated experimentally, but field evidence suggests that it occurs.

Hyperkeratotic mange, which is the prevalent form in mature swine, may occur also in growing pigs that fail to develop the typical hypersensitivity response. The lesions are characterized by thick scabs and scurf that are loosely attached to the subjacent skin and by the presence of numerous mites. These lesions occur most frequently in the ears, but they may also spread over the back, neck, and other parts of the body. The lesions are often accompanied by a thick, ceruminous, and odiferous exudate.

Diagnosis

Scabies has potential to be present in many herds unless some special measures to eradicate the mites have been followed. Many seedstock suppliers and production systems have eliminated mange, but reintroductions occur.

Scabies should be suspected when growing pigs with small red papules on the body are rubbing. Rubbing can be evaluated by observing a group of pigs for 15 minutes. It has been suggested that if more than 1 rubbing event per 10 pigs occur within this time frame, scabies may be present (Pointon et al. 1995). Finding the mites by means of skin scrapings confirms the diagnosis, but oftentimes the mites are difficult to find due to burrowing in skin. The majority of pigs in an infested herd may be subclinical and may not manifest pruritus (Kessler et al. 2003). Only a small percentage of growing pigs will harbor significant populations of mites. The ear is more likely to be positive than other skin sites (Bogatko 1974).

The best method to find the mites is to examine the inner surface of the ears of the breeding stock for encrustations. About 1–2 cm^2 of the lesion should be removed by means of a knife blade or chisel for examination for mites. The encrustations can be broken up onto a sheet of black paper and allowed to sit for a few minutes. When the crusts are carefully moved aside, mites may be seen with a magnifying glass against the dark background. Alternatively, the exudate should be minced finely with a scalpel blade, and the resulting material can be immobilized in a drop of immersion oil and examined microscopically. A more sensitive technique is to macerate the scabs in 10% potassium or sodium hydroxide. Low heat may be used to accelerate the maceration. The chitinous exoskeleton of the mites is impervious to hydroxide, so the exoskeletons can be found under low magnification. A third technique is to place the ear scrapings in a petri dish and incubate under low heat overnight. Mites will emerge in great numbers or adhere to the bottom of the dish (Sheahan and Hatch 1975).

Examination of carcasses at necropsy or slaughter provides information on the scabies status of herds. Skin lesions are scored, and categories defined according to the severity of the dermatitis. Minor spots must be disregarded, because they may be caused by reactions to bedding or insect bites. However, the grading of dermatitis about the shoulders, underline, and rump is of interest in evaluating scabies (Cargill et al. 1997).

Several enzyme-linked immunosorbent assays (ELISAs) have been used to detect antibodies to *S. scabiei* in serum (Bornstein and Wallgren 1997; Bornstein et al. 2000; Deckert et al. 2000; Zalunardo et al. 2000). Individual sensitivity varies from 29 to 64%, but as a herd test sensitivity approaches 95%. Specificity in individual pigs varies from 78 to 97% (Smets and Vercruysse 2000). Specific antibodies are not detectable until 5–7 weeks after infestation or approximately 3–4 weeks following the onset of clinical scabies (Bornstein and Zakrisson 1993). Detectable antibodies may persist for 9–12 months (Smets and Vercruysse 2000). Although antibodies can persist several months in sows following treatment, the half-life of antibody levels appears to be less than 2 months (Bornstein and Wallgren 1997). ELISA may have some use in evaluating the efficacy of eradication programs (Cargill et al. 2004).

Differential diagnosis from other skin diseases is important. Conditions that can be confused with scabies include parakeratosis, exudative epidermitis, deficiencies of niacin and biotin, swinepox, dermatomycosis, sunburn, photosensitization, and insect bites. Occasionally, in scabies-free herds, ear scrapings may reveal mites and/or mite eggs that can be attributed to pseudoparasites living in old bedding.

Treatment

Scabies may go unnoticed because farmers may consider rubbing by pigs to be a normal activity, but the recognition of the presence of scabies is essential to instituting the proper control measures. Several strategies have been reported to reduce the economic effects of mange. Treatment and control of mange is an option on some farms, whereas elimination is often a preferred option.

Successful treatment of scabies is dependent upon the correct use of acaricides. The large majority of registered products will keep scabies under control, and some even eradicate it, provided the correct dosage and treatment schedules are used. Oil mixtures are more effective than aqueous ones because oil helps to soften the hard scab surrounding the mites. Today oil mixtures continue to be used occasionally either by themselves or in conjunction with modern acaricides.

Older remedies (e.g. crankcase oil, diesel fuel, and lime sulfur) are very limited in efficacy and not recommended. Likewise, the chlorinated hydrocarbons (lindane, toxaphene) or organophosphates (malathion, trichlorfon, diazinon) were once used as acaricides in swine, but because of their toxicity, dwindling efficacy, or persistent tissue levels, they are no longer recommended.

More modern acaricides (see Table 65.1) are safer, have higher efficacies, and are easier to administer. If the active ingredient is not ovicidal, it must be readministered in 10 days in order to kill the emerging larvae. Instructions on dilutions, withholding periods, and other precautions by the manufacturer must be followed carefully. The availability of certain products depends on the legislation of a particular country. Effective modern acaricides include phosmet, used as a pour-on; amitraz, used as a spray; and the avermectins (ivermectin, doramectin, and moxidectin), which are given as injection. It is recommended that when using phosmet, a small amount of the product be placed in the inner aspect of each ear. The avermectins are broad-spectrum antiparasiticides effective against most internal parasites as well as lice and sarcoptic mange mites. Current products have varying levels of persistence. They are more efficient because of their systemic action and ease of administration.

Table 65.1 Products labeled for chemical treatment of external parasites of swine.

Chemical	Form	Parasites affected	Directions for use
Amitraz	Liquid concentrate	Mites	Spray pigs and surroundings, repeat in 7 days
Coumaphos	Liquid or solid concentrate	Lice, flies, ticks, mites, myiasis maggots	Spray premises and pigs
Dichlorvos	Liquid concentrate	Flies	Spray premises
Deltamethrin	Liquid concentrate	Lice, ticks, flies	Spray premises
Doramectin	Injectable solution	Lice, mites, larval flies	200 µg/kg body weight
Ivermectin	Injectable solution	Lice, mites	200 µg/kg body weight
Piperonyl butoxide	Liquid concentrate	Lice, fleas, flies, ticks	Spray premises
Permethrin	Liquid concentrate	Lice, ticks, mites, maggots, flies	Spray premises and pigs
Phosmet	Liquid concentrate	Lice, mites, ticks	Topical application or treatment of back rubbers. Do not apply to sick or suckling pigs
Tetrachlorvinphos	Feed concentrate	Fly maggots in manure	Add to feed according to label directions

Important: Always follow label directions, especially with insecticides that are toxic to humans and animals.

Control

Ongoing control of scabies involves identification of those animals that have chronic scabies so that they can receive regular treatment to prevent transmission to the young pigs. Therefore, control programs often focus on the breeding herd. Mercier et al. (2002) showed that a single dose of ivermectin (300 µg/kg body weight) given to sows 8 days before farrowing was very effective in preventing transmission of the mites to the piglets. Any sows with extensive hyperkeratotic lesions should be culled, and the rest of the sows should be treated before farrowing. The boars should be treated every 3–6 months to prevent transmission of mites during breeding. Pigs born to mite-free sows and housed in clean pens will remain free of mites unless they are exposed to infested pigs. If mange is present in both the breeding and growing pigs, the whole herd should be treated. However, successful elimination of mange from a two-site system can be achieved by treating animals at the breeding herd combined with hygiene and biosecurity measures at the growing facilities. All animals introduced into the herd must be treated before coming into the herd. Contaminated bedding should be removed, and the environment sprayed with an acaricide. Humans who handle infested pigs can act as mechanical vectors for the mites (Mock 1997), so it is important that workers with one herd must change clothes and shower before moving into another herd.

Elimination

The establishment and maintenance of mange-free herds and populations is facilitated by three important facts. First, piglets are born free of mites and become infested through contact with infested sows or older growing pigs. Second, the mites are highly host specific and survive poorly away from the pig host. Third, modern acaricides are very effective.

Mange-free herds can be established with Cesarean-derived pigs, by depopulation and repopulation from mange-free stock, by segregated rearing of treated pigs, and by eradication using avermectins and other products. Biosecurity measures that focus on careful scrutiny of minimal number of sources of incoming stock are usually adequate to prevent introduction of the parasite. In a number of countries, major breeding stock suppliers maintain mange-free herds, and large populations of pigs in integrated production systems have been kept free of the parasite for many years. This should be the goal of most farms.

Eradication of sarcoptic mange is possible with a variety of programs developed around the use of effective acaricides, provided the processes and procedures are properly executed. Documented successes and failures of eradication plans usually relate to the diligence of animal caretakers in following treatment and biosecurity protocols. Eradication programs involve several key points. If the whole herd is to be treated, all marketable pigs can be sold before each treatment to reduce the cost, and the withholding period for the chemical must be observed. All pigs are treated twice at the recommended interval for the acaricide used. If only the sow herd is to be treated, two options are available. All sows and boars can be treated at the recommended interval, or individual sows can be treated pre-farrowing and moved to clean pens or crates. If the latter approach is taken, boars should be treated every 3 months, and the progeny of treated sows must be isolated from the progeny of

untreated sows. Eradication is made easier in growing pigs by changing from a continuous-flow management system to an all-in/all-out system, such as age-segregated-rearing or multisite production. However, changing the management system must be combined with effective pre-farrowing treatment of sows. All of these approaches can be effective and are justified economically.

Economic importance

The effects of sarcoptic mange on production have been reviewed previously (Davies 1995). Deaths are unlikely, unless concurrent disease is present. The most significant effect of sarcoptic mange is reduced growth rate (4.5–12%) and feed efficiency in growing pigs. Other economic effects include downgrading and trimming of carcasses at slaughter, as well as damage to pens and fixtures caused by rubbing pigs. Field studies indicate that good control of scabies may increase lactation, reduce pig mortality due to overlying, and increase weaning weights (Hewitt and Heard 1982).

An interrelationship among immunity, inadequate nutrition, poor management, and hyperkeratotic scabies has been noted. Hyperkeratotic mange is considered to be a disease of poor management and of poorly fed pigs. Diets low in protein and iron are associated with reduced hypersensitivity and a greater proportion of animals with hyperkeratotic mange (Sheahan 1974). The overall clinical picture is substantially influenced by the effectiveness of treatments and herd management practices.

Demodectic mange

Demodectic mange (demodicosis, follicular mange) is relatively unimportant in swine, although it is quite common in the subclinical form as *Demodex* mites are thought to be normal skin flora in mammals. The causative agent is the mite *Demodex phylloides*, an elongate mite that resembles the head of an alligator. The mite spends its entire life inside hair follicles, alongside the hair shafts. To adapt to its narrow living space, the mite has stumpy legs and a fusiform shape. It is assumed that the life cycle of *D. phylloides* resembles that of other demodicid mites. Females live in the hair follicles and oviposit there. A series of larval and nymphal stages ensue, all in the same hair follicles. The next generation of adults matures in about 2 weeks, and the life span of the adult is 1–2 months. The rate of cycling slows down as the pig ages, so that eventually a small population composed almost entirely of adults is in the follicles. Neighboring follicles are colonized by mites crawling out of one follicle and into another.

Transmission of the mites probably occurs by direct contact with neonates while nursing or huddling. The mites can survive for several days in moist environments and up to 21 days under experimental conditions in pieces of skin kept cool and moist (Nutting 1976). However the mites can survive only 1 or 2 days if removed from the hosts' skin and are killed by desiccation in as little as 1 hour at 20 °C on the skin surface.

The sites on the body that are most commonly affected are the snout, eyelids, jowls, underside of the neck, mammary area, and inside surface of the thighs (Walton 1967). Early lesions are red pinpoint foci, but older lesions are hyperkeratotic and nodular. The nodules are distended hair follicles that contain mites, hyperkeratotic debris, and inflammatory cells. When incised, the nodules express a thick, white caseous material laden with mites. Such lesions may be confused with swinepox.

Diagnosis

The diagnosis of demodicosis is made by finding numerous mites in deep skin scrapings. Plucked hair may also reveal mites. Because the mites are present in nearly all swine, identification of a few mites does not necessarily prompt a diagnosis of demodicosis. A correlation between the presence of the mites, indications of an actively growing population of mites (i.e. large number of immature stages in the follicles), and visible lesions must be seen to justify the diagnosis of clinical demodicosis.

Treatment

There are no reports of successful treatment of swine demodicosis with any acaricide. Ivermectin and amitraz are successful in canine demodicosis. In dogs, there is a genetic predisposition to clinical demodicosis. While not proven in swine, it may be prudent to cull severely infected pigs.

Pediculosis (louse infestation)

The pig louse, *Haematopinus suis*, is among the largest of all lice and can be observed with the naked eye. *H. suis* has a worldwide distribution, is the only louse infesting swine, and is an anoplurid (sucking) louse. Accordingly, the head is long and narrow and contains stylets used for piercing skin and feeding on blood of the host. The thorax and abdomen are gray brown with black ornamentation. Females are about 6 mm in length, and the males are lightly smaller.

Haematopinus suis is strictly host specific, and their introduction does not involve rodents, birds, or any other pests. The entire life cycle takes place on the pig.

Adult females lay 3–4 nits (eggs) each day for up to about 25 days. Each nit is 1–2 mm in length and is cemented to a hair shaft. Nymphs hatch in 12–20 days. There are three nymphal instars, all of which suck blood. The third instar molts into an adult 23–30 days after the nit was laid. Lice are obligate parasites and cannot survive for more than 2–3 days off of the host. Lice are found on all parts of the body, but tend to be focused on the neck, jowls, flanks, and inner surface of the legs. They also seek shelter inside the ears where they may be found in clusters. The spread of lice is by direct contact.

The economic importance of lice has not been critically evaluated to the same extent as that for scabies. However it is known that severe pediculosis results in anemia in young pigs and may affect growth rates and feed efficiency. One estimate of reduced growth rate was 50 g/day, although others failed to demonstrate this effect. Lice have been considered to be vectors of swinepox and hides from pediculotic swine may be unsuitable for high-grade leather manufacturers.

Diagnosis

Lice should always be considered in the differential diagnosis of pruritus in pigs. Infestation can be confirmed by identifying lice and/or nits. Nits will be cemented to the proximal (lower) part of the hair shafts, which helps differentiate them from blow fly eggs, which are more diffuse in distribution. Nits are pearl white when first laid and are 1.5–2 mm in length. Adults have six legs and long sucking mouthparts (Figure 65.2).

Treatment and control

The treatment and control of pediculosis is aided by the fact that all stages of the parasite occur on the pig. Therapeutic agents may be administered to the swine in the form of sprays, pour-ons, or dusts (see Table 65.1).

Figure 65.2 *Haematopinus suis*, adult. Note the elongate mouthparts and claws adapted for grasping hair.

Control can be assisted by sprinkling insecticide granules in the bedding. Pour-ons and dusts have the advantage that they can be used in cold weather when spraying may not be indicated. If the active ingredient is not ovicidal, treatment should be repeated in 10 days.

Control and eradication strategies mentioned for scabies also are effective for pediculosis. This includes multiple treatment of sows prior to farrowing, segregation of clean and untreated animals, and treatment of newly introduced animals.

Fleas

Fleas (Siphonaptera) show a low level of host specificity; each species of flea may parasitize a range of host species of mammals or birds. Fleas may parasitize phylogenetically related species, or they may parasitize species that are related to one another by common habitat. All fleas have the same basic structure. They are wingless and are laterally flattened. The hind legs are enlarged and suited for jumping. The four fleas most often associated with swine are *Pulex irritans* (the human flea), *Echidnophaga gallinacea* (the sticktight flea), *Tunga penetrans* (the sand flea or jigger, not to be confused with chigger), and *Ctenocephalides felis* (the cat flea).

The life cycles are similar for all fleas. Adult fleas feed only on blood from the host and are the only stage found on the host. After mating the female oviposits eggs that drop off the host into the environment. The larvae that hatch in 2–16 days are hairy, wormlike creatures that feed on detritus and on the adult flea's blood-laden feces. Flea larvae require high humidity and warm temperature, allowing them to mature in 1–2 weeks. When ready to pupate, the larvae spin a cocoon, to which is stuck small particles from the environment, thus camouflaging the finished cocoon. Depending on environmental conditions, the cycle may be completed in 18 days or protracted to more than 1 year. Adult fleas depend on visual and thermal cues to locate hosts. They orient themselves toward a light source and are stimulated to jump when the light source is temporarily interrupted. Therefore, the shadow of a passing host stimulates the parasites' activity. Fleas can survive for many months awaiting the arrival of a host. How long these fleas can lie in wait depends on environmental conditions, but with optimum humidity and temperature, individuals may live for several weeks or longer off of the host. Environment is a major factor in survival of the flea population in the absence of the host.

Diagnosis

The diagnosis of flea infestation is often difficult unless the adult fleas can be found on the host. The eggs, larvae, and pupae are in the environment, not on the host, and

therefore are especially difficult to find. Adults, however, remain on the host but are somewhat elusive to observation, particularly in breeds of swine that have thicker hair coats. The adults of most flea species are found roaming on the host's skin, and bite lesions occur where fleas have fed. Bite lesions can occur anywhere, but they are most prevalent on the underline and inside surfaces of the legs. Flea bites are not particularly distinguishable from those of other insects. Allergic dermatitis (flea-bite dermatitis) similar to that seen in dogs has been described also in swine, and this may resemble scabies (Nesbitt and Schmitz 1978). Because fleas are not strictly host specific, humans in the same environment also may be attacked. Noticing attack by fleas on human observers may be the first indication of flea infestation.

A special case is *T. penetrans*, the sand flea, or jigger. This flea is found in Africa, the Caribbean, and tropical South America. The female of this tiny flea (1 mm) burrows into the dermis of the host and remains at that site for life. In the process of becoming ovigerous, she swells up to the size of a pea, causing a severe inflammation and ulceration. Humans may also be affected. Sites affected are the feet, snout, underline, and scrotum. Eggs are released through the ulcerated surface, and the immature stages develop in the environment. An unusual case involved the mammary gland, where the fleas obstructed the ducts and caused agalactia (Verhulst 1976).

Treatment and control

Treatment of fleas on the swine is readily accomplished by any of several products also used for other ectoparasites (see Table 65.1). However flea stages in the bedding are much more difficult to eradicate. Environmental control may be attained by the removal and burning of litter, dirt, and manure. Spraying of the environment with chlorpyrifos, pirimiphos, or malathion may be effective, but animal exposure and withholding periods must be considered.

Mosquitoes

While usually thought of as pests of humans, mosquitoes also attack swine and other livestock. Mosquito bites cause irritation and discomfort; the economic losses caused by these attacks have not been well documented, but in some cases it may be clinically important. Reports of mosquitoes attacking swine in large numbers exist (Becker and Gross 1987; Dobson 1973). Attacks by mosquitoes may be significant even in well-managed confinement operations.

All species of mosquitoes require some type of water to complete their cycle. Eggs are typically laid in water, and the larval and pupal stages occur there. In some cases, eggs are laid in areas that will eventually fill with water (egg holes in trees). The type of water suitable for oviposition varies according to each species. It may be salty, brackish, or fresh; stagnant or flowing; sunlit or dark; open or impounded in secluded niches; etc. Species of *Aedes* lay eggs in depressions (wheel ruts, hoof prints, tires) that are dry at the moment but will trap water during the next rain. Control measures involve treating or removing the offending water source. Because different genera utilize different aquatic environments, one method will not be effective against all species. Insecticides must be applied where the mosquitoes are and when the mosquitoes are present. Screened-in enclosures may provide relief but may be impractical. Effective mosquito control involves treatment of large regions, because mosquitoes can fly in from adjacent untreated areas.

In some localities, mosquitoes may transmit microbial pathogens to swine. The transmission of Japanese encephalitis virus, especially in rice-growing areas, was reported by Wada and Smith (1988). Also, mechanical transmission of porcine reproductive and respiratory syndrome virus (PRRSV) was documented (Otake et al. 2003). The virus survived in the mosquito's gut for up to 6 hours, but did not survive on the exoskeleton. Mosquitoes can also transmit *Mycoplasma suis* between pigs (Prullage et al. 1993).

Flies

Flies are important in swine production for several reasons. First, houseflies tend to be used as a measure of general sanitation. Second, some flies bite swine, leading to discomfort and disease (Figure 65.3). Further, some flies cause myiasis, which leads to significant disease including death. Flies express a moderate level of host specificity; therefore flies will also be pests of other livestock, wildlife, and humans.

Housefly

The most common fly in swine operations is the housefly, *Musca domestica*. It lays broods of eggs that develop into maggots in almost any moist organic matter (feces, carrion, garbage, rotting feed). The maggots crawl out of their larval environment and become thickened and darkened in color as they pupate in dry ground. Adult flies emerge from pupal cases and crawl to the surface in about 2 weeks, but that time is temperature dependent.

Musca domestica is nonbiting, but its crawling over an animal's skin is bothersome, and behaviors associated with fly avoidance may lead to decreased rate of gain. Experimentally, houseflies may act as mechanical vectors

Figure 65.3 Fly-bite dermatitis observed at slaughter after scalding.

for *Salmonella, Escherichia coli*, hog cholera, hemolytic streptococci, nematode eggs, and others. Flies may play a relatively small role in the ongoing transmission of such pests on a particular farm; the clinical relationship among flies and these pathogens is not well described. Dissemination of microbial pathogens occurs via the fly's exoskeleton (hairs, legs, proboscis), regurgitated from the fly's crop while dapping its proboscis on food, or in the fly's feces.

Removal of egg-laying sites has primal importance in housefly control. This is augmented by applying residual insecticide sprays and fogs to building surfaces where flies rest (floors, ceilings, partitions, etc.). Manure should be removed at least weekly and spread thinly on the ground to interrupt the life cycle. Electric light traps have not been found to be particularly useful for control.

Stable fly

The second most common fly in swine operations is the stable fly, *Stomoxys calcitrans*. This fly is a pesky biter. Although preferring sunlit areas, it will enter buildings. The adult flies strongly resemble houseflies, but can be distinguished by a long, pointed proboscis. The stable fly can vector hog cholera and *M. suis*. Stable flies deposit eggs in moist decaying vegetable matter such as stacks of straw, damp feed, or lawn clippings. The maggots crawl out of the larval brood site and burrow into the neighboring ground; adults emerge in about 2 weeks. The presence of stable flies is associated with annoyance and thus subtly reduces feed conversion. Control of stable flies is the same as for houseflies.

Tabanids

Tabanids (horseflies, breeze flies, deer flies, etc.) are large, robust flies with fierce biting mouthparts. Female tabanids require a blood meal for egg maturation, while the males feed on nectar and sap. Females lay egg masses on foliage that hangs over water. Hatched larvae drop into the water and feed on aquatic insects. Pupation occurs in dry ground, and adults emerge in 1–3 weeks. Tabanid bites are painful; the mandibles lacerate blood vessels, and blood trickles from bite sites for several minutes. These sites can attract muscid flies. Tabanids are capable of transmitting hog cholera and perhaps other pathogens that occur in the host's circulating blood. Control of tabanids is problematic, as insecticides and repellents work poorly. Screening of buildings or removal of swine to locations more remote from the fly's breeding site may be considered.

Gnats

Simuliids (buffalo gnats, black flies, turkey gnats) are small, stout-bodied, humpbacked biting flies that occur throughout the world. They breed only in running water, and the next generation of adults emerges from those streams in swarms. Most simuliids are ornithophilic, but many feed on a wide variety of mammals. Only the female bites. Black flies may attack in huge swarms and have been known to kill livestock. The exact mechanism leading to death (anemia, toxin, etc.) has not yet been resolved. Left undisturbed, females feed to repletion in about 4–5 minutes. Similar to tabanids, control is difficult. In areas with black fly problems, livestock may have to remain indoors until after sunset.

Myiasis

Screwworm flies cause primary myiasis. *Cochliomyia hominivorax* in South America (it has been eradicated from North America, although it has been recently reintroduced in the Florida Keys) and *Chrysomya bezziana* in Africa and southern Asia are the major causes. These flies oviposit on fresh wounds (fresh umbilical cords, surgical incisions, tabanid bites, nail scratches, fight wounds, etc.), and the larvae (known as screwworms) penetrate into living tissue. The excessive excavation caused by the larvae may be fatal. Ovipositing females attack any species of mammal. They lay 150–500 eggs at the edge of the wound, and the larvae spend 3–6 days in the wound. The larvae crawl out of the host and pupate in the ground, reaching adulthood in 3 days to several weeks. Clearing the environment of snags that may cause wounds, immediate attention to newly farrowed pigs, and similar husbandry methods to reduce oviposition sites are important. Repellent wound dressing may be applied before oviposition has occurred. If wounds are

already infested, pressurized larvicidal aerosols may be used if the larvae are still superficial. Eradication programs entail the release of irradiated sterile males. Screwworms mate only a single time, and mating with the irradiated males leads to nonviable offspring.

Blow flies (*Phaenicia*, *Calliphora*, *Phormia*, etc.) cause nonspecific myiasis in a wide range of host species. These differ from screwworm myiasis in that oviposition occurs in dead or decaying flesh. Some species of blow fly oviposit into wounds that are only slightly foul, whereas others are not attracted until the wound becomes very putrid, and still others are not attracted until the animal dies. Thus there is a succession of different blow flies during the development of the wound. This type of myiasis is much more common than the screwworm type, but it is less dangerous to the affected host, because the maggots keep within the bounds of necrotic tissue and do not wander deeper. As such, larvae from flies causing secondary myiasis have been called "surgical maggots" since they have been used to debride wounds.

Ticks

Domestic swine are susceptible to infestation by ticks but do not commonly come into contact with them. Certainly swine raised in modern confinement units are protected from most contact with ticks. Two kinds of ticks infest swine, ixodid (hard) ticks and argasid (soft) ticks. Ixodid ticks have a hardened scutum covering the entire dorsum of males and a small part of the anterior dorsum of females. Argasid ticks lack this shield altogether and resemble small moving pancakes as the mouthparts are not visible from above. Species of tick are adapted to particular geographic ranges and climatic ranges, so one should seek local assistance in identifying species of interest. In the United States ixodids occurring on swine are species of *Dermacentor*, *Ixodes*, and *Amblyomma*, and argasids are *Ornithodoros* and *Otobius*.

The life cycle of ixodids includes egg, larva ("seed tick"), nymph, and adult. Some ticks are so-called one-host ticks whereby all stages occur on the same individual. For other ticks, each stage requires a new host, and these organisms are aptly named "three-host ticks." Two-host ticks are relatively rare. Ixodid ticks position themselves on vegetation and extend their legs as they wait for a passing host, a technique known as "questing." A single large mass of eggs is laid by the ixodid female after it drops off of a host. In argasids, a small clutch of eggs is laid after each blood meal, and the adults are in the host's lair, not on the host. Argasids seek a host several times in their lives (plural-host ticks).

The main economic importance of ticks is their ability to transmit pathogens, such as protozoa, rickettsiae, and viruses. The recovery of African swine fever virus from *Ornithodoros moubata* nearly a year after experimental infection (Grieg 1972) speaks to the tick's importance as a vector and to the role ticks can play in transmitting agents from wild swine to domestic hosts.

The diagnosis of tick infestation is based upon the known geographic distribution of ticks and the access of pigs to these areas. Hard ticks are readily seen by visual inspection. Although they may occur on any portion of the host, ticks are more commonly attached to the ears, neck, and flanks. Soft ticks seldom occur on the body – they are found in the environment when not feeding. Spinose ear ticks, *Otobius megnini*, are an exception, as they are found in the ear canal.

If only a few ticks are present, they can be removed manually, and the pigs must be removed from the offending pasture. Many products are effective acaricides.

References

References to publications from older literature and reflecting general knowledge of helminths can be found in prior editions of this book.

Becker HN, Gross TL. 1987. *Agri-Pract* 8:8–10.

Bogatko W. 1974. *Med Weter* 30:38.

Bornstein S, Wallgren P. 1997. *Vet Rec* 141:8–12.

Bornstein S, Zakrisson G. 1993. *Vet Dermatol* 4:123–131.

Bornstein S, Eliasson-Selling L, Naslund K, et al. 2000. Evaluation of a serodiagnostic ELISA for swine sarcoptic mange. *Proc Congr Int Pig Vet Soc* 2000: 269.

Cargill CF, Dobson KJ. 1979. *Vet Rec* 104:11–14.

Cargill DF, Pointon AM, Davies P, et al. 1997. *J Parasitol* 70:191–200.

Cargill C, Sandeman M, Garcia R, et al. 2004. Three mange eradication programs based on breeding herd treatment only-Validated by slaughter check and ELISA assay. In Proc Congr Int Pig Vet Soc, Hamburg, Germany.

Davies PR. 1995. *Vet Parasitol* 60:249–264.

Davies PR, Moore MJ, Pointon AM. 1991. *Aust Vet J* 68:390–392.

Davies PR, Garcia R, Gross S. 1996a. Preliminary evidence of parasite aggregation in swine sarcoptic mange. *Proc Congr Int Pig Vet Soc* 14:354.

Davies PR, Bahnson PB, Grass JJ, et al. 1996b. *Vet Parasitol* 62:143–153.

Davis DP, Moon RD. 1990. *Vet Parasitol* 36:285–293.

Deckert A, Nixon R, Diagenault J, et al. 2000. The evaluation of Bommeli ELISA SARCOPTEST for *Sarcoptes scabiei* var *suis* and the prevalence of mange in Ontario, Canada. In Proc Congr Int Pig Vet Soc, Melbourne, Australia, September 17–20, 2000, p. 268.

Dobson KJ. 1973. External parasites of pigs: Mosquitoes. *Univ Sydney Post Grad Comm Vet Sci Proc* 19:349.

Grieg A. 1972. *Arch Gaesamte Virusforsch* 39:24C.

Hewitt GR, Heard TW. 1982. *Vet Rec* 111:558.

Kessler E, Matthes H-F, Schein E, et al. 2003. *Vet Parasitol* 114:63–73.

Mercier P, Cargill CF, White CR. 2002. *Vet Parasitol* 110:25–33.

Mock DE. 1997. *Lice, Mange and Other Swine Insect Problems*. Kansas State University.

Nesbitt GH, Schmitz JA. 1978. *J Am Vet Med Assoc* 173:282–288.

Nutting WB. 1976. *Cornell Vet* 66:214–231.

Otake S, Dee SA, Roger D. Moon et al. 2003. *Can J Vet Res* 67:265–270.

Pointon AM, Cargill CF, Slade J. 1995. Skin disease. In Ferguson J, ed. *The Good Health Manual for Pigs*. Canberra, Australia: Pig Research and Development Corp, pp. 113–115.

Prullage JB, Williams RE, Gaafar SM. 1993. *Vet Parasitol* 50:125–135.

Sheahan BJ. 1974. *Vet Rec* 94:202–209.

Sheahan BJ, Hatch C. 1975. *J Parasitol* 61:350.

Smets K, Vercruysse J. 2000. *Vet Parasitol* 90:137–145.

Smith HJ. 1986. *Can Vet J* 27:252–254.

Verhulst A. 1976. *Vet Rec* 98:384.

Wada Y, Smith WH. 1988. Strategies for the control of Japanese encephalitis in rice production systems in developing countries. In Proceedings of the Workshop on Research and Training Needs in the Field of Integrated Vector-borne Disease Control, Manilla, Philippines: International Rice Institute, pp. 153–160.

Walton GS. 1967. *Vet Rec* 80 (Clin Suppl) 9:11–13.

Zalunardo M, Cargill C, Sandeman RM. 2000. Serological confirmation of mange eradication in pigs. In Proc Congr Int Pig Vet Soc, Melbourne, Australia, p. 270.

66

Coccidia and Other Protozoa

David S. Lindsay, Jitender P. Dubey, and Mónica Santín-Durán

Coccidia (*Cystoisospora suis* and *Eimeria* spp.)

Coccidia are obligatory intracellular protozoan parasites. The number of valid species of intestinal coccidia (*Eimeria* and *Cystoisospora*) that infect swine is unknown because most are known only from the sporulated oocyst stage. Neonatal coccidiosis caused by *Cystoisospora suis* (synonym *Isospora suis*) is the most important protozoal disease of swine. Although the causative agent, *C. suis*, was described from pigs in 1934 (Biester and Murray 1934), it was not until the middle 1970s that clinical coccidiosis was recognized as a disease problem in nursing pigs (Sangster et al. 1976). In 1978, it was demonstrated that *C. suis* was the cause of piglet coccidiosis in natural cases and coccidiosis was experimentally reproduced in nursing pigs (Stuart et al. 1978). Neonatal piglet coccidiosis has a cosmopolitan distribution and is found anywhere pigs are raised in confinement.

Life cycle of *c. suis*

Coccidial life cycles are divided into three phases: sporogony, excystation, and endogenous development (Figure 66.1). Each coccidial phase is unique for each species, and knowledge of life cycle phases is important in diagnosis, treatment, prevention, and control of coccidiosis.

Sporogony is the process by which the oocyst (environmentally resistant stage) develops from the unsporulated noninfectious stage passed in the feces to the infective stage (Figure 66.2). Proper temperature and moisture must be present for sporulation to take place. The oocysts of *C. suis* sporulate rapidly at temperatures between 20 and 37 °C (Lindsay et al. 1982). The supplemental heat of between 32 and 35 °C provided by producers for newborn piglets favors rapid development (within 12 hours) of *C. suis* oocysts in the farrowing crate. Oocysts are most sensitive to killing when in the unsporulated state and during sporulation. Once the oocysts are sporulated, they are resistant to most disinfectants. When fully sporulated, the oocysts of *C. suis* and all other *Cystoisospora* species contain two sporocysts, each with four sporozoites.

Excystation is the phase of the life cycle that occurs immediately after the infectious oocysts are ingested. Passage through the stomach alters the oocyst wall and allows bile salts and digestive enzymes to activate the sporozoites. The activated sporozoites leave the sporocyst and oocyst and are freed into the intestinal lumen. The sporozoites then penetrate enterocytes and begin the endogenous phase of parasite multiplication.

The endogenous stages of the life cycle of *C. suis* occur in cytoplasm of enterocytes throughout the small intestine, with most stages being present in the jejunum and ileum. Occasionally, in heavy infections, parasites can be found in the cecum and colon as well. Coccidial stages are usually located on the distal portions of the villi and are in a parasitophorous vacuole below the host cell nucleus (Lindsay et al. 1980). In severe clinical or experimental cases, stages may also be located in crypt enterocytes. There are two distinct types of asexual stages in the endogenous life cycle of *C. suis*. Sexual stages consist of microgamonts, which produce microgametes, and uninucleate macrogamonts. The microgametes fertilize the macrogamonts, and an oocyst is formed. These sexual stages also may be seen 4 days post infection (PI), whereas oocysts are first seen in the feces 5 days PI (rarely 4 days).

Immunity to *Cystoisospora suis*

Pigs that have been infected with *C. suis* and recover are resistant to challenge infection. These challenged pigs excrete no or very few oocysts (in contrast to initial infection) and do not develop clinical signs. Administration of corticosteroids (methylprednisolone

Diseases of Swine, Tenth Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson.

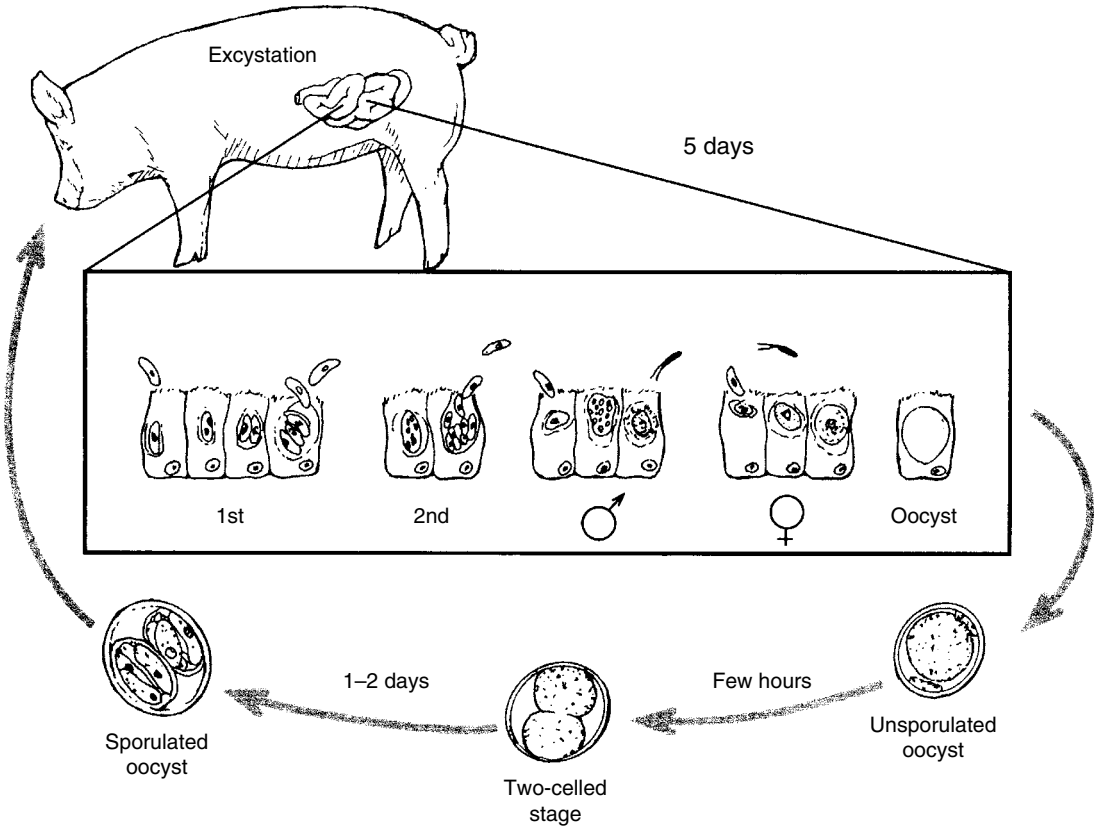


Figure 66.1 Life cycle of *Cystoisospora suis*.

acetate) does not cause these previously exposed pigs to reexcrete oocysts, suggesting good immunity has developed.

Experimental inoculation of sows with 1×10^5 *C. suis* oocysts 14 days before farrowing resulted in partial protection of piglets against clinical disease after experimental infection with 1×10^3 *C. suis* oocysts at 3 days of age (Schwarz et al. 2014). Protective substances were present in both colostrum and milk and highly correlated with IgA, IgM, and IgG antibody titers in the piglets during the first 2 weeks of life.

Pigs have age-related differences in susceptibility to experimental infection and disease (Koudela and Kucerova 1999). Pigs that are 1–2 days old develop much more severe disease than pigs inoculated with an identical number of oocysts at 2 or 4 weeks of age.

Clinical signs of *Cystoisospora suis*

Signs of disease occur in formerly healthy nursing pigs between 7 and 11 days of age (Stuart et al. 1978). Yellowish to grayish diarrhea is the major clinical sign. The feces are initially loose or pasty and become more fluid as the infection progresses. Piglets become covered with the liquid feces, causing them to stay damp and have a rancid odor of sour milk. The piglets usually continue to nurse, develop a rough hair coat, become dehydrated, and have depressed weight gains (Lindsay et al. 1985). Litters within the farrowing house vary in the degree to which they demonstrate clinical signs, and not all piglets within a litter are equally affected. Morbidity is usually high, but mortalities are usually moderate. Concurrent bacterial, viral, or other parasitic infections may lead to extreme mortalities and complicate diagnosis.

Occasionally *C. suis* oocysts are present in the feces of recently weaned pigs, some of which may have diarrhea. Diarrhea caused by *C. suis* can occur in 5- to 6-week-old pigs. Diarrhea begins 4–7 days after the piglets are weaned. It is likely associated with the stress of weaning and exposure or reexposure to environmental oocysts. Morbidity is high, but mortalities are rare. As with neonatal piglets, other causes of diarrhea besides *C. suis* need to be ruled out. *C. suis* infections do not cause disease in finishing pigs or in breeding stock.

Pathologic changes with *Cystoisospora suis*

The degree of disease is dependent on the number of sporulated *C. suis* oocysts that a piglet ingests (Stuart et al. 1980). Necropsy examination may demonstrate gross lesions of neonatal coccidiosis characterized by a fibrinonecrotic membrane in the jejunum and ileum, but this is seen

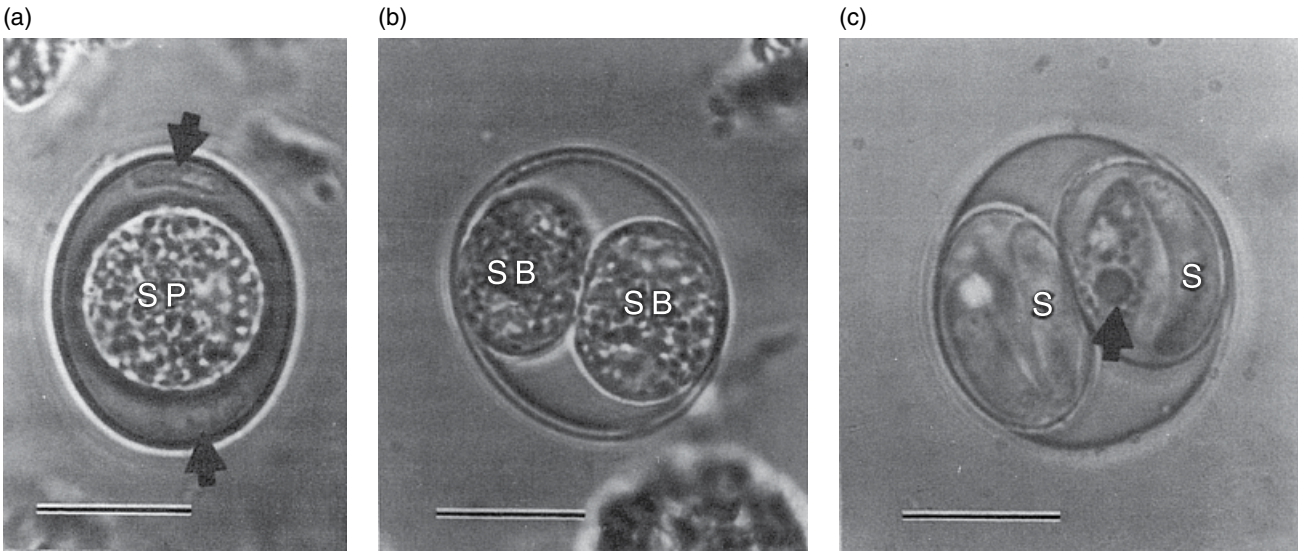


Figure 66.2 Oocysts of *Cystoisospora suis* in fecal flotation. Bar = 10 µm. (a) Freshly excreted unsporulated oocyst. Note the hazy bodies (arrows) and the sporont (SP). (b) Oocysts several hours after excretion. Note that two sporoblasts (SB) are present. (c) Sporulated oocyst approximately 1 day after excretion. Note the sporozoites (S) and residual body (arrow) in the sporocysts. *Source:* Adapted from Lindsay et al. (1982).

only in severely infected piglets. Hemorrhage is not seen even in extreme cases of natural infections or in experimental infections where large numbers of oocysts are given.

Microscopic lesions consist of villous atrophy, villous fusion, crypt hyperplasia, and necrotic enteritis (Stuart et al. 1980). The enterocytes at the tips of the villi may be destroyed, exposing the underlying lamina propria, or they may be replaced by flattened immature enterocytes. The functional ability for absorption is diminished in this altered epithelium, resulting in fluid loss and diarrhea. Lesions develop about 4 days PI and are associated with the presence of the asexual stages. In most natural cases few parasites are present in the sections, and most of these parasites are asexual stages. In severe cases piglets may succumb to coccidiosis before the sexual stages are produced.

Diagnosis of *Cystoisospora suis*

Diarrhea in nursing pigs 7–14 days of age that does not respond to antibiotic treatment is suggestive of neonatal *C. suis* infection. Other agents such as enteropathogenic *Escherichia coli*, TGE virus, rotavirus, *Clostridium perfringens* type C, and *Strongyloides ransomi* should be considered in the differential diagnosis.

Diagnosis of *C. suis* can be achieved by finding *C. suis* oocysts in the feces of clinically affected piglets (Figure 66.2a and b). This is the quickest method available for diagnosis. Fecal smears or fecal flotations should be made from several litters within the farrowing house that have been showing clinical signs for 2–3 days, because diarrhea starts about a day before oocysts are passed and peak oocyst production occurs about 2–3 days after clinical signs develop. Piglets excrete oocysts in several phases and may be negative during these phases. Pasty fecal samples are likely to contain more oocysts than liquid samples. The oocysts of *C. suis* have characteristic structures called "hazy bodies" between the oocyst wall and the sporont (Figure 66.2a). These are diagnostic for *C. suis* because none of the oocysts of the swine *Eimeria* species have this structure (Lindsay et al. 1982). Additionally, some of the oocysts may be in the two-celled sporoblast stage (Figure 66.2b), which is also diagnostic for *C. suis*. Fecal fat may make identification of oocysts in flotation preparations difficult. A solution of saturated sodium chloride and glucose (500 g of glucose in 1000 mL saturated sodium chloride solution) has been recommended as an alternative flotation medium (Henriksen and Christensen 1992).

Demonstration of developmental stages (Figure 66.3a–d) in mucosal smears can be used in the diagnosis of *C. suis* infection (Lindsay et al. 1983). The intestinal mucosa should be scraped with a scalpel or coverslip using just enough pressure to dislodge villi, and the scrapings should be prepared as a smear on a glass microscope slide. The smears are then stained with any of a number of routine blood stains.

The presence of paired type 1 merozoites (Figure 66.3d) is diagnostic. Other asexual stages (such as binucleated type 1 meronts or type 2 meronts and merozoites) and sexual stages (microgamonts and macrogamonts) will probably be present also, but their identification is more difficult and not needed for diagnosis. Histologic diagnosis of *C. suis* in tissue sections is possible (Lindsay

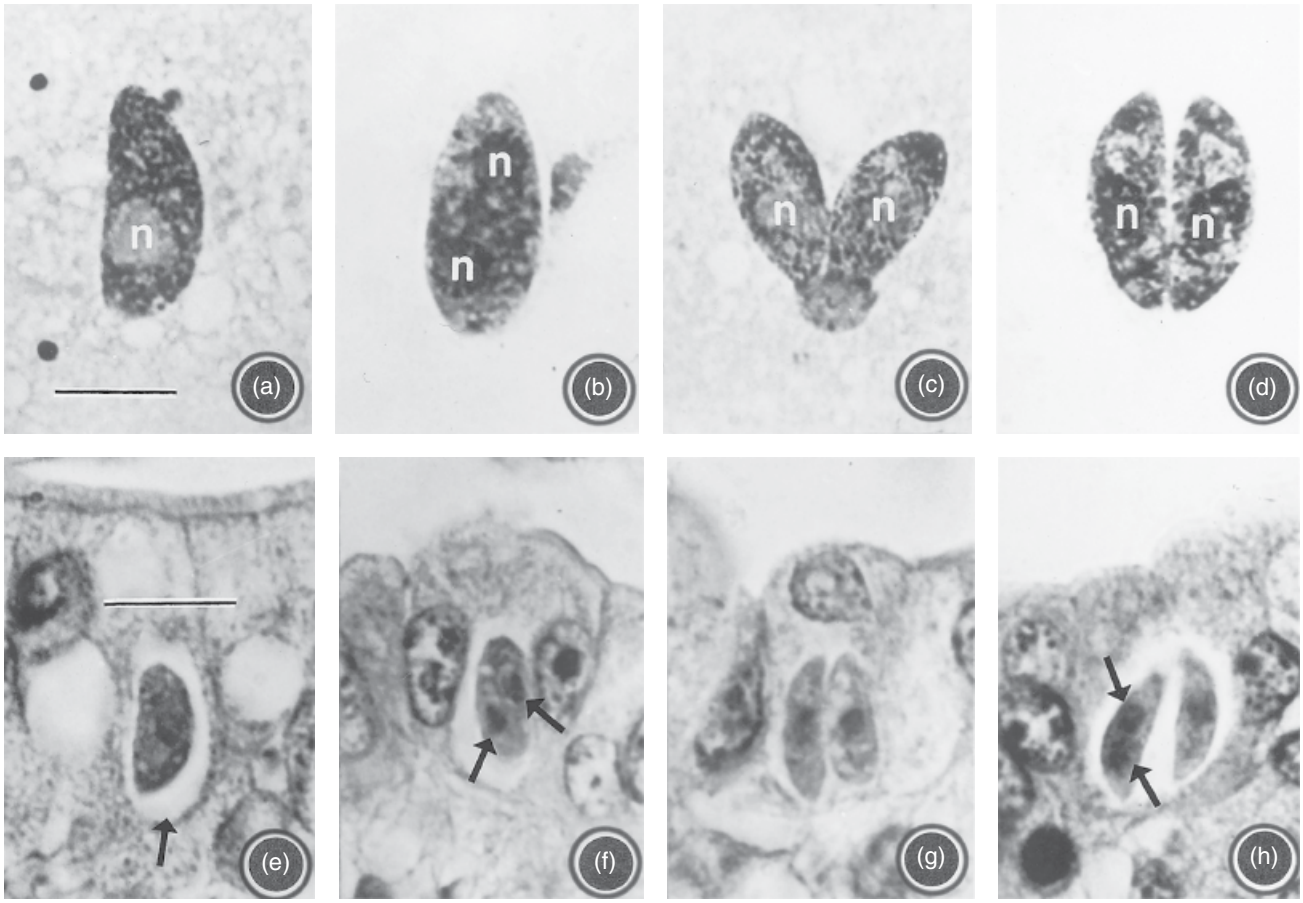


Figure 66.3 Diagnostic stages of *Cystoisospora suis* in Wright's Giemsa-stained intestinal smears (a–d: Bar = 5 µm) or hematoxylin-and eosin-stained histological sections (e–h: Bar = 10 µm). (a) Zoite with a single nucleus (n). (b) Type 1 meront, which has two nuclei (n). (c) Dividing type 1 meront. Note the nuclei (n). (d) Paired type 1 merozoites. Note that each has a single nucleus (n). (e) Zoite in a parasitophorous vacuole (arrow). (f) Type 1 meront. Note that two nuclei (arrows) are present. (g) Paired type 1 merozoites. (h) Host cell with a type 1 meront (arrows label nuclei) and a type 1 merozoite. *Source:* Adapted from Lindsay et al. (1980).

et al. 1983). As with mucosal smears, demonstration of paired type 1 merozoites is diagnostic (Figure 66.3e–h). The multinucleated type 2 meronts of *C. suis* are elongated and are often found in the same host cell. Finally, the macrogamonts of *C. suis* lack the characteristic prominent eosinophilic wall-forming bodies seen in *Eimeria* species.

Epidemiology: *Eimeria* species

Eight species of *Eimeria* occur in swine in the United States. Reports of coccidiosis in swine caused by *Eimeria* species are rare (Hill et al. 1985), but age-segregated rearing and changes in housing over the last few decades have increased the risk for outbreaks. Experimental studies have demonstrated that inoculation of large numbers of oocysts of *Eimeria debliecki*, one of the most common coccidian of swine, does not cause clinical disease (Lindsay et al. 1987). Reports of natural cases of *Eimeria spinosa*-associated disease in weaned pigs suggest that this species can cause disease under appropriate conditions in the field (Lindsay et al. 2002; Yaeger et al. 2003). Clinical coccidiosis can occur in finishing animals exposed to contaminated facilities and can occur in breeding stock that are born and reared in confinement and then exposed as breeding stock (Caudie et al. 2004; Henry and Tokach 2008).

Epidemiology: *Cystoisospora suis*

Once *C. suis* coccidiosis was recognized as a problem in nursing pigs, most veterinarians and researchers assumed that piglets were infected by ingesting *C. suis* oocysts from the sow's feces. However, studies have failed to confirm this assumption. Surveys of the swine population in the United States indicate that *Eimeria* infections are common (60–95%) in animals raised in lots or in the wild, but less than 3% of the animals sampled excrete oocysts of *C. suis* (Lindsay et al. 1984).

Another study examined the species of oocysts excreted by sows on farms with and without a history of *C. suis* infections in nursing pigs and reported that 82% of the sows on farms with a history of coccidiosis had *Eimeria* infections but no detectable *C. suis* infections, whereas sows from farms with no history of neonatal coccidiosis had an infection rate of 95% with *Eimeria* species and less than 1% with *C. suis* (Lindsay et al. 1984).

In the United States, Stuart and Lindsay (1986) examined the transmission of *C. suis* on two farms in Georgia. Daily fecal samples were collected rectally from sows 1 week prior to farrowing, the day of farrowing, and for about 1 week after farrowing. Colostrum and placentas from several sows were examined microscopically for parasites. A coccidiostat (amprolium HC1, Amprol 25% feed grade) was given to half of the sows on each farm. *Eimeria* species were the only coccidia seen in the feces of sows. None of the sows given amprolium HCl had oocysts in fecal samples at farrowing. No parasitic stages were seen in the colostrum or placentas examined. On Farm 1, 7 of 12 litters from nontreated sows and 9 of 12 litters from treated sows developed clinical coccidiosis. On Farm 2, all litters from nontreated sows and 11 of 12 litters from treated sows developed clinical coccidiosis. *C. suis* was the only species of coccidia seen in these piglets.

The results of these studies indicate that sows are not the primary source of *C. suis* infection for nursing pigs. It is still not known how *C. suis* becomes established on a farm; once it is established, it is probably transmitted through contaminated farrowing crates. The temperature (32–35 °C) and moisture in the farrowing crate favor rapid sporulation of *C. suis*.

Treatment and control

Anticoccidials

Sows do not appear to be a major source of infection for nursing pigs (Stuart and Lindsay 1986); therefore, using anticoccidial drugs in the sow's ration is of little value in controlling neonatal coccidiosis. Early studies that reported success with treating sows probably are due to improved sanitation once the producers were made aware that their pigs had coccidiosis. Studies that demonstrate anticoccidial activity of drugs in weaned or finishing pigs are of no value in predicting the ability of these drugs to control disease in nursing pigs.

Toltrazuril is an effective means of treating coccidiosis in nursing piglets. A single dose of 20 mg/kg given at 3 days of age decreases clinical signs (Skampardonis et al. 2010) and be cost effective (Scala et al. 2009). Toltrazuril's excellent activity is probably based on its ability to kill asexual and sexual stages of coccidia and because it is slowly released from tissues of treated animals.

Controlled studies conducted to date in nursing pigs have not identified other effective coccidiostats.

Sanitation

Improved attention to sanitation has been the most successful method for reducing losses due to neonatal coccidiosis in pigs (Stuart and Lindsay 1986). A good sanitation program entails thorough cleaning of the crates to remove organic debris, disinfection, and steam cleaning. In extreme cases, sealing or painting solid surfaces within farrowing crates can help break the cycle of reinfection by the hardy oocysts. Producers should limit access to farrowing crates by workers to avoid crate-to-crate contamination with oocysts carried on boots or clothing. Likewise, pets should be prevented from entering the farrowing house and spreading oocysts from crate to crate on their paws. Rodent populations should be controlled to prevent these animals from mechanically transmitting oocysts.

Facilities need to be sanitized after every farrowing. Producers should be made aware that even though clinical disease is under control, the potential for future outbreaks is still present.

Toxoplasmosis (*Toxoplasma gondii*)

Toxoplasmosis is caused by infection with *Toxoplasma gondii*, a protozoan parasite related to the coccidia. Infections are common in humans and warm-blooded animals (Dubey 2010). Postnatally, pigs or humans become infected by ingesting food and water contaminated with sporulated *T. gondii* oocysts or by consuming meat containing tissue cysts. Cats (and other felines) are the only animals that can excrete resistant oocysts in their feces and are important in the transmission of *T. gondii* to pigs and other animals (Figure 66.4). Tissue cysts are found in many edible tissues of infected pigs, and they contain bradyzoites, which are slowly multiplying stages (Figure 66.5b). Tissue cysts remain viable in pork for many years and probably the life of the pig. After ingestion, oocysts or bradyzoites can survive passage through the stomach. Once in the intestine of the host, sporozoites or bradyzoites change into a fast-multiplying stage called tachyzoites (Figure 66.5a). Tachyzoites multiply in the lamina propria of the intestine and eventually spread throughout the body. Prenatal infection may occur if the mother is infected during pregnancy. Tachyzoites from the mother's blood may cross to the fetus via the placenta. Tachyzoites cause tissue damage and eventually develop into the bradyzoite stage and form tissue cysts. Toxoplasmosis is a zoonosis, and pork is considered a source of *T. gondii* infection for humans in many countries (Dubey et al. 2005; Dubey 2009). Although the prevalence of *T. gondii* in feeder pigs in the

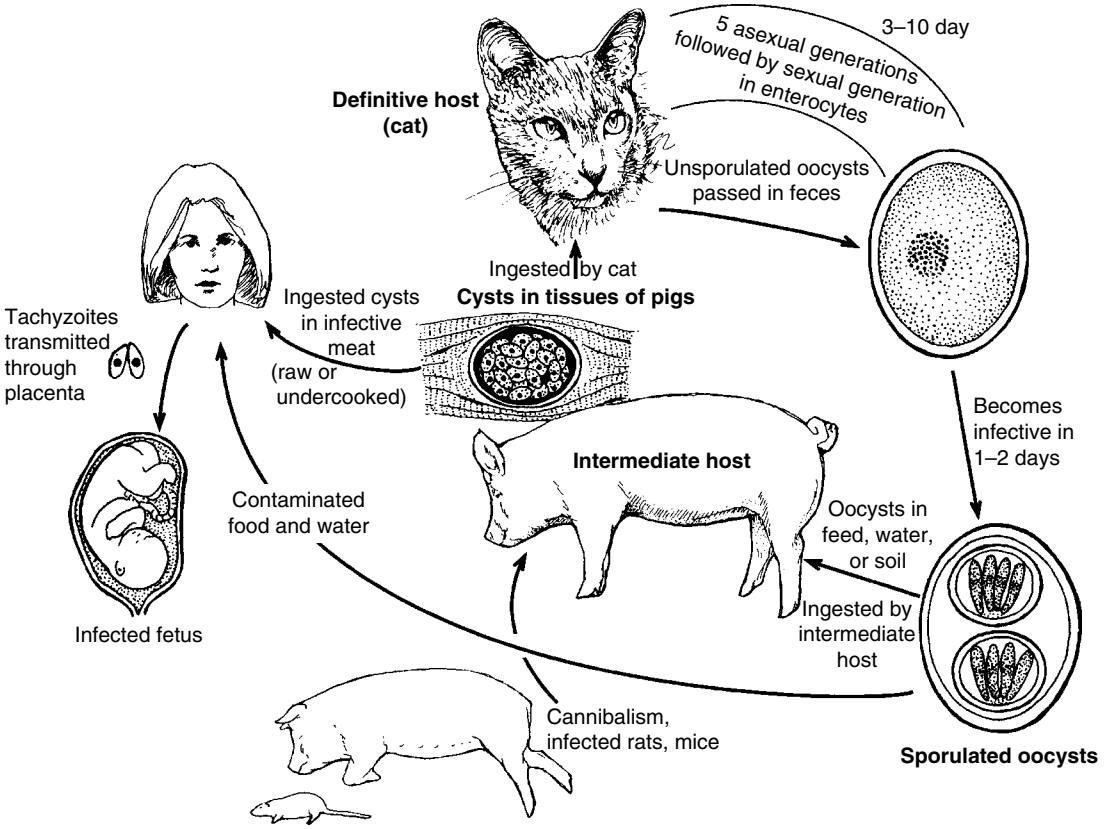


Figure 66.4 Life cycle of *Toxoplasma gondii*.

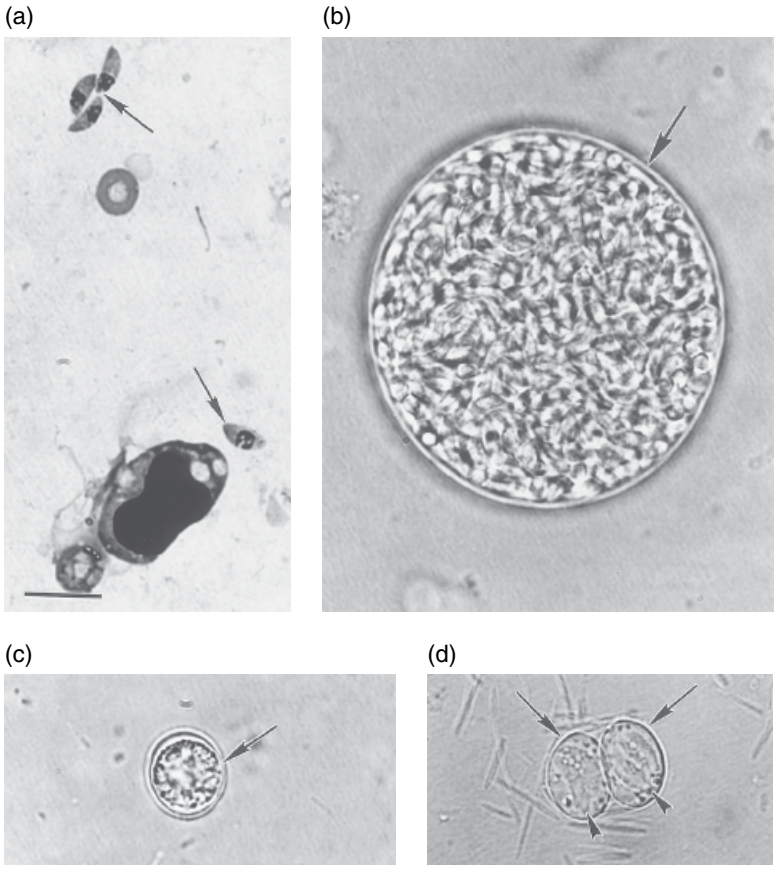


Figure 66.5 *Toxoplasma gondii* stages in smears of tissues of animals. Bar: 10 µm. (a) Tachyzoites from lung (arrows), Giemsa stain. (b) Tissue cyst from brain, unstained. Note hundreds of bradyzoites enclosed in a thin cyst wall (arrow). (c) Unsporulated oocyst (arrow) from cat feces, unstained. (d) Sporulated oocyst from cat feces, unstained. Note two sporocysts (arrows). The sporozoites are barely visible (arrowheads).

United States is declining, there are still small pig establishments with *T. gondii* infection.

Clinical signs

Infections in swine are usually subclinical (Dubey 1986). Abortions due to *T. gondii*, although uncommon, may occur in sows infected during pregnancy. Transplacentally infected pigs may be born premature, dead, or weak, or they may die soon after birth. Pigs that live may develop diarrhea, incoordination, tremors, or cough. Few reports exist on clinical disease in pigs that acquire infection postnatally, but epidemics of clinical toxoplasmosis have been observed in both young and adult pigs. Experimental studies indicate that ingestion of *T. gondii* oocysts by pigs is more likely to produce clinical disease than ingestion of tissue cysts (Dubey 1986). Severity of disease is dependent upon the number of oocysts ingested, and older pigs are less likely to develop clinical disease.

Pathologic changes

Pathologic changes are associated with necrosis of host tissue caused by the rapidly multiplying tachyzoites. Enteritis, lymphadenitis, splenitis, hepatitis, pneumonitis, and less frequently myositis and encephalitis are seen in naturally infected pigs (Dubey 1986, 2009).

Diagnosis

Several serologic tests are available for determining antibodies to *T. gondii* in pigs. The modified direct agglutination test is the most sensitive and specific for the detection of latent *T. gondii* infection in swine (Dubey et al. 1995a). Although finding *T. gondii* antibody in adult pigs only means exposure to *T. gondii*, finding antibody in a fetus indicates congenital infection because maternal antibodies are not transferred to the fetus in pigs. Histologic examination of tissues may be utilized for a presumptive diagnosis based on lesion characteristics and parasite structure (Figure 66.5a and b).

Epidemiology

The prevalence of congenital *T. gondii* infection in pigs is less than 0.01%. Prevalence of *T. gondii* antibodies in feeder pigs (younger than 6 months) is lower (<1%) than in sows (15–20%) based on large-scale surveys (Dubey et al. 1995b; Weigel et al. 1995). *T. gondii*-infected cats and infected rodents were identified as the main sources of *T. gondii* infection for pigs (Weigel et al. 1995). Cats become infected with *T. gondii* by ingesting infected animals (rodents, birds) soon after weaning. Therefore, infected juvenile cats are considered to be the main source of *T. gondii* for farm pigs.

Treatment and control

Because porcine toxoplasmosis is usually subclinical, little is known about the treatment of the disease in swine. Ponazuril (closely related to toltrazuril) is effective against acute toxoplasmosis in mice and should also be effective in swine.

Control of *T. gondii* infection in pigs is important because of public health concerns over human infections. Toxoplasmosis causes mental retardation and loss of vision in congenitally infected children. Following experimental infection, viable tissue cysts of *T. gondii* can be found in most commercial cuts of pork, and studies have shown that the tissue cysts will be viable infected pigs for at least 2.5 years (Dubey 1988). Cooking and freezing pork will inactivate tissue cysts.

Prevention of *T. gondii* infection in pigs can be achieved by practicing good husbandry. There is no vaccine. To prevent oocyst-induced infections, cats should never be allowed in buildings where pigs are housed or where feed is stored. Rodenticides should be used to control rodents and eliminate this possible source of tissue cysts. Any pigs that die should be removed promptly to prevent cannibalism. Uncooked garbage should never be fed to pigs. Feed should be kept covered to prevent cats from defecating in it.

Sarcocystis

Sarcocystis spp. are coccidian parasites that have a two-host life cycle. Three species use pigs as the intermediate host and form tissue cysts (sarcocysts) in the pig muscles. *Sarcocystis miescheriana* has a pig–dog life cycle and is the only species found in the United States. Dogs excrete infective stages (sporocysts) in their feces. The other species are *Sarcocystis suihominis*, which uses the human as the definitive host, and *Sarcocystis porcifelis*, which uses the cat as the definitive host (Dubey et al. 1989). Surveys indicate that from 3 to 18% of commercial breeding sows razed outdoors and 32% of wild swine examined in the United States have *Sarcocystis* infection (Dubey and Powell 1994). There are no reports of naturally occurring clinical disease due to *Sarcocystis* infection in swine (Dubey et al. 1989; Stentiford et al. 2016) (Figure 66.6).

Cryptosporidium

Cryptosporidium species are prevalent and ubiquitous parasites of humans and animals worldwide. These obligate intracellular protozoan parasites of vertebrates range from host-restricted species to species capable of infecting a wide range of hosts (Fayer 2010). Porcine cryptosporidiosis has been reported to occur worldwide.

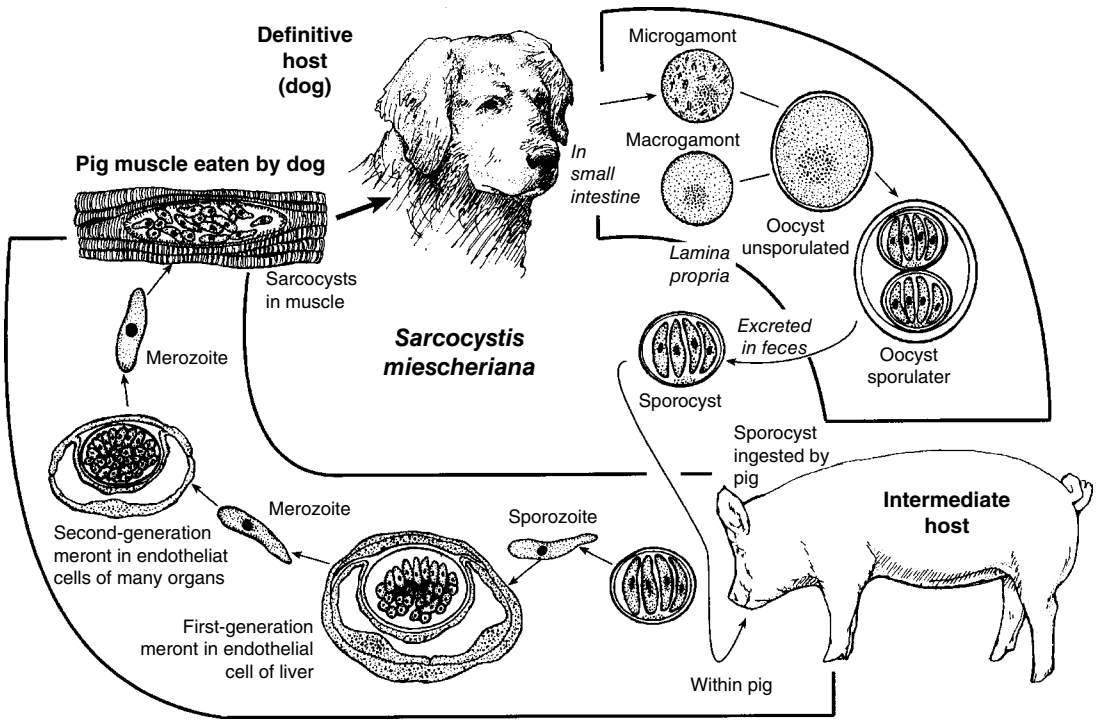


Figure 66.6 Life cycle of *Sarcocystis miescheriana*.

Although pigs have been reported to be susceptible to at least seven different species or genotypes, the most frequently found species in naturally infected pigs are host-specific species *Cryptosporidium suis* and *Cryptosporidium scrofarum*. Infections in pigs with the other species only occur occasionally.

Life cycle

Infection begins with ingestion of the oocyst stage after direct contact with feces or from contaminated food or water (Figure 66.7). Four sporozoites are released from each oocyst (diameter approximately 5 µm) in the intestinal lumen where they enter cells lining the small intestine. All endogenous stages are intracellular but extracytoplasmic, appearing to be on the luminal surface of the mucosal epithelial cells. There are two or more asexual generations, each generation producing merozoites that invade additional gut epithelial cells. Merozoites eventually give rise to male and female stages. Fertilized female stages develop into oocysts that mature internally and are infectious when passed in the feces. Generally, prepatent period ranges from 2 to 9 days with oocysts excreted for 9–15 days for *Cr. suis* infections, while prepatent period ranges from 4 to 6 days with oocysts excreted for more than 30 days for *Cr. scrofarum* infections.

Cryptosporidia: clinical signs

A wide range of clinical features has been reported for cryptosporidiosis in pigs, possibly reflecting differences among the *Cryptosporidium* isolates that cause infection in pigs (Santín and Trout 2008). Clinical signs, including inappetence, depression, vomiting, and/or diarrhea, were observed after experimental infection of piglets with oocysts derived from calves (most likely *Cryptosporidium parvum*). However, cryptosporidiosis in pigs does not always result in clinical signs. In the absence of molecular analysis, it is not possible to determine which *Cryptosporidium* species or genotypes were responsible for which infections. When the species or genotype was known, infections from *Cr. suis* or *Cr. scrofarum* were less severe than those from *Cr. parvum*. Clearly, identification of the species and genotypes is needed to establish the pathogenicity and host affinity of different *Cryptosporidium* isolates from pigs. Additionally, concomitant infection with other pathogens such as *C. suis* or viruses can influence the severity of infections. For example, incidental coinfection with rotavirus apparently resulted in severe clinical signs and the deaths of piglets experimentally infected with *Cr. suis*, whereas rotavirus-free piglets had mild signs.

Detection and diagnosis

Microscopy, fecal antigen ELISA, and PCR, with or without recovery and concentration of oocysts, have been used. However, recovery of oocysts from pig feces has been problematic with consistently lower recovery than from feces of other animals. Sucrose, cesium chloride, and other flotation media are effective in concentrating oocysts by density gradient centrifugation and reducing fecal debris.

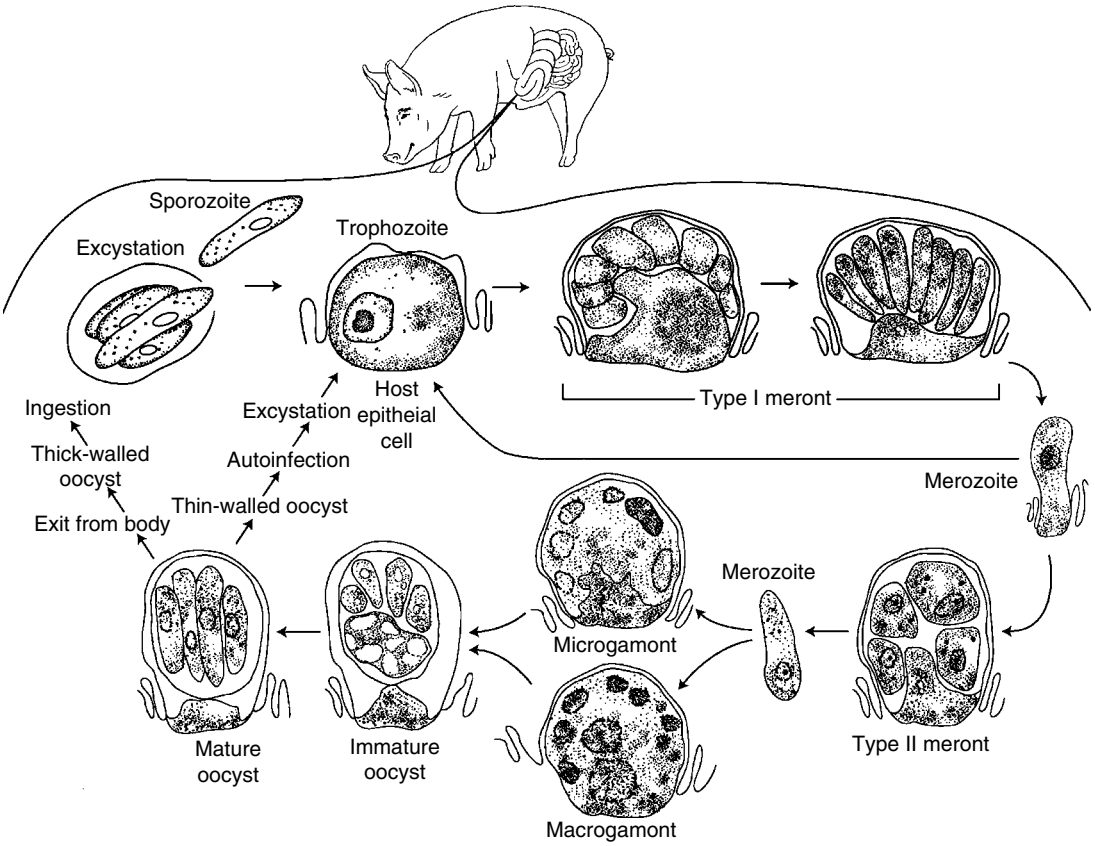


Figure 66.7 Life cycle of *Cryptosporidium*.

Because most oocysts of *Cryptosporidium* species and genotypes are nearly spherical, range in size from 4.5 to 5.5 µm in diameter, and have no distinguishing features, microscopy can be used only to determine the presence of oocysts. Definitive identification of species and genotypes requires the use of molecular techniques such as gene sequencing or PCR-RFLP. Pigs can harbor multiple species and genotypes, including *Cr. parvum*, which are infectious for humans. Therefore, molecular analysis is needed to evaluate the risk of human infection (Table 66.1).

Developing stages of *Cryptosporidium* have been reported throughout the intestinal tract, with lesions of varying degrees of villous atrophy, villous fusion, cellular infiltration of the lamina propria, and, infrequently, sloughing of epithelial cells. All species examined appear to cause similar types of damage, but lesions from *Cr. parvum* seemed most severe, reflected by the more significant clinical signs.

Epidemiology

Cryptosporidium has been found in pig of all ages. In the farm environment, pigs become infected predominantly with *Cr. suis* and *Cr. scrofarum*. Field studies have shown that *Cr. suis* and *Cr. scrofarum* differ in the age of pigs that they infect. *Cr. suis* appears to infect all age groups with lower prevalence in older pigs, while *Cr. scrofarum* appears to be specific for older pigs. Occasionally *Cr. parvum* has also been reported in natural infections. Pigs have been experimentally infected with *Cr. parvum*, *Cr. hominis*, and *Cr. meleagridis*.

Regarding zoonotic potential, *Cr.* parvum is not highly prevalent in pigs, and although sporadic cases of *Cr. suis* and *Cr. scrofarum* have been reported in humans, the risk that pigs pose as a source for human infections is apparently limited.

Treatment

Disinfection of the environment is difficult because oocysts are numerous, can remain infectious for months, and are extremely resistant to a broad range of chemicals. Heat, drying, and sunlight are most effective at inactivation oocysts. No drugs have been proven effective for treating *Cryptosporidium* in swine.

Other protozoa of minor importance or potentially transmissible to humans

Giardia

Giardia duodenalis (synonyms *G. lamblia* and *G. intestinalis*) is possibly the most common intestinal parasite of

Table 66.1 Species and genotypes of *Cryptosporidium*, *Giardia*, and *Microsporidia* found in swine and their zoonotic potential.

Species	Genotype/assemblage	Zoonotic
Cryptosporidium suis		Yes
Cryptosporidium scrofarum		Yes
Cryptosporidium parvum		Yes
Giardia duodenalis	A	Yes
Giardia duodenalis	E	No
Encephalitozoon cuniculi	III	Yes
Encephalitozoon intestinalis		Yes
Enterocytozoon bieneusi Infects many mammals including humans and pigs, 14 genotypes	D, I, O, BEB4, CALF1, CS-4, EbpA, EbpC, EbpD, Henan-III, Henan-IV, LW1, PigITS5, PigEBITS7	Yes
Enterocytozoon bieneusi Infects only pigs, 42 genotypes	CHN7-CHN10, CS-2, CS-3, CS-5-CS10, E1, EbpB, F1, HLJ-I-HLJ-IV, PigEBITS1- PigEBITS4, PigEBITS6, PigEBITS8, PigEb1-PigEb17	No
Enterocytozoon bieneusi Infects pigs and other mammals (no humans), four genotypes	CC-1, CS-1, G, H	No

humans and livestock worldwide. It is a species complex consisting of eight assemblages (A–H) that are morphologically indistinguishable, and molecular methods are used to determine assemblages. Only assemblages A and B infect humans, are zoonotic, and occur in a wide range of mammals. Other assemblages infect specific groups of animals: assemblage E infects swine and other livestock, while C and D infect dogs, F infects cats, G infects rodents, and H infects seals.

The life cycle involves two stages, the trophozoite and cyst stage. Trophozoites are flagellated, pyriform-shaped protozoa that attach to the brush border of cells lining the small intestine where they absorb nutrients and multiply by binary fission (Figure 66.8). Trophozoites encyst in the small or large intestine and pass in the feces as environmentally resistant cysts. Freshly excreted cysts are immediately infectious and can remain infectious under moist and cool conditions for weeks. Trophozoites excreted in feces do not survive outside the body. *Giardia* is transmitted by ingestion of food or water contaminated with cysts.

In the United States, no association was found between *Giardia* infections and clinical illness in pigs (Xiao et al. 1994). Likewise, on European farms, no clinical signs were associated with the presence of *Giardia* in pigs (Petersen et al. 2015).

Detection of motile trophozoites (10–20 µm long) can be seen in saline suspensions of loose or diarrheic feces. Ovoid to ellipsoid cysts (9–15 µm long) are best detected after concentration in feces using zinc sulfate flotation (specific gravity 1.18). Sucrose and sodium chloride flotation media are effective but hypertonic, distorting cysts if examination is not made quickly. Because cysts might be excreted intermittently, multiple fecal samples should be collected and examined at intervals within a week. Assemblages are determined based on genetic markers.

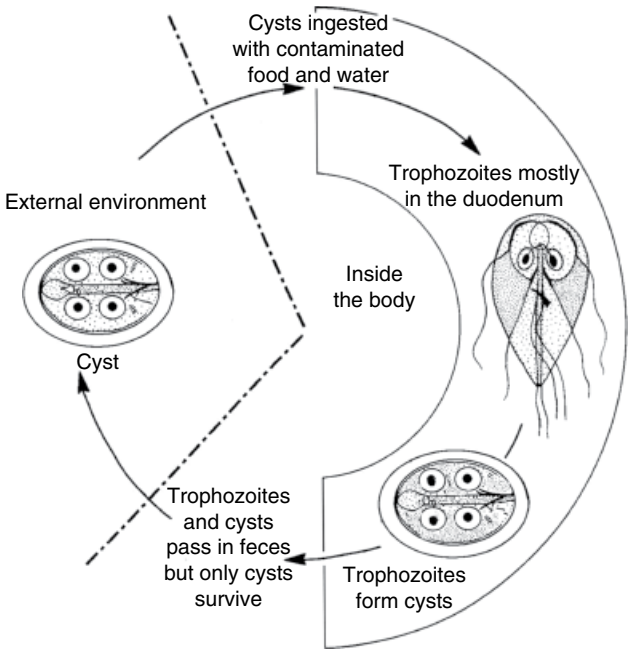


Figure 66.8 Life cycle of *Giardia*.

The role of swine and other domestic animals as sources for *G. duodenalis* in humans is unclear. *Giardia* infections have been reported in pigs in all age groups from nursing piglets to boars and sows in the United States, Canada, Europe, Australia, Africa, and Asia with the prevalence ranging between 0.1 and 20% (Armson et al. 2009). In Ohio *Giardia* was detected in 8.3% of

pre-weaned piglets, 2.6% of post weaned pigs, and 1.5% of sows (Xiao et al. 1994). Surveys worldwide indicate that most pigs infected with *Giardia* have assemblage E with few having other assemblages.

No drugs are approved for treating giardiasis in swine. However, fenbendazole and albendazole have been used to treat *Giardia* in dogs. Cysts are infectious when passed in the feces but can be rendered noninfectious by desiccation, many quaternary ammonium compounds, laundry bleach, and boiling water. After cleaning and treating contaminated spaces, thorough drying is recommended. Cysts in hog liquid manure holding tanks were found degraded, suggesting it is unlikely that distribution of liquid manure poses a serious threat for giardiasis from contamination of surface water (Guselle and Olson 1999).

Microsporidia

There are approximately 1200 named species of Microsporidia, a diverse group of obligate intracellular parasites once regarded as protozoa but now recognized as fungi. Most species infect invertebrates and fish, but at least 14 species in 10 genera have been reported to infect humans (Stentiford et al. 2016). Of these, *Enterocytozoon bieneusi* and *Encephalitozoon* spp. (*Encephalitozoon cuniculi* and *Encephalitozoon intestinalis*) are described as opportunistic pathogens in humans and have been reported in pigs, and other animals, suggesting the zoonotic potential of these parasites. *E. bieneusi* is the most frequently diagnosed species in swine and humans worldwide. It has been identified in swine in many countries, but its prevalence in pigs in the United States is not well studied. *E. bieneusi* is a complex species with multiple genotypes (Santín-Duran 2015). Surveys have demonstrated that pigs can serve as hosts for 60 *E. bieneusi* genotypes (Table 66.1). Most genotypes identified in swine are swine specific, but 14 genotypes identified in humans have also been identified in pigs. *E. cuniculi* genotype III has been reported in swine in Germany, and *E. intestinalis* in Mexico, Spain, and Slovakia; however none these two species have been reported in swine in the United States.

The life cycle stages of microsporidia are depicted using *E. bieneusi* as an example (Figure 66.9). All are intracellular, in direct contact with the host cell cytoplasm, and consist of binucleate cells, sporogonial plasmodia, uninucleate sporoblasts, and spores (Santín and Fayer 2011). Spores (1.5 × 0.5 µm) are the infective stage and are excreted in feces. Internally, spores have a polar tube with 5–6 coils, a single nucleus, and an anterior attachment complex extending to a polaroplast. After a spore is ingested, the coiled polar tube discharges, injecting the sporoplasm and nucleus into the host cell. Young proliferative stages become elongate and undergo nuclear division. Proliferative plasmodial cells contain

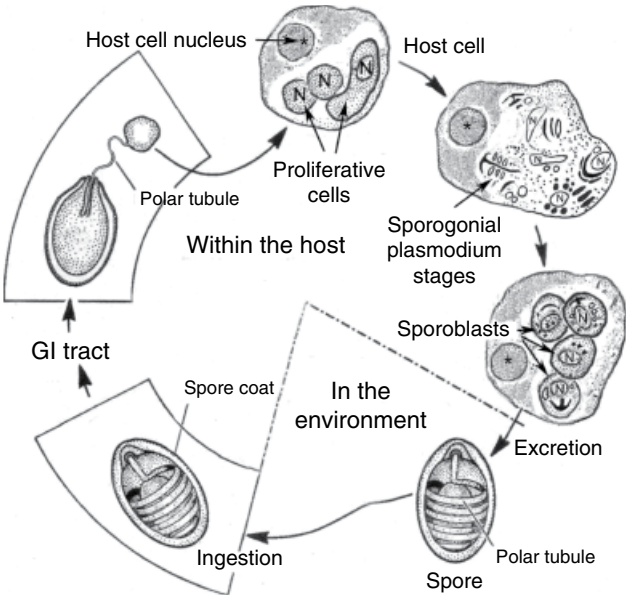


Figure 66.9 Life cycle of *Enterocytozoon bieneusi*.

multiple elongated nuclei. Later, sporogonial plasmodium develops disks, some in stacks or arcs in stages of polar tube formation. Individual nuclei with polar tube complexes segregate and mature into separate sporoblast cells that develop into mature spores. Spores released from host cells are excreted with feces into the environment where they can be ingested by the next host.

The role of microsporidia in clinical disease in swine is unclear. Disease due to microsporidia in humans occurs in immunocompromised patients. *E. bieneusi* has been detected in feces from asymptomatic pig and some pigs with diarrhea. No well-documented clinical cases have been reported. Whether certain genotypes are pathogenic whereas others are not is still unknown.

Detection of microsporidia infection is difficult because the spores are very small (1.5 × 0.5 µm). Spores can be detected by microscopic techniques used in specialized laboratories. Serological tests such as ELISA and indirect immunofluorescent antibody (IFA) microscopy can be used to detect antibodies against *E. cuniculi* and *E. intestinalis*. Specific and sensitive molecular methods are used to identify *E. bieneusi* and *E. cuniculi* genotypes. Although these tests are not routinely used in clinical diagnostic laboratories, they are widely used in research and some government public health laboratories.

Because a high prevalence of *E. bieneusi* has been reported in pigs and zoonotic genotypes detected, pigs are consider as reservoirs for potentially zoonotic microsporidia. Transmission of the spores in contaminated food and water maintains the parasites once they are introduced on a farm. Fortunately, the spores are readily killed by most common disinfectants and normal chemical

treatment of municipal water. Treatment of human patients using the anthelmintic albendazole is effective for *Encephalitozoon species* but not effective for treatment of *E. bieneusi*.

Balantidium coli

Balantidium coli is a ciliate found in pigs and in humans. Cysts that are excreted in the host's feces transmit it. The cysts are 50–70 µm in diameter and contain a macronucleus and a micronucleus. No division occurs in the cyst. Trophozoites are covered in short cilia, are up to 100 µm long, and also contain a macronucleus and a micronucleus. Trophozoites are usually found in the lumen of the large intestine.

Most infections in swine and humans are subclinical. Estimates of the prevalence in swine raised in the United States under modern production conditions are not available.

Studies conducted in the 1980s found that infection rates increased with pig age and that animals raised on pasture or dirt lots had higher infection rates than swine raised in confinement. Human infections with *B. coli* are more prevalent in areas where swine are present (Schuster and Ramirez-Avila 2008).

Entamoeba species

Amoeba in the genera *Entamoeba* has been reported in pigs in various parts of the world. The prevalence of these parasites in the US swine population is not known. Swine are not a significant reservoir for *Entamoeba histolytica*, the most pathogenic species in humans. Recent reports from Japan indicate that *E. polecki* is associated with chronic diarrhea and poor growth in young (50–70-day-old) pigs (Matsubayashi et al. 2015). This amoeba also infects humans, but little is known about the disease ecology of this parasite.

References

Armson A, Yaang R, Thompson J, et al. 2009. *Exp Parasitol* 121:381–383.

Biester H, Murray C. 1934. *J Am Vet Med Assoc* 85:207–209

Caudie CM, Done SH, Evans RJ. 2004. *Vet Rec* 155:647.

Dubey JP. 1986. *Vet Parasitol* 19:181–223.

Dubey JP. 1988. *Am J Vet Res* 49:910–913.

Dubey JP. 2009. *Vet Parasitol* 164:89–103.

Dubey JP.2010. *Toxoplasmosis of Animals and Humans*. 2nd ed. Boca Raton, FL: CRC Press.

Dubey JP, Powell EC. 1994. *Vet Parasitol* 52:151–155.

Dubey JP, Speer CA, Fayer R. 1989. *Sarcocystis of Animals and Man*. Boca Raton, FL: CRC Press.

Dubey JP, Thulliez P, Weigel RM, et al. 1995a. *Am J Vet Res* 56:1030–1036.

Dubey JP, Weigel RM, Siegel AM, et al. 1995b. *J Parasitol* 81:723–729.

Dubey JP, Hill DE, Jones JL, et al. 2005. *J Parasitol* 91:1082–1093.

Fayer R. 2010. *Exp Parasitol* 124:90–97.

Guselle N, Olson ME. 1999. Human pathogens in Alberta Hog Operations. Report to Alberta Hog Industry Development Fund, Alberta Pork Producers.

Henriksen SA, Christensen JPB. 1992. *Vet Rec* 131:443–444.

Henry SC, Tokach LM. 2008. *Swine Health Prod* 3:200–201.

Hill JE, Lomax LG, Lindsay DS, et al. 1985. *J Am Vet Med Assoc* 186:981–982.

Koudela B, Kucerova S. 1999. *Vet Parasitol* 82:93–99.

Lindsay DS, Stuart BP, Wheat BE, et al. 1980. *J Parasitol* 66:771–779.

Lindsay DS, Current WL, Ernst JV. 1982. *J Parasitol* 68:861–865.

Lindsay DS, Current WL, Ernst JV, et al. 1983. *Vet Med Small Anim Clin* 78:89–95.

Lindsay DS, Ernst JV, Current WL, et al. 1984. *J Am Vet Med Assoc* 185:419–421.

Lindsay DS, Current WL, Taylor JR. 1985. *Am J Vet Res* 46:1511–1512.

Lindsay DS, Blagburn BL, Boosinger TR. 1987. *Vet Parasitol* 25:39–45.

Lindsay DS, Neiger R, Hildreth M. 2002. *J Parasitol* 88:1262–1263.

Matsubayashi M, Kanamori K, Sadahiro M, et al. 2015. *Parasitol Res* 114:3069–3073.

Petersen HH, Jianmin W, Katakam KK, et al. 2015. Cryptosporidium and Giardia in Danish organic pig farms: Seasonal and age-related variation in prevalence, infection intensity and species/genotypes. *Vet Parasitol* 214(1–2):29–39.

Sangster LT, Seibold HR, Mitchell FE. 1976. *Proc Annu Meet Am Assoc Vet Lab Diagn* 19:51–55.

Santín M, Fayer R. 2011. *Res Vet Sci* 90:363–371.

Santín M, Trout JM. 2008. Livestock. In Fayer R, Xiao L, eds. *Cryptosporidium and Cryptosporidiosis*, 2nd ed. Boca Raton, FL: CRC Press, pp. 451–483.

Santín-Duran M. 2015. *Enterocytozoon bieneusi*, pp. 149–174. In Xiao, L., Ryan, U., Feng, Y. (eds.) *Biology of Foodborne Parasites*. Boca Raton, FL: CRC Press. 507pp.

Scala A, Demontis F, Varcasia A, et al. 2009. *Vet Parasitol* 163:362–365.

Schuster FL, Ramirez-Avila L. 2008. *Clin Microbiol Rev* 21:626–638.
Schwarz L. Worliczek KL, Winkler M, et al. 2014. *Vet Parasitol* 204:158–168.
Skampardonis V, Sotiraki S, Kostoulas P, et al. 2010. *Vet Parasitol* 172:46–52.
Stentiford GD, Becnel JJ, Weiss LM, et al. 2016. *Trends Parasitol* 32:336–348.
Stuart BP, Lindsay DS. 1986. *Vet Clin North Am Food Anim Pract* 2:455–468.
Stuart BP, Lindsay DS, Ernst JV. 1978. Coccidiosis as a cause of scours in baby pigs. *Proc Int Symp Neonatal Diarrhea* 2:371–382.
Stuart BP, Lindsay DS, Ernst JV, et al. 1980. *Vet Pathol* 17:84–93.
Weigel RM, Dubey JP, Siegel AM, et al. 1995. *J Parasitol* 81:736–741.
Xiao L, Herd RP, Bowman GL. 1994. *Vet Parasitol* 52:331–336.
Yaeger MJ, Holtcamp A, Jarvinen JA. 2003. *J Vet Diagn Invest* 15:387–389.

67

Internal Parasites

Helminths

Matthew T. Brewer and John H. Greve

Introduction

Internal parasites are common pests of swine worldwide. Controlled trials with nematode infections consistently demonstrate a reduction in average daily gain (ADG) and increase in feed to gain ratio (F/G) of infected pigs compared with their controls. In addition, internal parasites can generally compromise vigor and may act synergistically with other endemic potential pathogens. The extent of these losses depends on the quality of nutrient intake, type of housing, climate in the geographic area concerned, genetics of the swine, and veterinary costs associated with these infections. Premises heavily contaminated with thousands of infectious eggs or larvae from previous endemic infections can cause severe disease when encountered by a naïve animal. More often, subtle subclinical infections lead to insidious production losses that are substantial over time. This chapter addresses helminths, whereas ectoparasites and protozoa are addressed in Chapters 65 and 66, respectively.

Historically, schemes for parasite control incorporate sanitation, anthelmintics, and management practices aimed to reduce condemnations and production losses. While modern swine production facilities have decreased parasitism by denying pigs access to soil and/or parasite intermediate hosts, some helminths still persist due to their high fecundity and environmental stability. For example, despite the availability of efficacious anthelmintics, *Ascaris suum* remains as one of the most prevalent swine parasites throughout the world. On the other hand, pigs raised with access to soil continue to serve as hosts for a variety of internal parasites. Recent resurgence of extensive or outdoor production practices offers risk for reemergence of parasite concerns. Application of specific management practices from the last century and previous editions of this book may have merit, particularly because effective alternatives to licensed anthelmintics have not been documented.

Digestive system

The digestive system offers the most convenient entry into and exit from the host; therefore a myriad of parasites have evolved using this system as their niche. The mouth is a chaotic environment and hence is not often parasitized. However, all other levels for the digestive tract harbor helminth parasites.

Esophagus

Gongylonema pulchrum, the esophageal worm, is a spiruroid nematode occurring in tunnels burrowed into the epithelium covering the esophagus and occasionally the lingual or buccal mucosa. The tunnels are usually aligned with the longitudinal axis of the esophagus and form a sine wave appearance along their length. The males are 60 mm long, and the females about 90 mm long when removed from their tunnels. Oval-shaped eggs (55–65 × 30–35 µm) are transparent, contain a developed larva, and are passed in the host's feces. Infective L3 larvae develop when the eggs are ingested by coprophagous beetles or cockroaches, and pigs become infected when they ingest the insect intermediate host. *Gongylonema* cause minor inflammation as they glide back and forth in their tunnels. Their main importance is at slaughter, where tissue is trimmed if the lesion is discovered. Ruminants and humans are also susceptible to *G. pulchrum*, but they must ingest the intermediate host insect to become infected, so trimming of affected tissue is for appearance sake.

Stomach

Five nematode genera occur within the stomach. One, *Hyostrongylus*, is moderately common, but the other

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

four (*Ascarops*, *Physocephalus*, *Gnathostoma*, and *Simondsia*) are less common and limited geographically.

Hyostrongylus

Hyostrongylus rubidus, the red stomach worm, is a trichostrongyloid nematode occurring unattached on the mucosa of the lesser curvature of the stomach. Adults are the width of a hair and less than 10 mm in length. The eggs have typical strongyle structure (ovoid, thin shelled, transparent, 60–76 × 30–38 µm) containing the 16- to 32-cell stage when laid. These strongyle eggs resemble those of *Oesophagostomum* and *Globocephalus*; their differentiation requires careful measurement of eggs and harvest of infective ensheathed L3 larvae from incubated fecal cultures (Honer 1967). Eggs are passed in the feces and develop to infective larvae in about 7 days. The larvae migrate away from feces and onto grass where they are subsequently ingested by swine. Thus, the life cycle is direct, and hyostrongylosis is a disease of pastured swine. The ingested larvae enter gastric glands, undergo two molts, and reemerge into the gastric lumen. Some larvae may remain in the gastric glands in a state of hypobiosis, causing nodular distension of the affected glands. Hypobiotic larvae may enter the gastric lumen at a later time and mature into adult worms.

Although *H. rubidus* is reported from many areas of the world, little is known about the pathogenicity of this parasite. They suck a small amount of blood, leading to catarrhal gastritis and potential mucosal erosion. *H. rubidus* may be a factor in the pathogenesis of gastric ulceration, and these changes in the gastric mucosa can impact feed conversion and weight gains (Stewart et al. 1985).

Spiruroid stomach worms

Other stomach worms, such as *Physocephalus sexalatus*, *Ascarops strongylina*, *Gnathostoma spinigerum*, and *Simondsia paradoxa,* are spiruroid nematodes. They are stouter in appearance than *Hyostrongylus* and approximately 20 mm in length. The adults are attached by mouth to the mucosa, but this attachment causes no visible damage except for excessive mucus production. Female *Simondsia* have anterior ends that enter the gastric glands so that only their bulbous posterior is visible.

The life cycles of these four spiruroids, so far as is known, are all similar to one another. The typical spiruroid eggs (thick shelled, transparent, ovoid, contain a larva) pass in the feces where they are ingested by coprophagous beetles. The eggs (30–40 × 15–20 µm) resemble, but are slightly smaller than, eggs of *Gongylonema* (55–65 × 30–35 µm). Infective L3 larvae develop in beetles that are subsequently ingested by pastured swine.

Small intestine

Strongyloides

Strongyloides ransomi, the minute threadworm, is a rhabditoid nematode that has a cosmopolitan distribution but is more rarely present in modern indoor swine production facilities. It is particularly pathogenic in suckling pigs and has a greater importance in tropical and subtropical climates.

These minute (3–5 mm long) hairlike nematodes live embedded in the epithelium of the small intestine. Parasitic females are parthenogenetic and lay thin-shelled, transparent, embryonated eggs that are shed in feces. Rhabditiform larvae hatch in a few hours and may progress to either infective third-stage female filariform larvae (homogonic cycle) or free-living rhabditiform males and females (heterogonic cycle). The offspring of the free-living heterogonic cycle may become either rhabditiform free-living individuals or filariform infective parasitic females. Male individuals are not parasitic. Factors that determine whether individual nematodes undergo heterogonic or homogonic development are studied but poorly defined, with environmental factors such as availability of food and moisture suspected to influence which developmental pathway is taken.

Infective larvae typically infect the next host by penetration of the skin or the oral mucosa to gain access to the capillaries and carried by the bloodstream to the lungs where they are coughed up and swallowed (tracheal migration). This process results in a patent infection in 6–10 days. The most important route of infection in neonatal pigs is transcolostral (Moncol 1975). In sows, larvae accumulate in a hypobiotic state in the mammary fat until the time of parturition, whereupon the larvae become mobilized, enter mammary alveoli, and are shed in colostrum and milk. *S. ransomi* acquired by the lactogenic route produce a patent infection in 2–4 days. It is thought that piglets may also acquire larvae transplacentally; however, the lactogenic route is probably the most important. Piglets separated from their dam at birth are worm-free as opposed to nursing littermates.

Lesions are dependent on the number of infective larvae acquired and on the host's resistance. It is not uncommon to find a small number of *Strongyloides* without associated lesions. However, the adult nematodes are so small that they may be difficult to identify amid ingesta. Heavy infections in juvenile animals can lead to poor rate of gain, hemorrhagic diarrhea, and death. Immunity develops following exposure to larvae and is typically age related.

Diagnosis of patent infections is by observation of embryonated (larvated) eggs in fecal flotation procedures (Figure 67.1). However, these larvated eggs must be differentiated from other parasites such as spiruroids. This can be accomplished by culturing eggs to allow

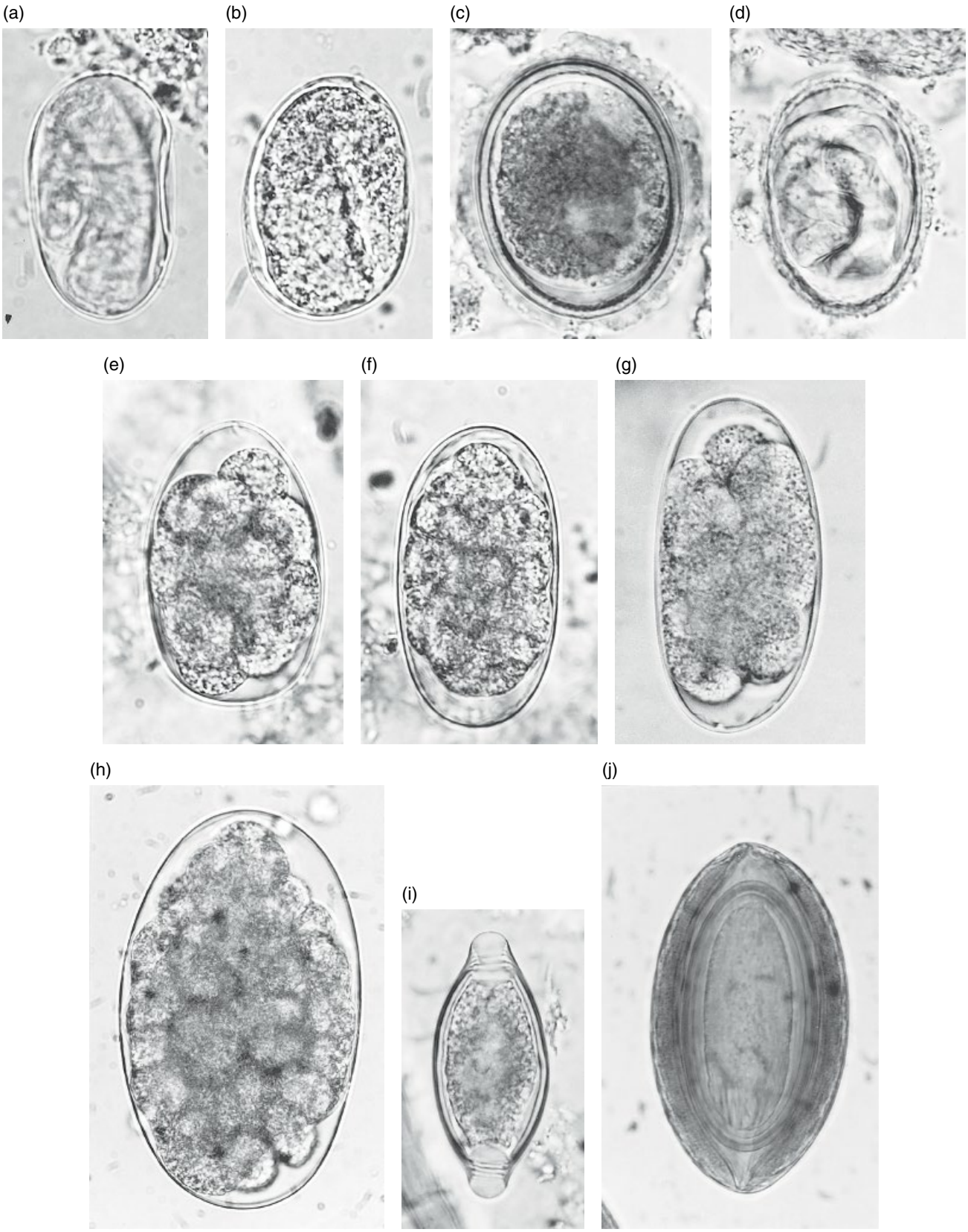


Figure 67.1 (a) *Strongyloides* egg, thick shelled, lacking one of three layers, and larvated. (b) *Ascarops* egg, larvated and similar morphologically to those of *Physocephalus* and *Gongylonema*. (c) The *Ascaris* egg has an outer proteinaceous layer, often missing. (d) *Metastrongylus* egg. (e) *Oesophagostomum* egg. (f) *Hyostrongylus* egg. (g) *Globocephalus* egg. (h) *Stephanurus dentatus* egg passed in the urine. (i) *Trichuris* egg. (j) *Macracanthorhynchus* egg. (All eggs photographed and printed at the same magnification.)

hatching and observation of rhabditiform L1 larvae. Adult *Strongyloides* may be found in mucosal scrapings, but the adults are very small (3–5 mm) and may be confused with larval stages of other nematodes. Squash preparations revealing typical eggs help address this problem as *Strongyloides* larvae do not have eggs.

Ascaris

Ascaris suum is the large roundworm and most cosmopolitan parasite of swine. *Ascaris* has persisted despite decades of pharmaceutical intervention and modern confinement systems. *A. suum* is a close relative to the human ascarid, *Ascaris lumbricoides*, and it is proposed that these two nematodes are a single species. *Ascaris* isolated from pigs is capable of infecting humans, and recent analysis of nuclear and mitochondrial genes from worms obtained from pigs suggests that human, swine, and hybrid genotypes are present (Jesudoss et al. 2017). If swine have access to soil, one can assume they are infected with *Ascaris* because of the ubiquitous nature of the nematodes and the extreme longevity of the eggs in the environment. Producers often assume that pigs are infected and administer anthelmintics, even without specific proof.

Ascaris suum adults can be easily observed at necropsy due to their large robust size (Figure 67.2): females are up to 40 cm in length, and males can be up to 25 cm. Adults live unattached in the lumen of the small intestine, swimming against peristalsis. It is proposed that *A. suum* maintains its position in the intestine by pushing up against opposing sides of the lumen. Adults mate and produce eggs that are thick and barrel shaped (50–80 × 40–60 µm). The colorless thick shell is coated with a sticky, brownish, mammillated proteinaceous layer and contains a single large cell. Females shed thousands of eggs over their lifetime of approximately 6 months. The eggs are resilient and long lived, so the environment is likely to be heavily contaminated wherever swine exist.

Life cycle

The life cycle is direct, with eggs passing in feces and developing infectivity over a period of approximately 3–4 weeks. The infective larva remains inside the protective egg, sheltered from potentially lethal environmental extremes until the egg is ingested. After ingestion, larvae hatch from the egg, penetrate the jejunal wall, and are carried by portal circulation to the liver. A few larvae may be found wandering in the mesenteric lymph nodes, peritoneal cavity, and elsewhere, but such wandering larvae probably do not complete their life cycle. Most larvae reach the liver 1–2 days postinfection and are then carried to the lungs via the blood 4–7 days postinfection. After molting and spending a few days in the lungs, larvae exit the pulmonary capillaries, breaking into the airways. Larvae are coughed up, carried to the pharynx by the mucociliary escalator, and then swallowed. Adult nematodes can be found in the small intestine 10–15 days after ingestion of infective eggs. Oviposition begins around 6–7 weeks after infection.

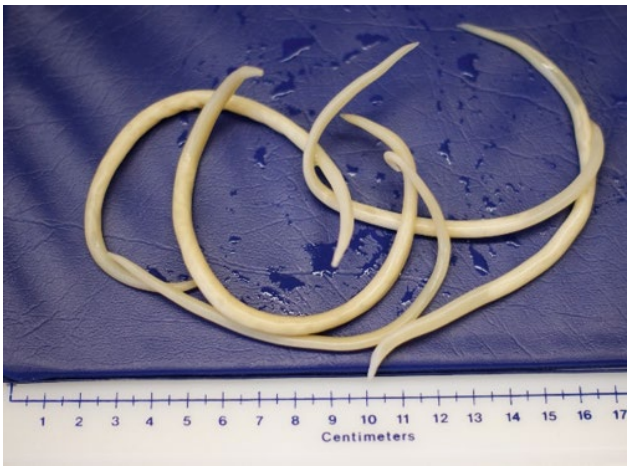


Figure 67.2 *Ascaris suum* adults removed from the intestine of a pig.

Eggs are very resistant to temperature extremes and may remain infective for years. Most chemicals have no effect on eggs, but steam may affect their viability. Because of the sticky outer layer of the egg, eggs are easily transported by boots, insects, and other means. It is thought that most piglets are exposed when they ingest eggs in manure stuck to the sows' udder.

Senescent ascarids are expelled in feces, but swine may continue to carry a small number of worms for several months. Ascarids often occur in young pigs, and by the time swine are 5–6 months old, they are often resistant to infection due to previous exposure to migrating larvae and/or by reason of age resistance. Pigs previously exposed mount an immune response, leading larvae to be trapped in foci of granulomatous inflammation. The larvae of *A. suum* are aggressive migrators and can cause visceral larva migrans in accidental hosts. The migrating larvae may cause serious verminous pneumonia or other inflammatory disease due to a prolonged migration or larval death, for example, in cattle housed in facilities previously dedicated to swine.

Pathology

Adult ascarids compete with the host for nutrients and probably interfere with nutrient absorptions as they browse on the tips of the villi, thereby causing insidious production losses. Adult ascarids swim against peristaltic waves to maintain presence and can occasionally wander up and occlude the bile duct or even approach the liver capsule. It has been hypothesized that bile duct occlusion can occur postmortem; however, ascarid occlusion of the bile duct should be considered as a potential cause of icterus in swine.

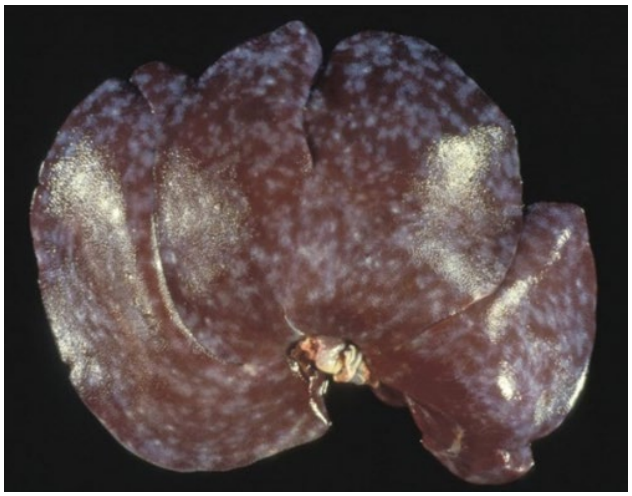


Figure 67.3 Multiple foci of fibrosis, the result of ascarid larval migrations ("milk spot liver").

In contrast to adult worms, the pathology damage associated with the migrations of larval stages of *A. suum* to the liver and lungs is substantial. Repeated waves of migrating larvae lead to an exuberant inflammation and immune response against larval antigens. In the liver, larvae migratory paths are infiltrated by eosinophils and fibrotic connective tissue that appears grossly as "milk spots." Lesions in the liver become visible 7–10 days postinfection and can expand or be variable in size if continued migrations occur. In low infestations, the "milk spots" can regress within 25 days if the liver does not experience further larval insult (Figure 67.3). Severe and ongoing infections result in diffuse liver fibrosis. Liver enzymes may be increased as a result of infection. In the lungs, larval migration is associated with petechial hemorrhages due to larvae breaking out of the capillaries and into the alveoli. Interstitial pneumonia, bronchiolitis, and alveolar edema also occur in the areas of larval migration. In mild cases, pigs are asymptomatic; however, more severely affected pigs have a characteristic expirational abdominal lift known as "thumping," sometimes with increase in cough. Most pigs become infected early in life, with immune mitigation of clinical signs. If naïve pigs are 20 kg or larger before first exposure, the pulmonary reaction may be extreme with life-threatening clinical signs similar to those of respiratory disease complex. Indeed, ascarid larval migrations may compromise the lung and exacerbate impact of other endemic viral and bacterial pathogens in the herd. The ingestion of infective eggs often occurs at low level over time, so lesions of larval migration can occur simultaneously with the presence of adults, which will continue until an immunological response develops.

Diagnosis

The diagnosis of *Ascaris* is straightforward when dealing with patent infections since the prodigious oviposition rate of the female makes eggs easy to find by standard flotation methods. At necropsy, the presence of milk spots in the liver is evidence that the animal has been infected with ascarid eggs within the past month. Early punctate lesions gradually expand in size before resolving in about 30 days. In areas where the swine kidney worm, *Stephanurus dentatus*, is present, the milk spots due to ascarid migration must be differentiated from fibrosis associated with kidney worm migration. Scars of the latter are more extensive with similar lesions occurring in other organs such as the lungs and kidney. Milk spots may also be caused by other helminths such as *Toxocara* undergoing visceral larva migrans in swine (Helwigh et al. 1999). Adult *Ascaris* are usually found in the small intestine at the time of necropsy or slaughter, but occasionally are found wandering in other portions of the gastrointestinal tract. The adults are large, often 20–30 cm in length and the diameter of a pencil with three large lips. Adults can be visible or palpable through the wall of the intestine.

When naïve pigs are exposed to egg-contaminated premises, morbidity or mortality can occur, while the infection is in the prepatent phase in which case fecal flotation is of no diagnostic value. Diagnosis is by observation of abundant milk spot lesions at necropsy. Careful examination may reveal small immature ascarids in the jejunum. Larvae migrating in lungs are difficult to observe grossly, but histopathology can offer a tentative diagnosis. Larvae can be collected by suspending snips of lung tissue in water. The motile larvae will migrate out of the lung tissue but are not strong swimmers, so they sink and can be collected with a funnel and tubing system similar to the Baermann apparatus often used to detect lungworm larvae in other domestic animal species.

Economic importance

Numerous studies have detailed economic importance of various aspects of ascarosis in swine. The results of experimental infections demonstrate some compromise of ADG and feed efficiency, even at low infection levels. *Ascaris* infection may also decrease the host response to vaccination against other pathogens. Metabolic studies show detrimental effects on nitrogen metabolism during the rapid growth phase of ascarids, about 1 month postinfection. At slaughter, there are losses due to condemnation or trimming of livers as well as condemnation carcasses due to icterus. The monetary value of these losses is enormous, though difficult to quantify. Historical estimates of losses are in the hundreds of millions of dollars in lost revenue annually (Stewart and Hale 1988). *A. suum* has continued to persist in commercial swine; a

visit to a slaughterhouse confirms the presence of these parasites on a daily basis.

Trichinella

Trichinellosis occurs in most mammals, including humans. The life cycle of this parasite is confusing as the adult nematodes live in the intestine and are seldom seen. Detection efforts focus on the larvae that live within muscle and cause disease. *Trichinella* has been found to be a diverse genus, but most cases of trichinosis are caused by *Trichinella spiralis*. Molecular characterization has revealed potential of at least eight different species in the genus with several genotypes that may achieve species status (Gottstein et al. 2009). *T. spiralis* has a global distribution, is a swine-adapted species, and is often detected in rats. *Trichinella britovi* can be found in pigs in Europe, Asia, and Africa and grouped in a single clade because they induce a thick collagen capsule during the muscle phase of infection; other species produce a capsule only detectable by electron microscopy. Examples of non-encapsulating species infecting pigs are *Trichinella pseudospiralis*, which has a cosmopolitan distribution, and *Trichinella papuae*, which can be found in Southeast Asia. Reduced incidence of *Trichinella* infections is attributed to regulation of garbage feeding to swine, public health programs, and improved detection techniques. In developed countries, trichinosis cases are now more often associated with ingestion of poorly cooked wild game meat.

Life cycle

Adult *Trichinella* are tiny (2–4 mm long) nematodes that are short lived and typically never encountered. The adults tunnel within the villi of the intestine. Within 5 days after mating, the viviparous females continuously deposit larvae in the lamina propria for duration of life, which is 2–3 weeks. The larvae enter the circulation and are distributed throughout the body until opportunity to penetrate the sarcolemma of skeletal muscle cells. After entering the myocyte, the myocyte becomes a "nurse cell" that supports the quiescent larva for months or years (Figure 67.4). Circulating larvae that do not enter myocytes eventually are arrested within granulomas. When cysts from the muscle are ingested, the larvae excyst and develop into adults within 48 hours. Examples of transmission within swine herds include tail biting, scavenging (rats, raccoons, etc.), and eating of garbage containing uncooked meat.

Pathology

The intestinal adults may be associated with enteritis although this is not typically observed clinically. Pathology and disease is associated with the larvae in the nurse cell. As the nurse cells form, there is malaise, pyrexia, and myalgia accompanied by eosinophilia.

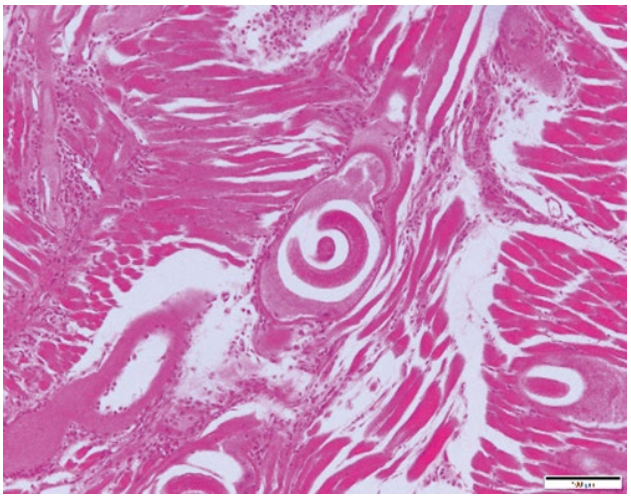


Figure 67.4 *Trichinella* larvae in a nurse cell.

Growth rates may be reduced, but the disease is often undetectable. Ten larvae per gram of body weight are thought to be a lethal infectious dose in a pig. As the nurse cell forms, the myocyte is walled off by collagen. Once the development of the nurse cell is complete, clinical signs regress, and normal growth rates resume.

Diagnosis

Traditional methods of diagnosis are aimed at finding nurse cells, which are not distributed homogeneously throughout the musculature but tend to be concentrated in particular muscle groups (diaphragm, extrinsic muscles of the eye, and muscles of posture). Both of the two main methods for detecting muscle cysts are labor intensive and produce false negatives. The first is by microscopic examination of bits of muscle compressed between glass plates, and the other is by digestion of several grams of muscle in artificial gastric juice (1% pepsin, 1% hydrochloric acid at 37 °C) and microscopy of sediment. These techniques have been largely replaced by a serum ELISA that detects pig antibodies directed at a larval secretory antigen (Murrell et al. 1986). False-negative ELISA results can occur when low populations (<5 larvae/g of muscle) of parasites are present.

Public health

Trichinella are zoonotic, infecting humans as well as other mammals. The source of *Trichinella* varies but can include pork products. Ground pork or sausage is particularly of concern because a single infected carcass may find its way into numerous sausages or can be used to dilute venison or beef sausage. In recent years, more cases of human trichinellosis in the United States have been caused by eating undercooked bear, wild game meat, or home-slaughtered pork. The incidence of swine trichinellosis in the United States has varied from 0.1 to

0.3% since the mid-twentieth century because the use of control measures (prohibition of feeding raw garbage, raising pigs in confinement, docking of tails, etc.) has practically eliminated trichinellosis in commercial pork. In the United States, there is a voluntary National Trichinae Certification Program aimed to continued efforts to eliminate trichinellosis.

Trichinella larvae cooked to a uniform temperature of 60 °C are not infective; therefore the US Department of Agriculture recommends that fresh pork be cooked to an internal temperature of 63 °C, allowing it to rest for 3 minutes between cooking and cutting. This temperature includes a safety factor that allows for variation in cooking methods (e.g. microwave ovens do not heat evenly). Freezing of fresh pork products less than 15 mm thick at −15 °C for 20 days or at −29 °C for 6 days will kill most larvae, but not isolates adapted to cold (e.g. *Trichinella nativa*, which is not a problem in commercial pork industry). Salt curing does not reliably kill larval *Trichinella*, so cured products should be cooked.

As a result of public health and veterinary food safety efforts, the number of cases of human trichinellosis in the United States has dropped from 450 cases per year in 1947 to about 12 cases per year 50 years later (Kennedy et al. 2009), and similar results have been achieved elsewhere. *Trichinella* is still present at a low level in swine as well as in wildlife in some regions, which is justification for continued vigilance.

Hookworm

Globocephalus urosubulatus is distributed throughout the world, but in North America it is limited to swine pastured in southern states. Adults attach to the jejunal mucosa by way of a large buccal capsule that lacks teeth or cutting plates. Young pigs are more likely to become anemic than older, more resistant individuals. However, clinical disease attributed to hookworms in pigs is rare. Adult *Globocephalus* are about 7 mm in length and lay strongyle-type eggs (52–56 × 25–35 µm) similar to those of *Hyostrongylus*. Infective larvae develop in the environment and infect other pigs by ingestion or skin penetration. Geographic distribution is limited to areas that do not freeze. Unlike hookworms infecting dogs and cats, *Globocephalus* is not associated with cutaneous larva migrans in humans.

Tapeworms

Although the swine gut is home to a myriad of helminth parasites, they are not typically definitive hosts for the adult stages of cestodes (tapeworms). Swine can, however, be important cestode intermediate hosts for *Taenia* and *Echinococcus* as discussed elsewhere in this chapter.

Acanthocephalans

Acanthocephala ("thornyhead") is a phylum distinct from Nematoda, characterized by parasites with protrusible proboscis. *Macracanthorhynchus hirudinaceus* is a large acanthocephalan up to 40 cm in length and has a dusty coral pink color, and the anterior end has a spiny proboscis with which it attaches to the jejunal wall. Its body may be turgid or wrinkled and flattened depending on hydration status and hence may be mistaken for an ascarid or a cestode. Ascarids lack a proboscis and do not attach, while cestodes have true segments (not mere wrinkles) and have a scolex that is not as dramatic as the proboscis of an acanthocephalan.

Eggs of *M. hirudinaceus* (70–100 × 40–65 µm) are passed in the hosts' feces and have a three-layered shell that is almond shaped, is brown, and contains a larva (acanthor). The acanthor has an ellipsoidal shape and needlelike hooks on one end. When ingested by white grubs of certain beetles (June bugs, dung beetles, etc.), an infective larva (cystacanth) develops in about 3 months, surviving metamorphosis to be present in the adult beetle. Pigs are infected by ingesting a grub or adult beetle while rooting. Once ingested, the cystacanths mature into adults in the small intestine, mate, and lay eggs after about 2–3 months. Since commercial swine are generally raised in confinement that denies pigs access to intermediate host, acanthocephalans have become rare. There are a few reports of *M. hirudinaceus* in humans in cultures that eat raw beetles. Because the life cycle is indirect, there is no danger in handling eggs or tissues of the adults.

Macracanthorhynchus hirudinaceus inserts its proboscis into the wall of the jejunum. The proboscis is large and capable of penetrating through the entire thickness of the intestinal wall, resulting in a perforation. This lesion may be encapsulated with nodular fibrous connective tissue that can be seen at necropsy, or they may rupture causing peritonitis. There are typically more nodules than adult worms, suggesting that release and reattachment may occur. Clinical disease associated with acanthocephalans is not reported often, but intestinal perforation may lead to abdominal pain, diarrhea, fever, and emaciation.

Large intestine

Two kinds of nematodes occur in the cecum and colon of pigs: nodular worms and whipworms. Both are very common, and both can lead to important clinical disease.

Whipworm

Trichuris suis, the swine whipworm, occurs in the cecum and colon. Much like ascarids, whipworms have persisted as an important parasite of pigs raised in confinement

facilities. Adult *Trichuris* are approximately 60 mm long, and about two-thirds of their length consists of a filamentous esophageal portion of the body that is stitched into the mucosa and not readily noticed. The thick posterior portion of the body protrudes from the mucosal surfaces. Intact nematodes are difficult to collect at necropsy because the delicate, long esophageal portion breaks. The eggs are thick shelled and lemon shaped (50–58 × 21–35 µm), each containing a single large cell. The egg shell is smooth and brown with a translucent plug filling an opening at the poles of each egg.

Life cycle

The life cycle of *Trichuris* is direct. Eggs pass in the feces, and once outside the body the first-stage infective larva develops within the egg in 3–4 weeks. The infective larva remains within the egg until ingested by a pig. Eggs can remain viable for several years in the environment. After ingestion, the plugs dissolve, perhaps stimulated by fimbria of intestinal bacteria. The first-stage larva penetrates the mucosa of the colon and undergoes four molts over a period of about 2 weeks. The posterior of the whipworm's body extends out into the lumen, and oviposition begins 6–7 weeks postinfection, while the worm's life span is approximately 4–5 months (Beer 1973).

Pathology

Low populations of adult *Trichuris* cause minimal lesions, although they may provide an entry point for other pathogens. Heavy infections with *Trichuris* are associated with ulceration of the mucosa, mucosal edema, and hemorrhage. Much of this tissue damage is caused by the histotrophic larvae, before adults are grossly present. Clinical signs include diarrhea, hematochezia, and rectal prolapse.

Diagnosis

The presence of adult *Trichuris* can be confirmed by demonstrating the characteristic eggs in fecal flotations; however, false negatives may occur due to sporadic egg laying by females, as well as the long (6–7 weeks) prepatent period. Adult worms can be detected at necropsy but are often overlooked due to their small size. Often, the most severe clinical signs, including dysentery, are produced by migrating larvae that may not be grossly visible for the first 3 weeks after infection. The tiny larvae and immature worms can be found in mucosal scrapings or recognized by histopathology. Whipworms can be recognized by their unusual esophageal structure of a single line of large glandular cells known as a stichosome. The uterus of a mature female contains eggs of typical morphology often visualized by histology. Commercial *Trichuris* antigen detection tests have been developed for use in other domestic animal species, but their utility has not yet been proven for *T. suis*.

Nodular Worms

Nodular worms (*Oesophagostomum* spp.) are common although less prevalent in swine raised indoors. These strongyloid nematodes inhabit the mucosal surface of the cecum and colon. Adults range from 8 to 15 mm in length, and males possess a copulatory bursa. There are several species, but their appearance and life cycle is similar. Eggs are typical strongyle eggs (70 × 40 µm) containing a morula stage.

The life cycle is direct; eggs passed in feces develop into first-stage larvae that hatch from the egg and molt twice in the environment to produce an infective third-stage larva that is ingested by swine. Infective larvae retain their last molted cuticle as a sheath, so they are moderately resistant to environmental extremes and can survive for several months or more. Ingested larvae exsheath and enter the mucosal glands of the ileum, cecum, and colon. They penetrate into the lamina propria, molt, and remain for about 2 weeks. They emerge into the intestine to become adults, and eggs begin to appear 3–6 weeks after infection.

Adults cause minimal damage to the mucosa and do not cause much in the way of clinical signs. The migration and molting of larvae in the lamina propria cause the characteristic nodule that is usually small (about 2 mm) but may be much larger, especially in animals with repeated infections. The larvae may remain within the nodules for several weeks before escaping into the lumen.

Antemortem diagnosis of nodular worms depends on finding the eggs in fecal flotations. However, this is complicated by the fact that eggs of *Hyostrongylus* and *Globocephalus* resemble them. For definitive identification, larval culture to the L3 stage is needed. At necropsy, the finding of nodules in the cecum and colon is suggestive but must be differentiated from other causes of inflammation, diverticulitis, or abscessed lymphoglandular complexes.

Respiratory system

Lungworms

Metastrongylus spp. have a worldwide distribution, with *Metastrongylus apri* being the predominant species, but mixed infections are common. The adults are slender, 40–50 mm in length, and occur in the bronchi and bronchioles, usually in the diaphragmatic lung lobes. Masses of entwined worms covered with mucus may occlude the peripheral airways. The eggs bear a thick rough coat, are colorless, contain a larva, and measure 50–60 × 35–40 µm.

The life cycle is indirect. Eggs are coughed up, swallowed, and passed in the feces. Certain earthworms, notably *Eisenia* and *Allolobophora* spp., ingest the eggs. Larvae hatch and invade the earthworm's tissues (calciferous

glands, heart, dorsal blood vessel, and crop). When swine eat the earthworm, the lungworm larvae migrate to the lungs via the lymphatic system and begin laying eggs in 4–5 weeks. Clinical signs are not pronounced, but heavy infections and infections complicated with bacterial infections cause coughing and "thumping."

Lungs with metastrongylosis have wedge-shaped areas of emphysema or atelectasis, usually at the tips and about midway along the length of the diaphragmatic lobes where major bronchi approach the lobe periphery. Often, hypertrophic bronchial muscle, hyperplastic epithelium, and nodular lymphoid hyperplasia are associated with the nematodes. Concurrent bacterial pneumonia is not uncommon.

Diagnosis of metastrongylosis is accomplished by finding the characteristic eggs on flotation, but the eggs do not float well. Postmortem diagnosis is accomplished by trimming 1 cm strips from the edge of the diaphragmatic lung lobe and squeezing to express adults from the bronchi.

Lung fluke

Paragonimus kellicotti is a trematode fluke that occurs in bronchial cysts of a wide range of hosts, including swine, in North America. *Paragonimus westermani* is a similar species occurring in Southeast Asia and a few areas of South America. *Paragonimus* are large, fleshy, brownish flukes that measure 8–12 mm long, 4–6 mm wide, and 3–4 mm thick. They are often found in pairs. The cysts are about 2–3 cm in diameter and are easily seen and palpated at necropsy. Usually there are few clinical signs because a few cysts do not compromise lung function. On the other hand, heavy infections can produce a productive cough with dyspnea and hemoptysis.

The life cycle is indirect. Miracidia hatch from the egg and penetrate the first intermediate host, a snail. Cercaria exit the snail and are consumed by the second intermediate host, which is a crayfish for *P. kellicotti* or either crayfish or crab for *P. westermani*. When eaten by a swine, the metacercariae in the crustacean excyst, penetrate the intestinal walls, burrow through the diaphragm, and enter the pulmonary parenchyma. They settle in pairs, mature, and begin oviposition in about 60 days. Pairs live in cysts that communicate with bronchi. Worms are hermaphroditic, but it is thought that they may either mate with another individual or self-fertilize. Eggs are coughed up, swallowed, and passed in feces.

At necropsy, the characteristic cysts can be found in the lungs. Adult flukes may be teased from the cysts or may be observed by histopathology. The eggs are relatively heavy but will float in a saturated sugar solution with centrifugation. The eggs are brown, vase shaped, and large (80–110 × 50–60 µm) and have a thickening at the junction of the operculum and shell. The number of eggs appearing at any one time varies because their presence in the feces depends on being coughed up and swallowed. Eggs may also be detected in smears of sputum. Some migrating excysted metacercariae wander, and adults may be found ectopically in lymph nodes, liver, peritoneum, etc.

Liver and pancreas

Aside from being the site of larval migration (e.g. *Ascaris* and *Stephanurus*), the liver and pancreas are the niches for only a few helminths.

Liver fluke

Fasciola hepatica is a cosmopolitan trematode fluke that has a broad host range that includes swine. The adult fluke is large (30 × 10 mm) and leaf shaped, with a conical anterior end. The eggs are large (130–150 × 65–90 µm), oval, yellowish brown, and operculated.

Eggs passed in the feces develop in water. A ciliated miracidium hatches and penetrates a lymnaeid snail that undergoes asexual reproduction and results in the release of hundreds of cercaria. Each cercaria encysts on vegetation to form an infective metacercaria. Metacercariae may remain infective for a long period of time but are killed quickly under dry conditions. After being ingested by the final host, the metacercaria penetrates the intestinal wall and the liver capsule and migrates in the liver parenchyma for 6 or more weeks. It eventually reaches the biliary duct system and migrates to the larger bile ducts and sometimes the gallbladder. The prepatent period is 10–12 weeks.

Migrating adolescent flukes cause necrohemorrhagic tracts in the liver parenchyma. These tracts regress once the flukes have entered the bile ducts. Adults cause bile duct hyperplasia and fibrosis, leading to the characteristic "pipestem liver" lesions that resemble the stems of clay pipes. Adult flukes and brownish exudate can be expressed if the affected bile ducts are incised. Clinical signs associated with fasciolosis are anemia and hypoproteinemia due to liver damage and hematophagia by adult flukes. Infected individuals may have weight loss or poor gains, but infections are often silent. The liver is condemned at slaughter. Ruminants and humans can also be infected. A similar fluke, *Fasciola gigantica*, infects swine in Africa and Asia.

Echinococcus (hydatid cyst disease)

Adult *Echinococcus* cestodes occur worldwide in carnivores, and their larval cysts (hydatids) occur in various herbivores and omnivores, including humans and swine. The adults are very small (3–6 mm) and typically occur

by the hundreds in the definitive host's intestine. Egg-laden proglottids are shed into the environment via feces. The eggs are identical to those of other taeniids (e.g. *Taenia*), and, when ingested by a pig, the oncosphere hatches from the egg, penetrates the intestinal wall, and enters the circulation. A large proportion becomes trapped in the liver where they nidate and develop into hydatids. Unilocular hydatid cysts (*Echinococcus granulosus*) have an external fibrous cuticle and an inner germinal membrane, while multilocular hydatids (*Echinococcus multilocularis*) have a germinal layer on both the inner and outer surfaces, which are capable of producing a more invasive lesion similar to that of a neoplasm. The germinal layers give rise to many thin-walled brood capsules, and each brood capsule contains several dozen protoscolices, often referred to as "hydatid sand," which can be visualized ultrasonographically. The end result is that numerous protoscolices are produced from each egg ingested. When ingested by a carnivore, each protoscolex develops into an adult cestode.

There are no clinical signs that imply hydatid cyst disease, and diagnosis is usually done at necropsy. Aspirates of hydatid cysts contain protoscolices that contain calcareous corpuscles and hooks. Histologically the brood capsules and protoscolices can also be identified. One challenge for diagnosis is that some hydatids developing in pigs, particularly those of *E. granulosus*, are "sterile" (e.g. contain no protoscolices), which makes for a more difficult diagnosis. Hydatid cyst disease is uncommon in the United States, but sylvatic cycles of the parasite mean that it is an ever-present threat. In areas of the world where pigs are free roaming or where offal is fed to carnivores, hydatid cyst disease is both an economic and a public health concern since humans can develop hydatids.

Urinary system

Kidney worm

Stephanurus dentatus adults occur in perirenal cysts that open by a fistula into the ureters. It is common to discover ectopic cysts in other organs such as the spleen, stomach, urinary bladder, and spinal cord. Renal parenchyma per se is seldom involved. Adult *S. dentatus* are large stout worms, measuring 20–40 × 2 mm with visible internal organs. Their eggs are typical for strongyles but can be differentiated from those of hookworms, nodular worms, and other strongyles because they are found in urine.

First-stage larvae develop in the egg, hatch, and molt twice into an infective third-stage larva. Infective larvae may survive on pasture for up to several months and can either be ingested or penetrate the skin, migrate to mesenteric lymph nodes, molt, and enter the liver via portal veins. There they migrate for several weeks, growing to a size of 5–6 cm before exiting the liver and migrating to retroperitoneal tissues where adults cluster within an inflammatory cyst that fistulates to the ureters or kidneys. The prepatent period is long (9–12 months), and adults may live for several years. Migrating larvae are aggressive and can be found in ectopic sites including the epaxial muscles and spinal column, and because early migration takes place in blood vessels, it is thought that some pigs may become infected *in utero*. Migrating larvae produce hemorrhagiconecrotic tracts wherever they go and hence produce considerable abscessation, eosinophilia, and fibrosis, including much more prominent hepatic "milk spots" than those caused by ascarids.

The infection is limited geographically to areas that do not experience severe winters, and confinement rearing has dramatically reduced the incidence of kidney worm. In systems where the problem persists, a "gilts-only" breeding system can be implemented in order to control the parasite. This measure is successful due to the long prepatent period; gilts are slaughtered prior to developing a patent infection.

Musculoskeletal system

The body musculature harbors immature stages of only a few helminths. Already mentioned above are the larvae of *Trichinella* in "nurse cells" and aberrantly migrating larvae of *S. dentatus*. In addition to these nematodes, the cysticercus of *Taenia solium* lives within muscle tissue.

Cysticercosis ("pork measles")

The larval stage (metacestode or cysticercus) of *T. solium* occurs in the skeletal muscles and myocardium of swine as a fluid-filled cyst. Prior to a complete understanding of the life cycle, this organism (stage) was named *Cysticercus cellulosae*; some authors still use this nomenclature to describe the metacestode of *T. solium*. The adult form of this cestode occurs in the intestine of humans. Although once widespread in distribution, the infection is now limited by methods of human hygiene and cooking of pork. However, human cases from endemic areas occur, and diaspora assures continued cases and public health concerns.

Taenia hydatigena, "the long-necked bladder worm," is another source of cysticercosis in swine. Unlike *T. solium*, *T. hydatigena* infects canine definitive hosts and does not develop in humans. The cysts are larger (approximately 8 cm) and found in the omentum and mesentery. The cyst bears a long armlike extension (neck) that holds the inverted protoscolex.

Life Cycle

Humans infected with adult *T. solium* shed proglottids in the feces. The egg-filled proglottids release eggs when the proglottids are desiccated or damaged as no uterine pore occurs in *Taenia*. The eggs or proglottids are ingested by swine. The oncospheres hatch from the egg, penetrate the small intestine, and are distributed throughout the body by the circulation. They become trapped in capillary beds associated with muscle tissue and develop into infective cysticerci. This condition is sometimes called "pork measles" by packers. The term measles refers to the spot that is the cysticercus. Cysticerci are subspherical, whitish, translucent, fluid-filled cysts about 1 cm in diameter. Visible through the surface is a white, 1–2 mm spot that is an inverted protoscolex that will become the scolex of the adult tapeworm when ingested. Cysts remain infective for up to 2 years, but eventually they die and undergo caseation and mineralization. Cysticerci develop in any muscle of the body but are most commonly found in the heart, psoas, masseter, tongue, and limbs. Humans can become accidental intermediate hosts and develop cysticercosis following ingestion of eggs from human feces.

Diagnosis

There are no characteristic clinical signs associated with cysticercosis. Risk factors for infection include pigs that are free roaming and have access to domestic waste and human feces. The presence of a cysticercus is revealed at necropsy or slaughter. Palpation of the tongue may reveal deeply seated cysts. Microscopy of squash preparations of cysticercus can reveal the rostellum containing hooklets; hooklets do not disintegrate despite caseation. Several ELISA assays have been developed to detect *Taenia* antigens as well as anti-*Taenia* antibodies. These assays have been applied in humans but are not regularly used for surveillance in swine herds.

Public health

Viable *T. solium* cysticerci are infective for humans, so meat inspectors search for them regularly. If inspection reveals only a few cysticerci, they may be excised and discarded. Finding several organisms requires the entire carcass be cooked at 170 °C for 30 minutes (which kills the cysts) prior to releasing for consumption; however, finding high numbers of cysticerci leads to condemnation of the entire carcass. Freezing cuts of pork is another method of killing cysticerci; the temperature and time held at that temperature depends on the size and thickness of the piece of pork. Discovery of cysticerci should prompt an investigation of how pigs are coming into contact with human waste.

Besides acting as the definitive host for *T. solium*, humans can become accidental intermediate hosts after ingesting the eggs. In humans the cysticerci have a predilection for the central nervous system; neurocysticercosis is thought to be the cause of cognitive dysfunction in humans wherever *T. solium* is endemic. Humans infected with adult *T. solium* can be treated with praziquantel. Swine intermediate hosts are not treated; elimination of the life cycle is accomplished by sanitation, treatment for infected humans, and proper disposal of human feces.

There are many other helminths reported to infect swine but are considered of lesser importance (Table 67.1).

Control of helminth parasites

Parasite control methods may be broadly categorized as preventative or therapeutic. Preventative methods focus on interrupting critical points in the parasite life cycle, whereas therapeutic approaches utilize pharmaceutical agents to remove parasites from the pig. Seldom will therapeutic approaches alone eliminate a helminth parasite from a herd.

Prevention

Those parasites requiring an intermediate host can be successfully prevented by removing pigs from contact with the intermediate host (e.g. dung beetles, earthworms, snails, etc.). Therefore, maintaining pigs on concrete will prevent infection with spiruroids, acanthocephalans, and metastrongyloids. An added benefit would also be the reduction or prevention of infection by strongyles, such as *Hyostrongylus*, *Globocephalus*, and *Trichostrongylus*, that are transmitted more efficiently under pasture conditions.

Good sanitation is a critical element for controlling parasitic infections. The major mode of transmission of internal parasites is through contaminated food, soil, or bedding with feces or urine. Since parasite eggs need moisture and warmth to develop and survive, direct sunlight or dry conditions shorten egg and larval survival. Thorough cleaning of buildings, pens, and equipment with detergent and steam is the best way to control parasite eggs and larvae. Steam penetrates cracks and crevices and kills the tiny eggs and larvae. The common disinfectants used on the farm do not kill eggs of *A. suum*.

Since parasites compete with the host for available nutrients, adequate nutrition aids in reducing the adverse effects on feed efficiency and ADG. Pigs on a good plane of nutrition are less likely to exhibit clinical parasitic disease as opposed to individuals with a negative energy balance. However, improved nutrition alone will not abrogate a serious helminth problem in a herd.

A management system in which gilts only are used as breeders has been shown to be effective in eradicating

Table 67.1 Gastrointestinal helminths of lesser importance.

	Locale	Description	Comments
Gastrodiscus aegyptiacus Gastrodiscoides hominis	Small and large intestine in Africa and southern Asia	Flaky paramphistome flukes 5 × 14 mm; operculated eggs 150 × 70 µm	Infects humans; infection acquired by ingesting metacercariae on vegetation
Fasciolopsis buski	Small intestine in southern Asia and India	Large fluke 20–75 mm long; operculated eggs 135 × 85 µm	Infects humans; cycle similar to that of *Fasciola hepatica*
Gnathostoma spp.	In nodules in gastric wall in Eurasia and Africa	20–40 mm long, spinose cuticle; eggs with one polar plug, 70 × 40 µm, brown pitted shell	Second intermediate host is small vertebrate (reptile, bird, etc.); prepatent period about 3 months
Ascarops strongylina	Mucosal surface of stomach	15–20 mm; eggs thick shelled, transparent, embryonated, ellipsoidal 30–40 × 15–20 µm	Intermediate hosts are coprophagous beetles; prepatent period 4–6 weeks
Physocephalus sexalatus	Mucosal surface of stomach	15–20 mm; eggs similar to those of *Ascarops*	Cycle similar to that of *Ascarops*
Simondsia paradoxa	Stomach mucosal glands	15–20 mm; posterior end of female bulbous and filled with eggs	Cycle similar to that of *Ascarops*
Eurytrema pancreaticum	Pancreatic ducts	Flukes 10–15 × 2 mm; eggs 40–50 × 25–35 µm; dark brown; contain miracidium	Second intermediate host is grasshopper
Clonorchis sinensis	Bile ducts	Flukes 10–25 × 4 mm; eggs 27–35 × 12–20 µm; light brown; contain miracidium	Second intermediate hosts are freshwater fishes

kidney worms. This is possible because of the lengthy prepatent period of 9 months or more. Selling breeders as soon as first litters are weaned and maintaining boars separately or replacing them with young stock will prevent contamination of the premises. Using the gilts-only breeding system was used to achieve eradication of kidney worm in less than 2 years (Stewart et al. 1964).

For some parasites, prevention is the most effective method for eliminating the organism. For example, elimination of feeding raw meat, scavenging, and cannibalism is effective in preventing the transmission of *Trichinella*. Similarly, denying pigs access to human fecal material is efficacious for halting the transmission of *T. solium*.

Treatment

Therapeutic anthelmintics present only a temporary solution unless the conditions under which the parasites were acquired are altered. No drug is effective against all stages of all parasites, and the tissue damage prior to therapy results in slower growth rates and increased nutrient requirements. A good management system will incorporate practices, such as sanitation, genetic selection, and nutrition, to prevent infections and will not use treatment as the sole method of controlling parasites. The choice of anthelmintic is dependent on the parasite species present and the relative cost of the products. Routine surveillance and strategic treatment for parasites are advisable on farms with a previous history of clinical disease. Treatment reduces the number of egg-producing parasites and keeps further premise contamination to a minimum. For pigs placed in high-risk environments, the use of prophylactic compounds pyrantel tartrate and fenbendazole fed for several weeks can control infections and reduce lesions from ascarids and nodular worms, reduce worm populations, or may enhance immunity against *A. suum* (Southern et al. 1989; Stankiewicz and Jeska 1990). Treatment of sows 10–14 days before farrowing has been shown to be effective in preventing transmission of nematodes and *Sarcoptes scabiei*.

Macrocyclic lactones

Macrocyclic lactones, such as avermectins and milbemycins, are fermentation products of the fungus *Streptomyces avermitilis* and are thought to act by preferentially binding glutamate-gated chloride ion channels, a class of ion channel that is only found in invertebrates, which leads to paralysis and death of susceptible invertebrates. Avermectins are available as injectable and pour-on formations or as feed additives and have stated slaughter withdrawals. In general, they control *A. suum* (adults and fourth-stage larvae), *H. rubidus* (adults and fourth-stage larvae), *Oesophagostomum* (adults and fourth-stage larvae), *S. ransomi* (adults), and

Metastrongylus (adults). Doramectin is labeled for *A. suum* (adults and fourth-stage larvae), *Oesophagostomum* (adults and fourth-stage larvae), *S. ransomi* (adults), *Metastrongylus* (adults), and *Stephanurus* (adults) and has variable efficacy for *Trichuris*.

Benzimidazoles

Benzimidazoles preferentially bind nematode beta-tubulin and disrupt cell division. This class of drugs is thought to have a wide margin of safety as it preferentially binds the nematode protein as opposed to mammalian tubulin. Thiabendazole is well known but less efficacious in swine than fenbendazole, which is the only benzimidazole labeled for helminths in swine in the United States, available for administration in an oral suspension or feed additive with a slaughter withdrawal. Fenbendazole products are labeled for the treatment of *A. suum*, *Metastrongylus*, *Oesophagostomum*, *Hyostrongylus*, *Stephanurus*, and *Trichuris* in the United States. It should be noted that fenbendazole is thought to be one of the few anthelmintics effective for removal of *Trichuris*.

Imidazothiazoles

Levamisole binds nematode nicotinic acetylcholine receptors, leading to paralysis and death. It has been used in water or feed. It is effective for the treatment of *Ascaris*, *Oesophagostomum*, *Metastrongylus*, *Strongyloides*, and *Stephanurus*. It has a slaughter withdrawal in the United States.

Tetrahydropyrimidines

Pyrantel tartrate is the only tetrahydropyrimidine labeled for use in swine; it also acts by binding nematode nicotinic acetylcholine receptors. It is available for incorporation into feed, most commonly used as a continuous dewormer for 30 days in starter and growing pigs as an aid in the prevention of larval migration and establishment of *A. suum* and *Oesophagostomum*. Pyrantel has a slaughter withdrawal in the United States.

Piperazine

Piperazine salts are an older generation of antiparasitic purge dewormer that causes a neuromuscular blockade by disrupting GABA neurotransmission. Piperazine is administered in feed or water and should all be consumed in an 8–12 hour period; withholding feed or water the previous night is beneficial for stimulating consumption. It is currently approved for treatment of adults of *Ascaris* and *Oesophagostomum*.

Organophosphate compounds

Dichlorvos is an organophosphate compound added to feed with good efficacy against *Ascaris, Oesophagostomum, Trichuris,* and *Hyostrongylus* with slightly lower efficacy against *Strongyloides* (Marti et al. 1978). It can be incorporated into slow-release polyvinyl chloride pellets that allows for continued effect in the cecum, producing the desired removal of whipworms.

References

References to publications from older literature and reflecting general knowledge of helminths can be found in prior editions of this book.

Beer RJS. 1973. *Parasitology* 67:253–262.

Gottstein B, Pozio E, Nockler K. 2009. *Clin Microbiol Rev* 22:127–145.

Helwigh AB, Lind P, Nansen P. 1999. *Int J Parasitol* 29:559–565.

Honer MR. 1967. *Z Parasitenkd* 29:40–45.

Jesudoss J, Murphy K, Snobl T, et al. 2017. *J Infect Dis* 215(1):131–138

Kennedy ED, Hall RL, Montgomery SP, et al. 2009. *Morb Mortal Wkly Rep* 58:55–59.

Marti OG, Stewart TB, Hale OM. 1978. *J Parasitol* 64:1028–1031.

Moncol DJ. 1975. *Proc Helminthol Soc Wash* 42:86–92.

Murrell KD, Anderson WR, Schad GA, et al. 1986. *Am J Vet Res* 47:1046–1049.

Southern LL, Stewart TB, Bodak-Koszalka E, et al. 1989. *J Anim Sci* 67:628–634.

Stankiewicz M, Jeska EL. 1990. *Int J Parasitol* 20:77–81.

Stewart TB, Hale OM. 1988. *J Anim Sci* 66:1548–1554.

Stewart TB, Hale OM, Andrews JS. 1964. *Am J Vet Res* 25:1141–1150.

Stewart TB, Batte EG, Connell HE, et al. 1985. *Am J Vet Res* 46:1029–1033.

Section VI

Noninfectious Diseases

68

Nutrient Deficiencies and Excesses

Steve S. Dritz, Robert D. Goodband, Joel M. DeRouchey, Mike D. Tokach, and Jason C. Woodworth

In modern swine production, the diagnosis of nutrient deficiencies or excesses is rarely a straightforward, linear process. A typical case usually starts with unremarkable or nonspecific clinical signs such as "elevated mortality," which may lead to a diagnostic investigation. The astute clinician will carefully collect a history and specific clinical description, coupled with necropsy findings, before determining the case definition. The case definition should provide the basis for a differential diagnosis that informs the collection of specific types and numbers of samples for a proper diagnostic investigation. Diagnostic investigation usually can identify disease processes involving pathogenic agents, but rarely will a specific diagnosis involving a nutritional component emerge with routine diagnostic investigation. A summary of clinical signs that may be associated with specific nutrient deficiencies (Table 68.1) and the base information in this chapter can be used to develop a list of rule-outs for further investigation from a nutritional perspective.

Pigs are a highly adaptable omnivorous species with a long domestication history of being fed many different ingredients. The majority of pigs today are fed grain and protein source-based diets with added vitamins and minerals. However, there are a wide variety of diet formulation practices due to local availability of ingredients and the capability of the pig to utilize a wide cross section of primary energy and protein sources. As with other deficiencies and toxicities in general, both the duration and dosage of specific nutrients are important determinants of effect on the animal. The transition between deficient, marginal, optimal, tolerable and toxicity is not always clearly defined (Figure 68.1).

Pigs may tolerate high dosages of a particular nutrient for short periods, but long-term feeding may have detrimental consequences. Likewise, low dosages of some nutrients can lead to either acute or chronic deficiency disease. Furthermore, pigs in some physiologic stages, such as young, old, reproducing, or disease-challenged states, may be more sensitive to deficiencies or toxicities. Few studies under modern pork production conditions are focused on quantifying clinical signs of deficiencies or toxicities, thus making early recognition of compromised pigs and the diagnosis of nutritional diseases difficult. Growth and reproductive performance are sensitive indicators of many nutrient deficiencies or excesses. Characterization of these criteria can be useful in confirming diagnosis or monitoring responses to dietary changes. Unfortunately, these indicators are closely associated with many other disease processes and so have limited use in the primary diagnosis of a nutrient deficiency or toxicity.

Most scientific studies evaluating nutrient requirements evaluate dose responses focused on optimizing growth, performance, and economic return. Seldom are these studies performed to characterize nutrient deficiencies or toxicity. Economic drivers sometimes lead to purposeful fortification of some nutrients at levels below or above the commonly acknowledged requirements to obtain maximal growth, in order to lower cost and increase profit. For example, feeding slightly below the amino acid requirement during the finishing phase will result in slightly slower growth and poorer feed efficiency but will usually result in optimized economic performance. Similarly, feeding excess feed to gestating sows has a negative impact on subsequent lactation performance and cost. Nutritional optimization is beyond the scope and not the objective of this chapter. This chapter will have a primary focus on clinical nutrient deficiencies or toxicities.

Investigation of nutrient deficiencies or excesses

Critical control points in feed production

The feed production chain in most modern swine systems is a highly integrated process that involves the procurement of ingredients, accurate weighing and mixing

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 68.1 Clinical signs with micronutrient deficiencies or excesses.

Clinical sign	Deficiencies	Excesses	Clinical history considerations
Anemia	Fe, B_{12}	Cu	Inadequate iron preweaning; little evidence to support inadequate dietary iron post weaning with anemia
Congenital defects	Vitamin A	—	Uncommon; requires deficiency for extended period; usually premix formulation errors
Coronary band separation	—	Se	Diet formulation or environment
Diarrhea	Niacin	Crude protein	Niacin deficiency is uncommon, but availability often is unknown; excess crude protein post weaning; other nutrients or ingredients may cause transient diarrhea when fed in excess
Fractures, lameness	Ca, P, vitamin D	Ca	No sunlight; phytase usage; formulation errors; mixing errors; equipment malfunctions
Growth plate/cartilage, kyphosis, rickets	Ca, P, vitamin D,	Vitamin A	
Hemorrhage, ecchymosis, or hematomas	Vitamin K	—	Aged premix; unstable source of menadione; heat in processing
Hair loss	I, Zn, B vitamins	Se	Goitrogens or low I (neonates); chronic high Se exposure through soils or feed
Neurologic (altered gait, ataxia, tremors, paresis)	Pantothenic acid; Ca, P, vitamin D	Vitamin A, Se, Na	Formulation errors; feedstuff origin; tetany can occur with severe deficiency Ca, P, or vitamin D; Na toxicity is due to water deprivation and will not occur when water access is adequate
Neurologic with polioencephalomalacia	Thiamin	—	Sodium metabisulfite; thermal processing; unprocessed fish meal
Skin lesions, parakeratosis	Zn, multiple vitamins	—	Low Zn; other minerals high; diet supplementation error
Sudden death	Se, vitamin E	Se, Fe	Geographic origin of feedstuff; errors; Fe sudden death potentially associated with injectable doses while there is little evidence with oral doses

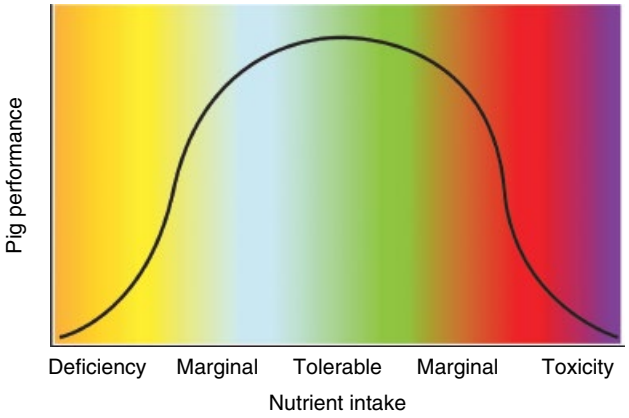


Figure 68.1 Responses to increasing nutrient fortification are on a continuum.

of ingredients, feed processing, and delivery of the correctly formulated diet to a specific group of pigs. With improved understanding of ingredient composition and current nutrient requirements of swine, gross deficiencies or excesses are less likely to be the result of failure to recognize the metabolic role of a specific nutrient and more likely a breakdown in the feed manufacturing chain. Therefore, the clinician needs to have a thorough understanding of the feed manufacturing process to help identify critical control points within the system. Critical control points in this process include:

1) Accounting for the potential variation of nutrient composition and bioavailability of feed ingredients based on their source or local conditions where they were grown. For example, variation in geographical distribution of soil selenium influences concentration in grain produced.
2) Recognition that processing of an ingredient before delivery to the feed mill can have impact on product quality. For example, an ingredient such as dried distillers grains with solubles (DDGS) can have compromised nutrient content if heat damaged in the drying process. Processing of DDGS is an example where removal of the starch results in a concentration of any mycotoxins initially present in the grain because mycotoxin activity is unaffected by the fermentation process.
3) Awareness and audits to verify that ingredient storage and handling maintains nutrient quality. For example, bins should be completely sealed or aerated to prevent moisture accumulation. Products with expiration dating, e.g. vitamin premixes, should be properly stored and used before expiration.

4) Verification processes to assure that the correct and accurate transfer of the diet formula from the nutritionist to the feed manufacturing center occurs. Errors in transcription and computer entry are not uncommon.
5) Proper calibration and periodic verification of accuracy of scales used to weigh macro- and micro-ingredients is critical. Many ingredients with low inclusion rates (less than 0.25 kg/ton [0.5 LB/ton]) are often used in many formulas. Both the scales and the augers for delivering such ingredients must be designed to handle low inclusion rates.
6) Assure that the mixer capability is adequate to properly mix the diet. Regular monitoring of feed for proper mixing and regularly conducted mixer efficiency tests is prudent.
7) Monitoring for excessive thermal processing (i.e. pelleting or extrusion) of complete diets; excess heat has been shown to inactivate some vitamins and enzymes.
8) Mitigation strategies for all-too-common errors in the delivery of the right diet to the right location. Feed bins should be easily identified, and drivers trained and reminded to be aware of the importance of making the correct delivery of diets.
9) Quality assurance on preparation and composition of the various premixes that contain supplemental vitamins and trace minerals. Vitamins and trace minerals are typically mixed into premixes, which are then added to the diet as a separate ingredient – thus another point in the manufacturing process where errors occur.

Guidelines for dietary nutrient inclusion rates

If nutritional involvement is still suspected after these critical control points are ruled out, then steps are taken to ensure that the diet formulation meets requirements for the intended stage of production. A standard reference for nutrient requirements in the United States is provided by the NRC (2012) and provides a good benchmark for supplementation practices. Such references indicate nutrient concentrations, but do not address ingredient selection. For example, NRC (2012) will not address important and common variations in diet formulation items. Examples such as a weanling pig diet that contains specialty protein sources like dried whey or fish meal or a finisher diet that may contain an excess of wheat middlings that limits growth performance are quite common. It should also be noted that the requirements suggested in NRC (2012) are suggested minimums. Suggested vitamin and mineral tolerances have also been provided in more detail by the NRC (1987, 2005). A more practical guide to typical dietary vitamin and mineral additions has been published by Flohr et al. (2016), which summarizes vitamin and mineral additions and their ranges as surveyed across a wide spectrum of swine production systems in the United States.

Role of feed analysis

Chemical analysis of ingredients and diets can be used for confirmatory diagnostic evidence. As with all diagnostic procedures, appropriate sampling technique is critical (AAFCO 2017; Gonçalves et al. 2016). In brief, sampling accuracy is improved using grain probes or other specialized sampling tools. It is important to obtain multiple samples from a free-flowing stream of ingredients or feed. These are pooled, thoroughly mixed, and then subsampled to assure that the final sample is representative. When sampling sacks, trucks, or feeders, at least 10 sampling points should be probed, pooled, and subsampled for analysis.

Feed manufacturing facilities following good manufacturing practices will retain samples of ingredients and diets. Many farms also retain feed samples from each delivery for some period of time, usually at least 1 month. Chemical analysis of final diets will have higher rates of variability compared with analysis of ingredients or premix mixtures (AAFCO 2015), but can confirm gross diet formulation or mixing errors. The testing of final diets is of less diagnostic value in those clinical cases where the quantity or quality of specific ingredients is compromised, particularly for low supplementation rate vitamins and trace minerals. Testing the premixes containing these low supplementation rate ingredients has considerable merit since they are in a more concentrated form that has better analytic accuracy.

Even if the feed or ingredients are sampled properly, there is analytical variation that impacts interpretation of the analytic results (Table 68.2). Analytic variation can be used as a guideline to interpret the expected concentration. For example, if the expected diet zinc concentration is 2000 ppm, a laboratory analysis result of 1600–2400 (±20%) is within the expected range. Note that analytic variation is in general higher for vitamins compared with other nutrients. Also, it is important to understand that analytic variation as a percentage is greater at low nutrient concentrations compared with high concentrations.

Another important point is to retain a sample in case further analysis is needed. These retained samples should be stored in a freezer to inhibit the growth of insects and molds. Also, if legal consequences are anticipated, establishing a chain of custody for the sample analyzed is important.

Testing of serum for specific nutrients is rarely definitive for diagnosis because most serum levels of nutrients are tightly regulated in a narrow range and abnormal values may not be noted until an advanced state of deficiency or toxicity is achieved. An example is calcium (and phosphorus) where mobilization of bone mineral stores will maintain normal values until an advanced stage of clinical disease. Another confounder is the paucity of recently published normal physiological ranges or

Table 68.2 Analytical variation.[a]

Item	Analytic variation (%)
Protein	±(20/*x* + 2)[b]
Fat	±10
Lysine	±20
Calcium	±(14/*x* + 6)
Phosphorus	±(3/*x* + 8)
Copper	±25
Zinc	±20
Selenium	±25
Sodium	±(7/*x* + 5)
Vitamin A	±30
Vitamin B_{12}	±45
Niacin	±25
Pantothenic acid	±25
Riboflavin	±30

[a] Adapted from AAFCO (2015). Note analytic variation only takes into account laboratory variation and does not take into account sampling variation.
[b] To obtain the expected variation, insert the expected concentration into the formula. For example, if the expected protein is 20%, the analytic variation is 3 (20/20 + 2), and if the laboratory result is between 17 and 23% and fails to indicate, the result is outside the expected range.

expected values for defining a truly deficient, healthy, or toxic status. Reference values from more recent studies where the specific nutrient was titrated are useful; however, these values are generally available only for a limited set of nutrients, age, genotype or diagnostic criteria. The selection of appropriate pigs for sampling is critical; slow-growing, unhealthy, or emaciated pigs selected for sampling may not accurately represent the primary problem because they may not have been consuming feed. Liver is usually a better sample for determining deficient or toxic nutrient status, particularly for trace minerals and fat-soluble vitamins. Despite best practices in selection and sampling, biological and sampling variation makes it challenging to confirm a herd diagnosis of suspected nutrient deficiency or toxicity from individual pig tissues or serum.

Common clinical nutrient concerns

Protein and amino acids

There are few distinguishable clinical signs of protein or amino acid deficiency other than moderate decreases in growth and performance. Because of the economic significance of amino acid requirements, there are excellent reference resources available as guidelines for amino acid fortification in swine diets (NRC 2012; PIC 2016). It is now common to supplement feed-grade crystalline lysine, methionine, threonine, tryptophan, and valine to swine diets, which adds complexity to proper diet formulation and preparation. Local conditions and availability of various by-products utilized in swine diets can vary greatly. It is important to account for availability of amino acids in these ingredients. Finally, supplementation rates for some amino acids may be quite low; feed manufacturing equipment must be sensitive enough to handle the low inclusion rates.

Excess of an individual amino acid is well tolerated when the basal diet contains adequate protein and other amino acids. An amino acid antagonism is when the addition of very high concentrations of one amino acid can interfere with absorption or utilization of another structurally similar amino acid, resulting in a dramatic decrease in feed intake (NRC 2012). Examples include decreased lysine absorption caused by high concentrations of arginine (structurally like lysine) due to competition of the amino acid transport channels through the small intestine or the interactions between the branch chain amino acids (leucine, isoleucine, and valine) where deficiencies and excesses of these amino acids are observed via decreased feed intake and growth (Baker 2005). Methionine and cysteine, if added at 2–3% of the diet, can compromise performance by decreased feed intake. Threonine appears to be the least toxic of the amino acids (Baker 2004). Importantly, imbalances and antagonisms with amino acids are rare and are not observed with typical practical diet formulations.

Excess crude protein is of little clinical significance except the period immediately after weaning (Goodband et al. 2014). High crude protein and high soybean meal-based diets have been shown to lead to an immune-mediated hypersensitivity that causes villous atrophy and reduced absorptive capacity in the small intestine (Li et al. 1991). High crude protein starter diets also can result in a large quantity of undigested nitrogen in the large intestine, which appears to be a factor in post weaning diarrhea (Heo et al. 2009). Lowering the quantity of protein in the diet decreases the ammonia concentration (Bikker et al. 2006), urea nitrogen, and volatile fatty acids in the small intestine (Nyachoti et al. 2006). It is thought that the decreased nitrogen concentrations going into the large intestine then reduce post weaning diarrhea due to reduced bacterial protein fermentation (De Lange et al. 2010). Nutrition in the post weaning transitional period has been managed by including high levels of lactose and highly digestible protein sources such as fish meal and spray-dried blood products (DeRouchey et al. 2010). However, a small amount of soybean meal in these diets has been useful to develop immune tolerance and mitigate the negative effects of delayed-type hypersensitivity

(Engle 1994; Friesen et al. 1993). The potential transmission of viral disease with animal products (Kim et al. 2008; Passick et al. 2014; Thimmasandra et al. 2015) has fostered current strategies using feed-grade amino acids, refined soy products, and reduced crude protein diets to manage the transition period around weaning

Sodium and chloride

Diets are typically supplemented with salt as a source of sodium and chloride due to relatively low cost and availability. Short-term Na and Cl deficiency in grain–vegetable protein source-based diets can cause marked reductions in feed intake and growth rate. Long-term salt deficiency will cause dehydration and mortality (NRC 2005). Chloride requirements are generally met with adequate supplementation of salt to meet the sodium requirement.

Most animal-derived products such as spray-dried blood products, fish meal, and dried whey typically have relatively high sodium content, so in some cases nutritionists will adjust the salt levels when these ingredients are used. A change in the composition of these products may lead to a deficiency, as seen in cases arising from the recent movement away from animal products in nursery diets. This change in nursery diets coupled with the relatively high requirement for sodium and chloride of the young pig has led to reevaluation and confirmation of their requirement estimates (NRC 2012; Shawk et al. 2017).

Sodium can cause toxicity when fed in excess with limited water availability (NRC 2005). The term "salt toxicity" is usually the result of limited water intake leading to increased osmolality in the brain (see Chapter 70). Rapid rehydration will lead to rapid swelling in the brain accompanied by neurologic signs and microscopic lesions (NRC 2005). Careful clinical history should focus on adequate access to water. Salt toxicity scenarios encountered include plugged water lines, large-scale facilities built with inadequate water supply lines, failure of wells or pumps during periods of high demand, or failure to open proper water valves after water medication, facility cleaning, or when restocking a room or barn.

Forms of added dietary salt can range from food-grade to rock salt. Salt available for animal diets must comply with standards as some forms of rock salt used for deicing roads may contain impurities or contaminants. Finally, water sources with high sodium or other mineral concentrations may be result in greater potential for toxicity than dietary salt and may influence water intake (NRC 2005).

Calcium, phosphorus, and vitamin D

Disorders of calcium, phosphorus, and vitamin D metabolism are among the most frequently encountered clinical nutritional disease in swine practice. Although these nutrients are involved in a wide array of metabolic functions essential for growth, the clinical signs most frequently presented are disorders of the musculoskeletal system, including fractures and pathology of the growth plates, or, less frequently, acute hypocalcemia tetany. Kyphosis in growing pigs associated with maternal vitamin D deficiency have been described (Amundson et al. 2016; Rortvedt and Crenshaw 2012). Clinical signs can also include lameness, which is clinically indistinguishable from that due to an infectious process. Due to the ability of the pig to mobilize calcium and phosphorus from bones while still maintaining growth rates, bone fractures and lameness are more sensitive clinical indicators than growth performance (Dritz et al. 2000), consistent with data indicating that calcium and phosphorus requirements are higher for bone mineralization than for growth performance (NRC 2012).

Phosphorus is the third most expensive component after energy and amino acids in swine diets, so diets are typically formulated to minimize its excess. Most phosphorus in plant-based ingredients is found in the form of phytic acid, a form low in bioavailability. As a result, swine diets are frequently supplemented with commercial phytases to improve the digestibility of phosphorus in plant-based ingredients. Because margins of safety are low with phosphorus in relationship to its requirement, anything that inactivates phytase, such as heat or prolonged storage (greater than 2–3 months), can result in phosphorus deficiency.

Calcium concentrations are low in most plant-based ingredients; however, addition of calcium to diets is relatively inexpensive. Limestone is a common supplemental source of calcium in swine diets and is also frequently used as a diluent for premixes and medications or as an additive to increase flow ability. If these supplemental sources are not accounted for, greater than expected calcium concentrations in diet can occur. Also, the calcium released by phytase is frequently not accounted for in formulations. When dietary available phosphorus is marginal, these unaccounted sources of calcium may result in a higher than expected calcium to phosphorus ratio. The increased ratio of digestible calcium to phosphorus will limit phosphorus absorption and lead to reductions in growth rate and bone mineralization (González-Vega et al. 2016). The ratio leading to clinical problems is not well defined, but an analyzed calcium to analyzed phosphorus ratio of more than 1.5 should be critically evaluated. An excess of calcium is of little consequence with adequate dietary phosphorus concentration (Létourneau-Montminy et al. 2012).

Vitamin D is important in the metabolic pathway to stimulate absorption of calcium and phosphorus from the gastrointestinal tract (Deluca 2014). There are different forms of vitamin D available for dietary additions, but vitamin D_3 (cholecalciferol) is the form most com-

monly supplemented in swine diets. Endogenous activation of vitamin D to D_3 is dependent on exposure to the ultraviolet spectrum from sunlight. Because many pigs are raised without direct exposure to sunlight, vitamin D_3 is routinely added to all swine diets, usually at levels above estimated requirements to provide a margin of safety. Dietary supplementation ranged from 5 to 10 times the suggested requirement (150–220 IU/kg; NRC 2012) in growing swine to 2 times or greater in adult swine (800 IU/kg) in a recent review of inclusion rates in the United States (Flohr et al. 2016).

Under short-term feeding conditions (less than 60 days), swine can tolerate as much as 33,000 IU D_3/kg of diet (NRC 1987). Higher doses will decrease feed intake. Feeding excess vitamin D for extended periods of time can result in calcification lesions in soft tissues and organs. For growing swine, the presumed maximum level of vitamin D_3 for long-term feeding conditions (more than 60 days) is 2200 IU D_3/kg of diet. The form of vitamin D supplemented may influence the toxicity, but there is only enough information available to set a minimum tolerance for vitamin D_3 supplementation (NRC 1987).

Special consideration for use of phytase is warranted. As phytase dosage increases, the release value of phosphorus increases at a diminishing rate, and the phosphorus release per unit of phytase varies across sources (Jones et al. 2010). Because phytase is an enzyme, it is susceptible to degradation during thermal processing; however, some forms have special coatings that provide resistance to heat degradation. The degradation has been shown to be accelerated by prolonged storage (greater than 60 days), increased ambient temperature, and the presence of other minerals and vitamins (Sulabo et al. 2011). Reduced amounts of phytate substrate found in certain ingredients, such as by-products of fermentation (e.g. DDGS) or grains that have high concentrations of endogenous phytase (e.g. wheat), can cause diminished phytase efficacy. Starter diet with high dose zinc supplements can reduce phytase efficacy and decrease calcium and phosphorus digestibility (Augspurger et al. 2004; Blavi et al. 2017). Added dietary phytase has dramatically decreased the need for inorganic phosphorus sources. As a result, feed manufacturing equipment may not be designed to handle the low inclusion rates, and errors in diet formulation can result from inappropriately sized or calibrated discharge augers for the calcium and phosphorus sources. Equipment malfunctions and failure to accurately add premixes or other micro-ingredients have also been implicated in clinical calcium and phosphorus disorder cases.

Chemical analysis of dietary phosphorus is typically consistent with laboratory variation of approximately 13% (AAFCO 2015). Calcium analysis, on the other hand, is more variable with a coefficient of variation of approximately 26% (AAFCO 2015). This variation in analytical accuracy must be kept in mind when comparing laboratory results with calculated composition. Also, this variance only accounts for laboratory analysis and does not account for sampling variance that emphasizes good sampling technique. Analysis of complete diets for vitamin D and phytase is rarely fruitful due to the large amount of analytic variability. However, in suspect cases where inclusion rates are confirmed, testing of a premix or phytase supplement is more appropriate because of the high concentrations in a premix or pure source lends itself to greater accuracy.

Bone ash is a common procedure used in a research setting as a measure of bone mineralization to determine the relative impact of dietary treatments (NRC 2012). Unfortunately, bone ash has not been useful as a diagnostic indicator. One reason for this is that bone ash cannot reliably predict the dietary mineral concentrations that the pig has been consuming nor reliably predict bone strength properties (Crenshaw 1986). Another reason is lack of standardization of the bone evaluated and analytic procedures such as whether the bones are defatted or not.

Serum calcium and phosphorus are tightly regulated by the body, so they may not be useful indicators of their physiological status until the advanced stages of a severe deficiency. For example, pigs affected with acute tetany or ataxia may have serum calcium levels from 4 to 8 µg/dL, which is diagnostic for acute hypocalcemia. Serum concentrations of 25-hydroxyvitamin D_3 offer the best indicator of vitamin D status. Extremely low or undetectable levels are diagnostically useful; however, clear diagnostically useful cutoff values are not available in swine. For example, research suggests that oral supplementation of vitamin D above that provided by the diet can influence serum 25-hydroxyvitamin D_3 across a wide range of values but without affecting bone mineralization or growth rate (Flohr et al. 2016). Urine chemistry values have been established but have not been validated across a variety of dietary exposures and are not practical in a field setting (Hagemoser et al. 2000).

Vitamin E and selenium

Acute death of rapidly growing young pigs is the classic clinical sign of mulberry heart disease, long associated with vitamin E or selenium deficiency. Cases are most commonly observed in the first few weeks after weaning (Pallarés et al. 2002). Gross lesions are those of acute heart failure, including excess fluid in pericardial and pleural cavities and multifocal to locally extensive hemorrhages on and in the myocardium. Microscopy confirms multifocal or diffuse acute myofibrillar necrosis, sometimes with mineralization. These lesions are consistent with the importance of these two nutrients in

cell membrane integrity (NRC 2012). Hepatomegaly and multifocal hepatic necrosis are less commonly observed. In the breeding herd, litter size has been shown to be improved in sows over four parities when supplemented with 0.15 or 0.3 ppm selenium compared with sows fed a basal diet without selenium supplementation that contained less than 0.1 ppm Se (Mahan and Peters 2004).

Natural vitamin E sources are more bioavailable in swine diets compared with the chemically synthesized vitamin E sources (Shelton et al. 2014). As with many other vitamins, placental vitamin E transfer does not occur, and colostrum intake is critical for providing vitamin E to the baby pig (Shelton et al. 2014). Toxicity from vitamin E has not been demonstrated in swine (NRC 1987).

Geographic location, both locally and regionally, has an influence on selenium content in grains. In the United States, grains from the Great Lakes region or east and southern coasts have lower selenium concentrations compared with those grown west of the Mississippi River (Mahan et al. 2005). Selenium-deficient regions occur globally, including large regions of China (Oropeza-Moe et al. 2015). In contrast, grains grown in the arid regions of the Dakotas, Wyoming, and the Prairie provinces of Canada have high selenium concentrations (Mahan et al. 2005; NRC 2005). This regional variation warrants attention to details of history, including exposure to soil and plants as well as sources of feedstuffs.

Selenomethionine from plant sources is more bioavailable than inorganic sodium selenite, which is typically added to swine diets (NRC 2005). The bioavailability of selenium in feed ingredients seems to vary widely depending on the ingredient and concentration in the ingredient (Henry and Amerman 1995). Bioavailability of selenium from organic yeast was greater than sodium selenite demonstrated by the increased transfer to colostrum and milk (Mahan and Peters 2004).

Selenium is the most toxic trace element added to swine diets. The maximum legal selenium addition in the United States is 0.3 ppm, and the maximum tolerable level in diets for swine is 4 ppm (NRC 2005). Decreased growth rate and feed efficiency are the most sensitive indicators of toxicity but are not specific. Clinical signs of more chronic exposures include hair loss and lameness resulting from separation of the coronary band at the hoof wall (Kim and Mahan 2001). Rapid development of paralysis resulting from bilateral spinal cord gray matter lesions has been reported with exposure to high dosages (NRC 2005). Kim and Mahan (2001) noted clinical signs of toxicity in the pigs fed either organic or inorganic selenium, but the former appeared to be better tolerated.

Although mulberry heart disease is often considered to be a result of vitamin E or selenium deficiency, in some cases the serum and tissue vitamin E and/or selenium concentrations are considered normal, and dietary concentrations are analyzed with at or above recommended levels (Pallarés et al. 2002; Shen et al. 2011). This and observations that suggest mulberry heart disease results from uncompensated stress suggest that vitamin E or selenium deficiency is not the only cause of this condition.

Supplemental vitamin E, in particular, is thought to aid in prevention of acute deaths. Appropriate physiological reference values derived from older literature may not properly reflect the status of modern production practices.

Other vitamins typically supplemented

Vitamin A

Vitamin A is an essential vitamin for growth and reproduction (NRC 2012). Because of liver storage levels, it has been difficult to create deficiency models in swine to clearly define requirements; consequently vitamin A deficiency has been difficult to accurately define. Several studies evaluating injection of vitamin A or its precursor, beta-carotene, have observed some reproductive benefit (Coffey and Britt 1993; Lindemann et al. 2008), whereas others have not (Tokach et al. 1994). Because the response is inconsistent, the wide-scale implementation of vitamin A injections in the breeding herd is not warranted.

Acute vitamin A toxicity is characterized by disturbances of nervous function (NRC 1987), whereas chronic signs of vitamin A toxicity include spontaneous fractures and bone malformation in growing pigs or osteomalacia in adults, as well as internal hemorrhage (Dittmer and Thompson 2015). Toxicity has been observed with dietary intake of approximately 10 times the estimated requirement of 20,000 IU/kg of diet for growing swine and 40,000 IU/kg of diet for breeding swine (NRC 1987). A survey across a broad section of the US swine industry indicates that supplementation rates on average ranged from 2.8 to 5.2 times recommended vitamin A requirement depending on the production stage (Flohr et al. 2016).

Although vitamin A deficiencies are rare, sporadic reports include congenital malformations such as arthrogryposis and eye defects, night blindness, or incoordination and altered gait. Diagnosis is by systematic and complete diagnostic investigation, including thorough examination of the nervous system and skeletal system. Analysis of livers from affected animals for vitamin A content can be useful to confirm deficiency.

Vitamin K

Deficiency of vitamin K results in increased clotting times and hemorrhages at multiple locations that fail to clot. Vitamin K deficiency in breeding females is most commonly reported as extended duration of newborn

pig navel cord hemorrhage. In contrast to other vitamins, few clinical signs outside of the association with decreased blood clotting time have been described. For example, in studies to establish vitamin K as an essential nutrient in the baby pig, normal growth was reported until signs of hemorrhage were observed (Schendel and Johnson 1962). Another mechanism of deficiency is intoxication with vitamin K antagonists such as warfarin, either from natural sources (sweet clover) or from rodenticides.

The normal gastrointestinal flora synthesis of vitamin K in growing pigs and adults can provide a significant amount of vitamin K to the point that pigs with access to fecal material may not require dietary supplementation (NRC 2012). However, as a precaution, vitamin K is routinely added to diets for all classes of swine. Menadione is the most commonly supplemented form of vitamin K in swine diets in the United States. Menadione pyrimidinol bisulfite (MPB) and menadione nicotinamide bisulfite (MNB) are the most commonly added forms of vitamin K. Menadione nicotinamide bisulfite has been observed to have the best stability characteristics of the vitamin K sources especially in the presence of choline or mineral mixes (Albers et al. 2002). Compared with other added vitamins, vitamin K is the least thermal-stable vitamin, and its oxidation can be as great as 50% after 30–60 days of storage in a premix; therefore it is routinely added above any suggested requirement to provide a margin of safety.

In swine, there has been little information to suggest any adverse effects from excess vitamin K or derivatives that provide vitamin K activity. A level of at least 1000 times the dietary requirement for menadione supplementation has been suggested as a minimum level to establish toxicity (NRC 1987).

Niacin (vitamin B_3, nicotinamide)

Niacin is a critical component of enzymes involved in energy metabolism. Severe niacin deficiency has been characterized by necrotic typhlocolitis, which resolved when supplemented (Dunne et al. 1949). Niacin is generally added to all swine diets because the bioavailability in feed ingredients is generally low (NRC 2012). The niacin requirement was increased from 10 ppm in the grower and 7 ppm in finisher pigs to 30 ppm in growing and finishing pigs in the latest edition of the NRC (2012). The requirement change was based on improved average daily gain and feed conversion when pigs were supplemented with niacin (Real et al. 2002). Ivers and Veum (2012) observed that 14 ppm niacin added to a diet maximized growth performance and prevented the occurrence of diarrhea, suggesting the niacin requirement may vary and be lower than 30 ppm in some situations. Tryptophan can be converted into niacin but inefficiently; hence it should not be relied upon. Few studies are available to define the niacin requirement in lactating and gestating sows; however, the NRC (2012) requirement is 10 ppm, while the average addition is 45 ppm in the United States (Flohr et al. 2016). Although data is limited, the maximum tolerable amounts of niacin in swine diets are at least 100 times the requirement (NRC 1987).

Riboflavin (vitamin B_2)

Riboflavin, like many of the other B vitamins, serves as a cofactor in energy and protein metabolism. In one of the few studies with riboflavin, an improvement in farrowing rate but not litter size was observed in sows supplemented greater than 60 mg/day compared with those supplemented 10 mg/day (Pettigrew et al. 1996). Riboflavin is deficient in typical corn–soybean meal diets (NRC 2012) and is routinely added at approximately two times the NRC (2012) requirement for all stages of production as a margin of safety (Flohr et al. 2016). High oral dosage at 10 and possibly 100 times the estimated requirement can be tolerated safely, but riboflavin does have a toxicity potential when administered parenterally (NRC 1987).

Pantothenic acid (vitamin B_5)

Toxicity with pantothenic acid, as with other water-soluble vitamins, is seldom an issue. Classic pantothenic acid deficiency signs are uncommon but described as "goose-stepping" or a gait with hyperextension of rear legs, which appears after approximately 7–10 days of feeding a purified diet devoid of pantothenic acid. Added dietary pantothenic acid is essential in diets for pigs up to approximately 15 kg body weight (Grinstead et al. 1998), but it appears that the pantothenic acid in corn and soybean meal may be sufficient to meet the requirements of 25–120 kg pigs (Groesbeck et al. 2007). When using cereal grains or ingredients other than corn and soybean meal, pantothenic acid may be less bioavailable; thus, added pantothenic acid may be needed (Southern and Baker 1981). To minimize any potential deficiency symptoms, pantothenic acid is typically added to diets fed to all classes of swine (Flohr et al. 2016).

Vitamin B_{12} (cyanocobalamin)

Anemia is one sign of vitamin B_{12} deficiency; however, there are few published papers available to establish a requirement estimate. Vitamin B_{12} is typically added to diets in all stages of production (Flohr et al. 2016). Plant products are practically devoid of vitamin B_{12}, but due to enteric microbial production, signs of deficiency are difficult to characterize (NRC 2012). No toxicity information is available in swine, but data from other species suggest that an upper safe level is at least 100 times the requirement (NRC 1987).

Other minerals typically supplemented

Copper

Copper is involved in a wide array of metabolic processes that range from hemoglobin production to a cofactor in several enzymes. Copper in a grain–soybean meal-based diet may be adequate to provide the nutrient requirement for growth performance in growing and finishing pigs (Gowanlock et al. 2013). However, copper is typically added to all swine diets with the suggested requirement ranging from 3 to 20 ppm (NRC 2012). In some countries, copper is added to diets for growth promotion at rates up to 250 ppm, particularly in nursery pigs (weaning to 22 kg).

Copper as sulfate or chloride salts and in organic forms have high bioavailability compared with other sources of copper (NRC 1998). Some have observed negative effects on growth from feeding 500 ppm copper for extended periods of time (NRC 2005) but not when feeding 250 ppm; therefore the maximum tolerable concentration has been set at 250 ppm added copper in swine diets (NRC 2005). Copper toxicity can cause hemolytic crisis similar to that described in other species, which is manifested as icterus, anemia, hemoglobinuria, and nephropathy. Feeding pigs added copper for growth promotion will lead to elevated liver concentrations.

Iodine

Iodine is an integral component of the thyroid hormones essential for metabolism (NRC 2012). Soil iodine varies by geographic location, which influences iodine content in crops and their respective feed ingredients. Iodine is a low-cost mineral, so it is typically supplemented in swine diets for all production stages (Flohr et al. 2016). Iodine deficiency results in thyroid hyperplasia ("goiter") from continued agonist effects of thyroid-stimulating hormone. Thyroid glands are grossly enlarged, and the characteristic lesion can be confirmed with histopathology. Nonspecific signs of reproductive failure, as well as piglets born with low viability, hairlessness, and grossly visible goiter, are reported with iodine deficiency.

Certain species of *Brassica*, which includes rapeseed, can increase the iodine requirement in swine diets. Older varieties of rapeseed or rapeseed meal contain glucosinolates, an anti-nutritional factor affecting thyroid metabolism; however, modern varieties of rapeseed (canola and other varieties) have been selected for low glucosinolate concentrations. Thermal processing can also mitigate the effects of these anti-nutritional compounds.

The suggested requirement for iodine is 0.14 ppm for all stages of swine (NRC 2012) with the maximum tolerable limit reported at 400 ppm (NRC 2005). One report indicates a moderate decrease in growth performance (Li et al. 2012); as dietary iodine concentration increased, the effect was greatest in grower pigs as opposed to finishing pigs. Iodine is typically included in the trace mineral premix for swine diets.

Iron

Iron is a critical component of hemoglobin and myoglobin for oxygen transport. Compared with other species, pigs are born with relatively little iron stored in the liver. This, coupled with low milk iron concentrations and lack of exposure to iron from the environment in modern production, can quickly lead to iron deficiency in suckling pigs. Supplemental iron by injection at birth or shortly thereafter has significant impacts on subsequent growth performance in the nursery phase. For example, in one study, pigs given a 200 mg injection of iron at birth were 3 kg heavier 28 days after weaning compared with those that were not treated with iron (Peters and Mahan 2008).

Iron deficiency in growing swine consuming typical diets is of little practical significance (NRC 2005). Animal products, grains, and oilseed meals typically include highly available forms of iron; hence supplementation of growing pig diets may not be necessary (Gowanlock et al. 2013). Limestone and phosphorus ingredients and other products mined from the ground also usually contain significant iron concentrations, as can water, which contributes to the total dietary iron concentration.

Dietary iron sources vary in bioavailability with sulfates the most available (NRC 1998), whereas oxides have virtually no absorption. Large excesses of other trace minerals can decrease iron absorption. Excess dietary iron in nursery pigs may facilitate the proliferation of pathogenic *Escherichia coli*. The maximum tolerable concentration of iron has been set at 3000 ppm in swine diets (NRC 2005); however, the maximum concentration tolerated is considerably lower when given either orally through water or parenterally. Also, some data suggest that pigs from sows with low vitamin E status are more susceptible to toxicity from iron injection (NRC 1987). Iron overload from parenteral injection causes acute necrosis and hemorrhage in multiple body systems, including the kidneys, muscle, liver, and stomach. While the molecular mechanisms are unknown, they appear to revolve around the breakdown of mitochondrial function (NRC 2005).

Manganese

Although typically added to swine diets, there are few reports that well characterize signs of a manganese deficiency well. Those cited report lameness, poor reproduction, and ataxia in offspring, but there is very little current data (NRC 2012). Gowanlock et al. (2013) observed that there was sufficient manganese in a corn–soybean meal–based diet to meet grower/finisher pig requirements for growth and hematological measurements. Reported requirements are considerably higher

in gestating and lactating sows (25 ppm) than growing pigs (4 ppm; NRC 2012).

Manganese is considered one of the least toxic essential elements (NRC 2005). Excess calcium, phosphorus, and iron can inhibit manganese absorption and may decrease tissue concentrations in some species. Pigs fed low iron concentrations are more sensitive to excess manganese, which may be the case with other trace minerals as well.

Zinc

Zinc is also essential for an array of metabolic processes and is a critical factor in protein metabolism. Zinc deficiency in swine is characterized by parakeratosis, especially on the legs, neck, and head. Nonspecific signs are slow growth and poor feed conversion. Due to zinc concentration variation and bioavailability in plant-based ingredients, zinc is typically added to all swine diets (Flohr et al. 2016). Requirement estimates for zinc in swine diets range from 50 to 100 ppm total zinc (NRC 2012) and sometimes at higher rates in boar diets to support spermatogenesis (Flohr et al. 2016). Since phytate reduces the bioavailability of zinc from plant sources, standard practice has been to provide concentrations more than the suggested requirement. In contrast, some reports suggest that added zinc is not needed in finishing pig diets (Gowanlock et al. 2013; Mavromichalis et al. 1999). Factors that may explain these responses in finishing pigs include mobilization of zinc from body stores (after adequate zinc concentrations have been fed in the nursery phase) and wide-scale adoption of phytase. Phytate reduces zinc bioavailability in plant-based ingredients, and inactivation of phytate with phytase improves trace mineral digestibility and reduces the need for dietary addition (Jolliff and Mahan 2012). High zinc dosages will decrease absorption of other minerals. In contrast, high calcium concentrations will decrease zinc absorption. This is especially important as some diets will contain relatively high calcium concentrations.

Supplemental zinc sulfate, zinc chloride, and organic sources are generally considered to have similar availability (100%) with zinc oxide considered to be 50–80% bioavailable (Baker and Ammerman 1995; NRC,1998). Supplementing zinc from zinc oxide at 1500–3000 ppm from weaning to approximately 12 kg is common practice in the United States and some other parts of the world to control diarrhea and improve growth performance (Flohr et al. 2016). Studies generally indicate that feeding high doses of zinc from zinc oxide is not additive with feeding growth-promoting concentrations of copper, but is additive when feeding antimicrobials (Hill et al. 2000, 2001; Shelton et al. 2011; Woodworth et al. 2005). The zinc dosage, rather than source, seems to be a more important factor for the growth-promoting response. However, serum zinc concentrations are increased when feeding highly bioavailable sources, which may potentiate toxicity (Hahn and Baker 1993). Feeding high zinc doses from zinc oxide reduces phytase efficacy and calcium and phosphorus digestibility (Augspurger et al. 2004; Blavi et al. 2017).

The maximum tolerable zinc concentration in swine diets has been set at 1000 ppm because toxicity signs have been noted when feeding 2000 ppm for long durations (NRC 2005). In one study, gilts were fed 0, 50, 500, or 5000 ppm zinc from zinc oxide from weaning through two parities. Sows fed 5000 ppm zinc weaned fewer pigs had greater rates of osteochondrosis and low liver concentrations of copper compared with sows fed the other concentrations (Hill et al. 1983). As a result, the maximum tolerable zinc level was left at 1000 ppm. The reasoning is that other sources of zinc used in diets may be more bioavailable and may be less tolerated. Despite this, feeding zinc from zinc oxide at 2000–3000 ppm for the first few weeks after weaning (up to approximately 12 kg) is common in the United States and other parts of the world. These high dose feeding regimens will lead to elevated liver zinc concentrations.

Added vitamins for specific situations

Biotin (vitamin B_7)

Biotin deficiency is commonly associated with hoof lesions, and the requirement for maintaining hoof integrity is between 0.05 and 0.1 ppm (Kopinski and Leibholz 1989). However, reports of improvements in reproductive performance and decreases in hoof lesions have not been consistent with biotin supplementation (NRC 2012). Nonetheless, biotin is commonly added to gestation, lactation, and boar diets. Because there are few reports consistently suggesting a biotin requirement for growing–finishing pigs (NRC 2012), it is infrequently added to diets for growing pigs (Flohr et al. 2016). Bioavailability appears to be low in plant-based ingredients (Kopinski et al. 1989). Data indicate that swine can safely tolerate dietary levels of 4–10 times the nutritional requirements and the maximum tolerable level of biotin may be much higher (NRC 1987).

Choline

Choline is an important source of labile methyl groups (NRC 2012). In most animals, the liver can produce sufficient amounts of choline needed for growth, but synthesis can be insufficient if diets are deficient in methyl groups. Betaine, pyridoxine, and methionine may spare choline as they also serve as methyl donors.

Choline is routinely added in gestation and lactation diets, but not routinely used in nursery and growing or finishing diets (Flohr et al. 2016). Data indicates that the pig has a high tolerance for choline with little evidence for toxicity (NRC 1987). An important consideration for

choline chloride addition to vitamin premixes is that it enhances the degradation of other vitamins and phytases in premixes or diets.

Folacin (vitamin B_9)

Folacin, supplemented as folic acid, is involved in metabolism of single carbon compounds such as methyl groups that have a wide range of metabolic functions. Although not all data agrees, folic acid supplementation has improved reproductive performance in several studies (Matte and Lauridsen 2013; NRC 2012). Folic acid supplementation is typically low cost; hence it is routinely added to all gestation and lactation diets (Flohr et al. 2016; NRC 2012). Deficiency signs in growing pigs have only been demonstrated using purified diets and inhibitors. There is little data to suggest any adverse effects for administration of high dosages relative to the requirement (NRC 1987).

Pyridoxine (vitamin B_6)

Pyridoxine is critical for nervous system function and tryptophan metabolism (NRC 2012). The requirement estimate for pyridoxine in the nursery pig is 7 ppm and is based on data indicating improved growth performance of weanling pigs (Matte et al. 2005; Woodworth et al. 2000; Zhang et al. 2009). Also, data suggests that pyridoxine may play a role in reproductive performance (NRC 2012). In a survey of US production systems, pyridoxine was added by approximately half the respondents in diets for the breeding herd and nursery pigs but by none in finishing pig diets (Flohr et al. 2016). Little information is available to determine maximum tolerable levels in swine (NRC 1987).

Thiamin (vitamin B_1, thiamine)

Thiamin is not routinely added to swine diets because typical feed ingredients provide ample amounts to meet requirements and there is no benefit to supplementation (Woodworth et al. 2000). However, thiamin is one of the most heat-labile vitamins and can also be inactivated in by-product ingredients such as unprocessed fish meal that contain thiaminase or sulfur-containing compounds (NRC 2012). Another notable exception is in diets containing sodium metabisulfite, which is used to reduce the effect of vomitoxin (Frobose et al. 2017). Metabisulfite has been shown to inactivate thiamin; hence when adding sodium metabisulfate to swine diets for vomitoxin mitigation, added thiamin may be necessary. Pigs affected with clinical neurologic signs and polioencephalomalacia lesions were responsive to thiamin injections after fed pelleted diets with a commercial feed additive containing sodium metabisulfite (Hough et al. 2015).

References

AAFCO. 2015. *AAFCO Official Publication*. Champaign, IL: American Association of Feed Control Officials.

AAFCO. 2017. *Feed Inspector's Manual*, 6th ed. American Association of Feed Control Officials.

Albers N, Gotferbarm G, Heirnbeck W, et al. 2002. *Vitamins in Animal Nutrition*. Bonn, Germany:AgriMedia.

Amundson LA, Hernandez LL, Laporta J et al. 2016. *Br J Nutr* 116:774–787.

Augspurger NR, Spencer JD, Webel DM, et al. 2004. *J Anim Sci* 82:1732–1739.

Baker DH. 2004. *J Nutr* 134:1646S–1650S.

Baker DH. 2005. *J Nutr* 135:1585S–1590S.

Baker DH, Ammerman CB. 1995. Zinc bioavailability. In Ammerman CB, Baker DH, Lewis AJ, eds. *Bioavailability of Nutrients for Animals*. San Diego: Academic Press, pp. 367–398.

Bikker PA, Dirkzwager J, Fledderus P, et al. 2006. *J Anim Sci* 84:3337–3345.

Blavi L, Sola-Oriol D, Perez JF, et al. 2017. *J Anim Sci* 95:847–854.

Coffey MT, Britt JH. 1993. *J Anim Sci* 71:1198–1202.

Crenshaw TD. 1986. *J Nutr* 116:2155–2170.

De Lange CFM, Pluske J, Gong J, et al. 2010. *Livest Sci* 134:124–134.

DeLuca HF. 2014. *J Anim Sci* 92:917–929.

DeRouchey JM, Goodband RD, Tokach MD, et al. 2010. Chapter 10: Nursery swine nutrient recommendations and feeding management. *National Swine Nutrition Guide*. U.S. Pork Center of Excellence, pp. 65–79.

Dittmer KE, Thompson KG. 2015. *Vet Pathol* 52: 851–861.

Dritz SS, Tokach MD, Sargeant JM, et al. 2000. *J Swine Health Prod* 8:121–124.

Dunne HW, Luecke EW, McMillen WN, et al. 1949. *Am J Vet Res* 10:351–356.

Engle MJ. 1994. *J Swine Health Prod* 2:7–10.

Flohr JR, DeRouchey JM, Woodworth JC, et al. 2016. *J Swine Health Prod* 24:290–303.

Friesen KG, Goodband RD, Nelssen JL, et al. 1993. *J Anim Sci* 71:2089–2098.

Frobose HL, Stephenson EW, Tokach MD, et al. 2017. *J Anim Sci* 95:327–337.

Gonçalves MAD, Dritz SS, Jones CK, et al. 2016. *J Swine Health Prod* 24:216–221.

González-Vega JC, Liu Y, McCann JC, et al. 2016. *J Anim Sci* 94:3321–3334.

Goodband RD, Tokach MD, Dritz SS, et al. 2014. *J Anim Sci Biotech* 5:12.

Gowanlock DW, Mahan DC, Jolliff JS, et al. 2013. *J Anim Sci* 91:5680–5686.

Grinstead GS, Goodband RD, Nelssen JL, et al. 1998. Kansas State Swine Day Report. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, Manhattan.
Groesbeck CN, Goodband RD, Tokach MD, et al. 2007. *J Anim Sci* 85:2492–2497.
Hagemoser WA, Goff JP, Sanderson TP, et al. 2000. *J Vet Diagn* 12:525–534.
Hahn JD, Baker DH. 1993. *J Anim Sci* 71:3020–3024.
Henry PR, Ammerman CB. 1995. Selenium bioavailability. In Ammerman CB, Baker DH, Lewis AJ, eds. *Bioavailability of Nutrients for Animals*. San Diego: Academic Press, pp. 303–336.
Heo JM, Kim JC, Hansen CF, et al. 2009. *J Anim Sci* 87: 2833–2843.
Hill GM, Miller ER, Stowe HD. 1983. *J Anim Sci* 57:114–122.
Hill GM, Cromwell GL, Crenshaw TD, et al. 2000. *J Anim Sci* 78:1010–1016.
Hill GM, Mahan DC, Carter SD, et al., 2001. *J Anim Sci* 79:934–941.
Hough SD, Jennings SH, Almond GW. 2015. *J Swine Health Prod* 23:143–151.
Ivers DJ, Veum TL. 2012. *J Anim Sci* 90:282–288.
Jolliff JS, Mahan DC. 2012. *J Anim Sci* 90:3012–3022.
Jones CK, Tokach MD, Dritz SS, et al. 2010. *J Anim Sci* 88:3631–3644.
Kim YY, Mahan DC. 2001. *J Anim Sci* 79:942–948.
Kim B, Song JY, Tark DS, et al. 2008. *Vet Rec* 162:12–17.
Kopinski JS, Leibholz J. 1989. *Br J Nutr* 62:761–766.
Kopinski JS, Leibholz J, Bryden WL. 1989. *Br J Nutr* 62:773–780.
Létourneau-Montminy MP, Jondreville C, Sauvant D, et al. 2012. *Animal*. 6:1590–1600.
Li DF, Nelssen JL, Reddy PG, et al. 1991. *J Anim Sci* 69:4062–4069.
Li Q, Christiane Mair C, Schedle K, et al. 2012. *Eur J Nutr* 51:685–691.
Lindemann MD, Brendemuhl JH, Chiba LI, et al. 2008. *J Anim Sci* 86:333–338.
Mahan DC, Peters JC. 2004. *J Anim Sci* 82:1343–1358.
Mahan DC, Brendemuhl JH, Carter SD, et al. 2005. *J Anim Sci* 83:852–857.
Matte JJ, Lauridsen C. 2013. Vitamins and vitamin utilization in swine. In Chiba L ed. *Sustainable Swine Nutrition*. Ames, IA: Wiley-Blackwell: pp. 139–172.
Matte JJ, Giguere A, Girard C. 2005. *Br J Nutr* 93:723–730.
Mavromichalis I, Hancock JD, Kim IH, et al. 1999. *J Anim Sci* 77:2700–2708.
NRC. 1987. *Vitamin Tolerance of Animals*. Washington, DC: National Academy Press.
NRC. 1998. *Nutrient Requirements of Swine*. 10th revised ed. Washington, DC: National Academy Press.
NRC. 2005. *Mineral Tolerance of Domestic Animals*. Washington, DC: National Academy Press.
NRC. 2012. *Nutrient Requirements of Swine*. 11th revised ed. Washington, DC: National Academy Press.
Nyachoti CM, Omogbenigun FO, Rademacher M, et al. 2006. *J Anim Sci* 84:125–134.
Oropeza-Moe M, Wisløff H, Bernhoft A. 2015. *J Trace Elem Med Biol* 31:148–156.
Pallarés FJ, Yaeger MJ, Janke BH, et al. 2002. *J Vet Diagn Invest* 14:412–414.
Pasick J, Berhane Y, Ojkic D, et al. 2014. *Transbound Emerg Dis* 61:397–410.
Peters JC, Mahan DC. 2008. *J Anim Sci* 86:2261–2269.
Pettigrew JE, el- Kandelgy SM, Johnston LJ, et al. 1996. *J Anim Sci* 74:2226–2230.
PIC. 2016. *Nutrient Specifications Manual*. Hendersonville, TN: PIC North America.
Real DE, Nelssen JL, Unruh JA, et al. 2002. *J Anim Sci* 80:3203–3210.
Rortvedt LA, Crenshaw TD. 2012. *J Anim Sci* 90:4905–4915.
Schendel HE, Johnson BC. 1962. *J Nutr* 76:124–130.
Shawk DJ, Moniz MM, Goodband RD, et al. 2017. *J Anim Sci* 95(Suppl. 5):106.
Shelton NW, Tokach MD, Nelssen JL, et al. 2011. *J Anim Sci* 89:2440–2451.
Shelton NW, Dritz SS, Nelssen JL, et al. 2014. *J Anim Sci* 92:4547–4556.
Shen H, Thomas PR, Ensley SM, et al. 2011. *Transbound Emerg Dis* 58:483–491.
Southern LL, Baker DH. 1981. *J Anim Sci* 53:403–408.
Sulabo RC, Jones CK, Tokach MD, et al. 2011. *J Anim Sci* 89:4262–4271.
Thimmasandra NA, Sooryanarain H, Deventhiran J, et al. 2015. *MBio* 6:e00593–15.
Tokach MD, Goodband RD, Nelssen JL. 1994. Kansas Swine Industry Day Report of Progress 717.
Woodworth JC, Goodband RD, Nelssen JL, et al. 2000. *J Anim Sci* 78:88–93.
Woodworth JC, Tokach MD, Nelssen JL, et al. 2005. *J Anim Vet Adv* 4:688–693.
Zhang Z, Kebreab EM, Jing M, et al. 2009. *Animal* 3:826–837.

69

Mycotoxins in Grains and Feeds

Steve M. Ensley and Scott L. Radke

Introduction

Mycotoxins are secondary metabolites of mold or fungal growth in grains or forages. They affect many body systems with a wide variety of signs, lesions, and impaired productivity. The annual cost of aflatoxin (AF), vomitoxin, and fumonisin to US agriculture has been estimated at $1.66 billion (CAST 2003a). Economic impact may vary between years, but it is estimated that approximately 25% of the world's crops are affected by mycotoxins annually (Dohlman 2003). Wu and Munkvold (2008) estimated that if all US swine feeds had 20% inclusion rate of distillers dried grains with solubles (DDGS), losses of $147 million annually could occur from weight gain reduction due to fumonisins alone.

Most swine mycotoxin problems involve feed grains (e.g. corn, wheat, milo, cottonseed, barley, and other cereals). Fungal growth requires readily available carbohydrate (grains), moisture, oxygen, and appropriate temperatures, often 12–25 °C (Wilson and Abramson 1992). Plant or fungal stressors (drought, high ambient temperatures, insect damage, mechanical harvest damage, and reduced plant vigor) predispose crops to toxigenic fungal infection with subsequent mycotoxin production (CAST 2003a).

Simple visual examination, spore counts, or culture of either grain or feed will not predict safety for animals. Physical appearance of grain is not an accurate indicator for presence of mycotoxins as toxigenic fungi can occur in grains without production of mycotoxins, and there is little correlation between spore counts or degree of fungal growth and presence or concentration of mycotoxins. Conversely, absence of molds does not assure absence of mycotoxins, since high temperature and pressure during milling/pelleting may kill molds but the heat-tolerant mycotoxin persists (CAST 2003a). This explains how mycotoxins can be concentrated in some distillers byproducts, sometimes up to 3× the concentration present in the corn used for fermentation. Control of mycotoxins and reduction of effects in animals depend primarily on environmental and climate factors, crop management, storage conditions, and appropriate use of mycotoxin binders in the diet. This chapter will emphasize the six high-risk mycotoxins for swine: aflatoxin B_1 (AFB_1), ochratoxin A (OTA), deoxynivalenol (DON), ergot, fumonisin B_1 (FB_1), and zearalenone (ZEA).

Mycotoxin formation

Fungi infect and grow in growing crops. Mycotoxins may be elaborated by fungi during the growing season (field formed) or after harvest and in storage (storage formed). Conditions that favor fungal growth and mycotoxin formation are summarized in Table 69.1.

Field fungi (e.g. *Fusarium* spp.) require high equilibrium relative humidity (>70%) or grain moisture (>22%) for growth and potential toxin production. They cause death of ovules, shriveling of seeds, and weakening or death of embryos, but they grow poorly in storage after harvest; therefore toxin production may not occur in stored dry grain even if remoistened (Christensen and Kaufmann 1965).

Storage fungi, which include most *Aspergillus* spp. and *Penicillium* spp., may produce mycotoxins even when grain moisture is 12–18% and temperatures are 10–50 °C. However, *Aspergillus flavus*, considered a storage fungus, often produces AF in crops prior to harvest.

Certain geographic regions are at high risk for specific mycotoxins (Pier 1981), but regional predilection is altered by local growth conditions (e.g. drought, insect damage, early frost), transport or blending of grains, and improper storage.

Environment and management influence mycotoxin production and animal exposure to mycotoxins. Mycotoxins are higher in damaged or broken grain (e.g. screenings or milled grain). Screenings fed on-farm or locally at harvest increase mycotoxin exposure. Grain

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

Table 69.1 Sources and conditions for selected mycotoxins important to swine.

Mycotoxin produced	Fungal source	Grains most affected	Optimal conditions	Agronomic influences
Aflatoxins B1, B2, G1, and G1; AFB_1 most toxic	*Aspergillus flavus* *Aspergillus parasiticus*	Corn, peanuts, cottonseed, milo	24–35 °C ERH 80–85% EMC 17%	Drought; insect damage; day–night temperature >21 °C; may produce toxin in storage
Deoxynivalenol	*Fusarium graminearum*	Corn, wheat, barley, other cereal grains	26–28 °C ERH 88% EMC 22%	Alternating warm and cool growing season; humid conditions; less likely increase in storage
Ergot alkaloids (ergotamine, ergovaline, others)	*Claviceps purpurea*	Rye, wheat, triticale, oats, barley	Moderate to cool when seed forms. Moist humid weather	Warm, humid conditions; wind and insects favor spread of infection
Fumonisin; toxins B_1 and B_2 most toxic; B_1 most prevalent	*Fusarium verticillioides*	Corn. Other commodities not reported	Likely <25 °C EMC > 20%	Dry, hot growing conditions, followed by moist autumn
Ochratoxin; ochratoxin A (OTA) is toxic fraction. Citrinin toxin	*Aspergillus ochraceus* *Penicillium viridicatum* *Penicillium citrinum*	Corn, wheat, barley, rye	12–25 °C; may produce toxin down to 4 °C. ERH 85% EMC 19–22%	Lower temperatures favor increased toxin yield; endemic in some parts of Europe; rare in the United States
T-2 toxin	*Fusarium sporotrichioides* *Fusarium poae*	Corn, barley, milo, wheat,	8–15 °C EMC 22–26%	Alternating cool and warm conditions; overwintered crops
Zearalenone	*Fusarium graminearum*	Corn, wheat	7–21 °C EMC 24%	Alternating high and low temperatures during maturation

ERH, equilibrium relative humidity; EMC, equilibrium moisture concentration.

above optimum moisture continues to respire in storage, producing water; alternating warm and cool temperatures during fall and spring may promote moisture migration and condensation during storage, reaching free moisture levels supportive of mold growth and toxin production. Feed in warm, humid conditions such as a nursery may mold and produce mycotoxins within a few days. For more extensive discussion and documentation of mycotoxin formation and sources beyond the scope of this chapter, see CAST (2003a). In addition, reputable federal, state, or commodity websites maintain information about crop risk from molds and mycotoxins. Computer models for estimating the probability of mycotoxin production based on rainfall, temperature, and insect damage have been developed to assist in predicting mycotoxin risk in crops (Dowd 2004; Prandini et al. 2009).

Intoxication by mycotoxins

Mycotoxicosis results from consumption of contaminated grain or feed by a susceptible animal. Dosage is usually expressed as parts per million (ppm) or parts per billion (ppb) in the diet. To convert from ppm in the diet to dose as mg/kg BW, use the following equation: %BW eaten × ppm (feed) = mg/kg BW where percentage is expressed as a ratio (e.g. 3% = 0.03).

Dietary deficiencies of protein, trace minerals, and vitamins may predispose to mycotoxicosis, and drugs that alter foreign-compound metabolism or detoxification could change metabolic response to mycotoxins (Coppock and Christian 2007).

Combinations of mycotoxins may be additive or synergistic in their effects. Mycotoxins that occur most frequently together are AFB_1 with FB_1 and DON with ZEA (CAST 2003b). Documented synergistic combinations are AFB_1 with FB_1, DON with FB_1, and experimentally AFB_1 with DON (Chaytor et al. 2011; Harvey et al. 1995a, 1996; Robertson et al. 2011).

Some mycotoxins are reported to alter immune function under certain conditions, enhancing development of infectious diseases (Bondy and Pestka 2000; Panangala et al. 1986; Pier 1981). Aflatoxins, trichothecenes, and OTA have been reported immunosuppressive in swine but with inconsistent conclusions. See individual mycotoxins for details. Immunosuppression is expressed indirectly; hence mycotoxin-facilitated infections are difficult to recognize or confirm and may sometimes be diagnosed incorrectly.

Clinical mycotoxicoses

Clinical response of swine to mycotoxins may be acute, subacute, or chronic and is both dose and time dependent. Response is usually subacute or chronic, and the presenting signs may be subtle or vague or expressed as alterations in feed intake, growth, feed efficiency, reproduction, or immunosuppression. Nevertheless, knowing the range of effects for specific mycotoxins is important in differential diagnosis and evaluation of clinical prognosis. Common mycotoxins affecting swine are summarized in Table 69.2 and discussed below.

Table 69.2 Characteristics of common mycotoxicoses in swine.

Toxin	Commodities	Clinical effects	Lesions, diagnosis, residues
Aflatoxins: B_1, B_2, G_1, G_2	Corn, cottonseed, wheat, peanuts, sorghum/milo	Reduced protein synthesis; hepatotoxicosis, cholangiohepatitis, hemorrhage, coagulopathy; chronic slow growth, poor feed conversion, reduced milk production, immune system dysfunction; classified a carcinogen	Hepatic necrosis, increased serum bile acids, bile duct hyperplasia; aflatoxin in feed; aflatoxin M_1 in liver or urine; return to normal 1–2 weeks post-exposure
Ochratoxin Citrinin	Corn, wheat, peanuts Wheat, rye, oats, barley	Nephrotoxicosis with polyuria and polydipsia. Gastric ulcers; anorexia and weight loss; reduced immunocompetence – for both ochratoxin and citrinin	Gastric ulcers and renal tubular damage or fibrosis; ochratoxin metabolites in kidney; high protein excretion in urine; residues may persist for weeks
Trichothecenes, macrocyclic (e.g. T-2 toxin; diacetoxyscirpenol) Relatively rare in North America	Corn, barley, wheat, rye sorghum	Hematopoietic suppression, anemia, leukopenia, hemorrhage, diarrhea, dermal irritation, or necrosis; reduced immunocompetence; self-limiting due to feed refusal	Test mycotoxin in feed; oral ulcers; lymphoid depletion; residues brief – 1–3 days; not common at clinical levels in North America
Deoxynivalenol (DON, vomitoxin)	Corn, wheat, barley, sorghum; common trichothecene worldwide	Feed refusal, vomiting, diarrhea, depression; variable effects on immunocompetence; rare reports of reduced litter size of stillbirths	DON concentration >0.5 ppm in feed – mild effect; clinical results 1–8 ppm; residues rapidly excreted (1–3 days); glucomannan binders variable effects on some aspects of toxicosis
Zearalenone	Corn, wheat, barley, sorghum	Estrogenic. Prepubertal gilts: vulvovaginitis, vaginal and rectal prolapses; signs of estrus. Mature sows: variable – nymphomania or anestrus; pseudopregnancy, retained corpora lutea – responsive to injection of PGF2α	Enlarged uteri/vulva (gilts), retained corpora lutea (sows); vaginal cornification; zearalenone >1 ppm in feed; excreted in urine, less in milk after 1–5 days
Fumonisins	Corn	Acute, fatal pulmonary edema (high dosages); hepatotoxicosis with icterus and liver necrosis (subacute exposure); some reports of chronic pulmonary effects; classified a carcinogen	Histologic lesions of massive pulmonary interlobular edema; liver apoptosis and bile retention; residues brief, mainly liver/kidney; serum increases in AST, GGT, bilirubin, and cholesterol are characteristic
Ergot alkaloids (ergotamine, ergocristine, ergonovine, ergocornine, ergovaline)	Cereal grains (barley, rye, triticale, wheat, oats) and grasses	Acute high doses: peripheral vascular necrosis with peripheral gangrene (feet, tail, ears); in late pregnancy causes reduced prolactin release with agalactia and piglet starvation	Ergot bodies should be <0.3% in feed; ergot alkaloids in urine 1–2 days post-exposure; ergot alkaloids recommended <100 ppb in feed; residues rapidly excreted; residues typically not a problem

Mycotoxin testing in grains and feed

A variety of diagnostic tests are available for the detection of mycotoxins in grains and feeds, which include bright green-yellow fluorescence, high-pressure liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), and liquid chromatography with mass spectrometry in tandem (LC/MS/MS). Fluorescence is typically utilized as a mycotoxin screening method at grain elevators. Although rapid, it is not a reliable test for mycotoxins other than AF and is suggestive of the presence of AF but not definitive. ELISA kits are now used for initial detection and quantification of suspect grains and feeds (CAST 2003b). LC/MS/MS is highly specific and can be used to identify and quantify multiple mycotoxins that may contaminate grains and feed at the same time (Di Muvunga et al. 2009).

Grain and feed samples should be representative of what is being or will be consumed by animals; however, due to the potential of mycotoxicosis resulting in chronic complications, suspect grains or feed may no longer be available at the time of clinical signs. A prudent practice is to save a representative sample from each batch of diet mixed for at least a month after feed is consumed or until swine are marketed. Samples of feeds or grain should be representative and taken after feed is well mixed by passing a cup through a moving auger stream at frequent intervals, mixing samples thoroughly, and saving a 2–4.5 kg (5–10 LB) sample for analysis (Davis et al. 1980). High-moisture samples should be either dried to 12% moisture or stored frozen. Long-term storage is recommended in paper bags permanently marked with the date and source of the feed or grain, and samples should be held in a dry, clean location.

Aflatoxins

Formation and Metabolism

Aspergillus flavus, *Aspergillus parasiticus*, and *Aspergillus nomius* produce AF (AFB_1, B_2, G_1, and G_2) before harvest and in storage. Aflatoxins B_1 and B_2 are more likely produced by *A. flavus* in corn and cottonseed, while *A. parasiticus* usually produces all four fractions in peanuts (Coppock and Christian 2007). AFB_1 is the most abundant and toxic fraction from natural contamination. Conditions supporting AF formation often occur in the southeastern United States (Bennett and Klich 2003) or during drought and insect infestation elsewhere. Aflatoxins are not destroyed during ethanol production and can be concentrated three- to fourfold in the distillers grains by-product (Rustemeyer et al. 2010). Contaminated corn sold to ethanol plants may increase mycotoxicosis risk, and because variation in mycotoxin occurrence in DDGS is expected, consistent sampling and testing is prudent.

Aflatoxin B_1 is metabolized by liver microsomal mixed-function oxidases to seven metabolites (Coppock and Christian 2007). The major toxic metabolite is an 8,9-epoxide that binds covalently to DNA, RNA, and proteins. The DNA N^7 adduct is resistant to repair and likely responsible for causing clinical signs, lesions, and hepatic cancer. Impaired protein synthesis and inability to mobilize fats cause early lesions of hepatic fatty change and necrosis, as well as reduced growth. Protein-deficient diets enhance AF effects on depression of weight gain (Coffey et al. 1989).

Toxicity

Aflatoxin effects in swine depend on dose, diet, exposure time, and age. The single acute oral dose LD_{50} for swine is 0.62 mg/kg body weight (BW) (equivalent to approximately 20 ppm in diet for 1 day); dietary levels of 2–4 ppm for extended time can cause fatal toxicosis; rations containing 260 and 280 ppb for several weeks cause reduced growth (Allcroft 1969; Marin et al. 2002). A diet containing 2.5 ppm AFB_1 fed to 17.5 kg barrows for 35 days decreased BW, rate of gain, and feed consumption (Harvey et al. 1995a,b), serum gamma-glutamyl transferase (GGT) and total iron concentration (TIC) increased, and blood urea nitrogen (BUN) and total iron-binding capacity (TIBC) decreased. Relative liver weight increased, and liver was pale, rubbery, and resistant to cutting. Microscopic lesions included hepatic necrosis and degeneration accompanied by bile duct proliferation.

Prolonged dietary AF affects productivity and immune function (Cook et al. 1989; Dilkin et al. 2003; Harvey et al. 1988, 1989b; Marin et al. 2002; Rustemeyer et al. 2010). Liver lesions are caused by dietary concentrations of 140 ppb for 12 weeks in 18–64 kg swine, and 690 ppb produced mild liver lesions in 64–91 kg finishing hogs (Allcroft 1969). AFB_1 fed at 280 ppb to weanling pigs for 28 days caused significantly decreased weight gain, but no effect on total erythrocyte count, differential leukocyte count, total globulin, albumins, or total protein concentration in serum. Rustemeyer et al. (2010) fed 0, 250, and 500 ppb to barrows for 7, 28, or 70 days. The 500 ppb AFB_1 diet depressed feed intake and average daily gain but 250 ppb did not. Serum aspartate aminotransferase (AST) was higher in AF groups, but BUN was lower than controls. Lower BUN may be from reduced protein turnover and/or reduced liver function. Both 250 and 500 ppb produced adverse effects on some measures of performance and health. Taken together, most studies suggest that a threshold for moderate effects on performance of swine is slightly above 200 ppb.

Clinical effects

Acute-to-subacute aflatoxicosis causes depression and anorexia, anemia, ascites, icterus, hemorrhagic diarrhea, and sometimes coagulopathy from hypoprothrombinemia (Coppock and Christian 2007; Osweiler et al. 1985). Hepatocellular enzymes are elevated, including AST, ALT, alkaline phosphatase (ALP), and GGT. Other serum clinical chemistry changes are decreased TIBC, total protein, albumin, cholesterol, BUN, and glucose (Harvey et al. 1989b). Total bilirubin, icterus index, sulfobromophthalein clearance, prothrombin time, and partial thromboplastin time are elevated (Panangala et al. 1986).

Lesions

Lesions of aflatoxicosis include pale tan or clay-colored liver with centrilobular hemorrhages, fatty change, subserosal petechial to ecchymotic hemorrhages, and intestinal and colonic hemorrhages. With time, the liver becomes yellow, and fibrosis develops with a firm parenchyma, accentuated lobular pattern, and generalized icterus. Microscopic alterations useful for diagnostic purposes include hepatocyte vacuolization, necrosis, and lipidosis, which are all predominant around the central veins. As disease progresses to subacute or chronic, hepatomegalocytosis, multiple nuclei, interlobular fibrosis, and biliary hyperplasia appear (Cook et al. 1989; Harvey et al. 1988, 1989a).

Reproductive effects

Abortion is not expected. Sows fed AF have maintained normal reproduction through four successive gestations at dietary concentrations of 500 and 700 ppb. Piglets nursing these sows had reduced growth from AF excretion in milk (Armbrecht et al. 1972; McKnight et al. 1983). Mocchegiani et al. (1998) observed reduced piglet birth weight where sows were fed 800 ppb AFB_1 from day 60 of gestation through 28 days post farrowing. Liu et al. (2015) observed a decrease in oocyte maturation *in vitro* following oocyte exposure to AFB_1.

Immunocompetence

Aflatoxin affects cell-mediated immunity and phagocytic cell function (Bondy and Pestka 2000). Common diseases influenced by AF under experimental conditions include swine erysipelas, swine dysentery, and salmonellosis (CAST 2003b). Immunocompetence was reduced in piglets nursing AF-exposed sows (Silvotti et al. 1997) where sows fed 800 or 400 ppb purified AFB_1 through gestation and lactation had AFB_1 and aflatoxin M_1 (AFM_1) residues in milk 5 and 25 days after parturition. Residues were approximately 1000-fold lower than that in the feed, with increases during the 25 days after parturition. Lymphoproliferative response to mitogens was reduced, and monocyte-derived macrophages failed to efficiently produce superoxide anions after oxidative burst stimulation *in vitro*. Mehrzad et al. (2014) observed that AFB_1 reduced phagocytic capacity of monocyte-derived dendritic cells as well as a reduction in these cells to induce T-cell proliferation. Ability of macrophages to phagocytose red blood cells was not compromised, but granulocytic cells had reduced chemotactic response *in vitro*. Marin et al. (2002) reported that 4-week feeding of 280 ppb AF reduced weight gain in swine while increasing leukocyte count and raising serum gamma globulin. Immune response to *Mycoplasma agalactiae* was reduced, and cytokine mRNA expression was associated with decrease of some proinflammatory factors (IL-1β and TNF-α), but increased anti-inflammatory IL-10 cytokine expression.

Residues

The AFM_1 metabolite can occur in tissues, milk, and urine of swine at relatively low concentrations and is not persistent. Dietary concentrations of 400 ppb resulted in tissue residues of 0.05 ppb or less, which rapidly disappear when AF feeding stops (Trucksess et al. 1982).

Diagnosis

Depression, hemorrhagic diarrhea, acute icterus, hemorrhages, and coagulopathy suggest acute aflatoxicosis, whereas chronic signs include slow growth, malnutrition, icterus, and persistent low-grade infections. A history of contaminated feed is important. Liver lesions, clinical chemistry changes, and chemical analysis of the ration and grain supply are important for confirmation. Effective and economical ELISA kits are used for detection and initial quantification of suspect grains. Use only USDA-GIPSA-approved test procedures; if positive by ELISA, use a confirmatory chemical analysis to verify the result (CAST 2003b). Make sure the ELISA kit used is valid for the feed analyzed. Many ELISA kits are only validated for whole shelled corn.

Therapy

Aflatoxicosis is generally not amenable to individual animal treatment as specific practical antidotes for affected animals are not available. Supportive intervention such as increased high-quality protein, selenium, and vitamins A, D, E, K, and B complex supplements have been recommended (Coffey et al. 1989; Coppock and Christian 2007). The potential of organic Se to reduce immunocompromising effects of AFB_1 by increasing antioxidant

ability and expression is reported by Hao et al. (2016). Supplemental therapy with choline and methionine has been recommended for aflatoxicosis in poultry, but has not been evaluated in swine (Cullen and Newberne 1994). Concurrent infectious diseases in animals with AF-compromised immune function can be appropriately medicated or immunized. Lincomycin and tylosin added to AF-contaminated diets neither decreased or enhanced the detrimental effects of aflatoxicosis in growing swine (Harvey et al. 1995b).

Prevention

No Food and Drug Administration (FDA) approved preventive additives for aflatoxicosis are available in the United States. Availability of preventive feed ingredients varies in other countries. One or more common anticaking agents for feed may provide effective physical binding for AF. Hydrated sodium calcium aluminosilicates (HSCAS) at 0.5% in the diet have consistently provided substantial protection against loss of gain and occurrence of lesions from dietary AF in swine (Harvey et al. 1989b; Phillips et al. 2002). Sodium or calcium bentonite also has value as an adsorbent (Schell et al. 1993). Clay- and yeast-based ingredients may have some ability to reduce the impact of mycotoxins (Weaver et al. 2013). Treatment of grain with anhydrous ammonia for 10–14 days has reduced AF concentration in grain, and swine accept ammoniated grain with growth comparable with controls. Meissonier et al. (2009) fed weanling pigs up to 1912 ppb AFB_1 for 28 days in combination with diet containing 0.2% glucomannans derived from yeasts in prevention of aflatoxicosis and found that those receiving dietary glucomannan had decreased severity of liver lesions, protected phase I metabolic enzymes, and restored ovalbumin-immunized specific lymphocyte proliferation compared with inhibition caused by AF.

Ochratoxin and citrinin

OTA and a related mycotoxin, citrinin, are fungal nephrotoxins. See Table 69.1 for sources and conditions of production. OTA is commonly found from eastern and northern Europe, Canada, and the northern United States (Juszkiewicz et al. 1992) and is also reported prevalent in Denmark, associated with the feeding of barley and oats (Carlton and Krogh 1979). Toxicosis has been documented in swine fed contaminated corn in the United States (Cook et al. 1986).

Toxicity is related to binding of OTA in specific renal organic ion transporters (Huessner et al. 2002) resulting from inhibition of phenylalanine-metabolizing enzymes, adenosine triphosphate (ATP) production, and lipid peroxidation (Marquardt and Frohlich 1992). Ochratoxin is a genotoxic carcinogen via oxidative DNA lesions coupled with DNA adducts (Pfohl-Leszkowicz and Manderville 2007). Immunosuppressive effects are a combination of suppressed lymphocyte proliferation and interference with the complement system (Bondy and Pestka 2000).

In swine, OTA at 1 mg/kg BW (0.03 ppm dietary) is lethal in 5–6 days. Concentrations of 1 ppm in the diet for 3 months cause polydipsia, polyuria, reduced growth, and lowered feed efficiency. Levels as low as 200 ppb for several weeks cause detectable renal lesions. Additional clinical signs are diarrhea, anorexia, and dehydration. Sometimes no clinical signs are noted, and the only effect observed is the appearance of pale, firm kidneys at slaughter, a not uncommon association with OTA in endemic areas of Eastern Europe, Denmark, and Sweden.

Clinical pathology changes are increases in BUN, plasma protein, packed cell volume, AST, increased urinary glucose, and proteinuria. Riley and Petska (2005) identified a correlation between histological renal damage from ochratoxin and high-level excretion of urinary proteins. Citrinin, ochratoxin, and penicillic acid are synergistic and produce nephrosis characterized by necrosis of the proximal convoluted tubules, followed by interstitial fibrosis. Liver damage with fatty change and necrosis may occur but is less severe than for other primary hepatic toxicities. Gastric glandular mucosal ulceration is a characteristic and consistent lesion in prolonged clinical cases (Carlton and Krogh 1979; Szczech et al. 1973). Boars given 20 µg OTA orally for 6 weeks had reductions in ejaculation volume, and sperm viability and motility after 24 hours storage were significantly reduced compared with controls (Biro et al. 2003). These were 250 kg boars given 20 µg, a dose of 0.08 µg/kg (ppb).

Spontaneous occurrence of dose-related clinical *Salmonella choleraesuis* infection occurred in piglets fed 1 and 3 ppm dietary ochratoxin (Stoev et al. 2000). In further studies, *Brachyspira hyodysenteriae* and *Campylobacter coli* infections were concomitant with OTA immunosuppression and delayed immunization response.

Diagnosis is confirmed by finding toxin and/or metabolites (ochratoxin alpha) in feed or fresh kidney combined with history and characteristic lesions. HPLC accompanied by tandem mass spectrometry can be used to detect OTA in renal tissue (Hou et al. 2015). The approximate half-life for OTA in swine tissue is 3–5 days, and little or no ochratoxin can be found in kidneys 30 days after ochratoxin exposure ceases (Carlton and Krogh 1979). Mildly affected animals may recover if removed promptly from the contaminated feed, but if clinical course is prolonged, recovery is slow.

Countries with known or potential ochratoxin contamination have regulations controlling the presence of OTA in food and animal feeds because of concerns for food contamination and potential carcinogenicity.

Trichothecenes

Trichothecenes include at least 148 structurally related compounds. Those of known veterinary importance are produced by *Fusarium*, especially *Fusarium graminearum* and *Fusarium sporotrichioides* (Table 69.1). This group of sesquiterpene toxins has an epoxide group responsible for most toxic effects. Those receiving most attention worldwide are T-2 toxin, diacetoxyscirpenol (DAS), and DON (vomitoxin). Even though much work has been done with the macrocyclic trichothecenes (T-2 and DAS) in swine, they rarely occur at toxic concentrations in North American grains. DON, however, is a common contaminant with potentially multiple effects (Mostrom and Raisbeck 2007).

Trichothecenes are metabolized in two phases. Phase 1 oxidation and hydrolysis is followed by conjugation with glucuronic acid. In the gut the epoxide ring is cleaved by gastrointestinal (GI) microflora (Bauer 1995). Serum, bile, urine, liver, kidney, and muscle may contain DON metabolites (mainly de-epoxy DON) (Doll et al. 2003; Goyarts et al. 2007). The glucuronide is rapidly excreted in urine and appears not to accumulate in plasma (Eriksen et al. 2003).

T-2 toxin in large dosages can cause direct skin irritation and necrosis, profound lymphoid depletion, gastroenteritis, diarrhea, shock, cardiovascular failure, and death from experimental direct dosing. Chronic administration causes hematopoietic suppression and eventual pancytopenia, and T-2 and DAS are immunosuppressants. Although T-2 and DAS are potent toxins, they occur at relatively low concentrations, and their tendency to induce feed refusal and/or vomiting in swine makes them somewhat self-limiting as toxins, except as potential causes of reduced feed consumption.

DON is a common mycotoxin of corn, barley, and wheat and is an economic issue because of feed refusal or reduced feed intake in swine (Bergsjo et al. 1993; Rotter et al. 1996; Trenholm et al. 1984). In corn, DON occurs at low levels during some harvest seasons, with prevalence as high as 50%. Contamination in other grains and in other parts of the world also occurs (CAST 2003a).

Clinical effects of DON

The effect of DON is dose related and starting at approximately 1 ppm, which can lead to a reduction in feed consumption in weaning-age swine; total feed refusal may occur at concentrations in excess of 10 ppm (Bergsjo et al. 1992; Pollman et al. 1985; Rotter et al. 1996; Young et al. 1983). DON at four dietary levels <1 ppm caused no reduction of feed intake (Accensi et al. 2006).

Studies of hematology and clinical chemistry for DON toxicosis are limited, with limited impact suspected at low or moderate dosages. Accensi et al. (2006) found that pigs accepted DON feeds containing <1 ppm and there were no changes in 9 standard hematological variables or 18 routine biochemical variables (cations, glucose, urea, creatinine, bilirubin, cholesterol, triglycerides, and plasma enzyme activity). Low concentrations caused no changes in piglet immune responses for immunoglobulin (Ig) subset concentration, lymphocyte proliferation, and cytokine production. Based on pair-fed control studies, altered clinical laboratory values appear due to differences in feed intake; thus hematologic and blood chemistry tests may be of limited value in diagnosing low-level dietary DON in swine (Accensi et al. 2006; Lun et al. 1985; Prelusky et al. 1994; Swamy et al. 2003).

There are many controlled studies of DON in swine in a dosage range of 2–8 ppm DON, a common level for naturally contaminated grains and for the variable effects on feed refusal. Generally, most experience shows that DON effects are transitory and any disturbed function is recovered shortly after DON is removed from the diet as compensatory or adaptation mechanisms are established (Rotter et al. 1994).

DON feeding studies with dietary levels from 2 to 8 ppm show linear reductions in feed intake and rate of gain, but variable results for feed efficiency (Dänicke et al. 2008; Doll et al. 2008). Signs include lethargy, restlessness, weight loss, cannibalism, and one report of increased skin temperature. Gross lesions are absent to variable but have included loss of body mass, empty GI tract, increased folding of the esophageal stomach, increased liver weights, and reduced thyroid size. The most consistent clinical laboratory changes have been decreased serum proteins, globulin, and alpha globulin with increased albumin/globulin ratio and reduced serum urea. Variable or inconsistent laboratory values have included decreased hematocrit, segmented neutrophilia, hypocalcemia, hypophosphatemia, increased serum thyroxine (T4) and changes in serum cortisol, or no consistent changes reported (Bergsjo et al. 1993; Dänicke et al. 2008; Diaz-Llano and Smith 2007; Doll et al. 2007, 2008; Rotter et al. 1994).

Laboratory evidence of immune dysfunction varies among swine studies. In general, DON appears to increase IgA concentration in blood, and nonspecific lymphocyte proliferation can be either increased or decreased (Doll et al. 2009; Frankic et al. 2008; Pinton et al. 2008; Tiemann et al. 2006). Pinton et al. (2008) fed pigs 2.2–2.5 ppm DON for 9 weeks. Results included increases in ovalbumin-specific IgA and IgG. Lymph nodes from treated pigs had reduced expression of TGF-β and IFN-γ mRNA, interpreted by the authors that DON may reduce immune response to vaccine. Doll et al. (2009) found *in vitro* changes that included the following: DON and LPS were synergistic for increased mRNA expression of TNF-α in hepatocytes,

DON stimulated a dose-dependent induction of IL-6 mRNA, supernatant concentrations of LPS-induced IL-6 were significantly decreased, and mRNA expression of the anti-inflammatory IL-10 was increased by DON. They concluded that DON has the potential to provoke and modulate immunological reactions of porcine liver cells.

Variable results from *in vitro* and *in vivo* studies are characteristic of DON response in other mammals as well. There is no clear dose–response relationship across available studies. There is need for continuing effort to refine and expand the clinical and laboratory knowledge of effects of DON in swine.

Feed refusal mechanisms

DON in swine causes conditioned taste aversion and flavoring agents did not correct that refusal (Osweiler et al. 1990). Low-level DON exposure (30 µg/kg intragastric) increases cerebrospinal fluid 5-hydroxyindoleacetic acid (5-HIAA). DON feeding sequentially elevates brain tryptophan, serotonin (5-hydroxytryptamine [5-HT]), and 5-HIAA. Hypothalamic dopamine (DA) is decreased and 5-HIAA:5-HT ratios are elevated. Brain norepinephrine levels decrease as well. Serotonin (5-HT) increases initially and then drops significantly at 8 hours (Prelusky 1993, 1996; Swamy et al. 2002, 2004). Fusaric acid (FA) is a less well-known mycotoxin that appears to interact with DON in the tryptophan–5-HIAA–serotonin relationship. It is considered an interacting or potentiating mycotoxin with DON for feed refusal and is likely an important part of feed refusal mechanisms (Swamy et al. 2002, 2004).

Diagnosis

Mycotoxin-related feed refusal presents a difficult problem to the clinician as a multitude of factors including other toxins, drugs, concurrent disease, inclement weather, and reduced water intake may contribute to feed refusal. There is no apparent detectable effect of DON on plasma levels of brain neurotransmitters at DON doses that cause vomiting in swine. Thus, sampling of peripheral blood would not predict the central serotoninergic effects associated with DON toxicosis (Prelusky 1994). Trichothecenes are rapidly metabolized, and diagnosis by analysis of tissue or blood samples is rarely practical. Variable blood and tissue values and rapid excretion of metabolites do not support diagnostic differentiation of animals fed low levels of DON (Doll et al. 2008). Fortunately, rapid metabolism and excretion reduces residue potential in edible swine tissues (Bauer 1995).

Often the DON concentration detected chemically in feed is insufficient to fully explain feed refusal. The discovery of conjugated trichothecene mycotoxins, including conjugated DON, has shown that standard chemical methods may not detect all DON present in grains, but they may be released by hydrolysis in the GI tract. Analytical laboratories are currently working to detect this portion of mycotoxins in feed sources (Berthiller et al. 2005; Zhou et al. 2007). When mycotoxins are bound to other compounds, they may become masked and potentially difficult to detect analytically.

Antiemetics that are specific serotonin (5-HT) receptor antagonists (ICS 205-930, BRL 43694 A) prevent DON-induced vomiting in swine, and anticholinergic compounds were moderately effective at high dosages acting directly at the emetic center. However, antihistaminic and antidopaminergic antiemetics were not effective against DON (Prelusky and Trenholm 1993).

Prevention

Attempts to prevent DON effects have been by feeding adsorbents or using chemical or physical means for detoxification (see Table 69.3). These have included calcium aluminosilicate, bentonite, and sodium metabisulfite. Few of these have been successful in an economic and practical way. Avantaggiato et al. (2004) developed an *in vitro* GI model for reduction in intestinal absorption of DON and nivalenol. With 2% activated carbon (charcoal), there was a reduction of absorption from 51 to 28% for DON and from 21 to 12% for nivalenol. Addition of 5% citric acid or 5% lactic acid solutions to feed has the potential to decrease trichothecene concentrations in feed (Humer et al. 2016). Addition of sodium metabisulfite to swine diets negates some of the adverse effects of DON (Dänicke et al. 2010). Sodium metabisulfite lessens the effects of DON on ADG and feed efficiency. Feeding of a polymeric glucomannan adsorbent (GMA) to starter pigs prevented some known effects of trichothecenes on brain neurochemistry and promoted increased serum Ig concentrations, but did not improve growth rate (Swamy et al. 2002). Swamy et al. (2003) also fed starter pigs diets contaminated with combinations of DON, FA, and ZEA to test the ability of GMA to control mycotoxin effects using pair-fed control group to account for effects of reduced feed intake and found that GMA prevented some toxin-related changes in metabolism; however, the growth depression was not corrected by GMA. Most of the adverse effects on laboratory values were caused by reduced feed intake. Diaz-Llano and Smith (2006) fed 5.5–5.7 ppm DON to pregnant gilts from gestation day 91 through farrowing. A comparable group fed DON was supplemented with 0.2% GMA. Those fed diets containing DON at 5.5–5.7 ppm with no GMA had reduced daily gain and a significant increase in

Table 69.3 Selected approaches to inactivate mycotoxins in swine feeds.

Mycotoxin	Agent or process	Details and comment	References
Aflatoxins	Ammoniation via anhydrous ammonia infusion or jack bean meal (urease)	Effectively destroys aflatoxins and is accepted by swine; not currently approved by FDA for use in food animals. Commercial products for aflatoxin destruction generally not available	CAST (2003c)
	Bentonite, zeolite	Effective in some studies; generally less effective than HSCAS (see below)	CAST (2003c)
	Hydrated calcium aluminosilicates (HSCAS)	Effective in improving performance (weight gain, feed efficiency) and protection against liver damage at 10 g/kg diet (range 5–20 g/kg). Available commercially as an anticaking agent (e.g. Novasil®) but not FDA approved for this use	CAST (2003c); Phillips et al. (2002)
Deoxynivalenol (DON)	HSCAS, bentonite, and zeolite	Generally ineffective for binding of trichothecenes including DON	CAST (2003c)
	Glucomannan adsorbents (GMA)	Benefits are variable for improving feed intake or rate of gain when DON is prominent. Some benefit occurred in studies where DON and/or zearalenol was associated with reduced fertility or live-born pigs or with increased serum ammonia	Avantaggiato et al. (2004); Diaz-Llano and Smith (2006, 2007); Diaz et al. (2010); Swamy et al.(2002, 2003)
	Physical decontamination	Abrasive pearling procedure removed 66% of DON with loss of only 15% of grain mass	House et al. (2003)
Ergot	Physical methods of cleaning to remove ergot bodies	Chemical binders generally have not been tried or have not been effective in feeds	CAST (2003c)
Fumonisins	Binding of fumonisins with glucose or fructose	Chemical inactivation of fumonisins has been accomplished with glucose or fructose; this method, while effective, has not yet been developed for commercial use	Fernández-Surumay et al. (2005)
Zearalenone	GMA binders	Some studies have shown benefits from GMA binders where zearalenone is present; more work is likely needed in this area	See references for DON above
	Activated charcoal or alfalfa meal	Activated charcoal in rations or high levels (≥20%) of alfalfa meal have also been effective against zearalenone	Avantaggiato et al. (2004) James and Smith (1982);
Mold growth	Prevention of mold growth	Keep storage conditions clean and moisture to recommended levels. For wet or damaged grains, organic acids (e.g. propionic acid) are available to control mold growth, but does not destroy preformed mycotoxins	CAST (2003c)

stillborn piglets; no effects on standard clinical chemistry values were observed.

Physical decontamination of DON from barley was demonstrated by use of an abrasive pearling procedure, which removed 66% of DON with loss of only 15% of the grain mass (House et al. 2003). This method could provide a practical means for reducing DON contamination to a manageable level in years of widespread crop contamination.

Xiao et al. (2013) detected increased proliferation of peripheral lymphocytes and an improvement in feed efficiency through the use of antimicrobial peptides in pigs dosed with DON. Four experiments conducted by Frobose et al. (2015) to evaluate preventatives for effects DON in swine diets found positive response to pelleting and a commercial additive containing a combination of preservatives, antioxidants, amino acids, and direct-fed microbials. Sodium metabisulfite has been reported to lessen the effects of DON in swine diets (Dänicke et al. 2005).

In 2011, the US FDA updated a nonbinding guidance statement on use of DON in animal feeds for a maximum DON of 5 ppm in grain and grain products with a maximum dietary inclusion of 20%, resulting in a 1 ppm maximum for finished feeds on an 88% dry matter basis (Table 69.4).

Table 69.4 Exposure guide to mycotoxin effects in swine.

Toxin	Category of swine	Dietary level	Clinical effect
Aflatoxins	Growing–finishing Brood sows and gilts	1) <100 ppb 2) 200–400 ppb 3) 400–800 ppb 4) 800–1200 ppb 5) >2000 ppb 6) 400–800 ppb	1) No clinical effect 2) Reduced growth and feed efficiency; possible immunosuppression; mild microscopic liver lesions 3) Microscopic liver lesions, cholangiohepatitis; elevated serum liver enzymes; immunosuppression 4) Reduced growth; decreased feed consumption, rough hair coat; icterus, hypoproteinemia 5) Acute hepatosis and coagulopathy; deaths in 3–10 days 6) No effect on conception; deliver normal piglets that grow slowly due to aflatoxin in milk
Ochratoxin and citrinin	Finishing Sows and gilts	1) 200 ppb 2) 1000 ppb 3) 4000 ppb 4) 3–9 ppm	1) Mild renal lesions seen at slaughter; reduced weight gain 2) Polydipsia; reduced growth; azotemia and glycosuria 3) Polydipsia and polyuria 4) Normal pregnancy when fed first month
Trichothecenes T-2 and DAS	Growing–finishing	1) 1 ppm 2) 3 ppm 3) 10 ppm 4) 20 ppm	1) No effect 2) Decreased feed consumption 3) Decreased feed consumption; oral/dermal irritation; immunosuppression 4) Complete feed refusal, vomiting
Deoxynivalenol (DON, vomitoxin)	Growing–finishing	1) <1 ppm 2) 2–8 ppm 3) 10 ppm	1) No clinical effect; minimal (10%) reduction in feed consumption at >0.5 ppm 2) 25–50% reduction in feed consumption; taste aversion to same diet. Limited and variable immunosuppression – humoral and cell mediated; occasional reports of stillbirths 3) Complete feed refusal
Zearalenone	Prepubertal gilts Cycling sows and gilts Pregnant sows Mature boars	1) 1–3 ppm 2) 3–10 ppm 3) >30 ppm 4) 200 ppm	1) Estrogenic; vulvovaginitis, prolapse in prepubertal gilts 2) Retained corpora lutea; anestrous; pseudopregnancy 3) Early embryonic death when fed 1–3 weeks post mating 4) No effect on fertility
Ergot	All swine Sows last trimester	1) 0.1% 2) 0.3% or >3 ppm ergot alkaloids 3) 1.0%	1) Reduced weight gain 2) Decreased feed consumption; agalactia, reduced piglet birth weight; piglet starvation 3) Gangrene of ears, tail, and feet
Fumonisins	All swine	1) 25 ppm 2) 50–75 ppm 3) 75–100 ppm 4) >100 ppm	1) Minimal changes in clinical chemistry – increased AST and AP 2) Minimal reduction in feed intake; possible mild hepatosis 3) Reduced feed intake, reduced weight gain; hepatosis with icterus and increased bilirubin and GGT 4) Acute pulmonary edema after 3–5 days consumption; survivors develop hepatosis

Zearalenone (f-2 toxin)

Sources and mechanism

Fusarium graminearum (*Fusarium roseum*) produces ZEA, an estrogenic mycotoxin in corn, milo, and wheat. *F. roseum* can produce either ZEA or DON (Diekman and Green 1992). High moisture (23–25%) is required for growth. Poorly dried ear corn and alternating high and low ambient temperatures favor ZEA production (Christensen and Kaufmann 1965). Often it is produced in the field prior to harvest with delayed harvest potentially playing a role in its production as well.

ZEA is a substituted resorcylic acid lactone that binds competitively to estrogen receptors of the uterus, mammary gland, liver, and hypothalamus. It causes hypertrophy of the uterus and cornification of vaginal epithelium. ZEA is rapidly absorbed from the intestine and is metabolized to alpha- and beta-zearalenol and then conjugated with glucuronic acid for excretion in bile and urine (Meyer et al. 2000).

Clinical signs

Response to ZEA varies with dosage and age of swine exposed. In prepubertal gilts, concentrations as low as 1–5 ppm in the ration cause vulvovaginitis, which is characterized by tumescence and edema of the vulva and vagina and precocious mammary development. Tenesmus is common, occasionally with resultant rectal prolapse (Osweiler 2000). ZEA at clinically effective dosage in sexually immature gilts causes ovarian follicle atresia and apoptotic-like changes in granule cells. Intensified cell proliferation occurs in both uterus and oviduct (Obremski et al. 2003). Prepubertal gilts fed 2 ppm for up to 90 days attained normal sexual maturity with no adverse effects on subsequent reproductive function (Green et al. 1990; Rainey et al. 1990). Doll et al. (2003) fed contaminated corn to piglets up to a diet maximum of 4.3 ppm DON and 0.6 ppm ZEA. BW gain was significantly reduced, and uterine weights compared to BW were increased by nearly 100%.

Reproductive effects of ZEA on mature cycling sows are quite different from effects seen in prepubertal gilts. As with other estrogens, ZEA is luteotropic in swine, and dietary concentrations of 3–10 ppm can induce anestrus in sows if consumed during the middle portion of the estrous cycle. Abortion is unlikely because estrogens are luteotropic in swine. Anestrus and elevated serum progesterone persist for several months long after exposure to ZEA has stopped (Edwards et al. 1987).

Fewer pigs per litter are seen in sows fed high concentrations of ZEA. The susceptible period for reduced litter size appears to be in the preimplantation stage at 7–10 days post mating (Diekman and Long 1989; Long et al. 1983). ZEA fed at 1 mg ZEA/kg BW (equivalent to approximately 30 ppm dietary ZEA) on days 7 through 10 after mating resulted in mild blastocyst degeneration by day 11 and advanced degeneration by day 13. Viability of individual embryos is apparently not maintained beyond 21 days. ZEA did not cause morphologic changes in the endometrium associated with hyperestrogenism (height of the endometrial luminal epithelium and morphology of secretory vesicles in the endometrial glandular epithelium) (Long et al. 1992). ZEA at 22.1 ppm in the ration of breeding gilts caused a decrease in number of corpora lutea, ovarian weight, and number of live embryos, but an increase in deadborn piglets (Kordic et al. 1992).

ZEA and its metabolites, alpha- and beta-zearalenol, are present in milk of exposed sows and may contribute to estrogenic effects in piglets, including enlarged external genitalia and uteri (Dacasto et al. 1995; Palyusik et al. 1980). A perinatal hyperestrogenic syndrome reported in swine herds and by experimental verification included lower conception rate, increased numbers of repeat breeders, decreased litter size, and increased numbers of stillbirths. Clinical signs in neonatal gilts were swelling of the vulva and teats and edematous infiltration of the perineal region, ventral abdomen, and umbilicus, usually accompanied by exudative crusted inflammation and necrosis of the teats. Lesions of hyperestrogenism included enlargement of the ovary and uterus, ovarian follicle maturation, glandular proliferation of the endometrium, and epithelial proliferation in the vagina (Vanyi et al. 1994). Swine diets containing 2 ppm ZEA from day 30 of gestation through weaning did not adversely affect reproduction in sows. Estrogenic effects on testes and on uterine and ovarian weights were observed in the piglets at 21 days of age, but subsequent breeding performance was not affected (Yang et al. 1995).

Preputial enlargement may occur in boars exposed to ZEA. Young boars may have reduced libido and decreased testicular size, but mature boars are unaffected by concentrations of ZEA as high as 200 ppm (Ruhr et al. 1983; Young and King 1983).

Diagnosis

ZEA toxicosis may resemble effects of estrogenic feed additives and natural estrogens such as coumestrol in mature alfalfa. Suspect rations of corn and feed should first be analyzed for the presence of ZEA and other estrogens. For a summary of ZEA effects in different classes of swine, see Table 69.4.

Treatment

Reversal of effects depends on the nature of the effect and the age and reproductive status of swine. Removal of the feed from prepubertal gilts will allow regression of signs within 3–7 days. Medical and surgical treatment of vaginal and rectal prolapse may be needed. For mature, nongravid sows with anestrus, administration of one 10 mg dose of prostaglandin F_{2a} or two 5 mg doses on successive days is useful in eliminating retained corpora lutea (B.N. Day, personal communication, 1982; Green et al. 1990).

Prevention

Dehydrated alfalfa has experimentally shown some protection from ZEA-induced enlargement of the uterus of gilts (James and Smith 1982) although the high concentrations needed (>20%) are not considered practical in swine diets. Activated charcoal or cholestyramine has been used at 2% in an *in vitro* GI model system to evaluate its binding effect on ZEA. Both activated charcoal and cholestyramine reduced absorption of ZEA from 32 to 5 and 16%, respectively (Avantaggiato et al. 2004). The dramatic reduction caused by activated charcoal could be useful for contaminated grain if feeding trials are effective.

Ergot

Ergot, the parasitic fungus *Claviceps purpurea*, infects cereal grains, especially rye, oats, and wheat. The fungus invades the plant seed and forms a sclerotium, a dark elongated body that produces ergopeptine alkaloids that cause gangrene and reproductive interference. Major toxic alkaloids have additive effects and include ergotamine, ergosine, ergocornine, ergocryptine, and ergocristine; total ergot alkaloid content commonly ranges from 0.2 to 0.6% of sclerotia weight. Tolerance levels of ergot bodies or alkaloid content vary among countries. In the United States, the allowable level of ergot in wheat and rye destined to become livestock feed is 0.3 ppm (Coufal-Majewski et al. 2016), which can be determined by analytical chemistry methods to quantify all the ergopeptines in feed.

Gangrenous ergotism is the result of a combination of vasoconstriction and endothelial damage, leading to prolonged ischemia and eventually gangrene of appendages. Because venous and lymphatic drainage remains intact, the gangrene is "dry" in nature. Signs occur over a period of days or weeks and include depression, reduced feed intake, rapid pulse and respiration, and general ill-thrift. Lameness may occur, most commonly in rear limbs, and in advanced cases necrosis and sloughing of the tail, ear tips, and hooves can occur. Signs may be exacerbated by cold weather. Reduced weight gain may be caused by as little as 0.1% ergots in the ration. Higher levels (3.0%) have been implicated in feed wastage and slow growth (Roers et al. 1974).

Ergot alkaloids from *C. purpurea* consistently cause sow agalactia, the result of stimulation of D_2 DA receptors leading to prolactin suppression in pregnant sows fed ergot sclerotia; piglets are born healthy but starve because of agalactia (Whitacre and Threlfall 1981). Pregnant gilts fed either 0.3 or 1% sclerotia during gestation had low piglet birth weights, low piglet survival, and poor piglet weight gains. Agalactia occurred in 50% of gilts fed 0.3% sclerotia in the gestation and lactation rations (Nordskog and Clark 1945). Recently Kopinski expanded on earlier work (2008), showing that *Claviceps africana* ergot sclerotia fed to sows up to 1.5% of the diet (equivalent to 7 ppm ergot alkaloids) 6–10 days prior to parturition caused agalactia and 87% of piglets died. Blood prolactin was reduced. The authors recommended no more than 0.3% ergot or 1 mg/kg dietary ergot alkaloids for multiparous sows and no more than 0.1% ergot for primiparous sows or avoid ergot completely in primiparous sows.

Differential diagnosis and treatment

Evaluation should include ZEA or other estrogenic factors, bacterial infections, and mastitis–metritis–agalactia syndrome. If the clinical signs suggest ergotism, grains should be examined for the presence of significant amounts of ergot sclerotia. Commonly, HPLC is used to analyze suspect ground or processed feeds for ergopeptines, which may be necessary to confirm the diagnosis (Coufal-Majewski et al. 2016).

Gangrenous areas should be cleaned and treated locally, and secondary infections controlled with broad-spectrum antibiotics. Removal of the feed is followed by improvement within 2 weeks for gangrenous effects. When agalactia has occurred, milk production returns 3–7 days after feed is changed. In the interim, supplemental nutrition and milk replacers may be used to save the piglets.

Fumonisins

Fusarium moniliforme and *Fusarium proliferatum* fungi are ubiquitous in white and yellow corn worldwide (Bezuidenhoudt et al. 1988; Gelderblom et al. 1988) and the source of the fumonisin mycotoxins. Swine consuming fumonisins can develop the disease generally known as porcine pulmonary edema (PPE). Fumonisins are produced when corn is stressed by moderate drought followed by persistent rainfall or high humidity late field development (P. Nelson, Personal Communication, 1989). Corn screenings are the most common source of fumonisin toxicosis (Harrison et al. 1990; Osweiler et al. 1992; Ross et al. 1991, 1992).

Fumonisins commonly present in corn are FB_1, fumonisin B_2 (FB_2), and fumonisin B_3 (FB_3). They are water-soluble, heat-stable, and alkaline-resistant aliphatic hydrocarbons with a terminal amine group and two tricarboxylic acid side chains (Steyn 1995). FB_1 and FB_2 are of approximately equal toxicity (P.F. Ross, personal communication, 1996), while FB_3 is nearly nontoxic to swine (G.D. Osweiler, unpublished data).

Mechanism of action and toxicity

Fumonisins are poorly absorbed orally (3–6% of ingested dose), and once absorbed, they are excreted readily and rapidly in bile and urine (Prelusky et al. 1994). Fumonisins inhibit the enzyme-mediated conversion of sphinganine to sphingosine, raising the sphinganine/sphingosine (SA/SO) ratio and potentially interfering with cell cycle control and cell function (Vos et al. 2007). FB_1 affects cell signaling proteins including protein kinase C (PKC), a serine/threonine kinase involved in a number of signal transduction pathways that include cytokine induction, carcinogenesis, and apoptosis (Gopee and Sharma 2004). FB_1 appears also to inhibit ceramide synthase in the sphingolipid signaling pathway on the ascending aortic impedance spectrum of pigs. This is associated with inhibition of myocardial L-type calcium channels with a decrease in cardiac contractility and mean systemic

arterial pressure, decreased heart rate, and increased pulmonary artery pressure leading to left heart failure and massive pulmonary edema and hydrothorax (Constable et al. 2003; Smith et al. 2000). Zomborszky-Kovács et al. (2002) reported that very low concentrations of FB_1 fed for 8 weeks resulted in chronic pulmonary changes of connective tissue proliferation in subpleural and interlobular connective tissue of lungs and in peribronchial and peribronchiolar areas.

More than 120 ppm dietary fumonisins for 4–10 days produce acute PPE (Colvin et al. 1993; Haschek et al. 1992; Osweiler et al. 1992). Surviving pigs develop subacute hepatic toxicosis 7–10 days later. Hepatosis results after feeding dietary levels >50 ppm for 7–10 days. Although 25 ppm or less causes no apparent clinical effects, mild microscopic hepatic lesions are documented for dietary levels as low as 23 ppm. The serum SA/SO ratio has been altered experimentally by diets containing 5 ppm fumonisins, although the clinical relevance of this change is not known (Moetlin et al. 1994; Riley et al. 1993). Fumonisins have been evaluated for their potential interactions with AF and DON. Effects of AF and fumonisins were found to be additive when fed together, except for the variables cholinesterase and ALP, which showed a synergistic response to AF and FB_1 (Harvey et al. 1995a). For a combination of FB_1 and DON, the effect on most variables was additive. However, for BW, weight gain, hepatic weight, and mean corpuscular hemoglobin concentration, the responses were interactive in a greater-than-additive manner (Harvey et al. 1996).

Clinical signs and lesions

Dietary fumonisins greater than 120 ppm are likely to cause acute interstitial pulmonary edema and hydrothorax, with attack rates up to 50% and case fatality rates of 50–90%. Initially there is lethargy, restlessness, depression, and dermal hyperemia. Mild salivation, dyspnea, open-mouth breathing, posterior weakness, recumbence, and moist rales develop rapidly, followed by cyanosis, weakness, and death. Initial signs begin after 4–7 days of continuous fumonisin consumption (Colvin and Harrison 1992; Osweiler et al. 1992). Once signs appear, death usually occurs in 2–4 hours. Survivors may develop hepatic disease. Feeding fumonisins at concentrations from 75 to 100 ppm for 1–3 weeks, without development of pulmonary edema, causes hepatic disease characterized by icterus, anorexia, ill-thrift, and weight loss (Osweiler et al. 1993).

Serum chemistry analyses include elevated concentrations of GGT, AST, ALP, lactate dehydrogenase (LDH), cholesterol, and bilirubin. Early increases in the serum enzymes and cholesterol are followed by increased GGT and serum bilirubin accompanied by clinical icterus (Colvin et al. 1993; Osweiler et al. 1992).

Based on current evidence, fumonisins are considered mild immunosuppressants in swine. One study has shown transient reduction in lymphocyte blastogenesis and delayed titer response to pseudorabies vaccine (Osweiler et al. 1993). Others have reported decreased lymphoblastogenesis (Harvey et al. 1995a, 1996). Tornyos et al. (2003) fed pigs a high FB_1 dose (100 mg/animal/day for 8 days) or low dose (1, 5, and 10 ppm) for 3–4 months and then vaccinated against Aujeszky's disease with inactivated vaccine, which had no significant effect on the humoral and cellular specific and nonspecific immune response.

Lesions of pulmonary edema and hydrothorax occur with 200–350 mL of clear, cell-free, straw-colored thoracic transudate. Lungs are heavy and wet with wide seams (3–10 mm) of interlobular edema. Bronchioles, bronchi, and trachea are relatively clear with little alveolar edema (Colvin et al. 1993; Haschek et al. 1992; Moetlin et al. 1994; Osweiler et al. 1992; Palyusik and Moran 1994). Acidophilic fibrillar material is found in alveoli and interlobular lymphatics, and hyalinized alveolar capillary thrombi may be present. Increased numbers of pulmonary intravascular macrophages (PIM) filled with osmiophilic material are reported by electron microscopy – possibly a result of phagocytosis of damaged cellular components. Pancreatic necrosis and hepatosis with disrupted hepatic architecture, increased mitotic figures in hepatocytes, apoptosis, and single-cell hepatic necrosis are seen with subacute fumonisin toxicosis (Haschek et al. 1992). Chronic exposure can include hepatic hyperplastic nodules and medial hypertrophy of small pulmonary arteries.

Abortions 1–4 days after acute spontaneous toxicosis presumably are due to fetal anoxia caused by severe pulmonary edema in the dam (Becker et al. 1995; Osweiler et al. 1992). Concentrations of 100 ppm FB_1 fed in the last 30 days of gestation caused no pulmonary edema and did not cause abortion, fetal abnormalities, or infertility in sows (G.D. Osweiler, unpublished data).

Diagnosis

Clinical signs of acute respiratory distress with high mortality and lesions of interstitial edema and hydrothorax suggest fumonisin toxicosis. A history of consumption of corn screenings or poor quality corn is typical. Serum chemistry changes and elevated serum SA/SO ratios are expected, and the acute liver enzymes usually peak at from 4 to 7 days after initial exposure, while bilirubin and GGT continue to increase for 1–2 weeks if sublethal exposure continues. Serum SA/SO ratio is the most sensitive indicator of fumonisin exposure and appears unique to the fumonisins (Moetlin et al. 1994; Riley et al. 1993). However, this assay is currently not widely available as a diagnostic test. Assay for fumonisins in feeds or corn is routine in many veterinary diagnostic and private laboratories. Chemical analyses to detect fumonisins in tissues

are not readily available, and the rapid metabolism and excretion rate of fumonisins generally precludes this mode of diagnosis (Prelusky et al. 1994).

Treatment and management

There is no antidote. The acute and massive changes of PPE do not allow for effective symptomatic and supportive therapy. Because clinical signs appear after days to weeks of exposure, oral detoxification is usually not useful. Liver damage from fumonisin toxicosis may be lessened by appropriate supportive care. Fernández-Surumay et al. (2005) demonstrated that a process binding fumonisins with glucose effectively prevents development of clinical signs, lesions, and clinical chemistry changes of fumonisins.

Analysis of corn or feeds for fumonisins can identify a source and help in estimating the risk from a specific feedstuff (Ross et al. 1991). Contaminated corn should be cleaned, and the good quality grain analyzed to demonstrate that a safe level of fumonisins has been reached.

As potential carcinogens in both humans and animals, fumonisins are often monitored or regulated (Table 69.3). Current FDA guidance for fumonisins in corn for swine is no more than 20 ppm in corn at a 50% inclusion rate for an effective feed concentration of 10 ppm on a dry matter basis (Federal Register 2001).

Prevention and management of mold and mycotoxin problems

When mycotoxicosis occurs or is suspected, corrective actions should include a change in the source of feed even when a specific mycotoxin has not been confirmed, and a thorough inspection of grain storage bins, mixing equipment, and feeders for caking, molding, or musty odors. Remove all contaminated feed, clean equipment, and sanitize storage areas with hypochlorite (laundry bleach) to reduce contaminating fungi.

Analyze representative samples of feed and feedstuffs for known mycotoxins. Visual observations of molds, spore counts, or fungal cultures alone do not confirm a diagnosis but may give some indication of the potential for mycotoxin production.

If storage conditions are adverse or grain moisture is high, use a mold inhibitor such as sodium or calcium propionate to reduce or delay mold growth; however, mold inhibitors do not destroy preformed toxins.

Dilution of contaminated grain with clean grain is commonly used to reduce mycotoxin effects, but care must be taken that wet or contaminated grain does not introduce new fungi and conditions of contamination.

Mycotoxins commonly compromise feed quality. The diagnosis and management of such present formidable challenges to the swine producer and veterinarian because diagnosis is sometimes difficult and effective therapies or prophylaxis are often lacking. Another current major diagnostic and scientific concern still unresolved is accuracy of current methods of detection and quantification of mycotoxins and closely related metabolites. This concern, articulated in a recent report, suggests that some mycotoxins can bind to other compounds and/or be completed masked, leading to inaccurate analytical results or perhaps even evade detection (Kovalsky et al. 2016). A sound, practical preventive program is a prudent part of every swine management system but requires continued vigilance to implement effectively.

References

Accensi F, Pinton P, Callu P, et al. 2006. *J Anim Sci* 84:1935–1942.

Allcroft R. 1969. Aflatoxicosis in farm animals. In Goldblatt LA, ed. *Aflatoxin*. New York: Academic Press, pp. 237–264.

Armbrecht BH, Wiseman HG, Shalkopf T. 1972. *Environ Physiol Biochem* 2:77–85.

Avantaggiato G, Havenaar R, Visconti A. 2004. *Food Chem Toxicol* 42:817–824.

Bauer J. 1995. *Dtsch Tierarztl Wochenschr* 102:50–52.

Becker BA, Pace L, Rottinghaus GE, et al. 1995. *Am J Vet Res* 56:1253–1258.

Bennett JW, Klich M. 2003. Mycotoxins. *Clin Microbiol Rev* 16:497–516.

Bergsjo B, Matre T, Nafstad I. 1992. *Zentralbl Veterinärmed A* 39:752–758.

Bergsjo B, Langseth W, Nafstad I, et al. 1993. *Vet Res Commun* 17:283–294.

Berthiller F, Asta DC, Schuhmacher R, et al. 2005. *J Agric Food Chem* 53:3421–3425.

Bezuidenhoudt SC, Wentzel A, Gelderblom WCA. 1988. *J Chem Soc Chem Commun* (11):743–745.

Biro K, Barna-Vetro I, Pecsi T, et al. 2003. *Theriogenology* 60:199–207.

Bondy GS, Pestka JJ. 2000. *J Toxicol Environ Health B Crit Rev* 3:109–143.

Carlton WW, Krogh P. 1979. Ochratoxins: A review. In *Conference on Mycotoxins in Animal Feeds and Grains Related to Animal Health*. Springfield, VA: National Technical Information Service, pp. 165–287.

CAST (Council for Agricultural Science and Technology). 2003a. Fungal growth and mycotoxin development by major mycotoxigenic fungi. In Richard JL, Payne GA, eds. *Mycotoxins: Risks in Plant, Animal, and Human Systems*. Task Force Report 139. Ames, IA: Council for Agricultural Science and Technology, pp. 58–85.

CAST (Council for Agricultural Science and Technology). 2003b. Mycotoxicoses of animals. In Richard JL, Payne GA, eds. *Mycotoxins: Risks in Plant, Animal, and Human Systems*. Task Force Report 139. Ames, IA: Council for Agricultural Science and Technology, pp. 58–85.

CAST (Council for Agricultural Science and Technology). 2003c. *Mycotoxins: Risks in Plant, Animal, and Human Systems*. Task Force Report 139. Ames, IA: Council for Agricultural Science and Technology.

Chaytor AC, See MT, Hansen JA, et al. 2011. *J Anim Sci* 89(1):124–135.

Christensen CM, Kaufmann HH. 1965. *Annu Rev Phytopathol* 3:69–84.

Coffey MT, Hagler WM, Cullen JM. 1989. *J Anim Sci* 67:465–472.

Colvin BM, Harrison LR. 1992. *Mycopathologia* 117:79–82.

Colvin BM, Cooley AJ, Beaver RW. 1993. *J Vet Diagn Invest* 5:232–241.

Constable PD, Smith GW, Rottinghaus GE, et al. 2003. *Am J Physiol Heart Circ Physiol* 284:H2034–2044.

Cook WO, Osweiler GD, Anderson TD, et al. 1986. *J Am Vet Med Assoc* 188:1399–1402.

Cook WO, Van Alstine WG, Osweiler GD. 1989. *J Am Vet Med Assoc* 194:554–558.

Coppock RW, Christian RG. 2007. Aflatoxins. In Gupta RC, ed. *Veterinary Toxicology: Basic and Clinical Principles*. New York: Elsevier, pp. 939–950.

Coufal-Majewski S, Stanford K, McAllister T, et al. 2016. *Front Vet Sci* 3:15.

Cullen JM, Newberne PM. 1994. Acute hepatotoxicity of aflatoxins. In Eaton DL, Groopman JD, eds. *The Toxicology of Aflatoxins. Human Health, Veterinary, and Agricultural Significance*. Toronto: Academic Press, pp. 2–26.

Dacasto M, Rolando P, Nachtmann C, et al. 1995. *Vet Hum Toxicol* 37:359–361.

Dänicke S, Valenta H, Gareis M, et al. 2005. *Anim Feed Sci Technol* 118:93–108.

Dänicke S, Beineke A, Goyarts T, et al. 2008. *Arch Anim Nutr* 62:263–286.

Dänicke S, Hegewald A, Kahlert S, et al. 2010. *Food Chem Toxicol* 48(8–9):2154–2162.

Davis ND, Dickens JW, Freie JW, et al. 1980. *J Assoc Off Anal Chem* 63:95–102.

Di Muvunga D, Monbaliu S, Scippo ML, et al. 2009. *Food Addit Contam Part A* 26:885–895.

Diaz-Llano G, Smith TK. 2006. *J Anim Sci* 84:2361–2366.

Diaz-Llano G, Smith TK. 2007. *J Anim Sci* 85:1412–1423.

Díaz-Llano G, Smith TK, Boermans HJ, et al. (2010). Effects of feeding diets naturally contaminated with Fusarium mycotoxins on protein metabolism in late gestation and lactation of first-parity sows. *J Anim Sci* 88(3): 998–1008.

Diekman MA, Green ML. 1992. *J Anim Sci* 70:1615–1627.

Diekman MA, Long GG. 1989. *Am J Vet Res* 50:1224–1227.

Dilkin P, Zorzete P, Mallmann CA, et al. 2003. *Food Chem Toxicol* 41:1345–1353.

Dohlman E. 2003. Mycotoxin hazards and regulations: Impacts on food and animal feed trade. International Trade and Food Safety: Economic Theory and Case Studies. US Department of Agriculture, Economic Research Service. Agriculture Economic Report, 828.

Doll S, Danicke S, Ueberschar KH, et al. 2003. *Arch Tierernahr* 57:311–334.

Doll S, Goyarts T, Tiemann U, et al. 2007. *Arch Anim Nutr* 61:247–265.

Doll S, Danicke S, Valenta H. 2008. *Mol Nutr Food Res* 52:727–735.

Doll S, Schrickx JA, Danicke S, et al. 2009. *Toxicol Lett* 190:96–105.

Dowd P. 2004. *Mycopathologia* 157:463.

Edwards S, Cantley TC, Rottinghaus GE, et al. 1987. *Theriogenology* 28:43–57.

Eriksen GS, Pettersson H, Lindberg JE. 2003. *Arch Tierernahr* 57:335–345.

Federal Register. 2001. Federal Register 66 (No. 218, 9 November 2001):56688–56689.

Fernández–Surumay G, Osweiler GD, Yaeger MJ, et al. 2005. *J Agric Food Chem* 18:4264–4271.

Frankic T, Salobir J, Rezar V. 2008. *Anim Feed Sci Technol* 141:274–286

Frobose H, Fruge E, Tokach M, et al. 2015. *J Anim Sci* 93(3):1074–1088.

Gelderblom WC, Jaskiewicz K, Marasas WF, et al. 1988. *Appl Environ Microbiol* 54(7):1806–1

Gopee NV, Sharma RP. 2004. *Life Sci* 74:1541–1559.

Goyarts T, Danicke S, Valenta H, et al. 2007. *Food Addit Contam* 24:369–380.

Green ML, Diekman MA, Malayer JR, et al. 1990. *J Anim Sci* 68:171–178.

Hao S, Hu J, Song S, et al. 2016. *J Agric Food Chem* 64(6):1385–1393.

Harrison LR, Colvin BM, Green JT, et al. 1990. *J Vet Diagn Invest* 2:217–221.

Harvey RB, Huff WE, Kubena LF, et al. 1988. *Am J Vet Res* 49:482–487.

Harvey RB, Kubena LF, Huff WE, et al. 1989a. *Am J Vet Res* 50:602–607.

Harvey RB, Kubena LF, Phillips TD, et al. 1989b. *Am J Vet Res* 50:416–420.

Harvey RB, Edrington TS, Kubena LF, et al. 1995a. *Am J Vet Res* 56:1668–1672.

Harvey RB, Edrington TS, Kubena LF, et al. 1995b. *J Vet Diagn Invest* 7:374–379.

Harvey RB, Edrington TS, Kubena LF, et al. 1996. *Am J Vet Res* 57:1790–1794.

Haschek WM, Moetlin G, Ness DK, et al. 1992. *Mycopathologia* 117:83–96.

Hou Y, Zhou J, Li Y, et al. 2015. *J AOAC Int* 98(6):1566–1570.

House JD, Nyachoti CM, Abramson D. 2003. *J Agric Food Chem* 51:5172–5175.

Huessner AH, O'Brien E, Dietrich DR. 2002. *Exp Toxicol Pathol* 54:151–159.
Humer E, Lucke A, Harder H, et al. 2016. *Toxins* 8(10): 285.
James LJ, Smith TK. 1982. *J Anim Sci* 55:110–117.
Juszkiewicz T, Piskorska M, Pliszczynska J. 1992. *J Environ Pathol Toxicol Oncol* 11:211–215.
Kopinski JS, Blaney BJ, Murray SA, et al. 2008. *J Anim Physiol Anim Nutr* 92:554–561.
Kordic B, Pribicevic S, Muntanola–Cvetkovic M, et al. 1992. *J Environ Pathol Toxicol Oncol* 11:53–55.
Kovalsky P, Kos G, Nahrer K, et al. 2016. *Toxins* 8:363.
Liu J, Wang QC, Han J, et al. 2015. *Mutagenesis* 30(4):527–535.
Long GG, Diekman MA, Tuite JF, et al. 1983. *Vet Res Commun* 6:199–204.
Long GG, Turek J, Diekman MA, et al. 1992. *Vet Pathol* 29:60–67.
Lun AK, Young LG, Lumsden JH. 1985. *J Anim Sci* 61:1178–1185.
Marin DE, Taranu I, Bunaciu RP, et al. 2002. *J Anim Sci* 80:1250–1257.
Marquardt RR, Frohlich AA. 1992. *J Anim Sci* 70:3968–3976.
McKnight CR, Armstrong WD, Hagler WM, et al. 1983. *J Anim Sci* 55(Suppl 1):104.
Mehrzad J, Devriendt B, Baert K, et al. 2014. *Toxicol In Vitro* 28(4):531–537.
Meissonier GM, Raymond I, Laffitte J, et al. 2009. *World Mycotox J* 2:161–172.
Meyer K, Usleber E, Martlbauer E, et al. 2000. *Berl Munch Tierarztl Wochenschr* 113:374–379.
Mocchegiani E, Corradi A, Santarelli L, et al. 1998. *Vet Immunol Immunopathol* 62:245–260.
Moetlin GK, Haschek WM, Ness DK, et al. 1994. *Mycopathologia* 126:27–40.
Mostrom MS and Raisbeck MF. 2007. Trichothecenes. In Gupta RC, ed. *Veterinary Toxicology: Basic and Clinical Principles*. New York: Elsevier, pp. 939–950.
Nordskog AW, Clark RT. 1945. *Am J Vet Res* 6:107–116.
Obremski K, Gajecki M, Zwierzchowski W, et al. 2003. *Pol J Vet Sci* 6:239–245.
Osweiler GD. 2000. Mycotoxins. *Vet Clin North Am Food Anim Pract* 15:33–46.
Osweiler GD, Carson TL, Buck WB, et al. 1985. Mycotoxicoses. In *Clinical and Diagnostic Veterinary Toxicology*, 3rd. Dubuque, Iowa: Kendall Hunt, pp. 409–442.
Osweiler GD, Hopper DL, Debey BM. 1990. *J Anim Sci* 68(Suppl 1):403.
Osweiler GD, Ross PF, Wilson TM, et al. 1992. *J Vet Diagn Invest* 4:53–59.
Osweiler GD, Schwartz KJ, Roth JR. 1993. Effect of fumonisin contaminated corn on growth and immune function in swine. (Abstract). In Midwestern Section of the American Society of Animal Science, 30 March, Des Moines, Iowa.
Palyusik M, Moran EM. 1994. *J Environ Pathol Toxicol Oncol* 13:63–66.
Palyusik M, Harrach B, Mirocha CJ, et al. 1980. *Acta Vet Acad Sci Hung* 28:217–222.
Panangala VS, Giambrone JJ, Diener UL, et al. 1986. *Am J Vet Res* 47:2062–2067.
Pfohl-Leszkowicz A, Manderville RA. 2007. *Mol Nutr Food Res* 51:61–99.
Phillips TD, Lemke SL, Grant PG. 2002. Characterization of clay-based enterosorbents for the prevention of aflatoxicosis. In DeVries JW, Trucksess MW, Jackson LS, eds. *Mycotoxins & Food Safety*. New York: Kluwer Academic/Plenum Publishers, pp. 157–171.
Pier AC. 1981. *Adv Vet Sci Comp Med* 25:185–243.
Pinton P, Accensi F, Beauchamp E, 2008. *Toxicol Lett* 177(3):215–222
Pollman DS, Koch BA, Seitz LM. 1985. *J Anim Sci* 60:239–247.
Prandini A, Silogo S, Filippi L, et al. 2009. *Food Chem Toxicol* 47:927–931.
Prelusky DB. 1993. *J Environ Sci Health B* 28:731–761.
Prelusky DB. 1994. *J Environ Sci Health B* 29:1203–1218.
Prelusky DB. 1996. *J Environ Sci Health B* 31:1103–1117.
Prelusky DB, Trenholm HL. 1993. *Nat Toxins* 1:296–302.
Prelusky DB, Gerdes RG, Underhill KL, et al. 1994. *Nat Toxins* 2:97–104.
Rainey MR, Tubbs RC, Bennett LW, et al. 1990. *J Anim Sci* 68:2015–2022.
Riley RT and Petska JJ. 2005. Mycotoxins: Metabolism, mechanisms and biochemical markers. In Diaz DE, ed. *The Mycotoxin Blue Book*. Nottingham: Nottingham University Press, pp. 279–294.
Riley RT, An NH, Showker JL, et al. 1993. *Toxicol Appl Pharmacol* 118:105–112.
Robertson AE, Munkvold G, Ensley S, et al. 2011. *Agron J* 103(1):1193–1199
Roers JE, Harrold RI, Haugse CN, et al. 1974. Barley rations for baby pigs. Farm Research November–December, North Dakota Agricultural Experiment Station.
Ross PF, Rice LG, Plattner RD, et al. 1991. *Mycopathologia* 114:129–135.
Ross PF, Rice LG, Osweiler GD. 1992. *Mycopathologia* 117:109–114.
Rotter BA, Thompson BK, Lessard M, et al. 1994. *Fundam Appl Toxicol* 23:117–124.
Rotter BA, Prelusky DB, Pestka JJ. 1996. *J Toxicol Environ Health* 48:1–34.
Ruhr LP, Osweiler GD, Foley CW. 1983. *Am J Vet Res* 44:483–485.
Rustemeyer SM, Lamberson WR, Ledoux DR, et al. 2010. *J Anim Sci* V1:2663.

Schell TC, Lindemann MD, Kornegay ET, et al. 1993. *J Anim Sci* 71:1209–1218.
Silvotti L, Petterino C, Bonomi A, et al. 1997. *Vet Rec* 141:469–472.
Smith GW, Constable PD, Eppley RM, et al. 2000. *Toxicol Sci* 56:240–249.
Steyn PS. 1995. *Toxicol Lett* 82–83:843–851.
Stoev SD, Goundasheva D, Mirtcheva T, 2000. *Exp Toxicol Pathol* 52:287–296.
Swamy HV, Smith TK, MacDonald EJ, et al. 2002. *J Anim Sci* 80:3257–3267.
Swamy HV, Smith TK, MacDonald EJ, et al. 2003. *J Anim Sci* 81:2792–2803.
Swamy HV, Smith TK, MacDonald EJ. 2004. *J Anim Sci* 82:2131–2139.
Szczech GM, Carlton WW, Tuite J, et al. 1973. *Vet Pathol* 10:347–364.
Tiemann U, Brussow KP, Jonas L, et al. 2006. *J Anim Sci* 84:236–245.
Tornyos G, Kovacs M, Rusvai M, et al. 2003. *Acta Vet Hung* 51:171–179.
Trenholm HL, Hamilton RMG, Friend DW, et al. 1984. *J Am Vet Med Assoc* 185:527–531.
Trucksess MW, Stoloff L, Brumley WC, et al. 1982. *J Assoc Off Anal Chem* 65:884–887.
Vanyi A, Bata A, Glavits R, et al. 1994. *Acta Vet Hung* 42:433–446.
Vos KA, Smith GW, Haschek WM. 2007. *Anim Feed Sci Technol* 137:299–325.
Weaver AC, See MT, Hansen JA, et al. 2013. *Toxins* 5(7): 1261–1281.
Whitacre MD, Threlfall WR. 1981. *Am J Vet Res* 42:1538–1541.
Wilson DM, Abramson D. 1992. Mycotoxins. In Sauer DB, ed. *Storage of Cereal Grains and Their Products*. St. Paul: American Association of Cereal Chemists, pp. 341–389.
Wu F, Munkvold GP. 2008. *J Agric Food Chem* 56:3900–3911.
Xiao H, Wu MM, Tan BE, et al. 2013. *J Anim Sci* 91(10):4772–4780.
Yang HH, Aulerich RJ, Helferich W, et al. 1995. *J Appl Toxicol* 15:223–232.
Young LG, King GJ. 1983. *J Anim Sci* 57(Suppl 1):313–314.
Young LG, McGirr L, Valli VE, et al. 1983. *J Anim Sci* 57:655–664.
Zhou B, Lin Y, Gillespie J, et al. 2007. *J Agric Food Chem* 55:10141–10149.
Zomborszky MK, Vetési F, Horn P, et al. 2002. *J Vet Med B Infect Dis Vet Public Health* V49(4):197–201

70

Toxic Minerals, Chemicals, Plants, and Gases

Steve M. Ensley and Scott L. Radke

Although modern confinement facilities, accurately formulated rations, and improved management practices have reduced some risks of poisoning, cases of poisoning in swine still occur. The occurrence of swine toxicoses associated with the environment, feed, or management practices is frequent enough to warrant their inclusion in differential diagnostic considerations for swine health problems. The following discussion summarizes the impact of potentially toxic agents to which swine may be exposed.

Essential minerals

Most formulated swine feeds are properly fortified with trace elements; however, for various reasons, some trace minerals may deliberately be added in excess, including copper (Cu), selenium (Se), zinc (Zn), and, occasionally, iron (Fe). The existence of concentrated premixes of these minerals raises the risk of feed mixing errors, resulting in the accidental feeding of high, potentially toxic levels of these elements.

Copper

Dietary requirements of 5–6 ppm Cu have been established for swine. A dietary level of 250 ppm is generally considered the maximum tolerable level (MTL) of Cu for swine. Ration levels ranging from 300 to 500 ppm cause reduced growth and anemia. The tolerance to Cu is related positively to dietary levels of Fe and Zn. For example, animals consuming feed containing 750 ppm Cu are essentially normal if also supplemented with 750 ppm Fe and 500 ppm Zn. Copper in both organic and inorganic forms has been added to growing swine diets at concentrations as high as 134 ppm with positive effects on growth and health. Additive effects of higher levels of dietary copper and zinc in diets for nursery pigs have been demonstrated. Copper sulfate or tribasic copper chloride (TBCC) at 125 ppm and zinc oxide at 0 or 3000 ppm from day 0 to 14 and 0 or 2000 ppm from day 14 to 28 have been shown to increase average daily gain, average daily feed efficiency, and feed/gain (Shelton et al. 2011). Limited information is available when using other sources of Cu. Reduced levels of dietary Zn and Fe or high calcium (Ca) can accentuate copper toxicity (NRC 2012).

Copper toxicity in swine can cause a hemolytic crisis characterized by icterus, anemia, hemoglobinuria, and nephropathy, although not as commonly as in sheep. Diagnosis can be suspected by clinical signs and a history of feeding excess Cu. Feed refusal was observed in swine when dietary Cu in the form of TBCC was detected in excess of 4000 ppm. Liver and kidney Cu levels greater than 250 and 60 ppm, respectively, on a wet-weight basis are diagnostically supportive.

Iron

The recommended dietary levels of Fe range from 40 to 150 ppm, the highest requirements being in the youngest pigs. Many factors influence the risk of Fe toxicosis. Elemental Fe and iron oxides are relatively nontoxic, whereas iron salts are more toxic. Dietary phytate, phosphate, cobalt (Co), Zn, Cu, manganese (Mn), and disaccharides competitively depress Fe absorption. Ascorbic acid, sorbitol, fructose, and several amino acids improve Fe absorption, which is facilitated by being chelated with citric, lactic, pyruvic, and succinic acids; Fe chelated by desferrioxamine is poorly absorbed.

Pigs fed 1100 ppm Fe as a salt have shown reduced weight gains. Animals fed 5000 ppm have displayed depressed feed intake and rates of gain as well as rickets characterized by hypophosphatemia and reduced bone ash. The condition has not been prevented by providing 0.92% dietary phosphorus (P). High single doses of iron salts will cause gastroenteritis, followed by apparent recovery and then, frequently, collapse and death within

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

2 days. Diagnosis may be facilitated by consideration of history, clinical signs, and necropsy changes. Feed and serum should be analyzed for Fe. Normal serum Fe levels are approximately 100 mg/dL and will increase during toxicosis.

Injections of Fe, usually as the dextran, have caused intoxications characterized by cardiovascular shock and death within hours after administration as well as staining at injection sites and in regional lymph nodes, liver, and kidneys. Piglets originating from vitamin E-deficient mothers are reported to be more susceptible to Fe toxicosis (Arpi and Tollerz 1965). Excess Fe in the feed may also interfere with vitamin E absorption or increase the catabolism of vitamin E, potentially causing a deficiency.

There is no practical individual treatment for Fe toxicosis. Desferrioxamine (Desferal) may be used in selected cases. Dietary imbalances or injection dosages should obviously be corrected.

Selenium

The recommended dietary level of Se varies from 0.1 to 0.3 ppm. Selenium, as the selenate or selenite, is approved for addition to swine feeds at up to a legal limit of 0.3 ppm by the Food and Drug Administration (FDA) because of the narrow margin of safety. Oversupplementation of swine feeds with selenium premixes has been a sporadic problem due to mixing errors, especially when responding via additional supplementation to a diagnosis of mulberry heart disease.

Selenium levels of 5–8 ppm fed to growing swine have caused anorexia, alopecia, separation of hooves at the coronary band, and degenerative changes in the liver and kidney. Liver lesions may look remarkably like those described for vitamin E–selenium deficiency (hepatosis dietetica). A level of 10 ppm fed to breeding sows has caused decreased conception and pigs dead or weak at birth. Misformulated feeds containing from 10 to 27 ppm Se produced a paralytic disease in growing swine characterized by quadriplegic or posterior paralysis while the pigs remained mentally alert and continued to eat and drink. Focal symmetrical poliomyelomalacia was found in affected swine (Casteel et al. 1985; Harrison et al. 1983).

Several injectable products containing varying concentrations of Se are currently available for treatment or prevention of Se-responsive diseases. Death losses have approached 100% when Se overdose occurred from the mistaken use of a more concentrated product or from miscalculation of the recommended dosage. The minimum lethal dose of injectable Se is about 0.9 mg/kg body weight, with pigs that are Se deficient being the most susceptible to toxicosis (Van Vleet et al. 1974). Weakness and dyspnea progressing to irregular gasps and death occur within 24 hours of the parenteral overdose. Caution should be used when using injectable Se-containing products, particularly those used for vitamin E supplementation, as vitamin E is never a toxicity issue but Se can be.

Diagnosis of Se toxicosis in swine can be made by consideration of a history of Se supplementation, clinical signs, necropsy findings, and chemical analysis of tissues and feeds. Liver and kidney Se concentrations greater than 3 ppm (wet weight) are expected with toxicosis.

Zinc

Recommended dietary levels of Zn for swine vary from 15 to 100 ppm based on age, sex, stage of production, and other ration components. A level of 2000 ppm zinc carbonate produced growth depression, arthritis, intramuscular hemorrhage, gastritis, and enteritis. The MTL is probably less than 300 ppm, possibly because zinc salts in large concentration are unpalatable. Zinc interacts competitively for absorption with Fe, Ca, and Cu. Pigs fed 268 ppm Zn developed arthritis, bone and cartilage deformities, and internal hemorrhages. However, feeding 3000 ppm Zn as zinc oxide for 14 days has shown increased weight gains and reduction of post weaning scours without adverse signs. Toxicity of Zn depends largely on its source as the MTL for dietary Zn in swine is 1000 ppm with the exception of zinc oxide (NRC 2012). Zn sulfate and Zn methionine have not consistently demonstrated positive effects. Zn toxicosis has been observed when other highly absorbable sources of Zn, such as zinc carbonate, have been used. Diagnostic considerations should include clinical signs, history, and chemical analyses of feed and tissues. Normal kidney and liver levels of Zn are 25–75 ppm (wet weight) and may increase during toxicosis. However, excretion is quite rapid.

Nonessential minerals

Arsenic

Inorganic arsenicals, which are distinctly different from phenylarsonic feed additives discussed later, have been used in antiquated ant baits, herbicides, insecticides, and some animal medications. Pigs are relatively resistant to inorganic arsenic (As) poisoning with 100–200 mg/kg body weight of sodium arsenite being a lethal oral dose. This is equivalent to about 2000–4000 ppm in the feed. However, pigs have refused to consume 1000 ppm in the feed. Clinical signs of acute As poisoning are colic, vomiting, diarrhea, dehydration, collapse, convulsions, and death within hours to days. Prominent necropsy findings are dehydration and severe hemorrhagic gastritis and enteritis with sloughing of mucosa and edema. Diagnostic considerations should include history, clinical signs,

lesions, and chemical analyses. Kidney and liver tissue levels of 10 ppm (wet weight) are significant. Prognosis is generally poor and depends on the degree of tissue damage and dehydration.

Fluorine

Although uncommon, fluorosis may be observed in animals consuming water or forages contaminated by nearby industrial plants or eating crops raised on soils high in fluorine (F). Inhaled F does not appear to accumulate in animals (NRC 2005). A common source is consumption of minerals high in F. Feed-grade phosphates by law must contain no more than 1 part F to 100 parts P (Thompson 1980). It is recommended that swine be fed feeds containing no more than 70 ppm F during their lifetime to prevent fluorosis. Sodium fluoride has been used as an ascaricide at levels of 500 ppm but is no longer used for this purpose in swine. Other signs of acute toxicosis are diarrhea, lameness, tetany, collapse, and death.

A tentative diagnosis of chronic fluorosis may be difficult, since the lameness may appear similar to rickets, mycoplasmosis, and erysipelas. Normal bone F levels in swine are 3000–4000 ppm. Higher levels are associated with fluorosis. Normal urine F levels are 5–15 ppm; higher levels are diagnostically significant. Necropsy findings may reveal exostoses on the long bones and tooth mottling. Treatment should be aimed at reducing dietary F and feeding aluminum or calcium (Ca) mineral supplements.

Lead

Swine are quite resistant to elevated lead (Pb) exposure; consequently field cases of lead poisoning in swine are extremely rare. Experimentally, pigs fed 35.2 mg lead (as the acetate)/kg body weight for 90 days did not die from lead poisoning despite blood lead concentrations as high as 290 µg/dL (Lassen and Buck 1979). If toxicosis is suspected, a thorough diagnostic workup including blood, kidney, and liver analyses should be performed to ensure food safety. It is recommended that animals with blood lead levels exceeding 0.1 ppm not enter the food chain until levels are found to be below 0.1 ppm in two consecutive blood samples at least 30 days apart.

Mercury

Mercury (Hg) has been used in paints, batteries, paper, and fungicides, but most uses have now been restricted. All mercurial compounds are toxic, but organic forms are the most toxic to all animal species. Mercury is cumulative, and toxicity depends on form, dose, and duration. Swine have been poisoned after consuming seeds treated with organic mercurial fungicides.

Initially, signs of gastroenteritis may be evident, followed by uremia and central nervous system (CNS) disturbance, including ataxia, blindness, aimless wandering, paresis, coma, and death. Mercury toxicosis may be confused with erysipelas, cholera, or poisoning by pigweed or phenylarsonics. Clinical signs, history, necropsy findings, and chemistry should aid in the diagnosis. The kidney and liver normally contain less than 1 ppm Hg but will contain much higher levels following Hg toxicosis. Treatment is usually unrewarding.

Feed additives

Adverse effects of drug additives are rare except in cases of misuse, misformulation of rations (Lloyd 1978), or unanticipated adverse interactions with other compounds that are simultaneously administered to pigs. Details of specific drug effects have been reviewed (Adams 1996).

Phenylarsonic compounds

Phenylarsonic compounds, occasionally referred to as organic arsenicals, have at times been used as growth promotants or historically as treatments of swine dysentery or eperythrozoonosis (*Mycoplasma suis*). Arsanilic acid and roxarsone (3-nitro-4-hydroxyphenylarsonic acid) have been used in swine rations, and their sodium salts have been used in drinking water. Arsanilic acid use in complete swine rations ranges from 50 to 100 ppm (45–90 g/ton). As of 2013, arsenical compounds are no longer utilized in animal feed in the United States following withdraw of FDA approval (US FDA 2015). With no approved use for swine and current limited availability, toxicosis would not be expected unless old sources are used off-label.

Onset of clinical signs involving arsanilic acid toxicosis occurs within a few days at feed levels of 1000 ppm, 2 weeks at 400 ppm, and 3–6 weeks at 250 ppm. Clinical signs include ataxia, posterior paresis, blindness, and quadriplegia. Paralyzed animals will continue to live and grow if provided food and water. Swine that receive lower doses for extended periods are prone to develop hypermetria or "goose-stepping" and total blindness from sciatic and optic nerve damage, respectively. Swine that receive very large doses, for example, 10,000 ppm in the ration, may exhibit a gastroenteritis resembling poisoning by inorganic arsenic compounds.

Roxarsone could once be used continuously in swine rations at levels of 22.7–34.1 ppm or at 181.5 ppm for 5–6 days. Poisoning may result with feed roxarsone levels of 250 ppm or more from 3 to 10 days. Clinical signs include uncontrolled urination and defecation as well as muscle tremor and convulsive seizures, all of which are

induced by physical stimulation. Ataxia may be observed, although not as severe as the "drunken sailor" incoordination of arsanilic acid toxicosis. In advanced stages, pigs show paraparesis and paraplegia, but will continue to eat and drink.

Clinical signs and a history of accidental exposure or administration of arsenicals in feed or water may be the best basis for suspecting phenylarsonic toxicosis. Necropsy findings are generally unremarkable, but histopathologic examination of the peripheral nerves, especially the sciatic nerves, may reveal demyelination. Chemical analysis of tissues for the specific phenylarsonic compounds may not be helpful since the compounds are excreted within a few days after withdrawal; however, analysis of the kidney, liver, muscle, and feed for As may assist in the diagnosis. Elemental arsenic levels (wet weight) greater than 2 ppm in kidney and liver and 0.5 ppm in muscle are illegal and indicative of excess As intake. Further analysis of feed for the specific phenylarsonic compound will provide more diagnostic evidence. Deficiencies of B complex vitamins, especially pantothenic acid and pyridoxine, may cause a similar demyelination of peripheral nerves, as can the effects of a delayed organophosphate such as *ortho*-cresyl phosphate. Chronic phenylarsonic toxicosis may also resemble rickets. The CNS signs of phenylarsonic compound toxicosis may be confused with sodium ion toxicity, organic mercurial poisoning, and certain viral diseases. Clinical signs of toxicosis are reversible if arsenicals are promptly removed from the feed and water.

Carbadox

Carbadox (Mecadox), where available, is added to feed at 10–25 ppm as a growth promotant or at 50 ppm to control swine dysentery or bacterial enteritis. A feed level of 100 ppm has caused decreased feed consumption and growth retardation. Higher levels have caused feed refusal and emesis. Mild lesions in the glomerular zone of the adrenal cortex are reported with 50 ppm carbadox in the feed for 10 weeks, while more extensive lesions are seen at feed levels of 100–150 ppm after 5 weeks of consumption (Van der Molen 1988). When fed a ration containing from 331 to 363 ppm carbadox, recently weaned pigs refused to eat and showed poor weight gains, posterior paresis, the passing of hard, pelleted feces, and death in 7–9 days (Power et al. 1989). Swine that have ingested carbadox are required to be withheld from slaughter for a minimum period of 42 days (Lundeen 2017).

Dimetridazole

Dimetridazole, listed as an anti-histomoniasis drug used in turkey rations, was once used for treatment and prevention of swine dysentery. Dimetridazole, suspected to be a carcinogen, and other imidazole compounds are now illegal for use in swine in the United States. A level of 1,500 ppm has caused no toxicosis, but 17,000 ppm has caused diarrhea in swine. Large overdoses of dimetridazole would cause ataxia, bradycardia, dyspnea, salivation, muscle spasms, prostration, and death. Death or recovery would be rapid.

Ionophores (monensin, lasalocid, and narasin)

Narasin (trade name Skycis) is fed at levels from 15 to 30 ppm in swine to increase rate of gain and feed efficiency and as a coccidiostat in chickens when fed up to 100 ppm (Lundeen 2017). Narasin toxicosis occurs as a result of mixing errors, errant feed deliveries, and the severe negative interaction with tiamulin, an antibiotic commonly used in swine.

In 9-week-old pigs, the median oral lethal dose of narasin has been reported to be 8.9 mg/kg of body weight. Clinical signs of anorexia, dyspnea, weakness, ataxia, and recumbency have been reported in cases when approximately 90 ppm, nearly three times the recommended dose, was included in the diet (Carpenter et al. 2005). Risk of narasin toxicity exists when fed at >45 ppm or when tiamulin is concurrently administered with narasin. Pigs offered tiamulin in either feed or water at any approved therapeutic level concurrently with approved levels of narasin are at high risk to develop adverse clinical signs.

Swine poisoned from this combination of narasin and tiamulin show acute ataxia with pain due to massive necrosis of skeletal muscles leading to myoglobinuria and acute death. Histopathological lesions of ionophore toxicity exacerbated by tiamulin include myonecrosis of skeletal muscle characterized by clustered necrotic myofibers with loss of striations and hyaline degeneration and cellular infiltrates (Sturos et al. 2016). Monensin is marketed as Rumensin for use in cattle or as Coban as poultry coccidiostat. Use levels are up to 120 ppm for poultry and 44 ppm in cattle feeds; some premixes may contain up to 440 ppm. Swine may be fed monensin by mistake, but the drug is not highly toxic to them; pigs fed monensin levels ranging from 11 to 120 ppm in the feed for 112 days were not affected, nor was feed consumption or weight gain altered. Gilts fed 110–880 ppm had a transient anorexia for 14 days; thereafter, only weight gains were depressed. The LD_{50} of monensin in swine is 16.8 mg/kg. Pigs suffering from monensin toxicosis showed open-mouth breathing, frothing around the mouth, ataxia, lethargy, muscle weakness, and diarrhea. These signs were visible within 1 day of exposure and persisted for about 3 days. Myocardial and skeletal muscle necrosis was present in pigs receiving 40 mg monensin/kg.

The greatest risk of poisoning from monensin and narasin in swine appears to be with the concurrent administration of the antibiotic tiamulin that potentiates the effect of monensin (Van Vleet et al. 1987) or narasin. Studies have demonstrated that tiamulin inhibits the oxidative drug metabolism via the formation of a cytochrome P450 metabolic intermediate complex (Monshouwer et al. 1996).

Lasalocid is a polyether antibiotic marketed as Bovatec for feedlot cattle and Avatec for poultry to improve feed efficiency and weight gains. Swine fed lasalocid at 2.78 and 21 mg/kg showed no adverse effects. However, transient muscle weakness occurred at a dose of 35 mg/kg (equivalent to about 1000 ppm of lasalocid in the feed), and death occurred at 58 mg/kg when fed for 1 day. Additional ionophores used as feed additives include salinomycin marketed as Bio-Cox or Sacox and laidlomycin marketed as Cattlyst.

In the event of ionophore toxicosis, stopping all administration of tiamulin and immediate removal of suspect feed and cleaning of feed bins should be performed to prevent further intoxication. Prevention includes ensuring correct mixing of rations and cleaning of equipment at feed mills; especially mills catering to various species can aid in reducing intoxications along with vigilance for concurrent use of any ionophores, particularly narasin and tiamulin. Computerized feed dosing and batching systems have been involved in some cases involving mixing errors.

Sulfonamides

Overdoses of sulfonamide antibacterials will cause crystalluric nephroses. Pigs are not likely to be intoxicated from drinking water containing sulfonamides because of the lack of palatability, but overdosing in the feed, coupled with low water intake, may cause nephrosis and uremia. The only feed-grade sulfonamides labeled for swine in the United States are sulfathiazole and sulfamethazine. Nephrotoxic mycotoxicoses such as those caused by citrinin and ochratoxin will predispose sulfonamide toxicosis. Sulfonamide residues in pork are related to persistence of the drugs in feed and excreta and are not a toxicosis. The only feed-grade sulfonamide is sulfamethazine, labeled for use only in combination with tylosin (Lundeen 2017).

Urea and ammonium salts

Swine may be fed cattle feeds containing nonprotein nitrogen compounds such as urea and ammonium salts. Urea is relatively nontoxic for swine, a level of 2.5% causing only reduced feed intake and growth rate, elevated blood urea nitrogen (BUN), polydipsia, and polyuria. Higher levels of urea should not cause signs of acute toxicosis. Ammonia and ammonium salts are toxic for swine, however, with individual doses of 0.25–0.5 g/kg body weight causing intoxication and doses of 0.54–1.5 g/kg being lethal. Considering that growing swine consume feed equal to 5–10% of their body weights, the expected toxic and lethal levels of ammonium salts are 0.25–1% and 1.5–3%, respectively. Pigs poisoned with ammonia and ammonium salts would be expected to become depressed, have tonoclonic convulsions, and either die or recover within a few hours.

Ractopamine

Ractopamine is a beta-2 agonist used as a feed additive (4.5–9 g/ton of complete feed) during the last 6 weeks of finishing to stimulate muscle growth in swine. Beta-2 agonists have the potential for adverse effects, including tachycardia, hypotension, tremors due to overstimulation of skeletal muscle beta-2 receptors, behavioral changes of anxiety or restlessness, weakness or lethargy, and hypokalemia (Rosendale 2004).

Pesticides: insecticides

Concurrent production of both livestock and crops on the same premises may provide a unique opportunity for exposure of swine to agricultural chemicals. Among the chemicals presenting the greatest potential hazard of poisoning are the organophosphorus (OP), carbamate, and the older chlorinated hydrocarbon (CH) insecticides.

Poisoning may occur when insecticides are accidentally incorporated into swine feed. Discarded or unlabeled portions of granular insecticides can be mistaken for mineral mixes or dry feed ingredients and added to swine feeds. When farm equipment used for feed handling is also used for insecticide transportation, contamination of this equipment may result in insecticides being inadvertently mixed into animal feeds. In addition, swine may have accidental access to insecticides when they are stored or spilled on the farm premises. Improperly operating back rubbers and oilers may provide an additional source of these insecticides for livestock.

Miscalculation of insecticide concentrations in spraying, dipping, and pour-on procedures may also result in toxicosis. Re-treating animals with OP or carbamate preparations within a few days' time may result in poisoning.

Organophosphorus and carbamate insecticides

OP and carbamate insecticides are discussed together because of their similar mechanisms of action. Cholinergic nerves utilize acetylcholine as a neurotransmitter, and under normal conditions, acetylcholine released at the

synapses of parasympathetic nerves and myoneural junctions is quickly hydrolyzed by cholinesterase enzymes. When the hydrolyzing enzymes are inhibited, the continued presence of acetylcholine maintains a state of nerve stimulation and accounts for the clinical signs observed with poisoning from these insecticides.

The clinical syndrome produced by OP and carbamate insecticides is characterized by a rapidly progressing overstimulation of the parasympathetic nervous system and skeletal muscles. Earliest clinical signs of acute poisoning frequently include mild to profuse salivation, defecation, urination, emesis, stiff-legged or "sawhorse" gait, and general uneasiness. As the toxicosis progress, signs observed include profuse salivation; gastrointestinal hypermotility resulting in severe colic and vomiting (especially common in swine); abdominal cramps; diarrhea; excessive lacrimation; miosis; dyspnea; cyanosis; urinary incontinence; muscle tremors of the face, eyelids, and general body musculature; and acute death. Hyperactivity of the skeletal muscles is generally followed by muscular paralysis, as the muscles are unable to respond to continued stimulation. Swine may exhibit increased CNS stimulation but rarely, if ever, convulsive seizures. More commonly, severe CNS depression occurs. Death usually results from hypoxia caused by excessive respiratory tract secretions, bronchoconstriction, and erratic, slowed heartbeat. The onset of clinical signs of acute poisoning may appear within a few minutes in severe cases to several hours in milder ones.

Lesions associated with acute OP or carbamate toxicosis are usually nonspecific but may include excessive fluids in the respiratory tract as well as pulmonary edema.

Diagnosis is usually by history of exposure to OP or carbamate insecticides associated with clinical signs of parasympathetic stimulation, which warrants a tentative diagnosis of poisoning with these compounds. Chemical analyses of animal tissues for the presence of insecticides are usually unrewarding because of the rapid degradation of OP and carbamate insecticides, resulting in low tissue residue levels. Finding the insecticide in the stomach contents and feed or suspect material is valuable in establishing a diagnosis. Cholinesterase enzyme activity is inhibited in whole blood and brain tissue of poisoned animals. A reduction of whole blood cholinesterase activity to less than 25% of normal is indicative of excessive exposure to these insecticides, and animals dying from these insecticides will generally have less than 10% of normal brain activity. Well-chilled whole blood and brain samples along with stomach contents and the suspect feed or material should be submitted to a laboratory for chemical analysis.

Treatment of animals poisoned by OP or carbamate insecticides should be considered an emergency because of the rapid progression of respiratory distress in the clinical syndrome. Initial treatment for poisoned swine should be atropine sulfate at approximately 0.5 mg/kg body weight. One-quarter of this dose may be given intravenously for a quick response in especially severe cases. Atropine does not counteract the insecticide–enzyme bond but blocks the effects of accumulated acetylcholine at the nerve endings. Although a dramatic cessation of parasympathetic signs is generally observed within a few minutes after administration of atropine, it will not affect the skeletal muscle tremors. More atropine at approximately one-half the initial dose may be required but should be used only to control recurring parasympathetic signs. Although the use of atropine alone is generally adequate, especially if vomiting has occurred, specific cases may warrant the use of pralidoxime chloride or activated charcoal.

Oral activated charcoal is recommended for treatment of any ingested insecticide to reduce continued absorption of the insecticide from the gut. Although a useful treatment, the need for activated charcoal in swine may be reduced if vomiting has occurred. The use of the oximes (e.g. TMB-4, 2-PAM, pralidoxime chloride) in large animals, although efficacious, may be economically unfeasible. If used, pralidoxime chloride is recommended at a dose of 20 mg/kg body weight; however the oximes are of no benefit in treating carbamate toxicoses.

Dermally exposed animals should be washed with soap and water to prevent continued absorption of these compounds.

Morphine, succinylcholine, and phenothiazine tranquilizers should be avoided in treating OP poisoning.

Chlorinated hydrocarbons

CH insecticides (e.g. toxaphene, chlordane, aldrin, dieldrin, and lindane) produce toxicosis in swine by acting as diffuse but powerful stimulants of the CNS. These products have been restricted from the market for over 35 years, yet leftover products may remain in old barns or storage areas.

Clinical signs often appear 12–24 hours after exposure. Initially, animals may appear apprehensive with a period of hyperexcitability and hyperesthesia characterized by exaggerated responses to stimuli and spontaneous muscle spasms observed. The spontaneous tremors and fasciculation are usually in the facial region and involve lips, muscle, eyelids, and ears, progressing caudally to involve the heavy muscles of the shoulder, back, and hindquarters. These spasms may progress into a tonoclonic convulsive seizure. Abnormal posturing, elevation of the head, and chewing movements may be observed. Varying degrees of respiratory paralysis occur during the seizures, with periods of depression and inactivity between successive seizures. The rapidity of onset and severity of clinical signs provide a poor index of the prognosis of the episode in individual animals. Occasionally, animals will die during

seizures, while others may completely recover following several severe episodes.

Diagnosis is tentatively made by observation of clinical signs of hyperexcitability and tonoclonic convulsive seizures, along with a known exposure to CH insecticides. Demonstrating the presence of significant levels of CH insecticide in the liver, kidney, or brain tissue or in stomach contents or feed is essential for confirming diagnosis. Samples of these tissues as well as stomach contents and suspect material such as feed or spray should be submitted to a laboratory. Avoid contamination of specimens with hair or gut contents to prevent erroneous analytic results.

Treatment is symptomatic since there is no specific antidote for CH insecticides. Sedation with long-acting barbiturates to control convulsive seizures, washing off dermal residues, and administration of activated charcoal are indicated.

Animals dying from CH insecticide toxicosis are a source of contamination for feed ingredients such as tankage, meat and bone meal, and fats because of the persistence of CH insecticides and their concentration in fat deposits of the carcasses. Tissue residues of these chemicals in swine surviving an episode of insecticide exposure should be an important consideration in market animals. In some cases the time required for excretion of these residues is too long to make decontamination economically feasible.

Pyrethroids, formamidines, neonicotinoids, and phenylpyrazoles

Several synthetic pyrethroids (e.g. permethrin, fenvalerate) are commercially available for fly and external parasite control. As a class, synthetic pyrethroids are relatively nontoxic to mammals. Amitraz is a formamidine pesticide with insecticidal and acaricidal properties. It is available in the United States as Taktic for control of lice and mange on swine. The neonicotinoids were developed in the late 1970s and are used as insecticides. Imidacloprid is the most common insecticide in this class and has a wide safety margin. These insecticides act on the postsynaptic nicotinic receptors swine. Fipronil is a member of the phenylpyrazole class of pesticides. These compounds target the GABA-regulated chloride channels. These are all very safe compounds and are unlikely to produce toxicosis in swine.

Fungicides

Captan has been widely used as a seed treatment although some newer fungicides are now being employed. Field corn seed produced commercially may be treated with captan at a level of approximately 1000 ppm, but consumption of captan-treated seed corn represents little hazard of poisoning, as the acute lethal dose of captan for livestock is greater than 250 mg/kg body weight.

Organomercurials include phenyl mercuric chloride, phenyl mercuric acetate, various aliphatic compounds such as ethyl mercuric chloride, and complex aromatic derivatives like hydroxy mercuric cresol. The toxicoses associated with mercury-based seed treatments are discussed above, in the section on mercury.

Pentachlorophenol (PCP) has been employed for over 45 years as a wood preservative and fungicide. PCP- or "penta"-treated wood has found application in livestock handling and housing facilities, where wood is in contact with soil, manure, or moisture. Acute poisoning is not a major problem from PCP-treated wood, although toxicosis, including stillborn pigs, may occur when livestock have contact with surfaces that have been freshly treated with PCP preparations (Schipper 1961). A single oral dose of 80 mg/kg was not fatal to a weanling pig. If toxicosis occurs, depression, emesis, muscular weakness, accelerated respiratory rate, and posterior paralysis are clinical signs that may be observed. A problem of greater concern may be the recognition of blood and tissue PCP residues in swine that have been in contact with PCP-treated facilities. Finding from 10 to 1000 ppb PCP in whole blood is apparently unrelated to manifestations of toxicosis.

Chromated copper arsenate (CCA) is widely used as a wood preservative in consumer lumber intended for outdoor use. CCA-treated wood generally presents a negligible hazard to swine, as the metallic salts are bound in the wood fibers. However, the residual inorganic arsenic in the ashes of burned CCA wood can produce poisoning in swine. Since 2003, no wood can be treated with CCA for residential uses according to the EPA.

Herbicides

Organic and selective herbicides are commonly used for control and elimination of noxious weeds. Toxicity from consumption of treated plants or overspray is rare; most are associated with human error or accidental ingestion of concentrates or sprays.

Chlorophenoxy herbicides (e.g. 2,4-D; 2,4,5-T; MCPA, Silvex, dicamba) are selective herbicides widely used in crop production and pasture and range management. Because the toxic dose of 2,4-D and 2,4,5-T is greater than 300 mg/kg body weight for several days, the hazard of poisoning by these compounds under normal conditions of use is very low.

Amide herbicides (e.g. thioamide, allidochlor, propanil) may cause anorexia, salivation, depression, and prostration. The toxic doses are quite high, making intoxication under typical field conditions quite rare. Other classes of

herbicides (e.g. glyphosates, triazines, and benzoic acid derivatives) are relatively nontoxic.

Dipyridyl herbicides (e.g. diquat, paraquat) are plant-desiccant types of herbicide and have found widespread application in no-till farming. Accidental as well as malicious poisoning of swine with paraquat has resulted in toxicosis. An approximate lethal dose of paraquat for swine is 75 mg/kg. Acute effects involving necrosis and erosion of the oral and gastric mucosa are attributed to the carrier solvent. The more classic effects, however, occur 7–10 days after ingestion and are characterized by pulmonary congestion and edema. The pulmonary lesions progress to a severe diffuse interstitial pulmonary fibrosis. Initial clinical signs include emesis and diarrhea, and the later stages are characterized by respiratory distress. Once clinical signs suggesting pulmonary involvement develop, therapeutic measures are usually futile.

Rodenticides

Rodenticides are used to control rat and mouse populations in or around farmsteads, feed storage areas, and swine production facilities. Accidental access to these compounds constitutes the usual route of exposure, although malicious poisoning of swine with rodenticides has also occurred.

Anticoagulant rodenticides

Anticoagulant rodenticides (e.g. warfarin, diphacinone, chlorophacinone, bromadiolone, brodifacoum, pindone) compose the largest group of rodenticides available through retail outlets. Swine are quite susceptible to this class of compound, as evidenced by toxicosis occurring after a single oral dose of warfarin at 3 mg/kg body weight. Repeated oral doses of only 0.05 mg/kg per day for 7 days also produced toxicosis in swine (Osweiler 1978). These rodenticides produce lowered prothrombin levels by interfering with vitamin K utilization. The physiologic result is increased blood clotting time, which is manifested clinically as mild to severe hemorrhage. The clinical signs – including lameness, stiffness, lethargy, recumbency, anorexia, and dark tarry feces – are related directly to extravasation of blood. Observed lesions include hematoma, articular swelling, epistaxis, intermuscular hemorrhage, anemia, and melena.

Anticoagulant intoxication may have food health and safety implications due to potential of relay toxicosis and prolonged volatile residues in tissue. A study involving bromadiolone, a second-generation anticoagulant, showed that liver residues persisted for a period of weeks in cases involving controlled high dose administration (Enouri et al. 2015).

A diagnosis of anticoagulant rodenticide toxicosis should include demonstration of a defect in the clotting mechanism as evidenced by increases in clotting time, one-stage prothrombin time, or activated partial thromboplastin time. The chemical detection of the rodenticide in samples of blood, liver, or suspect baits is also warranted. Injectable vitamin K and oral vitamin K supplements are included in a successful treatment regime.

Strychnine

Strychnine, an indole alkaloid, is available commercially, often as either a green- or red-dyed pellet or grain or as a white powder. This alkaloid acts by selectively antagonizing certain types of special inhibitory neurons, thereby allowing uncontrolled and relatively diffuse reflex activity to proceed unchecked. The approximate oral lethal dose of strychnine for swine varies from 0.5 to 1 mg/kg body weight.

Clinical signs appear within 10 minutes to 2 hours after ingestion and are characterized by violent tetanic seizures that may occur spontaneously or in response to external stimuli such as touch, light, or sound. The intermittent seizures are usually separated by periods of relaxation. Death occurs from anoxia and exhaustion during the seizures, often in less than 1 hour.

Diagnosis is best confirmed by detection of the strychnine alkaloid in either the stomach contents or urine. Treatment consists of controlling seizures with long-acting barbiturates and other muscle relaxants.

Cholecalciferol

Rodenticides containing cholecalciferol (vitamin D_3) are commercially available as Agrid3, Quintox, or Ortho Rat-B-Gone. Toxic doses of these products produce vitamin D poisoning with hypercalcemia, mineralization of soft tissues, and clinical signs of depression, weakness, nausea, anorexia, polyuria, and polydipsia.

Bromethalin

Bromethalin-based rodenticides, marketed as Assault, Vengeance, or Tomcat, produce cerebral edema and signs of rear leg ataxia and/or paresis and CNS depression.

Toxic plants

Amaranthus retroflexus (redroot pigweed)

A distinct disease syndrome of swine called perirenal edema occurs during the summer and early fall months. Its onset is associated with sudden access to pastures,

barn lots, or fencerows containing moderate amounts of *Amaranthus retroflexus* (redroot pigweed). Clinical signs appear suddenly 5–10 days after access to the pigweed. Initial signs are weakness, trembling, and incoordination. The disease rapidly progresses to knuckling of the pastern joints and finally to almost complete paralysis of the rear legs. Affected pigs usually lie in sternal recumbency, and if disturbed, attempts to walk will be in a crouching gait or with the rear legs dragging. The body temperature is usually normal and the eyes are bright. Coma and death generally occur within 48 hours of the onset of clinical signs, but affected swine may live from 5 to 15 days, with progression from signs of acute nephrosis to those of chronic fibrosing nephritis. In affected herds, new cases may appear for as long as 10 days after removal from the source. Morbidity ranges from less than 5% in some herds to 50% in others, and the mortality is usually about 75–80% in those showing clinical signs.

Gross necropsy findings are dramatic and characterized as edema of the connective tissue around the kidneys. The amount of fluid in the perirenal area varies, at times occupying the greater portion of the abdominal cavity. The edematous fluid may contain considerable blood, although the kidney itself is usually of normal size and pale. Edema of the ventral body wall and perirectal areas as well as ascites and hydrothorax may be observed. Histologic lesions of affected swine are characterized by hydropic degeneration and coagulative necrosis of both proximal and distal convoluted tubules. Glomeruli may be shrunken, with dilation of Bowman's capsules. Proteinaceous casts are numerous in distal and collecting tubules.

As a consequence of severe renal disease, there are elevations in BUN, serum creatinine, and serum potassium. The electrocardiograph of affected swine is characteristic of hyperkalemic heart failure (Osweiler et al. 1969). Immediate removal of affected pigs from the source of the weeds is the only definite therapeutic recommendation that can be made at this time.

Xanthium spp. (cocklebur)

Cockleburs, including *Xanthium strumarium* and other species, are annual herbs that reproduce only from seed. They may be found throughout the world in cultivated fields, fencerows, and ditches and may heavily infest pastures as a result of being washed in from adjacent cropland.

The greatest potential for cocklebur poisoning arises when the more toxic two-leaf seedling stage or ground seeds are ingested. The unpalatable more mature plant contains less of the toxic principle, carboxyatractyloside. Within 8–24 hours after ingestion, swine develop signs of depression, nausea, weakness, ataxia, and subnormal temperature. Spasms of the cervical muscles, vomiting, and dyspnea may occur. Death occurs within several hours after the onset of signs.

Lesions typically include ascites with large fibrin strands on the surface of the liver and other viscera and congestion and centrilobular accentuation of the liver. Microscopically acute centrilobular hepatic necrosis is observed (Stuart et al. 1981).

Treatment includes mineral oil orally to delay absorption of the carboxyatractyloside. Intramuscular injection of 5–30 mg physostigmine may produce a dramatic response in some cases (Link 1975).

Solanum nigrum (black nightshade)

Even though black nightshade is easily recognized and found in woods, permanent pastures, and fencerows, actual cases of poisoning are rare. The alkaloid solanine is found principally in the leaves and green berries, but the plant is generally not palatable and is usually consumed under conditions of its abundant growth and lack of other suitable forage.

Affected animals display anorexia, constipation, depression, and incoordination. Poisoned swine may vomit. Dilation of the pupils and muscular trembling are neurologic signs observed. Animals may be seen lying on their sides and kicking with all feet, progressing then to coma and death. Necropsy may reveal some degree of gastrointestinal irritation. The toxic alkaloid is rapidly eliminated through the urine (Kingsbury 1964).

Nitrates and nitrites

As monogastric animals, swine are relatively resistant to the effects of nitrate, especially when compared with cattle. Nitrate or nitrite toxicosis occurs most commonly when these ions accumulate in either plants or water sources. Some fertilizers, such as ammonium nitrate or potassium nitrate, may also be a source of nitrate for animals. Several different plants may accumulate nitrate, depending on varying climatic and soil fertility conditions. Nitrate may accumulate in the lower stalk and perhaps leaves of corn and other plants, but not in the fruit or grain.

The nitrates from both water (see the section on water quality below) and plant sources are additive and should be evaluated together. The nitrate ion (NO_3) itself is not particularly toxic and may produce no more than gastrointestinal irritation. However, nitrite (NO_2), the reduced form of nitrate, is quite toxic. The nitrite ion oxidizes ferrous iron in hemoglobin to the ferric state, forming methemoglobin, which cannot accept and transport molecular oxygen. The result is tissue hypoxia from poorly oxygenated blood.

Pigs given single oral doses of greater than 10–20 mg nitrite-nitrogen (as potassium nitrite)/kg body weight developed clinical signs of poisoning but recovered, whereas those given doses greater than 20 mg nitrite-nitrogen/kg body weight died within 90–150 minutes after ingestion (London et al. 1967). Clinical signs became apparent when approximately 20% of the total hemoglobin was present as methemoglobin; death was associated with methemoglobin levels of approximately 80%.

Clinical signs observed with acute nitrite toxicosis include increased respiratory rate, salivation, miosis, polyuria, weakness, ataxia, and terminal anoxic convulsive seizures. The blood and tissues are a chocolate brown color from the methemoglobin. Treatment of acute nitrite toxicosis consists of intravenous injection of 10 mg methylene blue/kg body weight in a 4% solution (Link 1975).

Water quality

The availability of adequate quantities of good quality water is essential for successful swine production. Water as the cause of poor performance and vague disease conditions in swine suggests that water evaluation should be part of a thorough diagnostic investigation. A detailed history of the animals as well as the water source, careful clinical evaluation, and submission of representative animal and water specimens should be included in the investigation. Results of water tests should be evaluated in view of existing standards for livestock water quality. Some general guidelines for evaluating water quality parameters for livestock are presented in Table 70.1.

Information about the source of the water should be recorded. Ponds, wells, and regional rural water systems are the most common water sources, and each may influence the quality of the water supplied. The depth of wells may also be helpful, as deeper wells tend to have a higher mineral content, while shallower wells are more likely to have higher nitrate levels and coliform counts. Sometimes the age of the well and type of pumping device may suggest certain mechanical problems, including a cracked casing or defective sanitary seal. Estimates of the amount of water consumed using water meters may also be helpful when investigating potential water problems.

Table 70.1 Water quality guidelines for livestock.

Item	Maximum recommended limit (ppm)
Major ions	
Calcium	1000
Nitrate + nitrite	100
Nitrite alone	10
Sulfate	1000
Total dissolved solids	3000
Heavy metals and trace ions	
Aluminum	5.0
Arsenic	0.5[a]
Beryllium	0.1[b]
Boron	5.0
Cadmium	0.02
Chromium	1.0
Cobalt	1.0
Copper (swine)	5.0
Fluoride	2.0[c]
Iron	No guideline
Lead	0.1
Manganese	No guideline
Mercury	0.003
Molybdenum	0.5
Nickel	1.0
Selenium	0.05
Uranium	0.2
Vanadium	0.1
Zinc	50.0

Source: Canadian Task Force on Water Quality (1987).
[a] 5.0 if not added to feed.
[b] Tentative guideline.
[c] 1.0 if fluoride is present in feed.

Microbiologic standards

Microbiologic examination of water samples determines the general sanitary quality of the sample and indicates the degree of contamination of the water with waste from human and animal sources. These examinations usually do not attempt to isolate pathogenic bacteria but rather detect the presence of indicator organisms. The coliform groups of bacteria are used to assess the degree of water pollution and thus the sanitary quality of the particular sample. The differentiation of fecal coliforms as a subgroup within the general category of coliforms is increasingly available. In the United States, the EPA (1973) proposed that acceptable levels for water to be used directly by livestock should not exceed 1000 cfu/100 mL. Since animals are allowed to range freely and drink surface waters, these proposed limits are unenforceable and of doubtful value. The standard plate count, which enumerates the number of bacteria multiplying at 35 °C, is of doubtful significance in evaluating livestock water sources other than helping judge the efficiency of various water treatment processes.

Salinity

Salinity, or total dissolved solids (TDS), generally expressed in milligrams per liter, is an expression of the amount of soluble salts in a particular water sample and is one of the most important parameters used to evaluate water quality. The ions most commonly involved are calcium, magnesium, and sodium in the bicarbonate, chloride, or sulfate form. Hardness is sometimes confused with salinity, but the two are not necessarily correlative. Hardness is expressed as the sum of calcium and magnesium reported in equivalent amounts of calcium carbonate. Although hardness of water may affect the mechanical function of valves and waterers because of the formation of mineral deposits, hardness itself has a minimal impact on animal performance.

Salinity is estimated by mg of soluble salts/L. Water with less than 1000 mg/L is not a hazard; between 1000 and 5000 mg/L may cause mild temporary diarrhea or be refused at first by swine not accustomed to it (Anderson and Strothers 1978; NRC 1974; Paterson et al. 1979); between 5000 and 7000 mg/L may present a health risk for pregnant, lactating, or stressed animals; and more than 7000 mg/L should be considered unsafe for swine.

In some regions, sulfates are a major portion of the TDS in water. Veenhuizen et al. (1992) demonstrated that except for an increase in fecal moisture content, water containing up to 1800 mg of sodium and/or magnesium sulfate per liter had no effect on nursery pig performance. An epidemiologic study of water on swine farms did not find an association between sulfate concentrations and the prevalence of diarrhea, although water sulfate levels increased with the depth of the wells tested (Veenhuizen 1993). A study of water quality on 173 Iowa swine farms found a mean TDS of 343 mg/L (range 100–2500), but measured no significant effects of elevated TDS on several performance parameters (Ensley 1998). However, drinking water sulfate concentrations >1000 ppm can cause an osmotic diarrhea in pigs.

Nitrates and nitrites

Nitrates and nitrites are water soluble and thus may be leached from the soil or soil surface into groundwater. Animal wastes, nitrogen fertilizers, decaying organic matter, silage juices, and soils high in nitrogen-fixing bacteria may be sources of contamination through surface-water runoff to adjacent poorly cased, shallow, or low-lying wells or reservoirs.

The upper limit for nitrate in human drinking water is 45 mg nitrate/L (USEPA 1975), established as preventive for the methemoglobinemia of "blue baby" syndrome in human infants who receive formulas made from high-nitrate waters. There is no evidence to suggest that neonatal swine have greater susceptibility to elevated nitrates, and Emerick et al. (1965) concluded that 1-week-old pigs are no more susceptible to nitrite-induced methemoglobinemia than older growing swine. A review of water quality for livestock (NRC 1974) proposed 440 mg nitrate/L as the maximum nitrate that could safely be allowed in livestock water.

Reports of experimental production of a chronic or low-level nitrate-poisoning syndrome in livestock have been extensively reviewed (Emerick 1974; Ridder and Oehme 1974; Turner and Kienholz 1972). The bulk of the evidence indicates that sublethal or chronic effects are extremely rare and difficult to verify. London et al. (1967) fed growing pigs up to 18.3 mg nitrite-nitrogen/kg body weight for 124 days without serious effects developing. No effect on the performance of growing–finishing swine or on reproductive performance of gilts was observed when the drinking water contained 1320 ppm nitrate (Seerley et al. 1965).

Miscellaneous toxicants

Sodium ion toxicosis

Sodium ion toxicosis, also called water deprivation or salt poisoning, is a common problem in swine. The occurrence of sodium ion toxicosis is inversely related to water intake and is almost always related to water deprivation caused by inadequate or interrupted water supply and oversights in husbandry. The likelihood of toxicosis will also increase with increased dietary salt, but the condition occurs when rations contain normal levels of added salt, for example, 0.25–1%. It has also been associated with the feeding of whey and other milk by-products. Sodium ion toxicosis may occur after water deprivation of a few hours, but in most cases the time exceeds 24 hours.

The initial clinical signs are thirst, anorexia, and constipation, followed by CNS involvement. Intermittent convulsions start within 1 to several days after water deprivation and are often exacerbated by rehydration. The frequency of the characteristic tonoclonic convulsions with opisthotonos, often starting from a "dog-sitting" position, increases with time. Affected animals are afebrile and may also wander aimlessly, head-press, and appear to be blind and deaf. Moribund pigs become comatose, often lying on their sides with continuous paddling. Most affected animals die within a few days. Some pigs that appear to be unaffected may succumb later from polioencephalomalacia. Salt poisoning from eating excess salt or consuming brine usually will cause vomiting and diarrhea.

Diagnosis is best accomplished by establishing that water deprivation occurred, which may be difficult in some cases. Necropsy findings may reveal an absence of ingesta, gastritis, constipation, or very dry feces; rehydration-exacerbated

deaths may have stomach distended with water. Chemical analysis of serum and cerebrospinal fluids may confirm a hypernatremia with levels of Na above 160 mEq/L (Osweiler and Hurd 1974). However, after rehydration, normal values of 140–145 mEq/L may exist. Fresh brain tissue is required for accurate analysis of brain sodium concentration. Brain sodium levels above 1800 ppm (wet weight) are consistent with a diagnosis of sodium ion toxicosis. Histologic examination of brain tissue, especially the cerebrum, often reveals a nearly pathognomonic eosinophilic meningoencephalitis characterized by cuffing of meningeal and cerebral vessels with eosinophils. However, when pigs live several days, eosinophils may disappear or be replaced by mononuclear cells. Brains of pigs affected subacutely may have a laminar subcortical polioencephalomalacia. Analysis of feed for sodium is usually of limited value. Gradual rehydration in cases of known water interruption is critical to prevent clinical manifestations. The prognosis is poor for animals showing clinical signs.

Coal tar pitch

Coal tars are a mixture of condensable volatile products formed during the destructive distillation of bituminous coal. The phenolic portions of these products have the greatest acute toxicity. Sources of these substances for swine are "clay pigeons," lignite tar flooring slabs, tar paper, and tar used in waterproofing and sealing. Because of the rapid clinical course, sudden death is often the first sign observed. Weakness, depression, and increased respiratory rate can be observed in animals that may live for several hours or even days. Icterus and a secondary anemia may develop. Necropsy of pigs poisoned by coal tar pitch reveals a greatly enlarged friable liver. The hepatic lobules are very distinct grossly; some are darkened in color, and others are yellowish orange. Microscopically, this lesion is observed as severe centrilobular necrosis with subsequent intralobular hemorrhage. Ascites and large turgid kidneys may also be observed. There is no specific treatment for this condition. Removal of animals from the source of the coal tar is important to prevent recurrence of poisoning.

Ethylene glycol

Many permanent antifreeze/coolant mixtures for liquid-cooled engines contain approximately 95% ethylene glycol. A hazard of poisoning exists when animals have accidental access to antifreeze solutions during periods of engine maintenance or when these solutions are used in plumbing systems to prevent freezing. Swine may be poisoned by ingesting 4–5 mL ethylene glycol/kg body weight. Ethylene glycol toxicosis is exhibited in two clinical phases. Initially the glycol may enter the cerebrospinal fluid, producing a narcotic or euphoric state of intoxication. Subsequent clinical signs of acidosis and renal failure are associated with the highly toxic metabolites of the glycol and formation of calcium oxalate crystals in the kidney tubules. Renal tubular blockage with development of uremia is observed 1–3 days after ingestion.

Clinical signs generally include emesis, anorexia, dehydration, weakness, ataxia, convulsions, coma, and death. The entire course of illness may be as short as 12 hours following consumption of large quantities of ethylene glycol. Oxalate nephrosis can be demonstrated histopathologically and is characterized by finding pale yellow birefringent oxalate crystals in the tubules. Polarizing filters greatly aid in the detection of oxalate crystals in kidney sections or in impression smears of freshly incised kidney.

Once clinical signs of renal failure are evident, treatment is usually of no avail. If treated within the first 6–12 hours after ingestion, reasonable response has been achieved in ethylene glycol–poisoned dogs by using 5.5 mL/kg body weight of 20% ethyl alcohol intravenously and 98 mL/kg body weight of 5% sodium bicarbonate intravenously.

Gossypol

Cottonseed meal (CSM), a by-product of the cotton fiber and cottonseed oil industries, is an important protein supplement for livestock rations in cotton-producing regions. Its use as a protein supplement for swine, however, is limited by gossypol content, which varies with the strain of the cotton plant, its geographic location, climatic conditions, and the oil extraction procedure used. Gossypol, a polyphenolic binaphthalene, is a yellow pigment in glands of decorticated cottonseed. The toxic "free" gossypol becomes partially inactivated (bound) during the extraction and milling processes, as well as spontaneously in the prepared meal. Toxicity of gossypol depends on the species and age of the animal and on various components of the diet, particularly the protein, lysine, and iron concentrations (Eisele 1986).

Toxicosis only follows prolonged feeding (weeks to months) of CSM with a high content of free gossypol and may be manifested simply as ill-thrift or as an acute respiratory problem followed by death. The main pathologic changes are cardiomyopathy, hepatic congestion and necrosis, skeletal muscle injury, and severe edematous changes throughout the animal. A decrease in hemoglobin total serum, protein concentration, and packed cell volume is seen in pigs fed a diet containing >200 mg of free gossypol/kg (Haschek et al. 1989).

Recommendations for growing and fattening swine include feeding no more than 9% CSM in the diet, with less than 100 mg (0.01%) of free gossypol/kg, in a 15–16% protein diet. Tolerance to gossypol can be induced by adding $FeSO_4$ (400 mg/kg) at a 1 : 1 weight ratio with free

gossypol. Increasing the amount of crude protein or supplementing with lysine can also induce tolerance (Pond and Maner 1984).

Disinfectants

Cleaning compounds like the quaternary ammonium and glutaraldehyde products need to be used with care as these compounds can be in contact with animals and result in severe dermal burns. In general, intoxication with disinfectants is rare.

Ventilation failure and consequences

Confinement of swine in closed structures dependent on mechanical ventilation increases the risk of hyperthermia or impact of toxic gases. Fortunately, concentrations of ammonia and hydrogen sulfide, the two most dangerous gases associated with manure decomposition, remain below toxic levels even at relatively low ventilation rates used during cold weather. However, accidents, poor design, power failure, mechanical failure, and improper operation may result in insufficient ventilation and increase the concentration of poisonous gases to toxic levels. It is useful to be reminded of some of the terminology used when investigating ventilation failures: hyperthermia (elevated body temperature), asphyxiation (displacement of oxygen by another gas such as CO_2), intoxication (toxic effect of gases such as hydrogen sulfide, ammonia, or carbon monoxide on structure or function), and suffocation (physical obstruction of air passages).

Ammonia and hydrogen sulfide are the most important gases released by the decomposition of urine and feces; carbon dioxide and methane are also produced but seldom reach significant concentrations. A number of vapors responsible for the odors of manure decomposition are also produced, including organic acids, amines, amides, alcohols, carbonyls, skatoles, sulfides, and mercaptans. Concentrations of toxic gases are usually expressed as parts of the gas per million parts of air (ppm) by volume.

Swine deaths associated with ventilation failures are usually due to mechanical failures or power loss rather than simple accumulation of toxic gases. Losses, sometimes disastrous, may occur at any time of year in part or all of a confinement facility. Similar dynamics and death losses occur in tightly sealed insulated trucks used to transport pigs.

Hyperthermia

When ventilation fails because of storms, power outages, or mechanical failure, the dynamics of air movement, heat dissipation, and high humidity in the confined space may quickly become critical. These factors greatly suppress evaporative cooling, which often and quickly leads to fatal hyperthermia in confined swine. Death losses may approach 95% under these circumstances; frequently only the smallest pigs survive. Although there is no method to specifically confirm hyperthermia as a cause of death, a history of ventilation failure, rapid carcass decomposition, a pale "cooked" appearance to the muscles, and blood-tinged foam in the trachea are usually seen. Hyperthermia is often an overlooked contributor to deaths while in pursuit of "toxic gas" diagnosis.

Ammonia

Ammonia (NH_3) is the toxic air pollutant most frequently found in high concentrations in animal facilities, and production is especially common where excrement can decompose on a solid floor. This gas has a characteristic pungent odor that humans can detect at approximately 10 ppm or even lower. The NH_3 concentration in enclosed animal facilities usually remains below 30 ppm even with low ventilation rates; however, it may frequently reach 50 ppm or higher during long periods of normal facility operation.

Ammonia is highly soluble in water and as such will react with the moist mucous membranes of the eye and respiratory passages. Consequently, excessive tearing, shallow breathing, and clear or purulent nasal discharge are common signs of aerial NH_3 toxicosis. At concentrations usually found in practical animal environments (<100 ppm), the primary impact of this gas is as a chronic stressor that can affect the course of infectious disease as well as directly influence the growth of healthy young pigs. The rate of gain in young pigs was reduced by 12% during exposure to aerial NH_3 at 50 ppm and by 30% at 100 or 150 ppm (Drummond et al. 1980). Ammonia at 50 or 75 ppm reduced the ability of healthy young pigs to clear bacteria from their lungs (Drummond et al. 1978). At 50 or 100 ppm, aerial NH_3 exacerbated nasal turbinate lesions in young pigs infected with *Bordetella bronchiseptica* but did not add to the infection-induced reduction in growth rate (Drummond et al. 1981a). In another study, aerial NH_3 at 100 ppm reduced the rate of gain by 32%, and when ascarid infection was imposed simultaneously, the rate of gain was reduced by 61% (Drummond et al. 1981b). More extensive reviews of aerial NH_3 and its effect on animal production are provided by Curtis (1983) and the National Research Council (1979a).

Hydrogen sulfide

Hydrogen sulfide (H_2S) is a potentially lethal gas produced by anaerobic bacterial decomposition of protein and other sulfur-containing organic matter. The source of H_2S that presents the greatest hazard to swine is liquid manure holding pits. Most of the H_2S, which may be continuously produced, is retained within the liquid of the pit. However, agitation of waste slurry to suspend solids prior to being pumped out causes the rapid release of much of the H_2S that may have been retained within it. Hydrogen sulfide is heavier than air and consequently accumulates in pits, tanks, and other low areas of a facility. The concentration of H_2S usually found in closed animal facilities (less than 10 ppm) is not toxic, but the release of gas upon agitation may produce concentrations of H_2S up to 1000 ppm or higher within the facility.

Acute H_2S poisoning is directly responsible for tragic deaths of humans and swine in closed animal facilities, particularly during pit manure agitation and removal. Humans can detect the typical "rotten egg" odor of H_2S at very low concentrations (0.025 ppm) in air. Exposures to these low concentrations have little or no importance to human health, and thus the olfactory response is a useful warning signal of its presence. However, at higher concentrations (greater than 200 ppm), H_2S presents the distinct hazard of a paralyzing effect on the olfactory apparatus, thus effectively neutralizing the warning signal (NRC 1979b).

Hydrogen sulfide is an irritant gas. Its direct action on tissues induces local inflammation of the moist membranes of the eye and respiratory tract. When inhaled, the action of H_2S is more or less uniform throughout the respiratory tract, although the deeper pulmonary structures suffer the greatest damage. Inflammation of the deep lung structures may appear as pulmonary edema. If inhaled at sufficiently high concentrations, H_2S can also be readily absorbed through the lung and can produce fatal systemic intoxication (O'Donoghue 1961). At concentrations in air exceeding 500 ppm, H_2S must be considered a serious imminent threat to life; between 500 and 1000 ppm, it produces permanent effects on the nervous system via polioencephalomalacia. If spontaneous recovery does not occur and artificial respiration is not immediately provided, death results from asphyxia or from respiratory paralysis of intoxication.

Management is the most important part of preventing animal deaths from H_2S. When manure stored in a pit beneath a building is agitated, animals should either be moved out of the building or other steps taken to protect the animals during agitation. In mechanically ventilated buildings, the fans should run at full capacity; in naturally ventilated buildings, manure pits should not be agitated unless there is a brisk breeze blowing. Immediate rescue of affected swine should not be attempted for the rescuer may quickly become a victim of H_2S toxicosis.

Carbon dioxide

Carbon dioxide (CO_2) is an odorless gas present in the atmosphere at 300 ppm. It is given off by swine as an end product of energy metabolism and by improperly vented, though properly adjusted, fuel-burning heaters. It is also the gas evolved in the greatest quantity by decomposing manure. Despite all this, CO_2 concentration in closed animal facilities rarely approaches levels that endanger animal health (Curtis 1983).

Methane

Methane (CH_2), a product of microbial degradation of carbonaceous materials, is not a poisonous gas. It is biologically rather inert and produces effects on animals only by displacing oxygen in a given atmosphere, thereby producing asphyxiation. Under ordinary pressures, a concentration of 87–90% CH_2 in a given atmosphere is required before irregularities of respiration and eventually respiratory arrest due to anoxia are produced. The chief danger inherent in this material is its explosive hazard as concentrations of 5–15% by volume in air are reached (Osweiler et al. 1985). Recently, the US Midwest has experienced increased propensity for methane-containing foam to form in pits. In the last decade, changes in ration constituents have increased the occurrences of pits generating gas and methane-laden foam, resulting in accidently explosions and fires on swine farms, some of which have claimed human lives (Yan et al. 2014).

Carbon monoxide

Carbon monoxide (CO), which is produced from the inefficient combustion of carbonaceous fuel, is also potentially lethal to swine. Poisoning occurs when improperly adjusted and improperly vented space heaters, furnaces, or equipment are operated in tight, poorly ventilated buildings such as farrowing houses. Ambient background levels of CO are 0.02 ppm in fresh air, 13 ppm in city streets, and 40 ppm in areas with high vehicular traffic.

Carbon monoxide acts by competing with oxygen for binding sites on a variety of proteins, particularly hemoglobin. The affinity of hemoglobin for CO is some 250 times that for oxygen. Therefore, CO is preferentially bonded to the heme group, which forms carboxyhemoglobin, thereby reducing the oxygen-carrying capacity of heme. Cellular and tissue hypoxia occurs, but the carboxyhemoglobin will often cause blood and tissues to appear "cherry red" by gross examination.

High concentrations of CO (>250 ppm) in swine farrowing houses can produce an increased number of

stillborn piglets. Stillbirths are often accompanied by a clinical history that includes very limited inadequate ventilation due to blocked apertures of natural systems or reduction to minimal winter rates for mechanical systems; use of unvented or improperly vented LP gas-burning space heaters; a high percentage of near-term sows delivering dead piglets within a few hours of being put in an artificially heated farrowing facility; sows that appear clinically normal but that produce whole litters born dead; and negative laboratory results for the detection of infectious causes of abortion (Carson 1990). Exposure to high levels of CO can be confirmed by actually measuring the CO level in the air or by measuring the percentage of carboxyhemoglobin in the blood of the affected animals. In addition, perhaps most common diagnostic technique is to demonstrate carboxyhemoglobin concentration of greater than 2% in fetal thoracic fluid for diagnosis of CO-induced stillbirth in swine (Dominick and Carson 1983).

Anhydrous ammonia

On occasion, swine may be exposed to anhydrous ammonia (gas-NH_3) used as an agricultural fertilizer nitrogen source. This gas presents a unique risk of exposure to both animals and people because of its presence on farms and the fact that it is stored, transported, and applied under high pressure. Poisoning with gas-NH_3 is associated with gas release from broken hoses, failure of valves, and errors in operating transport or application equipment. Once released, gas-NH_3 rapidly combines with water and forms caustic ammonium hydroxide. The cornea, mouth, and respiratory tract are high in moisture and especially susceptible to the resulting strong alkali burns. Acute death from laryngospasm and accumulation of fluid in the lungs can occur within a matter of minutes. Blindness from corneal opacity and sloughing epithelium in the respiratory tract may be seen in swine surviving initial exposure. Residual respiratory damage and secondary bacterial invasion may not allow affected animals to regain full productive status.

References

Adams HR. 1996. In Adams HR, ed. *Veterinary Pharmacology and Therapeutics*, 7th ed. Ames, IA: Iowa State University Press.

Anderson DM, Strothers SC. 1978. *J Anim Sci* 47:900–907.

Arpi T, Tollerz G. 1965. *Acta Vet Scand* 6(4):360–373.

Carpenter JA, Charbonneau G, Josephson G. 2005. *J Swine Health Prod* 13(6):333–336.

Carson TL. 1990. Carbon monoxide–induced stillbirth. In Kirkbride CA, ed. *Laboratory Diagnosis of Livestock Abortion*, 3rd ed. Ames, IA: Iowa State University Press, pp. 186–189.

Casteel SW, Osweiler GD, Cook WO, et al. 1985. *J Am Vet Med Assoc* 186:1084–1085.

CCREM (Canadian Council of Resource and Environment Ministers). 1987. Canadian water quality guidelines.The Task Force on Water Quality Guidelines of the Canadian Council of Resource and Environment Ministers.

Curtis SE. 1983. *Environmental Management in Animal Agriculture*. Ames, IA: Iowa State University Press.

Dominick MA, Carson TL. 1983. *Am J Vet Res* 44:35–40.

Drummond JG, Curtis SE, Simon J. 1978. *Am J Vet Res* 39:211–212.

Drummond JG, Curtis SE, Simon J, et al. 1980. *J Anim Sci* 50:1085–1091.

Drummond JG, Curtis SE, Meyer RC, et al. 1981a. *Am J Vet Res* 42:963–968.

Drummond JG, Curtis SE, Meyer RC, et al. 1981b. *Am J Vet Res* 42:969–974.

Eisele GR. 1986. *Vet Hum Toxicol* 28:118–122.

Emerick R. 1974. Consequences of high nitrate levels in feed and water supplies. *Fed Proc* 33:1183.

Emerick R, Embry LB, Seerly RW. 1965. *J Anim Sci* 24:221–230.

Enouri S, Dekroon K, Friendship R, et al. 2015. *J Swine Health Prod* 23(6):298–305.

Ensley SM. 1998. Relationships of swine water quality to cost and efficiency of swine production. Master of Science thesis, Iowa State University, Ames, Iowa.

Harrison LH, Colvin BM, Stuart BR, et al. 1983. *Vet Pathol* 20:265–273.

Haschek WM, Beasley VR, Buck WB, et al. 1989. *J Am Vet Med Assoc* 195:613–615.

Kingsbury JM. 1964. *Poisonous Plants of the United States and Canada*. Englewood Cliffs, NJ: Prentice-Hall.

Lassen ED, Buck WB. 1979. *Am J Vet Res* 40:1359–1364.

Link RP. 1975. Toxic plants, rodenticides, herbicides, and yellow fat disease. In Dunne H, Leman AD, eds. *Diseases of Swine*, 4th ed. Ames, IA: Iowa State University Press, p. 861.

Lloyd WE. 1978. Feed additives toxicology. Unpublished data, Iowa State University.

London WT, Hendersen W, Cross RF. 1967. *J Am Vet Med Assoc* 150:398–402.

Lundeen T. 2017. Feed Additive Compendium. http://www.feedadditivecompendium.com/.

Monshouwer M, Renger T, Witkamp M, et al. 1996. *Toxicol Appl Pharmacol* 137:2:237–244

National Research Council (NRC). 1974. *Nutrients and Toxic Substances in Water for Livestock and Poultry*. Washington, DC: National Academy Press.

National Research Council (NRC). 1979a. *Committee on Medical and Biologic Effects of Environmental*

Pollutants, Subcommittee on Ammonia. Ammonia. Baltimore: University Park Press.
National Research Council (NRC). 1979b. *Committee on Medical and Biologic Effects of Envi ronmental Pollutants, Subcommittee on Hydrogen Sulfide. Hydrogen Sulfide*. Baltimore: University Park Press.
National Research Council (NRC). 2005. *Mineral Tolerance of Animals: Second Revised Edition*. Washington, DC: The National Academies Press.
National Research Council (NRC). 2012. *Nutrient Requirements of Swine: Eleventh Revised Edition*. Washington, DC: The National Academies Press.
O'Donoghue JG. 1961. *Can J Comp Med Vet Sci* 25:217–219.
Osweiler GD. 1978. *Am J Vet Res* 39:633–638.
Osweiler GD, Hurd JW. 1974. *J Am Vet Med Assoc* 64:165–167.
Osweiler GD, Buck WB, Bicknell EJ. 1969. *Am J Vet Res* 30:557–577.
Osweiler GD, Carson TL, Buck WB, et al. 1985. *Clinical and Diagnostic Veterinary Toxicology*, 3rd ed. Dubuque, Iowa: Kendall/Hunt.
Paterson DW, Wahlstrom RC, Libal GW, et al. 1979. *J Anim Sci* 49:664–667.
Pond WG, Maner JH. 1984. *Swine Production and Nutrition*. Westport, CT: AVI Publishing Co.
Power SB, Donnelly WJC, McLaughlin JG, et al. 1989. *Vet Rec* 124:367–370.
Ridder WE, Oehme FW. 1974. *Clin Toxicol* 7:145.
Rosendale M. 2004. Bronchodilators. In Plumlee KH, ed. *Clinical Veterinary Toxicology*. Philadelphia, PA: Mosby Inc, pp. 305–307.
Schipper IA. 1961. *Am J Vet Res* 22:401–405.
Seerley RW, Emerick RJ, Embry LB, et al. 1965. *J Anim Sci* 24:1014–1019.
Shelton NW, Tokach MD, Nelssen JL, et al. 2011. *J Anim Sci* 89(8):2440–2451.
Stuart BP, Cole RJ, Gosser HS. 1981. *Vet Pathol* 18:368–383.
Sturos MJ, Robbins RC, Moreno R, et al. 2016. *J Swine Health Prod* 24(4):205–211.
Thompson DJ. 1980. *J Anim Sci* 51(3):767–772
Turner CA, Kienholz EW. 1972. Nitrate toxicity. *Feedstuffs* 44:28–30.
U.S. Environmental Protection Agency (USEPA). 1973. Proposed criteria for water quality: Quality of water for livestock. *Environ Rep* 4(16):663.
U.S. Environmental Protection Agency (USEPA). 1975. Primary drinking water proposed interim standards. *Fed Regis* 40(51):11990.
U.S. Food and Drug Administration (FDA). 2015. FDA announces pending withdrawal of approval of nitrasone. https://wayback.archive-it.org/7993/20170406075820/https://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm440668.htm.
Van der Molen EJ. 1988. *J Comp Pathol* 98:55–67.
Van Vleet JF, Meyer KB, Olander HJ. 1974. *J Am Vet Med Assoc* 165:543–547.
Van Vleet JF, Runnels LJ, Cook JR, Scheidt AB. 1987. *Am J Vet Res* 48:1520–1524.
Veenhuizen M. 1993. *J Am Vet Med Assoc* 202:1255–1260.
Veenhuizen M, Shurson GC, Kohler EM. 1992. *J Am Vet Med Assoc* 201:1203–1208.
Yan M, Kandlikar G, Jacobson L. et al. 2014. *Trans ASABE* 57:907–914.

Index

Note: Pages references in *italics* refer to Figures; those in **bold** refer to Tables

a

Abdominal cavity, necropsy 104–105
Abdominal surgery 188–190, *189*
Abortion
  diagnosis 384–91, **389–390**
  fetal tissue sampling 387, **387**
  serology 386–387
  specimen collection 106–107, **106**
Abscesses
  Staphylococcus aureus 930–931
  Streptococcus *porcinus* 946
  Trueperella (Arcanobacterium) pyogenes 997–998
Acanthocephala 1034
Acceptable level of risk (ALOP) 140
Acepromazine 173, **174**
Acquired immune response 264, 268, 269, 270
Actinobacillus equuli 763
Actinobacillus lignieresii 758
Actinobacillus minor 750, 763
Actinobacillus pleuropneumoniae 283–284, 749–762
  clinical signs 754–755
  diagnosis 756–760
  epidemiology 750–752
  etiology 749–750
  immunity 754
  lesions 755–756, *755*, *756*
  pathogenesis 752–754
  prevention and control 760–762
  relevance 749
  treatment 760
Actinobacillus porcinus 750, 763
Actinobacillus porcitonsillarum 750, 763
Actinobacillus rossii 763
Actinobacillus suis 762–763
Actinobaculum (*Eubacterium*) *suis* 981–982
Actinomyces hyovaginalis 982–983
Actinomyces pyogenes, *see Trueperella pyogenes*
Actinomyces suis 188
Adaptive immunity 271–272
  in fetus 281–282
Additives, feed 1074–1076
Adenomatosis, *see* Proliferative enteropathy
Adenoviruses 438–441
  clinical signs 440
  diagnosis 440–441
  epidemiology 439
  etiology 438–439, *439*
  immunity 441
  pathogenesis 439–440
  prevention and control 441
  public health 439
  relevance 438
Adenylate cyclase toxin (ACT) 770
Adhesins 746
Adjuvants 286, **286**
Adverse effects **160–161**
Affective states 20
Aflatoxicosis 257, 1058–1060
Aflatoxins 1055, 1058–1060, **1063**, **1064**
African swine fever virus (ASF) 443–50, 1013
  clinical signs 446–447, *447*
  diagnosis 448–449
  epidemiology 444–446
  etiology 443–444, *444*
  immunity 449
  lesions 447–448, *448*
  pathogenesis 446
  prevention and control 449–450
  public health 443
  relevance 443
Agalactia. *See* Mammary system
Agar gel immunodiffusion (AGID) 75
Aggression 27–28
  maternal behavior 21–22
  miniature pet pigs 216–217
  savaging 22
  tail biting 27
  See also specific behaviors
Air quality 402
Akabane virus 461–462
Alopecia. *See* Integumentary system
Alpha-2 receptor agonists 173–174
Alphaviruses. *See* Togaviruses
Amaranthus retroflexus (pigweed) 1079–1080
Amino acids 1046–1047
Aminocyclitols **160**
Aminoglycosides **160**
Amitraz 1078
Ammonia 1084, 1086
Ammonium salts 1076
Amoeba 1026
Amoxicillin 943
Amperozide 31
Amplicons 88, 91
Amputation,
  rectal 192
  of uterus 188
Anatomy and physiology
  cardiovascular system 223–225
  digestive system 234–236
  feet and claws 306
  hematopoietic system 223, **224**
  mammary glands 313, *314*
  milk production 316–324, *316*, *319*

Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson, and Jianqiang Zhang.

nervous and locomotor system 339–341, **340**
respiratory system 393–394, *394*
urinary system 408–409, *409*
Androstenone 32
Anelloviruses 453–455
clinical signs and lesions 454–455
diagnosis 455
epidemiology 453
etiology 453
immunity 455
pathogenesis 453
prevention and control 455
public health 454
relevance 453
Anemia 230–232, **231**
causes **67**, 231–232, **231**
Mycoplasma suis 876–878
types 231–232
Anemia of inflammatory disease (AID) 231
Anesthesia 171–179
epidural and regional anesthesia 177–178
inhalation agents 176–177
injectable 174–175
IV catheterization and support therapy 172–173
local anesthesia 177–178
malignant hyperthermia 171–172
miniature pet pigs 218
pain management 178–179
reversal agents 175-
see also Drug therapy
Aneurism, arterial 229
Angiotensin I converting enzyme (ACE) 47
Anguillina coli, *see Brachyspira pilosicoli*
Anhydrous ammonia 1086
Animal cruelty, defined 18
Animal Medicinal Drug Use Clarification Act (AMDUCA) 164, 198
Animal welfare. *See* Welfare and behavior
Ankylosing spondylitis 358
Ankylosis 193
Anorexia, weaning 235
Anthelmintics 163, 168, **168**, 1039–1040
Anthrax 132, 983–985
Antibodies
buffered *Brucella* antigen test 81
chicken egg yolk 167
ELISA tests 82–83
fluorescence detection 83–84
humoral immunity 285
measurement 117, 120
sample collection 99
serum virus neutralization 94–95
See also specific diseases; Immune system and immunity; Mammary system
Antibody-dependent cell-mediated cytotoxicity (ADCC) 268
Anticholinergics 172
Anticoagulant rodenticides 1079
Anticoccidials 1019
Antigen-presenting cells (APCs) 267, 268–9, 272–3
Anti-inflammatory drugs 169
Antimicrobial drugs
AASV guidelines **166**
classes of 158–62, **160–161**
prophylaxis 164
See also specific bacteria
Antimicrobial peptides (AMPs) 264
Antimicrobial resistance (AR) 165, 207
Antiparasitics 168, **168**
Aplasia cutis 305
Apophysiolysis 365
Arsanillic acid 1074
Arsenic 1073–1074
Arteriviruses 685–702
clinical signs 692–694
diagnosis 695–698
epidemiology 688–690
etiology 685–688
immunity 698–700
lesions 694–695
pathogenesis 690–692
prevention and control 700–702
public health 688
relevance 685
See also Porcine reproductive and respiratory syndrome (PRRS) virus
Arthritis 220, 359–361
acute fibrinous 360
erysipelas 299, *299*, 352, 361–2, 835–841
Glässer's disease 352, 844–851
infectious 359, 366
mycoplasmosis 871–873
polyarthritis 361
specimen collection 107–109
streptococcosis 935, 940, 946–947
surgical procedures 193
Trueperella (Arcanobacterium) pyogenes 997–998
Arthrogryposis 351
Arthrosis/arthropathy 366
Articular epiphyseal cartilage complex (AECC) 358–359, 365
Artificial insemination (AI)
semen collection and evaluation 384, **384, 385**
Ascariasis 168
Ascaris suum 1028, 1031–1033, *1031–1032*
egg *1030*
Ascarops strongylina 1029, *1030*
Aspergillus spp. 1058–1060
Astroviruses 457–460, *459*
diagnosis 460
epidemiology 458
etiology 457, *458*
pathogenesis, clinical signs, lesions and immunity 458
prevention and control 460
public health 458
relevance 457
Asymmetric hindquarter syndrome 363
Ataxia 353, 354
Atherosclerosis 229
Atipamezole 174
Atresia ani 191
Atrophic rhinitis
non-progressive (NPAR). *See* Bordetellosis
progressive (PAR). *See* Pasteurellosis
Atropine sulfate 172
Attaching and effacing *E. coli* (AAEC) 810, *810*, 819–20
Aujeszky's disease virus (ADV) 117, *128*, 131, 551–561, *552*, *557*, *558*, 737, 1067
See also Pseudorabies virus
Auricular vein. *See* Ears and ear veins
Australis infection 856
Autogenous vaccines 286–287
Autopsy. *See* Necropsy
Average daily gains (ADGs) 322, 324, 330, 1028
Avermectin 1039
Avidin–biotin complex (ABC) 85
Azaperone 24, 31, 173

b

B cells 275
  development *275*, *276*
Bacillus anthracis 132, 983–985
Bacteria, overview 745–747
  Gram-negative **746**, **748**
  Gram-positive **746**, **747**
  See also specific bacteria
Bacteriophages 167
Balantidium coli 1026
Barbiturates 175
Barriers, immune system 267, *267*
Basophils 268
Behavior
  drinking 26, 27
  feeding 26, 27
  nursing sows 323
  oral and locomotor 27–29
  sickness 33
  welfare. *See* Welfare and behavior
Belly nosing 27–28
Benchmarking
  animal welfare 20–21
  herd evaluation 4, 4
Benzimidazoles 1040
Benzodiazepines 173
Best linear unbiased prediction (BLUP) 45
Beta-lactams **160**
Bifidobacterium 239
Big data approach 118
Bioassay 81
Biological risk management (BRM) 136–137
Biosecurity 7, 136–154
  disease management 127, 142
  downtime 145
  evidence-based principles 153, *154*
  failures 141–54
  feed 148
  international level 140–141
  mechanisms of disease occurence. *See* Mechanisms of disease occurrence
  national level 139–140
  pathogens, emergence of **137**
  planning 136–141
  quarantine 144
  respiratory disease control 403
  risk analysis framework *140*
  show pigs 212
  transportation 144–145
  zoonotic diseases **31**. *See also* Zoonotic diseases
Biotin (vitamin B7) 310, 1052
Black nightshade 1080
Blackleg 801–802
Bladder displacement 183
Blood sampling 14–15, *14*, *15*, 229
Blood values, see Cardiovascular system
Bloody gut 249–250, 251
Blow flies 1013
Blue eye disease 352, 594–599, *595*, *598*
B-lymphocytes 271–272
Boar
  overuse 383–384
  reproduction 382–384
  semen collection **384**
  semen quality 384, **385**
Body cavity diseases 230, **230**
Body condition score (BCS) 8, 9
Body fluid parameters **230**
Bone ash analysis 1048
Bone density 103
Bones
  diseases affecting **342**, 350
  examination 342
  normal structure and function 339–41
  overview of 339–41
  physeal closure times **340**
  rickets, metabolic disease 342, 355–356, **356**
Border disease viruses (BDV) 633–636
  See also Pestiviruses
Bordetella bronchiseptica 767–775
Bordetellosis, pneumonia and non-progressive atrophic rhinitis (NPAR) 767–775
  clinical signs 771
  diagnosis 773
  epidemiology 768–769
  etiology 767–768
  immunity 773–774
  lesions 771–773, *772*
  pathogenesis 769–773
  prevention and control 774–775
  public health 768
Botulism 364, 803–804
Bovine tuberculosis (bTB)
Bovine viral diarrhea virus (BVDV). *See* Border disease virus (BDV)
Brachyspira (species overview) 951–3, *952*, **953**
Brachyspira hampsonii. See Swine dysentery
Brachyspira hyodysenteriae. See Swine dysentery
Brachyspira innocens **953**
Brachyspira intermedia 951, **953**
Brachyspira murdochii 951, **953**, 962
Brachyspira pilosicoli 961–966
  See also Porcine Intestinal/Colonic Spirochetosis (PIS/PCS)
Brachyspira pilosicoli. See Porcine Intestinal/Colonic Spirochetosis (PIS/PCS)
Brachyspira suanatina. See Swine dysentery
Brachyspiral colitis 961–966
Brambell Commission 17
Breeding, show pigs 215
Bromethalin 1079
Bronchopneumonia 398–400, *398*, **399**
Bronchus-associated lymphoid tissues (BALTs) 279
Brucella-containing vacuole (BCV) 783
Brucellosis (*Brucella* spp.) 778–788
  abortion 388
  buffered antigen test 81
  clinical signs 784
  diagnosis 785–786
  epidemiology 779–782
  etiology 779, **780**, **781**
  immunity 786–787
  lesions 784–785
  pathogenesis 783–784
  prevention and control 788
  public health 782–783
  relevance 778
Buffered *Brucella* antigen test (BBAT) 81
Bungowannah virus 630–633
  See also Pestiviruses
Bunyavirusesy 461–462
Burkholderia pseudomallei 985–986

c

Cachexia 226
Calcium 1047–1048
Caliciviruses 464–471, *468–469*
  sapo- and noroviruses 466–471, *466*, *468–469*
  St-Valérian virus 471
  vesicular exanthema of swine virus 464–466

Calluses 303–304
Campylobacter spp. 200, 239, 986–988
Canadian Integrated Program for Antimicrobial Resistance (CIPARS) 165
Candida albicans 301
Canicola infection 856–857
Canine tooth. *See* Tusk
Capsular polysaccharide (CPS) 934
Captan 1078
Carbadox 1075
Carbamate insecticides 1076–1078
Carbon dioxide 1085
Carbon monoxide poisoning 232, 354, 388, 1085–1086
Cardiac pathology 225–226, **225**
Cardiogenic shock 230
Cardiovascular system
  anatomy and physiology 223–224
  body cavities, diseases of 230
  cardiac pathology 225
  compensatory mechanisms 228
  conduction system, diseases of 228
  congenital anomalies 225–226, **225**
  endocardium, diseases of 228
  heart failure 228–229
  myocardium, diseases of 226–228
  neoplasia 232
  pericardium, diseases of 225
  postmortem exam 224–225
  vascular pathology 229–230
  See also Hematopoietic system
Castration 23–24, 179–180
Catheterization 172–173, *172*
Causality 112–122
Cell-mediated immunity
  overview 272–274
  respiratory system 395
Cellulitis and gas gangrene 799–802, *800*
  See also Clostridiosis
Central nervous system (CNS), necropsy 107
  See also Nervous and locomotor system
Cereals and fiber 239–240
Cerebellar abiotrophy 350
Cerebellar hypoplasia 350
Cerebellum 344
Cerebral cortex 344
Cesarean section
  anesthesia 173
  surgical procedure 184–187, *186*
Chemical hazards, pork consumption 198–199
Chemistry testing 92–93
Chlamydia spp. 989–991
Chlamydophila spp. 989–991
Chloride 1047
Chlorinated hydrocarbons (CH) 1078–1079
Chlorine dioxide 242
Chlortetracycline 869
Cholecalciferol 1079
Choline 1052–1053
Chromated copper arsenate (CCA) 1078
Chrysomya bezziana 1012
Circoviruses 473–483
  epidemiology 475–476
  etiology 473–475, *474*
  immunity 482–483
  pathogenesis, clinical signs and lesions 476–482, **477**, *478*, *479*, *481*
  porcine circovirus type, 2 reproductive disease 480–481
  porcine circovirus type, 2 systemic disease 300, 473–475, 476–480
  porcine dermatitis and nephropathy syndrome (PDNS) 300–301, *300*, *301*, 412, 481–482, *481*
  prevention and control 482
  public health 475
  relevance 473
Citrinin **1057**, 1060, **1064**, 1076
Classical swine fever (CSF) 623–630. *See also* Pestiviruses
  clinical signs 625
  diagnosis 627–628
  enteric component 255
  epidemiology 623–624
  etiology 623
  immunity 628–629
  integumentary system 292
  lesions 626, *626*
  nervous and locomotor system 343, 349
  pathogenesis 624–625
  prevention and control 629–630
  relevance 623
Claviceps purpurea 229
Claw conformation 308–309
Claws and feet
  lesions
    classification, pathology and scoring 307
    lameness and 308
    prevalence 307–308, 308–310
  structure and function 306
Cleft palate 242
Clinical and Laboratory Standards Institute (CLSI) 80, 162
Clostridiosis (*Clostridium* spp.) 132, **252**, 792–804
  pathology and diagnosis **253**
Clostridium botulinum (botulism) 364, 803–804
Clostridium botulinum neurotoxin (BoNT) 803–804
Clostridium chauvoei (blackleg) 800, 801–802
*Clostridium chauvoe*i toxin A (TccA) 801
Clostridium difficile 204–205, 797–799, *798*, *799*
Clostridium difficile ADP-ribosylating toxin (CDTa) 798
Clostridium difficile toxin A (TcdA) 798, 799
Clostridium difficile toxin B (TcdB) 798–799
Clostridium difficile-associated disease (CDAD) 797–799
Clostridium novyi (sudden death) 799, 800
Clostridium novyi alpha toxin (TcnA) 799, 800
Clostridium perfringens 792–797, **793**, *793–795*
  gas gangrene 799–801
  type A enteritis **793**, 796
  type C enteritis 792–796, **793**
Clostridium perfringens alpha toxin (CPA) 792, **793**, 795, 797, 800
Clostridium perfringens beta toxin (CPB) 792, **793**, 794, 795
Clostridium perfringens beta-2 toxin (CPB2) 794, 797
Clostridium perfringens endotoxin (CPE) 796–797
Clostridium perfringens epsilon toxin (ETX) 792, **793**
Clostridium perfringens iota toxin (ITX) 792, **793**

Clostridium septicum (malignant edema) 363, 799–800, *800*
Clostridium septicum alpha toxin (ATX) 799, 800
Clostridium sordelli hemorrhagic toxin (TcsH) 800
Clostridium sordelli lethal toxin (TcsL) 800
Clostridium tetani (tetanus) 364, 802–803
Clostridium tetani tetanospasmin (TeNT) 802
Coagulation disorders 232
Coal tar pitch 1083
Coccidia 1015–1019, *1016*, *1018*
Cochliomyia hominivorax 1012
Cocklebur 1080
Colibacillosis (*E. coli* diseases) 234, 807–832
  classification, pathotypes 808–809, **808**
  coliform mastitis (CM) 828–830
  diarrhea, neonatal 812–817, **813**, **814**, **817**
  diarrhea, post weaning 817–826, **808**, **813**, **814**, **817**
  E. coli causing fatal shock 826
  E. coli systemic infections 827–828
  edema disease 817–826, **808**, **813**, **814**, **817**
  epidemiology 812
  etiology 807–811
  genetics of virulence 811
  public health, food-borne illness 199, 811–812
  relevance 807
  urinary tract infections 830–832
Coliform mastitis. *See* Colibacillosis
Colonic spirochetosis. *See* *Brachyspira pilosicoli*
Colony-stimulating factors (CSFs) 274
Colostrum,
  milk production 323–324
  composition 317–318
  passive immunity 280
  See also Mammary system and immune system
Commensal pathogens 133–134
Common immune system 279
Community-acquired *Clostridium difficile*-associated disease (CA-CDAD) 797–799
Community-associated MRSA (CA-MRSA) 930
Complement fixation (CF) 82
Complement fixation test (CFT) 81
Complement system 269–270
Complete blood counts (CBCs) 81–82
Conduction system diseases 228
Confounders 135
Congenital anomalies
  cardiac pathology 225, **226**
  cardiac rhabdomyoma 232
  integumentary system 304–305
  locomotor 347–351
  neurological 351–354
  reproductive system 374
Congenital tremor (CT) 349, **350**
Congestive heart failure 226, 228
Conjugated linoleic acid (CLA) 237–238
Conjunctivitis 352, 355
Constipation 328–329
Copper and copper sulfate 168, 229, 231, 1051, 1072
Corium 306
Coronary band abscesses (bush foot) 307
Coronaviruses 488–516, *489*
  hemagglutinating encephalomyelitis virus 513–515
  overview and types 488–492, *489*
  porcine epidemic diarrhea virus 504–510
  porcine respiratory coronavirus
  porcine torovirus 516
  transmissible gastroenteritis and porcine respiratory coronavirus 492–504
Corynebacterium equi. *See* *Rhodococcus equi*
Corynebacterium pyogenes. *See* *Arcanobacterium pyogenes*
Cottonseed meal (CSM) 1083–1084
Cresol toxicity 257
CRISPR technology 45
Critical control points (CCP) 138, 144
  in feed production 1043–1045
Cross-foster piglets 325
Cruelty, defined 18. *See also* Welfare and behavior
Cryptorchidism 180–181, 219
Cryptosporidium spp. 1022–1023, *1023*, **1024**
Cutaneous spirochetosis 995–996
Cutoff threshold (CT) 120
Cutoff values 115, 120
Cyanosis *299*
Cystic kidney. *See* Urinary system
Cysticercosis 1037–1038
Cysticercus cellulosae 1037–1038
Cystitis–pyelonephritis complex 417–4, *418*
  See also Urinary tract infection (UTI)
Cystoisospora (previously *Isospora*) *suis* 1015–1019, *1016*, *1018*
  life cycle 1015, *1016*
  immunity to 1015–1016
  clinical signs 1016
  pathologic changes 1016–1017
  oocysts *1017*
  diagnosis of 1017–1018, *1018*
  epidemiology 1018–1019
  treatment and control 1019
Cystotomy 183
Cysts, valvular 228
Cytokines 269, 271
Cytolethal distending toxin (CDT) 845
Cytomegalovirus. *See* Herpesviruses
Cytopathic effect (CPE) 437
Cytotoxic necrotizing factor (CNF) 811
Cytotoxic T lymphocytes (CTLs) 269

d

DAMNIT approach 11
Dantrolene sodium 172
Decision tree *113*
Defensins 269
Deficiencies, nutrient 1043–1053
Degenerative joint disease 343, 358
Delayed-type hypersensitivity reaction (DTH) 786
Demodectic mange (follicular mange) 302, 1009
Dendritic cells (DCs) 268
Dentition
  development, feeding and drinking and 26
  disease, in miniature pet pigs 220
  tusk removal 194
  tusk trimming 24–25
Deoxynivalenol (DON), **1057**, **1063**, **1064**
Dermatitis, see Integumentary system
Dermatosis vegetans 305

Desferrioxamine (Desferal) 1073
Desmoglein (Dsg1) 928
Dewclaws (DCs) 306
Dexamethasone 185
Diacetoxyscirpenol (DAS) 1061
Diagnosis
  application and examples 116
  differential. *See* Differential diagnosis
  herd evaluation 11–13, *12*, *13*
  multiple tests 118
  process 114–15
  sensitivity and specificity 115–16
  submissions 116–118
Diagnostic testing 75–96, 112–122. *See also* Sample collection
  agar-gel immunodiffusion 75
  bacterial culture and anitmicrobial sysceptibility testing (AST) 75–81
  buffered Brucella antigen test (BBAT) 81
  chemistry testing 94
  clinical pathology 81–82
  complement fixation 82
  cutoff values 115, 120
  DNA sequencing 91
  electron microscopy 82
  ELISA 82–83
  fecal flotation 87
  fluorescence polarization assay 81
  fluorescent antibody detection 83–84
  fluorescent microsphere immunoassay 84
  hemagglutination inhibition 84–85
  immunohistochemistry 85
  immunoperoxidase monolayer assay (IPMA) 85–86
  in situ hybridization 86–87
  indirect fluorescent antibody (IFA) 85–86
  individual vs population 99–100
  microscopic agglutination test 86
  necropsy 101–110
    external examination 102
    internal examination 102–109, *106*
    safety 110
    specimen collection **104**, **105**, **106**, **108**, *109*
  parallel, testing in 118
  parasite (internal) identification 87
  problem identification 98–99
  polymerase chain reaction (PCR) 87–90, 431
  prevalence estimation 119–120
  selection and collection 100–109
    antemortem 100–101
    postmortem 101–102
  sequencing (nucleic acid) 91–4, **93**
  serial, testing in 118
  serum virus neutralization 94–95
  testing purpose 100
  toxicology/chemistry testing 94
  virus isolation 94
Diarrhea
  causes, by age **60–61**
  herd evaluation 6, 10, *13*
  oral rehydration 242
  mechanisms **251**
  specimen collection **105**
Diazepam 173, **173**, **174**
Dichlorvos **168**, 1040
Diet. *See* Nutrition
Dietary fiber 239–40
Differential diagnosis 59–74
  digestive system 59, **60–62**
  gastrointestinal conditions **252**
  hemopoietic system 59, **67**
  integumentary system 59, **65**, **66**
  nervous and locomotor system 64, **68–69**, **70**
  overview 59
  reproductive system 64, **71**, **72–73**
  respiratory system 59, **63–64**
  urinary system **421**
  zoonotic diseases 64, **74**
Digestive system 234–258
  anatomy and histology 234–236
  diarrhea **251**. *See also* Diarrhea
  dietary interventions 239–242
  esophageal diseases 243
  gastric ulceration 243–246, *244*
  gut flora 238–239
  helminths 1028–1035, **1039**
  immunology 236–239
  intestinal tract 247–256, *229*, *250*, **251**, **252**, **253**
  liver conditions 256–257, *257*
  oral cavity diseases 242–243
  oral rehydration, piglets 242
  pancreatic conditions 258
  pathology and diagnosis **253**
  peritoneal cavity 255–256
  physiology 236
  stomach diseases 246–247
  suckling pigs, diseases in 252–254, *254*
  weaned pigs, diseases in 254–255
Digit amputation 193
Dimetridazole 1075
Dioxins 199
Direct-fed microbials (see probiotics) 167
Discharge, vulvar 374
Disease ecology 124–125. *See also* Transmission
Disease transmission. *See* Transmission
Disinfectants 1084
Distillers dried grains with soluble (DDGS) 1055
DNA sequencing 91
DNA viruses 428, *430*, 431, 433, 437
Doramectin **168**, 1040
Dried distillers grains with solubles (DDGS) 1044
Drinking, behavior development 26–27
Drug therapy 158–169, **159.** *See also* Anesthesia
  anti-inflammatory drugs 169
  antimicrobials 158–162, **160–161**
  antiparasitics 168
  bacteriophages 167
  direct-fed microbials (probiotics) 167
  hormones 168–169
  nutrients 167–168
  objectives 158–159
  options and swine physiology 159–162, **160–161**
  outcomes, assessment of 166
  regimens, establishment of 163–166
  S.P.A.C.E.D. **159**
Dyschondroplasia 358
Dysmetria 344
Dystocia 171, 184, 381–382
  See also Cesarean section

e

Ear necrosis 297–298, *297*
  See also Staphylococcus hyicus; Treponema pedis
Ears and ear veins 15, *15*, 354
  fluid therapy *172*, 185
  See also Integumentary system

Eastern equine encephalitis virus (EEEV) 740–741
See also Togaviruses
Ebola viruses. *See* Filoviruses
Echinococcus spp. 1036–1037
Ecology of disease 124–125
Ectopic testicle 180–181
Edema 230, 294
of stomach wall 247
Edema disease (ED) 226
Edema disease *E. coli* (EDEC) 807, 808, **808**, 810–811, 812, 817–826, *820*
See also Colibacillosis
Eimeria spp. 1015
epidemiology 1018
oocysts 1017
See also Coccidia
Electron microscopy (EM) 82–3
viruses 431
Embolic nephritis 416–417, *416*
Embolic pneumonia **399**, 400–401
Emotional states 20
Encephalitis, see Nervous and locomotor system
Encephalitozoon spp. 1025–1026
Encephalomyocarditis (EMC) virus 351–352, 645, 664–668, *667*
Endemic disease 126–127, *128–129*
Endocardium, disease of 228
Endometritis 373, 374–375, 376, 380, 381
Entamoeba spp. 1026
Enteroaggregative heat-stable toxin 1 (EAST1) 809
Enterococcus spp. 992
Enterocytozoon bieneusi 1025–1026, *1025*
Enterohemorrhagic *E. coli* (EHEC) 808, **808**, 810–811
Enteropathogenic *E. coli* (EPEC) 808, **808**, 810, *810*, 811, 812, 818, 819–820, *820*
Enteropathy, proliferative 898–908
clinical signs 903
diagnosis 905–906
epidemiology 900–901
etiology 898–899
immunity 906–907
lesions 903–905, *904*
pathogenesis 901–903
prevention and control 907–908
public health 899
relevance 898
Enterotoxigenic *E. coli* (ETEC). *See also* Colibacillosis
control **817**
diagnosis 815
dietary practices 239, 240
differential diagnosis **252**
etiology **808**, 809–810, *809*
immune system development 237, 252
immunity 816
neonatal 812–817
pathogenesis 812–814, 818–820
pathology and diagnosis **253**, 821, 822–3, 826, 827
post weaning 817–826
rehydration 242
Enterovirus. *See* Picornaviruses
Environment, effect on health 50–56
coccidia 1018
feeder space 54, **54**
flooring and foot/claw lesions 309–310
humidity 51
noise 56
pen space 53–54
postpartum dysgalactia syndrome 326–327
respiratory transmission 401–402
skin necrosis and trauma 303
stray voltage 56
temperature 50–51, *51*
ventilation **51**, **52**, 1084–1086
ventilation failure 51–53
water 54–56, *55*
weight gain 52, 53
Environmental enrichment (EE)
aggression, redirecting 28
suckling devices 28
Enzootic pneumonia. *See Mycoplasma hyopneumoniae*
Enzyme-linked immunosorbent assay (ELISA) 120, *121*, 122, 431, 432, 1059. *See also specific diseases*
Eosinophils 268
Eperythrozoon suis. *See Mycoplasma suis*
Epididymectomy 182
Epidural anesthesia 177–178
Epiphysiolysis 365
Epitheliogenesis imperfecta 305
Ergot 229, **1057**, **1063**, 1066
Erysipelas 299, *299*, 352, 360–361, 835–841
clinical signs 837
lesions 838–839, *838*, *839*
diagnosis 840–841, **840**
epidemiology 836
etiology 835
immunity 841
pathogenesis 836–837
prevention and control 841
public health 835–836
relevance 835
Erysipeloid 836
Erysipelothrix inopinata. *See* Erysipelas
Erysipelothrix rhusiopathiae *228*
Erysipelothrix rhusiopathiae. *See* Erysipelas
Erysipelothrix tonsillarum. *See* Erysipelas
Erythropoiesis 231
Escherichia coli. *See* Colibacillosis
Esophagus
diseases of 243
helminths in 1028
Essential minerals 1072–1073
Essential oils 241
Estimation, prevalence 119–120
Estrogens 314
Ethylene glycol 1083
Eubacterium suis. *See Actinobaculum suis*
Euthanasia 34–35, 102
Evaluation
of herds. *See* Herd evaluation
of individual pigs 6–9, 7, 8, 9
Examination. *See* Evaluation
Excessive nutrients 1043–1053
Exogenous hormones 322–323
Exotoxins 747
External parasites 1005–1013, **1008**
demodectic mange 1009
fleas 1010–1011
flies 1011–1013
lice (pediculosis) 1009
mosquitoes 1011
sarcoptic mange (scabies) 1005–1009
Extraintestinal pathogenic *Escherichia coli* (ExPEC) 808, 811, **813**, 827, *828*
Extra-label drug use) 164, 198

Exudative epidermitis (EE) impetigo contagiosa, seborrhea contagiosa) 297, *297*, 926–930, *929*
See also Staphylococcosis
Eyes 355
clinical signs and diseases **346**
neurological examination 344

f

Facial necrosis (facial pyemia) 298–299
Farm Animal Welfare Council 17
Farrowing
pre-farrowing behavior 21
rates 375, 378, 379–80
Fasciola hepatica 1036
Fat sow syndrome (FSS) 326
Fatigued pig syndrome 29–30
Fatty acids, dietary 236, 238
Fecal flotation 87
Feed
additives 1074–1076
analysis 1045–1046, **1046**
production, critical control point in 1043–1045
Feed to gain ratio (F/G) 1028
Feedback 21
Feeders *52*
space 54
Feeding. *See also* Nutrition
behavior development 26–27
drug treatment route 163–164
milk production 322
Feet and claws
structure and function 306
lesions
classification, pathology and scoring 307
prevalence 307–308, 308–310
lameness and 308
overgrown hooves 310
Fenbendazole **168**, 1039, 1040
Fermented liquid feeds 241–242
Fetuses and neonates. *See also* Piglets
immunity 281–282
pain management in 25
Fever, diagnostic considerations 99–100
Fiber and cereals 239–240
Fibrinoid necrosis 229
Field investigation process *12*
Filoviruses 524–528
Ebolavirus 527–528
etiology 524–5, *525*
public health 525
relevance 524
Reston virus 525–526
Fipronil 1078
'Five freedoms 17
Five production inputs model 11–13, *12*, *13*
Flank biting. *See* Integumentary system
Flaviviruses 431, 530–540. *See also* Pestiviruses
Japanese encephalitis virus 206, 384, 531–535, *533*
Murray Valley encephalitis virus 538–538
overview 530–531
West Nile virus 535–538
Fleas 302, **1008**, 1009–1011
Flies **1008**, 1011–1013
Flooring, foot/claw lesions 309–310
Fluid therapy 172, *172*
Flunixin meglumine 169
Fluorescence polarization assay (FPA) 81
Fluorescent antibody (FA) 431
Fluorescent antibody (FA) detection 83–84
Fluorescent focus neutralization (FFN) assay 95
Fluorescent *in situ* hybridization (FISH) 957, 963
Fluorescent microsphere immunoassay (FMIA) 84–85, 432
Fluorine 1074
Fluoroquinolones **161**, 162
Folacin (vitamin B9) 1053
Folic acid 1053
Food Animal Residue Avoidance and Depletion (FARAD) Program and Databank 164, 198
Foodborne disease risks 197–203
biological hazards 199–203
chemical hazards 198–199
physical hazards 197–198
safe practices 207
Foot-and-mouth disease (FMD) 645–659
clinical signs 652–654
diagnosis 655–656
epidemiology 647–651, *650*
etiology 646, 733, 735
immunity 656–657
landscape epidemiology 124
lesions 654–655, *653*
pathogenesis 651–652
prevention and control 657–659
public health 646–647
relevance 645–646
Foot-and-mouth disease virus (FMDV 270
Forebrain 344
Foreign bodies, gastric 247
Formamidines 1078
Four-circle approach, herd evaluation 5–11, 5, 7, 8, 9
Fracture repair 193–194
Fractures 365–366
Frequency, of disease 125–126, **125**
Frostbite 303
Fumonisins 1055, **1057**, 1066–1068, **1063**, **1064**
Fungal diseases 301–302
Fungi. *See* Mycotoxins
Fungicides 1078
Fusarium 1061, 1064, 1066

g

Galactopoiesis *316*
Gas gangrene 799–801
Gases, toxic 1084–1086
Gastric ulceration
diet and 239
differential diagnosis **252**
disease and pathology 243–246
pathology and diagnosis **253**
surgery 191
Gastric venous infarction 247
Gastric volvulus 249–250
Gastritis 247
Gel-based polymerase chain reaction 88
Genetic factors
disease resistance 43–44
genetic advancements 44–45
immune response 44
longevity 45–47
nursery-finisher mortality 43–45
pre-weaning mortality 42–43
sow productive lifetime 45–47
Genitourinary surgery 179–184, *180*
bladder displacement 183
castration 23–24, 179–180
epididymectomy 182
oopherectomy 183–184
persistent frenulum 182
preputial diverticulum 181
preputial prolapse 181–182

Genitourinary surgery (*cont'd*)
prolapsed penis 181
urethral obstruction 183
vaginal prolapse 182–183
vasectomy 182
Getah virus (GETV) 741
Giardiasis 1023–1025, *1023*
Glässer's disease 352, 844–851
clinical signs 847–848
diagnosis 849
epidemiology 845–847
etiology 844–845, **845**
immunity 849–851
lesions 848–849, *848*
pathogenesis 846–847
prevention and control 851
relevance 844
Globocephalus urosubulatus *1030*, 1034
Glomerulonephritis (GN) 409, 411–413, 4*13*
Glutamine 237
Glutathione peroxidase 227
Gnathostoma spinigerum 1029
Gnats 1012
Gnotobiotic pigs 187
Gongylonema pulchrum 1028, 1029, *1030*
Gossypol 1083–1084
Granulocyte–macrophage CSF (GM-CSF) 274
Granulocytes 267
Greasy pig disease 297, 926–930, *929*. *See also* Staphylococcosis
Grippotyphosa infection 857
Guaifenesin 175
Gut flora 238–239
Gut-associated lymphoid tissues (GALTs) 272

h

Haematopinus suis 302, 1009–1010
Haemopericardium 225–226
Haemophilus parasuis. *See* Glässer's disease
Haemophilus pleuropneumoniae. *See Actinobacillus pleuropneumoniae* (App)
Hardjo infection 857
Hazard analysis and critical control point (HACCP) methodology 137–138, 139, 144, 151
Healthcare-associated MRSA (HA-MRSA) 930
Heart failure 228–229
Heart rate 8
Heart, postmortem examination of 224–225
Heat stress 50
Heat-liable enterotoxin (LT) 809
Heat-stable enterotoxin (ST) 809
Heel lesions 307
Helicobacter suis 246
Helminths 1028–1040
digestive system 1028–1035, *1030–1033*, **1039**
liver and pancreas 1036–1037
musculoskeletal system 1037–1038
prevention 1038–1040
respiratory system 1035–1036
treatment 1038–1040
urinary system 1037
Hemagglutinating encephalomyelitis virus (pHEV) 488, 513–515. *See also* Coronaviruses
Hemagglutination (HA) test 433
Hemagglutination inhibition 75, 83, 84–85
Hemangiomas 232
Hemangiosarcomas 232
Hematopoietic system
anatomy and physiology 223
diseases of 230–232
neoplasia 232
Hemoglobin disorders 232
Hemolytic anemia 231–232
Hemopericardium 225–226
Hemorrhage
postpartum 382
urinary system 410–411, *411*
vitamin K and 1049–1050
Hemorrhagic anemia (HeA) 231–232
Hemorrhagic bowel syndrome 249–250, 251
Hendra virus. *See* Nipah virus (NV); Paramyxoviruses
Hepatic encephalopathy 353
Hepatic lymphoma *257*
Hepatitis E virus (HEV) 205–206, 544–546
clinical signs 545
diagnosis 545–546
epidemiology 545
etiology 544
immunity 546
lesions 545, *546*
pathogenesis 545
public health 544–545
relevance 544
Hepatosis dietetica 256
Herbicides 1078–1079
Herd evaluation 3–15
benchmarks 4, 4
biosecurity 5
context 3
diagnosis, approaches to 11–13, *12, 13*
history and records 3–4, 10–11
integumentary system 293
outcomes, monitoring 13
preparation for 3–5, 4
reporting structure 4–5
reports 13
respiratory transmission 402–403
sample collection 14–15, *14, 15*
site visits, four circles of 5–11, *5, 7, 8, 9*
Herd immunity 285
Hernia 364
diaphragmatic 364
inguinal 189–90, *190*, 249
perineal 364
scrotal 189–190
umbilical 188–189, *189*, 364
Herpesviruses 548–571
classification **549**
malignant catarrhal fever 569–571, *571*
overview 548–551
porcine cytomegalovirus 561–565, *562, 563, 564*
porcine lymphotrophic herpesviruses 565–569, *566, 568*
replication cycle 550–551, *550*
structure *549*
Syud herpesvirus, 1 (pseudorabies; Aujeszky's disease) 551–561, *552, 557, 558*
High performance liquid chromatography (HPLC) 972
Histiocytes 268
Histopathology
fetal tissue sampling 387, **387**
specimen collection 101–102
History and records. *See also* Herd evaluation
herd evaluation 3–4

integumentary system 293
site visits 3–4, 10–11
Hoof trimming, miniature pet pigs 219
Hoof, overgrown 310
Hookworm 1034
Hormones 168–169
hoof integrity and 309
immune system and 282
milk production 319–320
Housefly 1011–1012
Housing. *See* Environment
Human chorionic gonadotropin (HCG) 168
Human health interface 197–207
Humidity 51
Humoral immunity 274–277
Hydatid disease 1036–1037
Hydrocephalus 350
Hydrogen sulfide 1085
Hydronephrosis 420
Hydropericardium 226
Hyostrongylus rubidus 1029, *1030*
Hyperkeratosis 294
Hypermetria 344
Hyperthermia 1084
Hypoalbuminemia 226
Hypoglycemia 349–350
Hypothalamic–pituitary–endocrine responses 282
Hypovolemic shock 230
Hypoxia 349
Hysterectomy 184, *184*

i

Icterohemorrhagiae infection 857
Ileitis. *See* Proliferative enteropathy
Imidazothiazoles 1040
Immune system and immunity 264–288
acquired immune response 264, 268, 269, 270
adaptive immunity 271–272, 281–282
antibodies. *See* Antibodies
digestive system 236–238
dysfunction and infectious agents 283–284
humoral immunity 274–277
immune dysfunction and infectious agents 283–284
immune modulation 277
in fetus and neonates 281–282, 325
innate defense mechanisms 267–269
innate immune factors 267–26
lymphocyte circulation 272–277
mucosal barrier and microbiome 264–265
mucosal immunity 277–80, **277**, *278*, *280*
natural killer cells 269–270
nutritional influences 284
passive 166–167
spray-dried animal plasma 166
stress, immunosuppression, and nutrition 282–283
swine lymphoid system 266–267
tolerance 277, 280
Toll-like receptors 270–271
vaccines 166, 284–288, **286**, 736, 737–738
Immunocastration 24
immunoglobulins (Igs) 273, 274–275
IgA 237, 238, 264, 273, 275, 276–7, 395
IgE 273, 275, 277
IgG 86, 237, 273, 275, 276, 325, 395
IgM 86, 237, 239, 273, 275, 276
classes of 276–277
Immunohistochemistry (IHC) 85
Immunoperoxidase monolayer assay (IPMA) 85–86
impetigo contagiosa 297, *297*, 926–930, *929*
See also Staphylococcosis
In situ hybridization (ISH) 86–87
Incidence risk and rate 125–126, **125**
Indirect fluorescent antibody (IFA) detection 83–84, 85–86
Infarction 411
Infection routes 123
In-feed medication 163–164
Inflammatory heart disease **226**
Influenza viruses 576–588
clinical signs 583–584
diagnosis 584–586, *585*
epidemiology 578–580
etiology 576–578
geographic distribution 580–582
immunity 586–587
lesions 584, *584*
pathogenesis 582–583, *582*
prevention and control 587–588
public health 578, *579*
relevance 576
structure *577*
transmission 580
type A virus 205, *577*
Inguinal hernia 189–90, *190*
Inhalation anesthesia 174–175
Injection sites 363
Innate immunity 267–269
cells 267–269
defense mechanisms 267–269
immune factors 269–270
neonates 281–282
physical, chemical and microbial barriers 267
Innate lymphoid cells (ILC) 270
Insecticides 254, 1076–1078
in situ hybridization 86–87, 432, 697
insulin-like growth factor binding protein (IGFBP1) 47
Integumentary system 292–310
bacterial diseases 297–301
clinical examination 293–294
congenital and hereditary diseases 304–305
examination and diagnosis 292–297
age of onset **65**, **66**, **295–296**
diagnostic tests 294–297
environmental diseases 302–304
feet and claws 306–310
fungal diseases 301–302
neoplastic diseases 305–306
nutritional diseases 304
parakeratosis and nutritional diseases 304
parasitic diseases 302
skin diseases,**65**, **66**, 297–302, *297–301*
structure and function 292
viral diseases 299–300
Interferon (IFN) inducer (poly I:C) 269
Interferons 271
IFN-α 269, 274
IFN-γ 269
interleukins
IL-1α 269
IL-2 274
IL-4 274
IL-6 271
IL-8 271
IL-10 282
IL-12 274, 282
Internal parasites, helminths 1028–1040, *1030–1033*

International Committee on Taxonomy of Viruses (ICTV) 428
Interstitial nephritis 415–416, *415*, *416*
Interstitial pneumonia **399**, 400
Intestinal adenomatosis. *See* Proliferative enteropathy
Intestinal emphysema 250–251, *250*
Intestinal hemorrhage syndrome 249–250, 251
Intestinal impaction 249
Intestinal obstruction 191, 249
Intestinal spirochetosis. *See* *Brachyspira pilosicoli*
intravenous catheterization 172–173
Intubation, orotracheal 175, 176, *176*
Involution, mammary glands 315–316
Iodine 1051
Ionophores 1075–1076
Iron 1051, 1072–1073
Iron-deficiency anemia (IDA) 231
Ischemic tubular necrosis 413
Isospora suis, see Coccidia; *Cystoisospora suis*
Ivermectin **168**

j

Japanese encephalitis virus (JEV) 206, 384, 531–535, *533*. *See also* Flaviviruses
Joints
  diseases **343**
  examination 343
  normal structure and function 341
Jugular vein 14

k

Keratosis 355
Ketamine **174**, 175
Kidney disease. *See* Urinary system
Kidney malformation 410
Kidney worms 1037
Klebsiella pneumoniae 992–993
Kyphosis/lordosis 357–358, 1047

l

Laboratory analysis. *See* Diagnostic data and analysis; Diagnostics
Lacrimation. *See* Conjunctivitis
Lactase 235
Lactational osteoporosis 356
Lactogenesis 316, *316*
Lameness
  behavior 28–9
  causes by age **70**
  foot lesions 308
  nervous and locomotor system 358–61, 364–366
  show pigs 214
  in sows 364–366
  specimen collection 107–109
  surgical procedures 192–193
Landscape epidemiology 124
Large intestine, helminths in 1034–1035, **1039**
Lasalocid 1076
Laws and legal considerations/regulation
  animal cruelty 18
  Animal Medicinal Drug Use Clarification Act (AMDUCA) 164, 198
  animal welfare 18
  food safety certification 207
  miniature pet pigs 220
Lawsonia intracellularis. *See* Proliferative enteropathy
Lead 1074
Leptin 283
Leptospira 117
Leptospirosis (*Leptospira* spp.) 86, 415, 854–861
  abortion 388
  clinical signs 858
  diagnosis 859–860
  epidemiology 855–857
  etiology 854–855
  lesions 858–859
  pathogenesis 856–857
  prevention and control 860–861
  public health 855
  relevance 854
Leukemia 232
Levamisole 1040
Levamisole hydrochloride liquid **168**
Lice (pediculosis) 302, **1008**, 1009–1010
Lidocaine 178, 179
Lincosamide **160**
Lipooligosaccharides (LOS) 953
Liquid feeding 241–242
Listeria monocytogenes 993–994
Litter size, piglet growth 324–325, **324**
Liver conditions 256–257, *257*
  helminths 1036–1037
Livestock-associated MRSA (LA-MRSA) 930–931
Local anesthesia 177–178
Locomotor system. *See* Nervous and locomotor system
Long-cycle pathogens 131–132
Lower motor neuron (LMN) disease 344
Lumbo virus 461–462
Lumbosacral epidural anesthesia 177–178
Lung flukes 1036
Lungs 394, *394*
  pathogens of 396–397, **397**
Lungworms 1035–1036
Luteinizing hormone (LH) 373
Lymphocyte circulation 272–277, *273*
  acquired immune defense mechanisms 274
  cell-mediated immunity 272–274
    antigen presentation 272–273
    clonal selection and expansion 273–274
    T1, Th2, and Treg cells 274
  humoral immunity 274–277
Lymphocytes. *See also* Immune system
  circulation 272–277, *273*
  populations 271–272
Lymphoid system 266–267
Lymphoid tissue inducer (LTi) cells 270
Lymphoma 232, 418–419, *419*
Lymphosarcoma 418, *419*

m

Macracanthorhynchus hirudinaceus *1030*, 1034
Macrocyclic lactones 1039–1040
Macrolides **161**
Macrophages 268–269
Magnesium deficiency 353
Major histocompatibility complex (MHC) 237, 271
Malignant catarrhal fever (MCF) 569–571, *571*
Malignant edema. *See Clostridium septicum*
Malignant hyperthermia 171–172
Mammary system 313–331
  mammary gland anatomy 313, *314*
  mammary gland involution 315–316

mammogenesis 313–315
mastitis 326
milk production
composition **317**, 321, **321**
control 319–321
manipulation of 322–323
physiology 316–323, *316*
piglets
growth 324–325, **324**
immune protection 325
mortality 324–325
postpartum dysgalactia syndrome 325–331, *326*, *327*
sow behavior 323
treatment, diseased sow 331
Mammogenesis and mammary development 313–316
Manganese 310, 343, 1051–1052
Mange (*Sarcoptes*) 1005–1009. *See also* Parasites
Marker-assisted selection (MAS) 45
Marmite disease. *See* Staphylococcosis
Mast cells 268
Mastectomy 188
Mastitis and coliform mastitis 325–6, 807, 811, 828–830
Mastitis-metritis-agalactia (MMA) syndrome 807
Maternal behavior 21–22
Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) 78–80
Matrix Assisted Laser Desorption/ Ionization-Time of Flight (MALDI-TOF) 78
Mechanisms of Disease Occurrence 125–126, **125**
commensal pathogens 133–134
long-cycle pathogens 131–132
resistant pathogens 132–133
short-cycle pathogens 130
vector pathogens 129–130
Medial saphenous vein 172, *172*
Melanomas 305–306
Melena 244, 251
Melioidosis 985–986
Meloxicam 169
Menadione pyrimidinol bisulfite (MPB) and menadione nicotinamide bisulfite (MNB) 1050
Menangle virus 350, 599–602, *600*
Mercury 1074
Mesenteric ossification 256
Metastrongylus spp. *1030*, 1035–1036
Methane 1085
Methicillin-resistant *Staphylococcus aureus* (MRSA) 203–204, 930–931
community-associated MRSA (CA-MRSA) 930
healthcare-associated MRSA (HA-MRSA) 930
livestock-associated MRSA (LA-MRSA) 930–931
Microangiopathic anemia 231
Microbiome 264–265, *265*
Microflora, gut 238–239
Microscopic agglutination test (MAT) 856, 859
Microsporidia (*Microsporum* spp.) 301, 1025–1026, *1025*
Midazolam 173, **173**, **174**
Milbemycins 1039
Milk ejection 318
Milk production, See Mammary system
composition **317**, 321, **321**
control of 319–321
manipulation of 322–323
measurement 318–319
milking frequency 319
patterns 319, *319*
physiology 316–323, *316*
piglet growth 323–324, **324**
sow behavior 323
Minerals
essential 1072–1073
non-essential 1073–1074
Minimum core genome (MCG) 941
Minimum inhibitory concentration (MIC) 162
MMA. *See* Mastitis, metritis, agalactia (MMA) syndrome
Modes of transmission 123
Mold. *See* Mycotoxins
Monensin 1075–1076
Monitoring of diseases 20–21, 119
Monoclonal antibodies 277
Monocytes 268–269
Mononuclear phagocytes 268–269
Mosquitoes 302, 1011. *See also* Vector-borne pathogens
MRSA. *See Methicillin*-resistant *Staphylococcus aureus*
Mucociliary apparatus impairment 395–396
Mucosal addressin cell adhesion molecule, 1 (MADCAM1) 279
Mucosal barrier 264
Mucosal defenses *266*
Mucosal immunity 277–80, **277**, *278*, *280*
common mucosal system 279
environmental influences, nutraceuticals and development of mucosal immunity 279–280
intraepithelial lymphocytes and LP immune cells 278–279
mucosal epithelium , role of 277–278
organized and diffuse mucosal lymphocytes (279
secretory Iga 279
Mucosal-associated lymphoid tissues (MALT) 279
Mulberry heart disease (MHD) 226–228, 1048, 1049
see also Cardiovascular system
Multifactorial diseases 134–135
Multilocus enzyme electrophoresis (MLEE) 953, 962
Multilocus sequence typing (MLST) 91, 953, 962
Multiple-locus variable-number tandem repeats analysis (MLVA) 953, 962
Multiplex polymerase chain reaction 89
Multispacer sequence typing (MST) 973
Murray Valley encephalitis virus (MVEV) 538–539. *See also* Flaviviruses
Musca domestica 1011–1012
Muscular hypertrophy of the ileum 250
Muscular steatosis 364
Musculoskeletal system
helminths in 1037–1038
surgery 192–194
Mutated transferrin binding protein (TbpB) 850
Mycobacterium spp. 971–979. *See also* Tuberculosis
Mycobacterium avium complex (MAC) 971, 972–973, 974, 976, 977, 978
Mycobacterium avium ssp. *silvaticum* (MAS) 973

Mycobacterium avium subsp. *avium* (MAA) 972, 973, 974, 975, 976
Mycobacterium avium subsp. *hominissuis* (MAH) 972, 973, 974, 975, 976, 979
Mycobacterium avium subsp. *paratuberculosis* (MAP) 972, 973, 975
Mycobacterium tuberculosis complex (MTBC) 971, 972, 974, 975, 976, 977
*Mycoplasma (*formerly *Eperythrozoon*) *suis* 876–878
Mycoplasma flocculare 878
Mycoplasma hyopneumoniae 283, 863–871. *See also* Porcine respiratory disease complex (PRDC)
  clinical signs 867
  diagnosis 868–869
  diagnostic test results 115
  epidemiology 864–865
  etiology 863–864
  host-pathogen environment 124
  immunity 866–867
  lameness, in show pigs 214
  lesions 867–868
  pathogenesis 865–866
  specimen collection 101
  treatment, control and eradication 869–871
Mycoplasma hyorhinis 354, 871–874
Mycoplasma hyosynoviae 874–876
Mycoplasma suis (formerly *Eperythrozoon suis*)
  hemolytic anemia 231
Mycoplasmosis 863–878
Mycotoxins 1055–1068. *See also* Feed-related disorders; Nutrition
  aflatoxins 1058–1060, **1063**
  characteristics **1057**
  clinical responses 1057
  ergot **1064**, 1066
  exposure guide to **1064**
  formation of 1055–1056
  fumonisins **1057**, 1066–1068, **1063**, **1064**
  inactivation of **1063**
  intoxication by 1056
  prevention and control 1068
  sources **1056**
  testing in grains and feed 1058
  trichothecenes **1057**, 1061–1063, **1064**
  zearalenone 388, **390**, **1057**, 1064–1065, **1063**, **1064**
Myelination 348–349
Myiasis 1012–1013
Myocarditis 226
Myopathies 362–364
Myositis ossificans 364
Myotatic reflex 344

n

Nanoparticles 286
Narasin 1075–1076
Nasal cavity 393–394
Natural killer (NK) cells 264, 269–270
Necropsy 98–110, 116
  abdominal cavity 104–105
  CNS tissue 107
  external examination 102
  heart examination 227
  internal exam 102–109, *106*
  locomotor tissue 107–108
  nervous and locomotor system 343, 344, 347, 356
  porcine septicemia **105**
  renal disease differential diagnosis **421**
  reproduction and abortion tissues 106–107
  safety 109–110
  specimen collection 103–108, **105**, **106**, **108**
  thoracic cavity 103–104
Necrosis, fibrinoid 228, 229
Necrosis, skin
  ear 297–298, *297*, *298*
  facial 298–299
Necrotic enteritis (NE) 796
Neonatal isoerythrolysis 231
Neonates. *See* Fetuses and neonates; Piglets
Neonicotinoids 1078
Neophobia 29
Neoplasia
  cardiovascular and hematopoietic 232
  hemangioma 232
  hemangiosarcoma 232
  leiomyoma 374
  lymphoma and lymphosarcoma 232, 418–19, *419*
  melanoma 305–306
  nephroblastoma (embryonal nephroma) 418
  rhabdomyoma 232
  rhabdomyosarcoma 306
Nephrotoxic tubular necrosis 413–14, *414*
Nervous and locomotor system 339–366
  bone disorders 355–359
  clinical examination of locomotor systems 341–343
  congenital conditions 347–351
  diagnostic investigation 344–347
  eye and vision 355
  hoof and claw 366
  joint disorders 359–360
  lameness 364–366
  muscle disorders 361–364
  neoplasms 366
  neurological conditions 351–354
  neurological examination 344
  normal structure and function 339–341
  vestibular disease 354
Neurocysticercosis (NCC) 203
Neurological illness **68–69**, 107, **108**. *See also* Nervous and locomotor system
Neutrophil extracellular traps (NETs) 268
Neutrophils 267
Next-generation sequencing (NGS) 431,432
Niacin (vitamin B3, nicotinamide) 1050
Nicotinamide adenine dinucleotide (NAD) 78
Nipah virus (NV) 206, 602–606, *605*
Nitrates and nitrites 1080–1081, 1082
Nodular worm 1035
Nomenclature, virus 428–30
Non-essential minerals 1073–1074
Non-esterified fatty acids (NEFAs) 329
Non-fimbrial adhesion involved in diffuse adherence (AIDA-I) 809
Non-progressive atrophic rhinitis (NPAR). *See* Bordetellosis
Non-starch polysaccharides (NSPs) 236, 238
Non-steroidal anti-inflammatory drugs (NSAIDs)
  postpartum dysgalactia syndrome 331
Noroviruses 466–471, *468–469*

Nucleic acid sequence-dependent approaches 431–432
Nucleic acid sequence-independent approaches 432
Nucleotide-binding oligomerization domain-like (NLR) 786–777
Nursing. *See* Mammary system; Milk production; Weaning
Nutrients
  drug therapy 167–168
  neurological conditions 353–354
Nutrition
  deficiencies or excesses 1043–1053, **1044**, **1046**
  dietary nutrient inclusion rates 1045
  digestive system 239–242
  hoof integrity 309–310
  immunity 284
  liquid feeding 241–242
  mammogenesis 313–314
  minerals 1072–1074
  mycotoxins. *See* Mycotoxins
  myopathy 362
  nutrient concerns 1046–1053
  postpartum dysgalactia syndrome 330
  post weaning *E. coli* and edema disease 825–826
  rickets, metabolic disease 342, 355–356, **356**
  show pigs 215
  skin diseases from 304
  weaning 235
Nutritional myopathy 362–363

o

Obesity 219
Occurrence
  of disease 125–126, **125**
  patterns 126–127, *128–129*
Ochratoxin **1057**, 1060, **1064**, 1076
Oesophagostomum spp. 1029, *1030*, 1035
Oligoystic ovarian degeneration (COD) 373
On-site records 10–11
Oopherectomy 183–184
Oral fluid collection 15
Oral medication 162, 163
Oral rehydration 242
Organic acids, *Salmonella* 241
Organomercurials 1078
Organophosphate compounds 1040, 1076–1077
Ornithodoros moubata 1013
Orotracheal intubation 175, 176
Osteochondrosis (OCD) 214, 358–359, **359**, 364
Osteodystrophies **356**
Osteomyelitis 365–366
Otobius megnini 1013
Ovariohysterectomy 219
Over-muscled sow syndrome (OMSS) 326, 329
Ovulation, failure of 380
Oxidative stress 227
Oxytetracycline 877
Oxytocin 168, 188, 382
Oya virus 461–462

p

P97 protein 865
Pain, recognition and management 25, 178–179
  See also Welfare and behavior
Palatoschisis 242
Pancreas
  conditions 258, 1036–1037
  helminths in 1036–1037
Pantothenic acid (vitamin B5) 1050
Paragonimus kellicotti 1036
Parakeratosis 304
Paralumbar fossa laparotomy 187
Paramyxoviruses 594–608
  Menangle virus 350, 599–602, *600*
  Nipah virus 206, 602–607, *605*
  overview 594
  porcine parainfluenza virus, 1 (PPIV-1) 607–608
  rubulavirus (blue eye) disease 352, 594–599, *595*, *598*
Parasites
  external 1005–1013, **1008**
    fleas (*Siphonoptera*) 1010–1011
    flies 1011–1013
    lice (*Haematopinus*) 1009–1010
    mange (*Demodex*) 1009
    mange (*Sarcoptes*) 1005–1009
    mosquitoes 1011–1013
    ticks 1013
  internal (helminths) 1028–1040
    ascarid (round worm) 1031–1033
      Ascarops (stomach worm) 1029
      cysticercosis (pork measles) 1037–1038
      Echinococcus (hydatid tape worm) 1036–1037
      Fasciola (liver fluke) 1036
      Globocephalus (hook worm) 1034
      Gnathostoma 1029
      Gongylonema 1028
      Hyostrongylus (red stomach worm) 1029
      Macracanthorhynchus (thornyhead) 1034
      Metastrongylus (lungworm) 1035–1036
      Oesophagostomum (nodular worm) 1035
      Paragonimus (lung fluke) 1036
      Physocephalus 1029
      Stephanuris (kidney worm) 1037
      Strongyloides (thread worm) 1029–1031
      Trichinella 1033–1034
      Trichuris (whipworm) 1034–1035
  internal (protozoan) 1015–1026
    amoeba 1026
    Balantidium coli 1026
    coccidia (*Cystoisospora*, *Eimeria*) 1015–1018, 1018–1019
    Cryptosporidium 1022–1023
    Giardia 1023–1025
    Microsporidia 1025–1026
    Sarcocystis 1021
    Toxoplasma 1019–1021
  neurological conditions 353
Paratyphoid nodules 920
Parity distribution **324**
Pars esophagea
  gastric ulceration 245–246
  specimen collection 105
Parvoviruses 611–619
  clinical signs 615
  diagnosis 617–618, *618*
  epidemiology 613–614
  etiology 611–613, *612*
  immunity 618–619
  lesions 615–617, *616*, *617*
  pathogenesis 614–615, **614**
  prevention and control 619
  public health 613
  relevance 611

Pasiviruses 676
Passive transfer in neonate 281–282
Pasteurella haemolytica-like 749. *See also Actinobacillus pleuropneumoniae* (App)
Pasteurella multocida toxin (PMT) 887–888, 892, 893–894
Pasteurella multocida. *See* Pasteurellosis
Pasteurellosis, pneumonia and progressive atrophic rhinitis (PAR) 884–895
  clinical signs 888–889, *889*, *890*
  diagnosis 892–893
  epidemiology 885–886
  etiology 884–885
  immunity 893–894
  lesions 889–892, *891*
  pathogenesis 886–888
  prevention and control 894–895
  public health 885
  relevance 884
Pathogen-associated molecular patterns (PAMP) 786
Pathogenicity island (PAI) 811
Pathogens. *See also specific pathogens*; Viruses
  commensal 133–134
  emergence of, recent decades **137**
  long-cycle 131–132
  resistant 132–133
  respiratory system 396–397, **397**
  short-cycle 130
  vector-borne 129–130
Pathology. *See specific diseases*
Pattern, disease occurrence 126–127, *128–129*
Pattern-recognition receptors (PRRs) 264
PCV2, 284
PDNS. *See* Porcine dermatitis and nephropathy syndrome
Pediculosis (lice) 302, **1008**, 1009–1010
Pelleting, of food 245
Pentachlorophenol (PCP) 1078
Pentobarbital 175
Pentobarbitone 175
Pericarditis 226, **226**
Pericardium, diseases of 225–226
Peritoneal cavity 255–256
Peritonitis 256
Persistent frenulum 182
Pesticides
  fungicides 1078
  herbicides 1078–1079
  insecticides 1076–1078
  rodenticides 1079
Pestiviruses 622–637. *See also* Flaviviruses
  atypical porcine pestivirus 636–637
  bovine viral diarrhea and border disease virus 633–636
  Bungowannah virus 630–633
  classical swine fever virus 247, 255, 292, 343, 623–630
  overview 622–623
Pet pigs, miniature 211–220
  behavior and training 216–217
  disease 219–220
  hoof trimming 219
  parasites 218
  porcine stress syndrome 218
  regulations 220
  restraint 217–218
  surgical procedures 219
  teeth trimming 218–219
  vaccination 218
Peyer's patches 266
Phages 167
Phagocytosis 267, 281, 284, 395
Phenicols **161**
Phenylarsonic compounds 1074–1075
Phenylpyrazoles 1078
Phosphorus 1047–1048
Photosensitization 303
Physocephalus sexalatus 1029, *1030*
Picornaviruses 641–676, *643*
  encephalomyocarditis virus 664–668, *667*
  foot-and-mouth disease 645–659, *650*
  molecular biology 641–645, **642**
  porcine enteroviruses 675–676
  porcine kobuvirus 675
  porcine picornavirus Japan 676
  porcine sapelovirus 675
  porcine teschovirus 352, 668–673, **670**
  Seneca Valley virus 673–675
  swine vesicular disease 659–664, **659**, *662*, *663*
Pietrain creeper syndrome 363
Pig endogenous retoviruses (PERV). *See* Retroviruses
Piglets. *See also* Fetuses and neonates
  cross-fostering 325
  growth 323–324, **324**
  immune protection 325
  mortality 324–325
Pigweed 1079–1080
Piperazine **168**, 1040
Pityriasis rosea 304–305, *305*
Planned exposure 286–287
Plant toxicity 354, 1079–1081
Plaque reduction neutralization (PRN) 95
Pleuritis 401
Pleuromutilin **161**
Pleuropneumonia 749. *See also Actinobacillus pleuropneumoniae* (App)
PMNs 267–268, 269
Pneumonia. *See also* Respiratory system
  bronchopneumonia 398–400, *398*, **399**
  classification **399**
  interstitial pneumonia 400
  patterns *398*
Poison hemlock 354
Poisoning, (see Toxicity)
Polioencephalomyelitis 670
Pollution 401–402. *See also* Toxicity
Polyarthritis 361
Polyclonal antibodies 271
Polycystic ovarian degeneration (POD) 373, *377*
Polymerase chain reaction (PCR) 87–90, 115, *117*, 118, 120, 431, 656, 735
  integumentary systen 296–297
  reverse transferase (RT-PCR) 655–656
  reverse transcription (RT-PCR) 645
Porcine alveolar macrophages (PAMs) 86
Porcine anelloviruses. *See* Anelloviruses
Porcine astroviruses 457–460, *459*
  diagnosis 460
  epidemiology 458
  etiology 457, *458*
  pathogenesis, clinical signs, lesions and immunity 458
  prevention and control 460
  public health 458
Porcine biochemistry reference intervals **224**

Porcine caliciviruses 464–471, *466–469*
sapo- and noroviruses 466–471, *466*, *468–469*
St-Valérian virus 471
vesicular exanthema of swine 464–466
Porcine circovirus-associated diseases (PCVADs), *See* Porcine circoviruses
Porcine circovirus type, 2 reproductive disease 480–481
Porcine circovirus type, 2 systemic disease 473–475, 476–480
Porcine circoviruses 133, 473–483
epidemiology 475–476
etiology 473–475, *474*
immunity 482–3
pathogenesis,clinical signs, lesions, and diagnosis 476–482
porcine circovirus type, 2 systemic disease 473–475, 476–480
porcine circovirus type, 2 reproductive disease 480–481
porcine dermatitis and nephropathy syndrome 300–301, *300*, 412, 481–482, *481*
prevention and control 482
relevance 473
Porcine cytomegalovirus (PCMV) 561–565, *562*, *563*, *564*
Porcine deltacoronavirus 510–513
Porcine dermatitis and nephropathy syndrome (PDNS) 300–301, *300*, 412, 481–482, *481*
Porcine enteric caliciviruses (PECs). *See* Sapoviruses
Porcine enteroviruses (PEVs) 675–676. *See also* Picornaviruses
Porcine epidemic diarrhea virus (PEDV) 284, 488, 504–510. *See also* Coronaviruses
Porcine hematological reference intervals **224**
Porcine hemorrhagic enteropathy (PHE) 898, 903, *903*, 905, 907
Porcine intestinal adenomatosis (PIA). *See* Proliferative enteropathy
porcine intestinal distension syndrome 249–250, 251
Porcine intestinal/colonic spirochetosis (PIS/PCS) 961–966. *See also Brachyspira pilosicoli*
clinical signs 963–964
diagnosis 965
epidemiology 962–963
etiology 962
immunity 965
lesions 964–965
pathogenesis 963
prevention and control 966
public heath 962
relevance 961–962
Porcine kobuvirus (PKV) 645, 675
Porcine lymphotrophic herpesviruses (PLHV) 565–569, *566*, *568*
Porcine multicentric lymphoma 232
Porcine parainfluenza virus, 1 (PPIV-1) 607–608
Porcine parvovirus (PPV) 284, 611–619
clinical signs 615
diagnosis 617–618, *618*
epidemiology 613–614
etiology 611–613, *612*
immunity 618–619
lesions 615–617, *616*, *617*
pathogenesis 614–615, **614**
prevention and control 619
public health 613
relevance 611
Porcine picornavirus Japan 676
Porcine proliferative enteropathy. *See* Proliferative enteropathy
Porcine reovirus. *See* Reoviruses
Porcine reproductive and neurological system (PRNS) 305
Porcine reproductive and respiratory syndrome (PRRS) virus (Porcine arteriviruses) 685–702
clinical signs 692–694
lesions 694–695
diagnosis 695–698
diagnostic test results 117
epidemiology 688–690
etiology 685–688
immunity 698–700
pathogenesis 690–692
prevention and control 700–702
public health 688
relevance 685
Porcine reproductive and respiratory syndrome virus (PRRSV) 117, 269, 284, 286, 287, 699
Porcine respiratory and encephalitis syndrome. *See* Nipah virus (NV)
Porcine respiratory coronavirus (PRCV) 488, 492–504. *See also* Coronaviruses
Porcine respiratory disease complex (PRDC) 396–397, **397**, 749, 767, 884
Porcine rotavirus. *See* Reoviruses; Rotaviruses
Porcine sapelovirus (PSV) 675
Porcine septicemia, specimen collection **105**
Porcine somatotropin (PST) 169
Porcine stress syndrome (PSS) 361–362, 361–362
miniature pet pigs 218
Porcine teschovirus 352, 668–673, **670**
Porcine torovirus 516
Pork measles 1037–1038
Pork, food safety
biological hazards 199–203
chemical hazards 198–199
physical hazards 197–198
Postmortem examination. *See* Necropsy
Postmortem knife 72–73
Postpartum dysgalactia syndrome (PDS) 323, 325–31, *327*, 807
Post weaning *E. coli* diarrhea (PWD) 817–826, *822*
See also Colibacillosis
Post weaning multisystemic wasting syndrome (PMWS). *See* Porcine circovirus (type 2 systemic disease)
Potassium diformate 241
Prebiotics 168, 238–239
Pre-farrowing behavior 21
Pregnancy detection 376, 380
Preputial diverticulum 181
Preputial prolapse 181–182
Prevalence. *See specific* diseases; Epidemiology
Prevalence estimation 119–120
Prevalence risk 125–126, **125**
Pre-weaning mortality 21–22
Probiotics 167, 239
Progestin 169

Progressive atrophic rhinitis (PAR). *See* Pasteurellosis
Prolapsed penis 181
Prolapsed rectum **62**, 192, 247–248
Prolapsed uterus 187–188, 382
Prolapsed vagina 182–183, 382
Proliferative enteropathy (PE) 898–908
  clinical signs 903
  diagnosis 905–906
  epidemiology 900–901
  etiology 898–899
  immunity 906–907
  lesions 903–905, *904*
  pathogenesis 901–903
  prevention and control 907–908
  public health 899
  relevance 898
Proliferative hemorrhagic enteropathy (PHE). *See* Proliferative enteropathy
Proliferative osteolitis 366
Prophylaxis 164
Propofol 175
Prostaglandin 168
Protein 1046–1047
  dietary 235, 236
Protozoa 1015–1026
  amoeba 1026
  Balantidium coli 1026
  Coccidia (*Cystoisospora*, *Eimeria*) 1015–1018, 1018–1019
  Cryptosporidium 1021–1023
  Giardia 1023–1025
  Microsporidia 1025–1026
  Sarcocystis 102
  Toxoplasma 1019–1021
Pruiritis, scabies 1006
Pruritis 302
Pseudopregnancy 373, 379, 380, 391
Pseudorabies virus , 352, 551–561, *552*, *557*, *558*. *See also* Aujesky's Disease; Herpesviruses
Public health 197–208. *See also specific diseases;* Zoonotic diseases
Puerperal mastitis 807
Pulse medication 164
Pulsed field gel electrophoresis (PFGE) 91, 767, 953, 962
Purified protein deratives (PPDs) 978
Pustular dermatitis 297
Pyelonephritis. *See* Urinary tract infection
Pyrantel tartrate **168**, 1039, 1040
Pyridoxine (vitamin B6) 1053

q

Quantitation of viruses 433–437
Quantitative trait loci (QTL) 45
Quinoxaline derivatives **161**

r

Rabies virus 736–738. *See also* Rhabdoviruses
Ractopamine 169, 1076
Reactive oxygen intermediates (ROI) 268
Real-time polymerase chain reaction (RtPCR) 88–89
Real-time ultrasonography (RTU) 373
Recombination–activating gene (RAG) 276
Records and history
  herd evaluation 3–4
  integumentary system 293
  site visits 3–4, 10–11
Rectal prolapse **62**, 192, 247–248
Rectal stricture 247–248
Redroot pigweed 1079–1080
Reference intervals, blood **224**
Regional anesthesia 177–178
Regulations. *See* Laws and legal considerations
Rehydration 242
Relative humidity 51
Relaxin 316
Renal function 409. *See also* Urinary system
Renal infarcts 411
Reoviruses 715–723, *722*
  rotaviruses **253**, 715–722
Reporting structure 4–5
Reproductive system, diseases of 373–391
  abortion and reprductive diseases 384–391, **387**, **389–390**, 654, 666
  artificial insemination **384**, **385**
  boar 382–384
  boar overuse 383–384
  conception and farrowing rates, problems associated with 379–380
  congenital anomalies in 374
  female 373–382
  heat stress or pyrexia 382–383, *383*
  herd level problems 375
  individual pig examination 375–376, **376**, *377*
  infectious pathogens 384
  mycotoxins 383
  non-puerperal diseases of vagina, cervix and uterus 374–375
  ovaries 373
  oviducts 374
  parturition problems 381–382
  puberty problems 376–378
  semen collection **384**
  semen quality 384, **385**
  vaginal and uterine prolapse 382
  vulvar discharge 374, 380–381
  wean-estrus interval (WEI) 378–379
Respiratory illness. *See also* Respiratory system
  causes by age **63–64**
  specimen collection **104**
Respiratory rate 8
Respiratory system 393–405
  control 401–402
  diagnosis and monitoring 404–405
  disease control 402–404
  defenses 394–395
  helminths in 1035–1036
  pathogens, lung 396–7, **397**
  pathology 397–401, *398*, **399**
  structure 393–394, *394*
Reston ebolavirus (REBOV) 206–207. *See also* Filovirus
Reston virus 525–526
Restraint
  for blood collection 14, *14*
  of miniature pet pigs 217–218
Retinoic acid-inducible gene I-like (RIG-I-like) 787
Retroviruses 728–731
Reversal agents 175
Rhabdomyosarcoma 306
Rhabdoviruses 733–738
  rabies virus 736–738
  vesicular stomatitis 733–736
Rhinitis 398
Rhodococcus equi 994–995
Riboflavin (vitamin B2) 1050
Ribs, strength assessment 103
Rickets 342, 355–356, **356**
Ringworm 301–302
RNA viruses 428, *430*, 431, 433, 437
Rodenticides 1079
Rose Bengal plate test (RBT)
Ross River virus (RRV) 742

Rotaviruses 252, 715–722
clinical signs 719
diagnosis 720
epidemiology 717–718, **717**
etiology 715–717, *716*, **717**
immunity 720–721
lesions 719–720, *720*
pathogenesis 718–719
prevention and control 721–722
Round worm (*Ascaris*) 1031–1033
Routes of infection 123
Roxarsone 1074–1075
Rubulavirus (blue eye) disease 352, 594–599, *595*, *598*
RYRL gene 362

S

Safety, necropsy 109–110
Sagiyama virus (SAGV) 741–742
Salinity 1082
Salmonellosis (*Salmonella* spp.) 167, 199–200, 299, 912–923
clinical signs 917–918
diagnosis 920–921
differential diagnosis **252**
epidemiology 913–916
etiology 912–913
lesions 918–921, *918*, *919*
organic acids 241
pathogenesis 916–917
pathology and diagnosis **252**
prevention and control 921–923
probiotics 238, 239
public health 913
relevance 912
Salt poisoning 353, 1082–1083
Sample collection
abortion **106**
blood 14–15, *14*, *15*
diagnostic considerations 98–99
diarrhea **105**
nasal swabs, picornaviruses 650, *650*
neurological disorders **108**
oral fluids 15
respiratory disorders **104**
septicemia **105**
Sample-to-positive (S/P) ratios 120
Sampling
antemortem 100–101, 346
postmortem 101–102, 346–347
Sapoviruses 466–471, *466*, *468–469*
Sarcocystis spp. 1021, *1022*
Sarcoptes 1005–1009, *1006*
See also Parasites
Sarcoptic mange (scabies) 302, 1005–1009, **1008**
Savaging 22
Scabies 302, 1005–1009, **1008**
Screwworm flies 1012–1013
Scrotal hernia 189–190
Seborrhea contagiosa) 297, *297*, 926–930, *929*
See also Staphylococcosis
Selenium 1048–1049
toxicity 353, 1073
Selenomethionine 1049
Semen
collection and evaluation 384, **384**, **385**
heat stress and fever 382–383, *383*
quality 384, **385**
Seneca Valley virus (SVV) 641, 645, 652, 655, 663, 673–675
Sensible heat transfer 51
Septic arthritis 193
Septicemia, specimen collection **105**
Serpulina spp. See Brachyspira
Serpulina hyodysenteriae. See Brachyspira hyodysenteriae
Serpulina pilosicoli. See Brachyspira pilosicoli
Serum tube agglutination test (SAT) 786
Serum virus neutralization (SVN) 94–95
Shewhart Cycle of Plan, Do, Study, Act 121
Shiga toxin-producing *Escherichia coli* (STEC) 201, **808**, 810–811, **813**
Shiga toxins (Stx) 810, 811
Shock 230, 826
Short-cycle pathogens 130
Show pigs 211–220
biosecurity 212
breeding management 215
drug use and testing 216
education 215–216
ethics 216
farrowing management 215
general health issues 214–215
individual animal medicine 214–215
nutrition 215
size and scope of industry 211–212, *212*
vaccination and health protocols 214
zoonotic implications 212–14
See also Pet Pigs
Simondsia paradoxa 1029
Single nucleotide polymorphisms (SNP) 44, 270
SIR epidemiological model 127
Site visits
benchmarks 4, 4
biosecurity 5
defined 3
diagnosis 11–13, *12*, *13*
four circles of 5–11, *5*, *7*, *8*, *9*
history and records 3–4, 10–11
reporting structure 4–5
sample collection 14–15, *14*, *15*
Skin biopsy 294–296
Skin diseases **65**, **66**, **295–296**. *See also* Integumentary system
Skin necrosis,
ear 297–298, *297*, *298*
facial 298–299
Skycis. *See also* ionophore; Narasin
Small intestine, helminths in 1029–1034, **1039**
Sneezing, causes of **64**
SOAP approach 11
Socialization 30
Sodium 1047
Sodium ion toxicosis 1082–1083
Solanium nigrum 1080
Sole lesions 307
Somatic cell count (SCC) 321
Space recommendations 53–54, **54**
Specimen collection. *See* Sample collection
Spinal reflex 344
Spinose ear ticks 1013
Spirochetosis, intestinal/colonic. *See Brachyspira pilosicoli*
Spiruroid stomach worms 1029
Splayleg 347–349, *349*
Splenic torsion 256
Spongiform encephalopathy 352–353
Sporadic disease 126–127, *128–129*
Spray-dried animal plasma 166, 237
STa protein 809
Stable flies 1012
Staphylococcosis (*Staphylococcus* spp.) 926–931
S. aureus 930–931
S. hyicus (exudative epidermitis) 297, 926–930, *929*

Statistical process control (SPC) chart 122
STb protein 809
Stephanurus dentatus *1030*, 1032, 1037
Stocking density 6, 6
Stomach diseases 246–247, 1028
  helminths in 1028–1029
  ulcer. *See* Gastric ulcer
Stomatitis 242
Stomoxys calcitrans 1012
Streptococcosis 934–947
Streptococcus dysgalactiae subsp. *equisimilis* 946–947
Streptococcus equi subsp. *zooepidemicus*. *See* *Streptococcus zooepidemicus*
Streptococcus porcinus (cervical lymphadenitis) 946
Streptococcus suis 934–945
  clinical signs and lesions 940
  diagnosis 107, 940–942
  epidemiology 936–938
  eradication 945
  etiology and prevalence 934–935
  pathogenesis 938–940, *939*
  prevention 943–945
  public health 204, 935–936
  treatment 942–943
  valvular endocarditis 228
Streptococcus zooepidemicus 947
Stress. *See also* Environment; Welfare and behavior
  gastric ulceration 245, 246
  immunity 282–283, *282*
  physical and psychological 282–283
  porcine stress syndrome 171–172
  responses to 30–31, 33
  socialization 30, 32
  transportation 235
Strongyloides ransomi 1029–1031, *1030*
Strychnine 1079
St-Valérian virus 471
Suckling pigs. *See* Fetuses and neonates; Piglets; Weaning
Sulfonamide– diaminopyrimidine combinations **160**
Sulfonamides **160**, 1076
Sunburn 302
Surgical procedures
  abdominal procedures 188–90, *190*
  atresia ani 191
  genitourinary surgery 179–184, *180*
  musculoskeletal surgery 192–194
  miniature pet pigs 219
  rectal prolapse 192
  uterine procedures 184–188, *184*, 186, *187*
Surveillance 118–122
  application and examples 120–122
  diagnostic data in process improvement 121–122
  monitoring versus 119
  prevalence estimation 119–120
  qualitative data and cutoff values 120
  quantitative data and time 120–121
Swine dysentery
  clinical signs 956
  diagnosis 251, 254, **953**, 957–958
  dietary considerations 237–238
  epidemiology 954–955
  etiology 953–954
  immunity 959
  lesions 956–957, *957*
  pathogenesis 955–956
  prevention and control 959–961, **960**
  public health 954
  recent developments 951
  relevance 953–954
  See also Brachyspira
Swine lice. *See* Lice (pediculosis)
Swine pasiviruses 676
Swine vesicular disease virus (SVDV) 645, 659–664, **659**, *662*, *663*
Swinepox virus (SWPV) 299–300, *300*, 709–713
  clinical signs 711–712
  diagnosis 712–713
  epidemiology 710
  etiology 709–710
  immunity 713
  lesions *711*, 712
  pathogenesis 710–711
  prevention and control 713
  public health 710
  relevance 709
Synthetic pyrethroids 1078
Syud herpesvirus 1. *See* Aujeszky's disease

t

T cells 264, *265*, 266, 267, 699
T lymphocytes 232, 268, 271–272
T-2 toxin 1061
Tabanids 1012
Taenia hydatigena 1037–1038
Taenia solium 202–203, 363, 1038
Tahyna virus 461–462
Tail biting 27
Tail docking 22–23, 25
Tapeworms 1034
Tarassovi infection 856
Target body condition score (BCS) 8, 9
Teat order 24
Teeth clipping/trimming 24–25, 218–219, 242
Telazol 175
Temperature
  environmental 50–51, *51*
  preparturient sows *327*
  respiration and heart rate 8
Terrorism. *See* Biosecurity
Teschen disease 352, 668–673, **670**
Testicular abnormality 180–181
Testicular atrophy 180–181
Testing. *See* Diagnostics; Sample collection
Tetanus (*Clostridium tetani*) 364, 802–803
Tetracyclines **161**
Tetrahydropyrimidines 1040
Th1/Th2 balance 283
Thiabendazole (TBZ) 1040
Thiamin (vitamin B1, thiamine) 1053
Thimbling 654
Thiopental 175, **174**
Thoracic cavity, necropsy 103–104
Thornyhead worm 1034
Thrombosis 229
Thymus 267
Ticks **1008**, 1013. *See also* Vector-borne pathogens
Tiletamine–Zolazepam 173
Tissue culture infective dose (TCID) 89, *90*
Togaviruses 740–742
  Eastern equine encephalitis virus 740–741
  Getah virus 741
  Ross River virus 742
  Sagiyama virus 741–742
Toll-interleukin-1 receptor (TIR) 787
Toll-like receptors (TLRs) 270, **270**
Toltrazuril 1019
Tonsils 243, 266–267
Torque teno sus virus (TTSuV). *See* Anelloviruses
Torsion
  intestinal tract 249–250, *249*
  liver 256

spleen 256
visceral 190–191
Total dissolved solids (TDSs) 1082
Toxic equivalency value (TEQ) 199
Toxicities and toxins, *See also* Toxicity
feed additives 1074–1076
gases and ventilation failure 1084–1086
herbicides 1078–1079
minerals 1072–1074
pesticides 1076–1078
plants 1078–1081
rodenticides 1078
Toxicity 1072–1086
abortion 388–391
aflatoxins 1058
coal tar pitch 1083
ethylene glycol 1083
feed additives 1074–1076
fungicides 1078
gossypol 1083–1084
herbicides 1078–1079
insecticides 1076–1078
liver conditions 257
minerals 1072–1074
mycotoxins. *See* Mycotoxins
plants 1079–1081
rodenticides 1079
sodium ion toxicosis 1082–1083
ventilation failure 1084–1086
water quality 1081–1082, **1081**
Toxoplasmosis (*Toxoplasma gondii*) 202, 1019–1021, *1020*
Trachea 393
Tracheal cytotoxin (TCT) 770
Tracheitis 398
Tramadol 179
Translocated intimin receptor (Tir) 819
Transmissible gastroenteritis virus (TGEV) 488–92, 492-–504, *493*, *495*, *498*
See also Coronaviruses
Transmissible spongiform encephalopathy (TSE) 352–3
Transmission 123. *See also specific diseases*
biosecurity 5, 136–154, **137**, *140*, *143*, *146*, *149*, *152*, *154*. *See also* Biosecurity
causal inference 134–136, **134**, *135*
causation 134–136, *135*
commensal pathogens 133–134
definition and overview 123
disease occurrence 125–126, **125**
disease persistence 127–134
evidence-based biosecurity 153, *154*
evolution of disease management **136**
host-pathogen environment 124–125
long-cycle pathogens 131–132
occurrence patterns 126–127, *128–129*
resistant pathogens 132–133
respiratory illness 402–403
routes of infection 123, 129
short-cycle pathogens 130
vector-borne pathogens 129–130
Transportation 56, 144–145, 235
Treatment. *See* Drug therapy
Treponema hyodysenteriae. See Brachyspira hyodysenteriae
Treponema pedis 995–996
Tribasic copper chloride (TBCC) 1072
Trichinella spiralis 201, 363, 1033–4, *1033*
Trichinellosis 1033–4
Trichophyton spp. 301
Trichothecenes **1057**, 1061–1063, **1064**
Trichuris suis *1030*, 1034–1035
Triune brain concept 20
Trueperella (Arcanobacterium) pyogenes 193, 352, 360, 997–998
Trueperella abortisuis 997
Trueperella pyogenes 997–998
TT virus (TTV). *See* Anelloviruses
Tuberculosis 971–979
clinical signs 976
diagnosis 977–978
epidemiology 974–975
etiology 971–973
lesions 976–977, *977*
pathogenesis 975–976
public health 973–974
prevention and control 978–979
Tubular diseases, of kidney 413–14, *414*
Tubulointerstitial diseases 414–418
Tumor necrosis factor-α (TNF-α) 269, 271, 274
Tumors. *See* Neoplasia
Tusk
removal 194
trimming 24–25

u

Ulcerative dermatitis 298
Ultrasound 376, *377*, 380
Umbilical hernia 188–189, *189*
Unilateral castration 180
Upper motor neurons (UPNs) 344
Urea, additive 1076
Urethral obstruction 183
Urinary system 408–422
anatomy 408, *409*
circulatory disturbances 410–411
developmental anomalies 410, *410*
differential diagnosis **421**
glomerular diseases 411–413, *413*
helminths in 1037
hydronephrosis 420
neoplasia 418–419, *419*
parasites 420–422
physiology 408–409
tubular diseases 413–14, *414*
tubulointerstitial diseases 414–418, *415*, *416*
urolithiasis 419–420, *420*
Urinary tract infection (UTI) 807, 811
Actinobaculum suis 981–982
nonspecific UTI (*E. coli)*, 830–832
Urine 409
Urolithiasis 419–420, *420*
Uterine prolapse 187–188, 382
Uterine surgery 184–188, 219
amputation, of uterus 188
cesarean section 184–187, *186*
gnotobiotic pigs 187
hysterectomy 184, *184*
prolapsed uterus 187–188

v

Vaccination. *See also respective causative agent chapters*
classical swine fever virus 623, 627
environment and 13
failure 287–288
foot and mouth disease (FMD) 646
gastrointestinal conditions 254–255
immunity 284–288, 737–738
intervals between 285
licensing **286**
minature pet pigs 218
planned exposure 286–287
postpartum dysgalactia syndrome 331
as preventive measure 166
program 284–285

Vaccination (*cont'd*)
respiratory pathogens 403–404
route of 285–286
show pigs 214
Vaccine-associated enhanced respiratory disease (VAERD) 584
Vaginal prolapse 182–183
Valvular cysts 228
Valvular endocarditis **226**, 228
Variable-number tandem repeats (VNTR) 899
Vascular pathology 229–230
Vasculitis 229, **229**
Vasectomy 182
Vasogenic shock 230
Vector-borne pathogens 129–130
African swine fever virus 443–50, 1013
flaviviruses 530–540
togaviruses 740–742
Vena cava, anterior 14, *15*
Venipuncture 15
Ventilation 51–53, 1084–1086
Verotoxigenic *E. coli* (VTEC). *See* Shigatoxigenic *E. coli* (STEC)
Verotoxins (VT) 810
Vesicular exanthema of swine (VES) 464–466
Vesicular stomach viruses (VSVs) 130, 733–736. *See also* Rhabdoviruses
Vestibular disease 354
Viral antigen-based detection methods 431
Viral diseases 299–300
abortion 388
neurological conditions 351–2
Viral genome characterization 433–437
Viral nucleic acid-based detection methods 431–432
Viral phenotypes 437
Virion. *See also specific viruses*
composition 427
structure *428*, 432–433
Virulence-associated trimeric autotransporters (VtaAs) 845, 846
Virus isolation (VI) 94
Virus neutralization (VN) 735
Viruses
characteristics 427–428
characterization 432–437
cultivation 431
detection 431–432
hierarchy and nomenclature 428–429
morphology 427–428, **429**, *430*
taxonomy 428–431, **429**, **434–436**
Vitamin A 342, 350, 355, 356, 363, 1049
Vitamin B12 (cyanocobalamin) 1050
Vitamin B_6, 353
Vitamin C
Vitamin D 229, 422, 1047–1048
Vitamin E 1048–1049
Vitamin K 1049–1050
Volvulus. *See* Torsion
Vomiting, causes by age **61–62**
Vomitoxin 1055, 1061
Vulvar discharges 374

w

Water deprivation 1082–1083
Watering
drinking, behavior development 26–27
drug treatment route 163–164
feeders and 6, 8, *52*
milk production 320–321
quality 1081–1082, **1081**
Weaning. *See also* Fetuses and neonates; Piglets
diarrhea 242, 807
digestive system 235
diseases 254–255
edema disease *E. coli* 807, 817–826, *822*
immune response 265
mammary gland involution 315–316
stress of 283
Weights and gain 7
Welfare and behavior 17–35. *See also* Environment
affective (psychological) state 20
aggression 27
belly nosing 27–28
caretaker interaction 29
castration 23–24
cruelty, defined 18
defined 17–18
disease 33
domesticated vs. wild pigs 19
euthanasia 34–35
feeding and drinking 26–27
lameness 28–29
maternal behaviors 21–22
monitoring and assessment 20–21
pain, recognition of 22
pain management 25
production and health 19–20
surgical procedures 171. *See also* Anesthesia; Surgical procedures
tail biting 27
tail docking 22–23, 25
teeth clipping 23
tusk trimming 24–25
West Nile virus 530, 535–538. *See also* Flaviviruses
Whey bloat 250
Whipworm 1034–1035
White spot liver 257
Wild pigs, vs. domesticated 19
'Wind up' phenomenon 22
Withdrawal, from drugs 164–165
WL (white line) lesions 307, 308

x

Xanthium spp. 1080
Xenotransplantation 728, 730
X-ray 198
Xylazine 179

y

Yersinia enterocolitica 200–201
diagnosis, prevention and control 1000
epidemiology, clinical signs and lesions 999–1000
etiology 999
Yersinia spp. 999–1000
Yohimbine 175

z

Zearalenone 388–391, **1057**, 1064–1065, **1063**, **1064**
Zinc 284, 309, 1052, 1073
Zinc oxide 167, 241, 825
Zooanthroponosis 153
Zoonotic diseases **31**
non-foodborne 203
show pigs 212–214