Pathogenesis and pathobiology of brucellosis in livestock

F.P. Poester [(1)*], L.E. Samartino [(2)] & R.L. Santos [(3)]

(1) Comitê Científico Consultivo sobre Brucelose Animal, Ministério da Agricultura Pecuária e Abastecimento, Esplanada dos Ministérios, Bloco D, Anexo A, 70043-900, Brasilia, DF, Brazil.
(2) Instituto de Patobiología, Instituto Nacional de Tecnología Agropecuaria (INTA) Castelar, Buenos Aires, Argentina
(3) Universidade Federal de Minas Gerais, Escola de Veterinária, Av. Antonio Carlos, 6627, 31270-901 Belo Horizonte, Brazil
* Corresponding author: poesterf@terra.com.br

Summary

Brucella species are facultative, intracellular, Gram-negative bacteria with marked tropism for the pregnant reproductive tract of domestic animals. All *Brucella* species establish persistent infection in the reticuloendothelial system of their natural hosts. The mechanisms of placenta localisation, trophoblast tropism and abortion are poorly understood. A complete picture of the molecular determinants and mechanisms of the cell internalisation process began to emerge only recently. Cyclic β-1,2-glucan is a molecule secreted into the periplasm of *Brucella* and is required for intracellular *Brucella* to avoid fusion of the phagosome with lysosomes. The type IV secretion system translocates *Brucella* effector proteins into host cells and is critical for both survival and replication of *Brucella* in infected host cells. Some aspects of the pathogenesis and pathobiology of brucellosis in productive domestic animals are discussed in this section.

Keywords

Brucella abortus – *Brucella melitensis* – *Brucella ovis* – *Brucella suis* – Brucellosis – Pathobiology – Pathogenesis – Zoonosis.

Introduction

Brucellae are intracellular bacteria that cause brucellosis, a chronic disease of domestic and wild animals and humans. The ability of these bacteria to invade, survive for long periods of time and multiply within host cells is critical for disease causation.

The facultative intracellular parasitism characteristic of *Brucella* spp. evolved through evolutionary selection to avoid the host immune system. Target cells and tissues include trophoblasts, fetal lung, macrophages, and the male and female reproductive organs (2). Subsequently, fetal viscera and placenta become heavily infected, and placentitis and abortions occur, with devastating economic effects on livestock production.

In spite of some recent significant advances, the virulence mechanisms of *Brucella* are not completely known. Identification and characterisation of the mechanisms that control expression of bacterial virulence genes inside the host will not only be important for understanding the intracellular behaviour of *Brucella* but will also be key factors in the design of improved attenuated live vaccines and new therapeutic tools. Here the authors describe the most important pathogenic mechanisms of *Brucella* spp. and manifestations of brucellosis in livestock.

Pathogenesis of brucellosis

The ability of *Brucella* spp. to cause disease requires a few critical steps during infection. *Brucella* spp. can invade epithelial cells of the host, allowing infection through mucosal surfaces: M cells in the intestine have been identified as a portal of entry for *Brucella* spp. (1, 53). Once *Brucella* spp. have invaded, usually through the digestive or respiratory tract, they are capable of surviving intracellularly within phagocytic or non-phagocytic host cells (21). *Brucella* has the ability to interfere with intracellular trafficking, preventing fusion of the *Brucella*-containing vacuole (BCV)

with lysosome markers, and directing the vacuole towards a compartment that has rough endoplasmic reticulum (RER), which is highly permissive to intracellular replication of *Brucella* (8, 55, 56).

The outcome of infection is dependent on the species of *Brucella* and host. The *Brucella* spp. that infect livestock are host restricted. For instance, *Brucella melitensis*, *B. abortus*, *B. suis* and *B. ovis* infect preferentially small ruminants, cattle, pigs and sheep, respectively (74). With the exception of *B. ovis*, these *Brucella* spp. have zoonotic potential, with *B. melitensis* being the most pathogenic for humans (77).

The mechanisms that allow host cell invasion by *Brucella* spp. are not completely clear, but although specific host receptors that interact with *Brucella* have not yet been identified, internalisation of *Brucella* into host cells requires cytoskeletal changes (31, 54). Interestingly, invasion through the digestive tract does not elicit any inflammatory response from the host (53). Therefore, *Brucella* spp. invade silently or unnoticed by the innate immune system of the host. In fact, *Brucella* spp. have mechanisms that prevent activation of the host innate immune system (10). Indeed, *Brucella* Toll/interleukin-1 receptor (TIR) domain-containing protein prevents Toll-like receptor (TLR) 2 signalling by interfering with MyD88, and also inhibits DC maturation, cytokine secretion and antigen presentation (25, 63). *Brucella abortus* also induces suppression of the transcription of pro-inflammatory mediators in trophoblastic cells at very early stages of infection (22). Trophoblasts are placental cells that are targeted during infection of pregnant cows. After an initial suppression of pro-inflammatory transcripts, *B. abortus* induces expression of pro-inflammatory chemokines by cultured trophoblastic cells, which correlates with the profile of expression observed *in vivo* in the placenta of infected cows (22).

Brucella spp. lack classical bacterial virulence factors such as exotoxins, cytolysins, a capsule, fimbriae, flagella, plasmids, lysogenic phages, endotoxic lipopolysaccharide (LPS), and inducers of host cell apoptosis (50). However, LPS plays an important role in *Brucella* virulence because it prevents complement-mediated bacterial killing and provides resistance against antimicrobial peptides such as defensins and lactoferrin (4, 38). Another important virulence mechanism of *Brucella* is the BvrR/BvrS two-component regulatory system, which is required for modulation of the host cell cytoskeleton upon *Brucella* invasion, and for regulation of the expression of outer membrane proteins, some of which are required for full virulence (39). Cyclic β-1,2-glucans, which are also part of the outer membrane, are also required for intracellular survival of *Brucella* (13). *Brucella* spp. express a type IV secretion system (T4SS), encoded by the components of the *virB* operon, that is crucial for intracellular survival in host cells and virulence *in vivo* (34, 51).

Unlike other type IV systems, which are expressed extracellularly, transcription of the *virB* operon is induced specifically within macrophages, and phagosome acidification is a key intracellular signal inducing *virB* expression (12).

The *Brucella* T4SS is required for persistence in mice and induction of the host immune response (59, 61). It is also essential for elicitation of inflammatory and immune responses during *Brucella* infection in mice (61), and it is required for microgranuloma formation during *Brucella* infection (60). The T4SS is required for *Brucella* to reach its intracellular replication niche (23), and its expression is regulated by the BvrR/BvrS two-component regulatory system (44). The T4SS delivers *Brucella* effector proteins into the host cell cytosol, but the identities and roles of these effectors have only recently begun to emerge (42).

A novel strategy used for screening proteins of unknown function that are involved in protein–protein interactions was used to identify *Brucella* effectors. Using this approach, four putative proteins (BPE043, BPE005, BPE275 and BPE123), which were translocated into mouse macrophages by *B. abortus*, are hypothesised to be candidates for modulation of host cell functions (26).

Pathobiology of *Brucella abortus* infection in cattle

Brucella abortus was initially found in exudates in the intervillous space of the placenta of a pregnant cow in which abortion was imminent (9). It is a facultative, intracellular, Gram-negative bacterium with great affinity for the pregnant uterus of ruminants. Localisation of *B. abortus* within the female and male reproductive tracts accounts for the most common clinical signs of infection: abortion and male infertility (69). In pregnant cows, it produces a chronic infection, replicating preferentially within the chorioallantoic trophoblasts of the placenta, resulting in placentitis, fetal death and abortion (28). Infected bulls may develop systemic signs of infection, but the most significant lesion produced by *B. abortus* in males is orchitis, often associated with seminal vesiculitis and epididymitis (37). Chronic orchitis and fibrosis of the testicular parenchyma of infected bulls are frequently followed by impairment of semen production, and partial or permanent infertility (57).

Venereal transmission is not a major route of infection under natural conditions, but artificial insemination with contaminated semen is a potential source of infection (57). Pneumonia has been described as the most common pathological manifestation in fetuses aborted as a result of *B. abortus* infection (75).

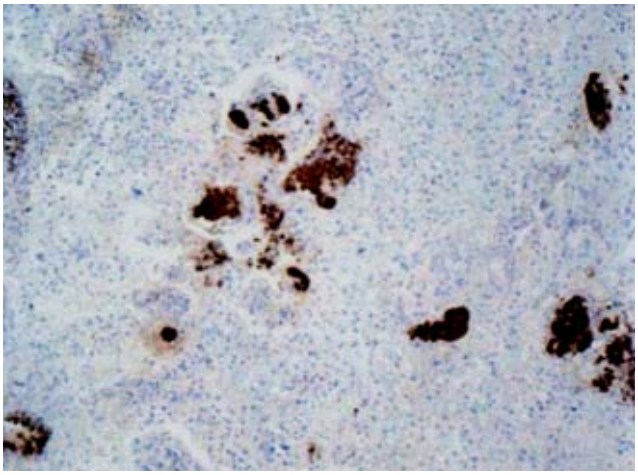


Fig. 1
Bovine placenta from a *Brucella abortus*-infected cow
Immunolabelling of several colonies of *B. abortus* with a multifocal distribution. Streptavidin–biotin–peroxidase complex

The most important ports of entry for *B. abortus* are the nasal and oral mucosae, from where the organisms are drained into the regional lymph nodes. From these foci, a bacteraemia occurs and the organisms localise in other lymphoid tissues such as the spleen and the iliac, mesenteric and supramammary lymph nodes, where they may induce a granulomatous reaction (68).

A second bacteraemia results in generalised infection affecting other target organs, such as the pregnant uterus and the udder, as well as their associated lymph nodes. Uterine lesions are primarily located in the placentomes. Colonisation of the trophoblastic cells of the placenta is often concomitant with abortion in pregnant cows (Fig. 1). Aborted fetuses and fetal membranes and fluids contain high bacterial loads and therefore cause environmental contamination, resulting in a high risk of infection of susceptible hosts (28).

Ruminant placentation is composed of multiple placentomes, which are formed by the combination of the maternal caruncular endometrium and fetal cotyledon. The presence of erythritol, a four-carbon polyol, in fetal tissues of ruminants has been demonstrated (36). Supposedly, erythritol plays an important role in the tropism of *Brucella* for the pregnant uterus of ruminants. High concentrations of erythritol have been shown to stimulate the growth of *B. melitensis* and *B. abortus* (36).

The host mechanisms responsible for the increased susceptibility to infection in advanced pregnancy are not known, but they may be related to the differential susceptibility of placental trophoblasts during the middle and late stages of pregnancy (64). The high concentrations of erythritol in uterine tissues, and the ability of *B. abortus* to utilise this rare sugar, suggest that it may be a determinant for the tissue tropism of this pathogen in cattle (36). However, experimental confirmation of this hypothesis has not been reported. Other strains, including *B. abortus* vaccine strain 19, are not stimulated by erythritol, even though they are capable of causing genital infections and abortions (28).

The mechanisms of *Brucella*-induced abortions are poorly understood. Placentitis, which prevents the delivery of nutrients to the fetus and results in fetal stress and death, has been hypothesised as being responsible for abortion in brucellosis (69). Researchers have speculated that hormonal influences contribute to the abortion process. A shift from the predominant production of progesterone to oestrogen

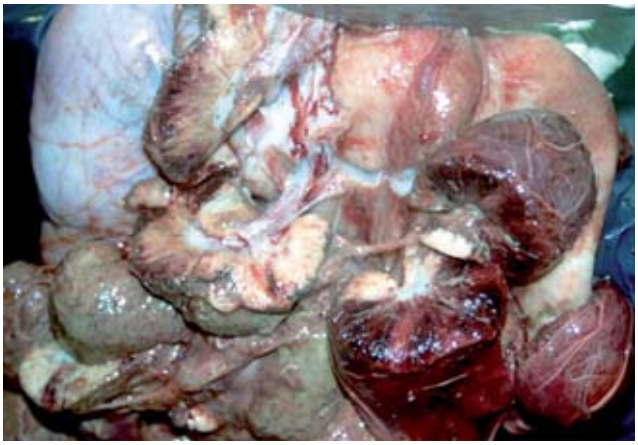


Fig. 2a
Uterus from a *Brucella abortus*-infected cow immediately after abortion
Several necrotic and haemorrhagic placentomes, characterising a severe and diffuse fibrinous–necrotising and haemorrhagic acute placentitis

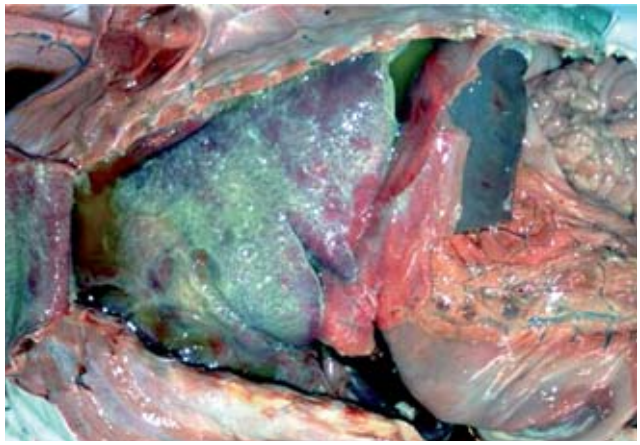


Fig. 2b
***Brucella abortus*-infected aborted fetus with an acute diffuse severe fibrinous pleuritis**

results in the endometrial production of prostaglandin F 2-alpha (PGF2α), which induces the normal or premature delivery process (29).

Brucella abortus modulates the innate immune response of trophoblastic cells, suppressing the expression of pro-inflammatory mediators during the early stages of infection. This is followed by a delayed and mild expression of pro-inflammatory chemokines in the placentomes of experimentally infected cows (22).

Gross lesions in the uterus of infected cows are characterised by brownish fluid, with exudate consistent with a necrotising placentitis, and the uterus can also show fibrinous necrotic exudates and multifocal haemorrhages (Fig. 2a). Necrotic neutrophilic placentitis with perivascular infiltrates is the most frequent microscopic change in experimentally infected cows, and inflammation is associated with large numbers of *B. abortus* cells inside macrophages and trophoblasts (75). Fetal lesions include fibrinous pleuritis (Fig. 2b) and peritonitis, bronchopneumonia and splenitis (33, 75). Fibrinous pericarditis has been described as a significant fetal lesion in brucellosis (75).

Pathobiology of *Brucella melitensis* infection in small ruminants

Brucella melitensis was first isolated by Sir David Bruce in 1887 (15), from the spleen of a British soldier on the island of Malta, and was denominated *Micrococcus melitensis*. Meyer and Shaw renamed the bacterium *Brucella melitensis* in 1920, in honour of Dr Bruce (41). This organism causes significant problems in humans and in goats and sheep throughout the world. Goats are very susceptible to infection and are the primary hosts for *B. melitensis*. Infected goats are the main cause of human brucellosis, a worldwide zoonosis.

As occurs in other ruminants, the main route of infection for goats is the oral mucosa. *Brucella melitensis* infection in pregnant goats affects the placenta and fetus. Late gestation abortion occurs in pregnant goats, and has been described as the main clinical sign of infection. Although not all infected goats abort, they all shed *Brucella* into the environment. In pregnant ruminants, over 85% of *Brucella* can be found in the cotyledons, placental membranes, and amniotic liquid, reaching up to 1×10^{10} colony-forming units (CFU)/ml in allantoic fluid and 1×10^{13} CFU/g of tissue in the cotyledons (44). Therefore, *Brucella*-infected goats pose a serious threat of transmission of the disease to other animals in the flock and to animal handlers.

Abortion may occur at different stages of gestation, and aborted fetuses often have a normal gross appearance. However, in many cases there is bronchopneumonia, haemorrhagic fluid in the thoracic cavity, and enlargement of lymph nodes, liver and spleen (5). In male goats, the infection can be located in the testis, epididymis, seminal vesicle and deferent ducts, resulting in inflammation of the genital organs. In the chronic stage, hygromas and inflammation of the joints can be observed. The most common manifestation of the disease in males is impairment of semen quality and consequent fertility loss (28).

Anderson *et al*. (8) demonstrated a unique mechanism for invasion of the chorionic trophoblasts of goats by *B. abortus*. The bacterium preferentially replicates within trophoblasts, highly metabolically active cells that show variable production of proteins and steroids throughout gestation. Trophoblasts play an important role in fetal development and in the maintenance of pregnancy in ruminants (32). Invasion of the placenta by *Brucella* occurs primarily through erythro-phagocytic trophoblasts, with subsequent spread to the cells of the chorioallantoic membrane. Later, *Brucella* organisms multiply within the RER of trophoblasts, causing hypertrophy of the RER and subsequent release into the uterine lumen. Vasculitis and other lesions lead to separation of placental trophoblasts and maternal epithelium, resulting in death of the fetus and consequent abortion (7).

In contrast to *B. abortus*, which is usually not isolated from vaginal samples prior to parturition or abortion, *B. melitensis* can often be isolated from the vagina of infected pregnant goats prior to delivery. Shedding of *Brucella* decreases within one month, but the organism can be isolated for up to one year after parturition. Similar to the situation in other ruminant species, *B. melitensis* localises in the reproductive and fetal tissues of pregnant goats, causing necrosis and exudation in placental tissues (45).

The pathogenesis of *B. abortus* infection of the mammary gland and lymph nodes of goats has been evaluated under experimental conditions (47). One study demonstrated the role played by macrophages in transporting *B. abortus* from the systemic circulation into the mammary glands and milk, the intracellular multiplication in alveoli and ducts, and the transport of the organism from the infected gland to supramammary lymph nodes. Gross and histopathological lesions observed in infected mammary glands have contributed to the understanding of the pathogenesis of *Brucella* infection in mammary tissues of ruminants. Another study demonstrated that failure to nurse or release milk resulted in increased multiplication of *Brucella* in the mammary glands of goats after parturition, which indicates that the mammary glands are an important site for persistence and transmission (46).

Pathobiology of *Brucella ovis* infection in sheep

Brucella ovis was originally isolated and recognised as a pathogen in New Zealand in 1952 (40) and as a *Brucella*-like organism in Australia in 1953 (67). It is an important cause of infectious subfertility in rams, and has a significant economic impact on the sheep industry (19). *Brucella ovis* infection results primarily in epididymitis and seminal vesiculitis in rams, with associated poor semen quality (20). Sexually mature rams are more often affected than young animals (72). Infection occurs by direct contact with mucosal surfaces, with sexual transmission being an important route (76). Ewes can be a mechanical vector for the transmission of brucellosis between rams (14). *Brucella ovis* infection is widely distributed in most significant sheep-raising countries, with flock prevalence ranging from 9.1% to 46.7% (16, 65).

Clinical diagnosis alone is not accurate, but a high frequency of clinical unilateral or bilateral epididymitis (Fig. 3) is suggestive of *B. ovis* infection (20, 74). The tail of the epididymis is most frequently affected (Fig. 4), but only approximately one-third of seropositive or bacteriologically positive rams have any clinical signs (35). Although seroconversion and shedding of bacteria in the semen occur, experimentally infected rams demonstrate mild or no detectable lesions during the acute phase of infection (20, 76). Asymptomatically infected rams may be subfertile or may have normal fertility, which contributes to dissemination of *B. ovis* because marked infertility is only perceptible in the flock when a very high percentage of rams are infected (35).

Fig. 3
Asymmetrical epididymal tails from a ram due to *Brucella ovis*-induced unilateral chronic epididymitis

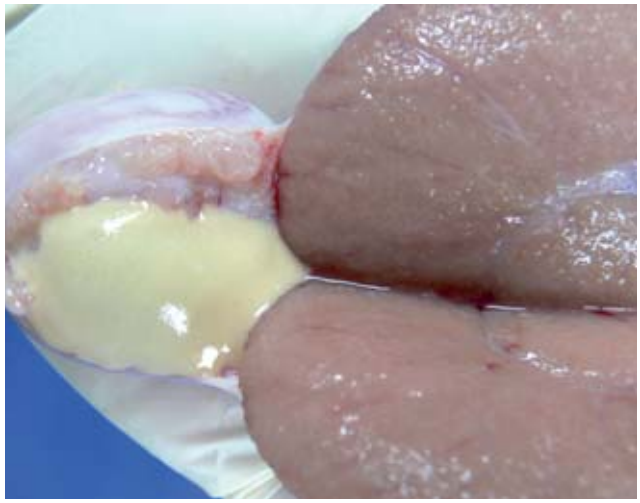
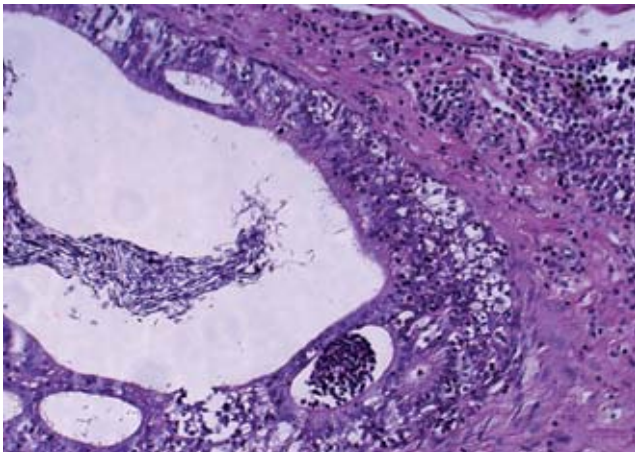


Fig. 4
Chronic epididymitis in a ram due to *Brucella ovis* infection
Although it appears that there is purulent exudate draining from the cut surface, the figure shows epididymal duct contents (mostly sperm) draining from a spermatic granuloma

Infected rams usually develop focal to diffuse interstitial lymphohistioplasmacytic epididymitis (Fig. 5a). These changes may be associated with rupture of the epididymal duct and formation of sperm granulomas (Fig. 5b). The epithelium of the epididymal duct frequently develops hyperplasia with marked vacuolisation (Fig. 5a). These epididymal changes are often associated with mild to moderate testicular degeneration. *Brucella ovis* also causes vesiculitis, which is characterised by interstitial infiltration of lymphocytes and macrophages and accumulation of neutrophils in the lumen of the epididymis (20). These lesions contribute to the poor spermatic quality and increased frequency of spermatic defects associated with infection (18), and to the variable infiltrates of inflammatory cells in the semen (20). The mechanism responsible for the strong tropism of *B. ovis* for the male genital tract is not clear.

Infection of pregnant ewes may result in abortion or birth of weak lambs with a high neonatal death rate (30). The highest susceptibility of pregnant ewes occurs around the 30th day of gestation (52). Although lactating ewes often excrete the organism in their milk, ingestion of contaminated milk does not seem to be a significant source of infection for young animals (30).

***a)* Epididymal duct epithelium with severe vacuolation, and intraepithelial infiltration of inflammatory cells associated with interstitial lymphocytic infiltration**



***b)* Sperm granuloma, with several multinucleated giant macrophages**

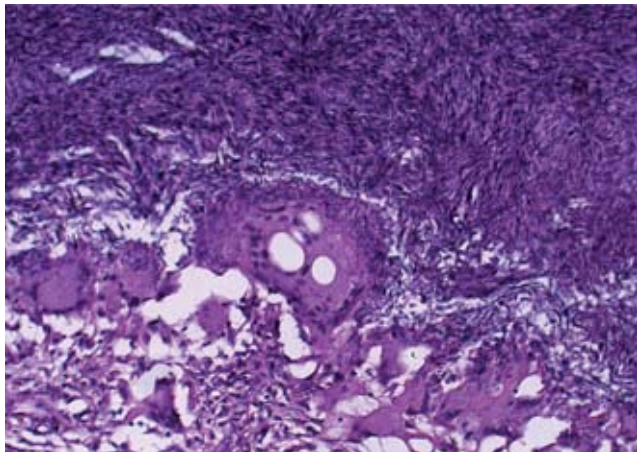


Fig. 5
Chronic epididymitis in a ram due to *Brucella ovis* infection

Although the sheep is by far the most important host species for *B. ovis*, the domestic goat, mouflon (*Ovis musimon*), white-tailed deer (*Odocoileus virginianus*) and red deer (*Cervus elaphus*) are also susceptible to infection (11, 17, 24, 58).

Molecular mechanisms in the pathogenesis of *B. ovis* have been poorly characterised. However, the *virB* operon-encoded type IV secretion system is critical for *B. ovis* infection and persistence, and for intracellular survival in murine models (62). Outer membrane proteins of the Omp25/Omp31 family also appear to play a role in intracellular survival of *B. ovis* (43). Genomic analysis of the *B. ovis* genetic sequence (71) has led to the identification of a *B. ovis*-specific genomic island (BOPI-1) that encodes an ATP-binding cassette (ABC) transporter that is required for *B. ovis* virulence (66).

Pathobiology of *Brucella suis* infection in pigs

Brucellosis in pigs is caused by *Brucella suis*, which was isolated from aborted swine fetuses in 1914 by Traum (70). This species includes five biovars, with biovars 1 and 3 being the most common in pigs worldwide. Biovar 2 occurs in Europe, where the hosts are pigs and hares. *Brucella suis* biovar 4 is enzootic in reindeer and caribou in Siberia, Alaska and Canada. Although biovar 4 is not pathogenic for pigs, it can cause human brucellosis. *Brucella suis* biovar 5 causes murine brucellosis (6).

Brucella suis is the only *Brucella* species recognised to cause systemic and generalised infection in pigs, which results in reproductive failure. Pigs can be infected with other *Brucella* species, but the infection is self-limiting and restricted to regional lymph nodes at the point of entry (27).

Porcine brucellosis is usually a more generalised and chronic disease than bovine brucellosis. Bacteraemia may persist for months, and the organisms may also persist in the uterus, leading to a chronic metritis. Genital infections are more frequent in boars than in bulls, and may induce lesions of necrotic orchitis. Infection in pigs is characterised by abortion, stillbirths, decreased litter size, weak piglets, infertility, orchitis and epididymitis in males, and focal abscess formation (48). Spondylitis is commonly seen and associated posterior paralysis is an occasional sequel, especially in older pigs (27). Nodules may occur in the spleen or liver, and abscesses may be observed in bones, joints and bursae in both sexes. Venereal transmission is an important route of spread in pigs (6).

Clinical signs in sows include oestrus after artificial insemination, vaginal discharge, and posterior paralysis. Splenic abscesses and small intestinal adhesions can be found on post-mortem examination (49). Abortions have been observed as early as 17 days following natural insemination by infected boars. Early abortions are usually unnoticed and the only evidence of infection is that the sow shows signs of oestrus 30 to 45 days after mating (48).

Histopathologically, there are inflammatory lesions characterised by accumulations of neutrophils, macrophages and giant cells, and hyperplasia of reticular tissue. Necrosis

can be observed associated with small abscesses within fibrous tissue (6, 73).

As in other species of the genus, *B. suis* requires the *virB* operon-encoded T4SS for intracellular invasion and multiplication within host cells; T4SS mutants are not able to survive and multiply in macrophages or epithelial cells (51).

Concluding remarks

The *Brucella* species that infect livestock cause significant clinical disease and economic losses. They are host restricted and, importantly, most of them have the potential to be zoonotic. Although the determinants of host specificity are unknown, significant advances in knowledge have occurred recently in regard to the mechanisms that *Brucella* spp. use in pathogenesis and pathobiology. These advances may help researchers to develop better livestock vaccines and measures to prevent human infections.

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Pathogénie et pathobiologie de la brucellose chez les animaux domestiques

F.P. Poester, L.E. Samartino & R.L. Santos

Résumé

Les brucelles sont des bactéries intracellulaires facultatives à Gram négatif présentant un tropisme génital marqué chez les femelles gestantes d'espèces animales d'élevage. Quelle que soit l'espèce de *Brucella* en cause, l'infection s'installe de manière persistante dans le système réticuloendothélial des hôtes naturels. Les mécanismes régissant la localisation placentaire, le tropisme pour le trophoblaste et les avortements sont encore mal connus. Les déterminismes moléculaires et les mécanismes d'internalisation cellulaire viennent tout juste d'être élucidés. Le glucane cyclique β-1,2 est une molécule extraite de l'espace périplasmique de *Brucella* grâce à laquelle la bactérie prévient la fusion du phagosome avec les lysosomes à l'intérieur de la cellule. Le système de sécrétion de type IV transfère les protéines effectrices de *Brucella* dans les cellules de l'hôte et s'avère indispensable pour que la bactérie puisse survivre et se multiplier dans les cellules hôtes infectées. Les auteurs examinent certains aspects de la pathogénie et de la pathobiologie de la brucellose chez les animaux d'élevage.

Mots-clés

Brucella abortus – *Brucella melitensis* – *Brucella ovis* – *Brucella suis* – Brucellose – Pathobiologie – Pathogenèse – Zoonose.

Patogénesis y patobiología de la brucelosis en el ganado

F.P. Poester, L.E. Samartino & R.L. Santos

Resumen
Las especies del género *Brucella* son bacterias gram negativas intracelulares facultativas, con un marcado tropismo por el tracto reproductor de las hembras grávidas de animales domésticos. Todas las brucelas se establecen en forma de infección persistente en el sistema reticuloendotelial de sus hospedadores naturales. No se conocen bien los mecanismos que intervienen en la localización de la placenta, el tropismo por el trofoblasto y el aborto. Solo últimamente se han empezado a describir el conjunto de los mecanismos y determinantes moleculares del proceso de interiorización celular. El glucano β-1,2 cíclico es una molécula secretada al interior del periplasma de *Brucella*, que la bacteria, una vez dentro de la célula, necesita para evitar que el fagosoma se fusione con lisosomas. El sistema de secreción tipo IV, que introduce las proteínas efectoras de *Brucella* en las células hospedadoras, es esencial para la supervivencia y replicación de *Brucella* en la célula del animal infectado. Los autores examinan ciertos aspectos de la patogénesis y la patobiología de la brucelosis en animales domésticos productivos.

Palabras clave
Brucella abortus – *Brucella melitensis* – *Brucella ovis* – *Brucella suis* – Brucelosis – Patobiología – Patogénesis – Zoonosis.

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