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Review

Porcine circovirus type 2 (PCV2) infections: Clinical signs, pathology and laboratory diagnosis

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ABSTRACT

Clinical signs and pathological features are still the corner-stones to suspect and diagnose overt disease associated with PCV2 infection. The clinico-pathological scope of this viral infection has been expanded over time. From the initial description of postweaning multisystemic wasting syndrome, some enteric, respiratory and reproductive disorders have been subsequently linked with PCV2. Porcine dermatitis and nephropathy syndrome, an immunocomplex disease, has also been associated with infection by this virus. All together, these conditions have been grouped under the name of porcine circovirus diseases (PCVD) or porcine circovirus associated diseases (PCVAD). The precise mechanisms by which a PCV2 infected pig develops a PCV2 subclinical infection or a clinical PCVD/PCVAD are still to be fully elucidated, but inferences based upon clinical, gross and histologic findings from field cases of disease have been useful to suggest the pathogenesis of this viral infection. The objective of the present review is to update the current knowledge on the clinical and pathological scope of PCV2 infections, as well as on their diagnosis. Moreover, a proposal on a unified PCVD/PCVAD terminology and clearly defined diagnostic criteria for these conditions are also given.

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1. Introduction

Postweaning multisystemic wasting syndrome (PMWS) was initially described in 1991 in Saskatchewan (Canada) as a sporadic disease characterized by wasting and jaundice (Clark, 1996; Harding, 1996). This condition was further observed from 1994 onwards by its discoverers (Dr. John Harding as clinician and Dr. Edward G. Clark as pathologist) and it was soon described in all continents, including Oceania (Grau-Roma et al., 2011). The new disease defined a clinical picture characterized by wasting, paleness of the skin, respiratory distress and, occasionally, diarrhea and jaundice in late nursery and fattening pigs (Segalés et al., 2005a). Affected animals displayed characteristic lesions in multiple tissues (multisystemic), mainly in lymphoid organs (Clark, 1997; Rosell et al., 1999).

Soon after initial descriptions of PMWS, a massive presence of porcine circovirus (PCV) antigen was demonstrated within lesions of affected animals (Clark, 1997; Segalés et al., 1997). Since PCV was considered non-pathogenic for swine (Allan et al., 1995; Tischer et al., 1986), an immediate reaction against the causality of PMWS by PCV occurred in the swine scientist and veterinarian communities worldwide. The following year, nucleotide sequence analysis of the PCV associated to PMWS revealed important

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differences compared to the previously known PCV derived from PK-15 cells (ATCC CCL-33) (Hamel et al., 1998; Meehan et al., 1998). Therefore these viruses were named PCV type 1 (PCV1) for the cell culture-derived virus, and PCV type 2 (PCV2) for the disease associated virus (Allan et al., 1999b).

After 1998, experimental studies with PCV2 were mainly focused on PMWS reproduction (Allan et al., 2004). It was soon realized that reproduction of the disease was rarely achieved by means of using only PCV2 in the inoculum (Bolin et al., 2001; Harms et al., 2001; Okuda et al., 2003). In most cases clinical disease was reproduced when PCV2 was inoculated together with another infectious or non-infectious agent (Allan et al., 1999a; Krakowka et al., 2000, 2001; Opriessnig et al., 2004; Rovira et al., 2002). Therefore, PCV2 was considered a necessary but not sufficient factor to develop the clinical disease (Ellis, 2003; Tomás et al., 2008).

The clinical and pathological scope of PCV2 infection has been expanded since 1991. Besides PMWS, PCV2 has also been implicated in other conditions. PCV2 has been suggested to play a role in reproductive disorders, the so-called porcine respiratory disease complex (PRDC), enteritis, porcine dermatitis and nephropathy syndrome (PDNS) and proliferative and necrotizing pneumonia (PNP) (Opriessnig et al., 2007; Segalés et al., 2005a). In addition, congenital tremor type A2 was initially linked to PCV2 infection (Stevenson et al., 2001), but subsequent studies suggested no association between the virus and the condition (Ha et al., 2005; Kennedy et al., 2003). It is worthy to remark that the role of PCV2 in all these conditions, as well as an unequivocal interpretation of what means to find the virus in a given lesion, has been a matter of controversy over the years. Such complicated scenario has caused that a number of terminologies have been used to name diseases linked to PCV2, being in some cases rather confusing. Confusion extended also to the establishment of unequivocal diagnostic criteria for PCV2 associated conditions.

The objective of the present review is to update the current knowledge on the clinical and pathological scope of PCV2 infections, as well as on their diagnosis. Moreover, a proposal on terminology and clearly defined diagnostic criteria for these conditions are given.

2. Clinical features associated to PCV2 infections

2.1. PCV2 subclinical infection

Based on PCV2 serological studies, it is assumed that PCV2 infection is ubiquitous all over the world (Segalés et al., 2005a), while prevalence of clinical disease is much lower. On the other hand, the first evidence of PCV2 infection was retrospectively found in Germany in 1962 (Jacobsen et al., 2009), while the first retrospectively established diagnoses of PMWS are from middle 80s (Jacobsen et al., 2009; Rodríguez-Arrioja et al., 2003). Therefore, the most common form of PCV2 manifestation is the subclinical infection. Even no overt clinical signs are seen, different field evidences indicate that PCV2 vaccination is able to improve productive parameters (average daily gain, percentage of runts, body condition and carcass weight) in PCV2 subclinical infection scenarios (Young et al., 2011). However, it must be emphasized that such effect of PCV2 vaccines on subclinical infection scenarios should be further studied.

It has also been demonstrated experimentally that subclinical PCV2 infection may be associated with decreased vaccine efficacy (Opriessnig et al., 2006c). In contrast, another experimental PCV2 subclinical infection was not able to establish detrimental effects upon pseudorabies vaccine immunological responses (Díaz et al., in press).

2.2. PCV2 clinical infection

A number of clinical syndromes have been linked to PCV2 infection. The most described one is PMWS, which has been referred with different names in the literature including porcine circovirosis (Rosell et al., 2000b), PCV2-associated systemic infection (Opriessnig et al., 2007) and porcine circovirus-associated disease (PCVAD) in some cases (Carman et al., 2008). Other pathological conditions linked to PCV2 have been porcine dermatitis and nephropathy syndrome (PDNS) (Allan et al., 2000; Rosell et al., 2000a; Wellenberg et al., 2004), reproductive failure (Madson et al., 2009a; Mateusen et al., 2007; O'Connor et al., 2001; West et al., 1999), proliferative and necrotizing pneumonia (Grau-Roma and Segalés, 2007; Szeredi and Szentirmai, 2008), respiratory disease (Cheng et al., 2011; Kim et al., 2003; Wellenberg et al., 2010) and enteritis (Kim et al., 2004; Opriessnig et al., 2007).

All these clinical conditions were initially referred as porcine circovirus diseases (PCVD) (Allan et al., 2002). This name was widely used in Europe, but in 2006, in North America, it was felt that any new term used in connection with PCV2 should include the word "associated", which led to the creation and introduction of the term (PCVAD) (http://www.aasv.org/). At the very end, and paraphrasing Dr. John Harding (University of Saskatchewan, Canada): "The use of PCVAD versus PVCD more clearly separates the Europeans, who collectively are the leaders in PCV research, from the North Americans, which except for a few individuals have lagged. Because we are dealing with a global disease with the same etiology, pathogenesis, and pathology, there must be one cohesive name for this syndrome." (Harding, 2007). In order to be cohesive, the author of this revision proposes the use of the first terminology proposed, porcine circovirus diseases – PCVD, to designate all the above mentioned conditions, including the PCV2 subclinical infection.

In addition, Table 1 configures a proposal on terminology of the different PCVDs described so far based on a number of publications, with special emphasis on the contribution of Opriessnig et al. (2007). These authors introduced a number of specific PCVD names, which have been used irregularly in the literature to date. The present review will use the proposed terminology (Table 1) from here onwards.

From a clinical point of view, PCVDs with overt clinical outcome may potentially include the following signs in broad sense:

PCV2 systemic disease (PCV2-SD): Morbidity in affected farms is commonly 4–30% (occasionally 50–60%) and mortality ranges from 4 to 20% (Segalés and Domingo, 2002). PCV2-SD is clinically characterized by wasting or weight loss, pallor of the skin, respiratory distress, diarrhea, and occasionally, icterus (Harding and Clark, 1997; Krakowka et al., 2004; Rosell et al., 1999). Enlarged subcutaneous lymph nodes are a common finding in the early clinical phases of the systemic disease (Clark, 1997; Rosell et al., 1999). More details on clinical signs of PCV2-SD have been described elsewhere (Chae, 2005; Harding, 2004; Harding and Clark, 1997; Madec et al., 2000; Segalés and Domingo, 2002).

PCV2 lung disease (PCV2-LD) and PCV2 enteric disease (PCV2-ED): Main clinical signs are respiratory distress (Harms et al., 2002; Kim et al., 2003) and diarrhea (Kim et al., 2004; Opriessnig et al., 2007), respectively. There is potential diagnostic overlapping between PCV2-SD and these two conditions (Opriessnig et al., 2007), since both clinical signs can be easily present in cases of the systemic disease. Their differentiation, even of limited interest by field veterinarians, must rely on histopathological findings and examination not only of lung and gut but also lymphoid tissues (which must not display microscopic lesions of PCV2-SD). Importantly, PCV2 is considered as one more pathogen potentially contributing to PRDC outcome (Hansen et al., 2010b; Kim et al., 2003). Taking into account that PRDC is diagnosed based on clinical signs (Dee, 1996), the involvement of PCV2 on it may lead to a

Table 1
Proposed terminology for porcine circovirus diseases (PCVDs) together with their case definition based on clinical and laboratorial findings.

PCVD proposed name (acronym)	Replaced terminology	Main clinical sign	Individual diagnostic criteria
PCV2 subclinical infection (PCV2-SI)	None	Decreased average daily gain without any evident clinical sign	1. Lack of overt clinical signs 2. No or minimal histopathological lesions in tissues (mainly lymphoid) 3. Low amount of PCV2 in few (lymphoid) tissues Criteria 2 and 3 can potentially be substituted by PCV2 detection techniques such as standard PCR
PCV2 systemic disease (PCV2-SD)	Postweaning multisystemic wasting syndrome (PMWS) Porcine circovirosis PCV2-associated systemic infection	Wasting, weight loss, decreased rate of weight gain clinically evident, ill thrift or poor-doer	1. Weight loss and paleness of skin (respiratory and/or digestive clinical signs may be present as well) 2. Moderate to severe lymphocyte depletion with granulomatous inflammation of lymphoid tissues (plus granulomatous inflammation in a number of other tissues) 3. Moderate to high amount of PCV2 in damaged tissues
PCV2 lung disease (PCV2-LD)	PCV2-associated respiratory disease Proliferative and necrotizing pneumonia (PNP)	Respiratory distress, dyspnea	1. Respiratory clinical signs 2. Lympho-histiocytic to granulomatous interstitial or broncho-interstitial pneumonia, peribronchiolar fibroplasia, mild-to-severe necrotizing and ulcerative bronchiolitis or proliferative necrotizing pneumonia in absence of lymphoid lesions as indicated for PCV2-SD 3. Moderate to high amount of PCV2 in lung Lymphoid tissues should not display microscopic lesions (otherwise, it would be into the scope of PCV2-SD)
PCV2 enteric disease (PCV2-ED)	PCV2-associated enteritis	Diarrhea	1. Diarrhea 2. Granulomatous enteritis and lymphocyte depletion with granulomatous inflammation in Peyer's patches but not other lymphoid tissues 3. Moderate to high amount of PCV2 in intestinal mucosa/Peyer's patches Lymphoid tissues (but Peyer's patches) should not display microscopic lesions (otherwise, it would be into the scope of PCV2-SD)
PCV2 reproductive disease (PCV2-RD)	PCV2-associated reproductive failure	Abortions or mummifications	1. Reproductive failure at late gestation 2. Fibrous to necrotizing myocarditis of fetuses 3. Moderate to high amount of PCV2 in heart The use of real time quantitative PCR on fetal tissues might be more sensitive to detect PCV2-RD
		Regular return-to-estrus	1. Regular return-to-estrus 2. PCV2 sero-conversion following the return-to-estrus and/or PCV2 PCR positivity around return-to-estrus occurrence
Porcine dermatitis and nephropathy syndrome (PDNS)[a]	None	Dark red papules and macules on skin, mainly in hind limbs and perineal area	1. Hemorrhagic and necrotizing skin lesions and/or swollen and pale kidneys with generalized cortical petechia 2. Systemic necrotizing vasculitis, and necrotizing and fibrinous glomerulonephritis

[a] PDNS is considered an immune-complex disease of not yet demonstrated etiology; link with PCV2 is still circumstantial, and detection of the virus is not considered into its diagnostic case definition. Therefore, no novel terminology has been suggested for this particular syndrome.

complex diagnostic scenario including clinical assessment of PRDC and laboratory confirmation of PCV2-LD or PCV2-SD (plus other respiratory/systemic pathogens).

PCV2 reproductive disease (PCV2-RD): PCV2 has been linked to late term abortions and stillbirths (Brunborg et al., 2007; West et al., 1999) as well as mummification (Madson et al., 2009a) resembling the one caused by porcine parvovirus (Mengeling, 2006). However, PCV2-associated reproductive disease under field conditions is rare (Pensaert et al., 2004). This is probably due to the fact that the seroprevalence of PCV2 in adult pigs is high and, therefore, most breeding herds are not suffering from the clinical disease. Affected herds are typically start-up herds, new populations in which the number of gilts is high or PCV2 seronegative herds (Opriessnig et al., 2007; West et al., 1999;Togashi et al., 2011). Although sows (Calsamiglia et al., 2007; Rodríguez-Arrioja et al., 2002; Shen et al., 2010) and boars (Larochelle et al., 2000; Madson et al., 2008; McIntosh et al., 2006) may be subclinically PCV2 infected, the precise disease outcome on their offspring is not yet known. Indeed, the amount of PCV2 DNA present in semen of experimentally infected boars, ranging from $10^{5.6}$ to $10^{5.8}$ viral genomic copies/mL (Madson et al., 2009b), is probably not sufficient to cause reproductive failure, seroconversion of the sow, or fetal infection. Further studies would be needed to establish the potential relevance and clinical implications of PCV2 infection of dams to their offspring during pregnancy. Additionally, experimental data are suggesting that PCV2 might be associated as well to return-to-estrus scenarios since PCV2 can replicate in embryos and might lead to embryonic death (Mateusen et al., 2007). Importantly, in a small proportion of embryos, PCV2 exposure did not have a detrimental effect on embryo development before the first 21 days of pregnancy (Mateusen et al., 2007).

Porcine dermatitis and nephropathy syndrome (PDNS): PDNS-affected pigs are clinically anorexic and depressed, with little or no pyrexia (Drolet et al., 1999). They may be prostrate, reluctant to move, and/or display stiff-gait. The most obvious sign is the presence of irregular, red-to-purple macules and papules in the skin, primarily on the hind limbs and perineal area, but sometimes more generally distributed. With time, lesions become covered by dark crusts. Cutaneous lesions gradually fade, sometimes leaving scars (Drolet et al., 1999; Segalés et al., 1998). PDNS affects nursery, growing, and adult pigs (Drolet et al., 1999). The prevalence of the syndrome is usually below 1% (Segalés et al., 1998), although higher frequency has been occasionally described (Gresham et al., 2000). Mortality may reach 100% in pigs older than 3 months of age versus approximately 50% of affected 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 to 10 days after the beginning of the syndrome (Segalés et al., 1998).

In 2009, pigs from PCV2-vaccinated herds in the U.S.A. experienced a peracute syndrome, referred as acute pulmonary edema (APE), which mainly affected nursery and younger finisher pigs (Cino-Ozuna et al., 2011). Mortality approached 20% in some affected groups, and clinical signs included a rapid onset of respiratory distress followed rapidly by death; in fact, pigs were often found dead with no previous indication of disease signs. The only consistent infectious agent detected in a number of examined tissues was PCV2 (Cino-Ozuna et al., 2011). A likely pathogenesis of this condition was speculated. Large quantities of virus would replicate in mononuclear cells and endothelial cells lining the blood vessels in the lungs of young pigs, which may be more susceptible to infection and could support higher levels of replication. Infection might be established in young pigs prior to vaccination and in the absence of protective levels of maternal derived antibodies. Vascular endothelial cell damage combined with cytokine release by monocytes would result in the loss of blood vessel wall integrity and outflow of vascular contents into the interstitium. APE-susceptible pigs would lack sufficient levels of preexisting antibody and would become infected prior to vaccination (Cino-Ozuna et al., 2011). Therefore, these authors speculated on the fact that vaccination was applied too late in these particular animals and an earlier administration of the vaccine may prevent the condition (Cino-Ozuna et al., 2011). However, this potential new condition linked to PCV2 has only been described once and it is too early to establish a likely pathogenesis and drawn sound conclusions about PCV2 causality.

3. Pathological features associated to PCV2 infections

Gross and microscopic pathology associated to PCV2 infections have been described elsewhere in a number of original (Brunborg et al., 2007; Clark, 1997; Kennedy et al., 2000; Krakowka et al., 2000; Nielsen et al., 2008; Rosell et al., 1999; Saha et al., 2010; West et al., 1999) as well as review papers (Chae, 2005; Opriessnig et al., 2007; Segalés et al., 2004). Moreover, detailed pathological descriptions of PDNS were performed even before the establishment of its link with PCV2 infection (Drolet et al., 1999; Helie et al., 1995; Ramos-Vara et al., 1997; Segalés et al., 1998; Thibault et al., 1998). Table 2 summarizes the most significant gross and microscopic lesions associated to PCVDs. In addition, the following paragraphs focus on these less frequently and more recently described lesions linked to PCV2 infection.

Necrotizing lymphadenitis can be found in at least one lymph node in around 10% of pigs suffering from naturally occurring PCV2-SD (Segalés et al., 2004). This lesion has been also observed under experimental conditions with pigs displaying lymphocyte depletion and granulomatous inflammation in lymph nodes (Opriessnig et al., 2006a,b). Such necrosis can affect wide areas of lymph node parenchyma or be mainly focused in follicular areas. PCV2 is widely found in the necrotic areas. Subsequent studies suggested that lymph nodes affected by such necrosis show hypertrophy and hyperplasia of high endothelial venules, over-expression of von Willebrand factor and secondary thrombosis (Galindo-Cardiel et al., 2011). Therefore, this study pointed towards a vascular accident with thrombus development as the pathogenic mechanism underlying necrotizing lymphadenitis in pigs with PCV2-SD. Apoptosis was not apparently involved in the observed necrosis (Galindo-Cardiel et al., 2011). Vasculitis has not been observed in necrotizing lesions, but overall findings suggest that necrotic areas are likely associated with damaged vessels (Galindo-Cardiel et al., 2011; Opriessnig et al., 2006b). On the other hand, PCV2-associated necrotizing lymphadenitis in individual lymph nodes of clinically healthy pigs with no lymphocyte depletion and granulomatous inflammation has been also described (Kim and Chae, 2005). This latter study concluded that necrotizing lymphadenitis might be a new clinical manifestation of PCV2 infection in pigs, but the authors could not rule out that presence of virus and necrotizing lymphadenitis would be completely independent (Kim and Chae, 2005). Also, necrotic foci in lymph nodes have been described in PCV2-SD affected animals concomitantly infected with pseudorabies virus (Rodríguez-Arrioja et al., 1999). However, the extensive presence of this latter virus in such necrotizing lesions, together with PCV2, pointed out as pseudorabies virus as the main cause of them (Rodríguez-Arrioja et al., 1999).

Heart failure has been described due to acute necrotizing or chronic fibrosing myocarditis, with chronic vasculitis in heart, kidney and lymphoid tissues from nursery pigs; PCV2 antigen was detected within the cytoplasm of myocardiocytes and within vascular endothelial cells in the myocardium (Opriessnig et al., 2006b). Moreover, severe diffuse segmental to circumferential

Table 2
Summary of the most frequent gross and histopathological features of PCVDs, as well as the amount of PCV2 usually found in these lesions and/or serum of affected animals.

PCVD	Gross lesions	Microscopic lesions	Amount of PCV2[a]
PCV2 subclinical infection (PCV2-SI)	• None	• None or slight lymphocyte depletion with granulomatous inflammation of lymphoid tissues	• *IHC*/*ISH*: none or low amount in lymphoid tissues • *qPCR*: $<10^5$ to 10^6 (A)
PCV2 systemic disease (PCV2-SD)	• Long rough hair coat, prominent backbone and relatively oversized head • Lymph node enlargement • Lack of pulmonary collapse and tan-mottled lungs • White spots on kidney's cortices • Atrophic and discolored liver, slightly rough hepatic surface • Catarrhal enteritis with or without mesenteric edema • Occasional spleen infarcts	• Moderate to severe lymphocyte depletion with granulomatous inflammation of lymphoid tissues; possible presence of intracytoplasmic botryoid inclusion bodies • Lymphohistiocytic to granulomatous interstitial pneumonia; occasionally, peribronchiolar fibroplasia, mild to severe necrotizing bronchiolitis and/or proliferative and necrotizing pneumonia • Interstitial nephritis • Variable degree of lymphohistiocytic hepatitis, with apoptotic bodies, disorganization of hepatic plates, and/or perilobular fibrosis • Granulomatous enteritis • Possibility of lymphohistiocytic inflammation in virtually whatever tissue	• *IHC*/*ISH*: moderate to high amount in lymphoid tissues; from none to high amount in non-lymphoid tissues, depending on lesional severity • *qPCR*: $>10^6$ (B)
PCV2 lung disease (PCV2-LD)	• Lack of pulmonary collapse and tan-mottled lungs	• Granulomatous bronchointerstitial pneumonia with or without bronchiolitis and bronchiolar fibrosis • Lack of PCV2-SD hallmark lesions in lymphoid tissues	• *IHC*/*ISH*: moderate to high amount in lung and negative or low amount in lymphoid tissues • *qPCR*: non-determined
PCV2 enteric disease (PCV2-ED)	• Catarrhal enteritis with or without mesenteric edema • Intestinal mucosa thickened • Enlargement of mesenteric lymph nodes	• Granulomatous enteritis • Lymphocyte depletion with granulomatous inflammation in Peyer's patches but not in other lymphoid tissues	• *IHC*/*ISH*: moderate to high amount in intestinal mucosa and Peyer's patches; negative or low amount in lymphoid tissues • *qPCR*: non-determined
PCV2 reproductive disease (PCV2-RD)[b]	• Fetal mummification or edematous fetuses • Fetal hepatic enlargement and congestion • Fetal cardiac hypertrophy with multifocal areas of myocardial discoloration • Ascites, hydrothorax and hydropericardium in fetuses	• Non-suppurative to necrotizing or fibrosing myocarditis of fetuses • Chronic, passive, hepatic congestion in fetuses • Mild pneumonia in fetuses	• *IHC*/*ISH*: moderate to high amount in fetal myocardium • *qPCR*: $>10^7$/500 ng DNA of myocardium, liver, and spleen from mummified or stillborn piglets; $>10^5$/500 ng DNA of in piglets with myocarditis (C)
Porcine dermatitis and nephropathy syndrome (PDNS)	• Irregular, red-to-purple macules and papules in the skin; subcutaneous hemorrhages and edema of affected areas • Enlarged lymph nodes, mainly inguinal superficial one • Cutaneous scars in animals that recovered from the acute phase • Bilaterally enlarged kidneys, small cortical petechiae and edema of the renal pelvis • Occasional spleen infarcts	• Systemic necrotizing vasculitis • Fibrino-necrotizing glomerulitis with non-purulent interstitial nephritis • Chronic, fibrous interstitial nephritis with glomerulosclerosis in animals that recovered from the acute phase • From none to mild/moderate lymphocyte depletion with mild granulomatous inflammation of lymphoid tissues	• *IHC*/*ISH*: none or low amount in lymphoid tissues • *qPCR*: $<10^6$ (D)

(A) Based on Brunborg et al. (2010), Cortey et al. (2011) and Grau-Roma et al. (2009); (B) based on Brunborg et al. (2004), Fort et al. (2007), Grau-Roma et al. (2009) and Olvera et al. (2004); (C) based on Brunborg et al. (2007) and Hansen et al. (2010a); (D) based on Aramouni et al. (2011) and Olvera et al. (2004).

[a] IHC/ISH: PCV2 antigen or nucleic acid detected by means of immunohistochemical or in situ hybridization methods, respectively, on formalin-fixed, paraffin-embedded tissues; qPCR: PCV2 nucleic acid detected by means of real time quantitative PCR methods in serum samples (expressed as PCV2 DNA copies/mL serum or per 500 ng of total DNA extracted for fetal tissues in PCV2-RD). In all cases, animals affected by these PCVDs give positive results by PCV2 PCR standard, non-quantitative methods in serum and/or tissues.

[b] Lesions have been so far described in fetuses but not in sows in the context of late term reproductive disease. No gross or microscopic lesions have been reported in return-to-estrus scenarios.

lymphohistiocytic and plasmacytic periarteritis and endarteritis were described in several organs of another nursery pig (Opriessnig et al., 2006b). Although only in the last pig it was confirmed the lack of PCV2-SD lymphoid lesions, it cannot be ruled out that such lesions might belong, in most of the cases, into the scope of PCV2-SD. On the other hand, lymphohistiocytic and plasmacytic vasculitis in pigs with experimentally reproduced PCV2-SD has been reported as well (Langohr et al., 2010; Opriessnig et al., 2006b). Overall, these unusual findings indicate that cardiovascular system and endothelial cells might play a role in the pathogenesis of PCV2 infections. In fact, PCV2 antigen and nucleic acid have been found in epithelial and endothelial cells, as described in early PCV2-SD reports (Kennedy et al., 2000; Krakowka et al., 2000; McNeilly et al., 1999; Rosell et al., 1999), and replication of the

virus in these cell types has been recently confirmed (Hamberg et al., 2007; Pérez-Martin et al., 2007).

Brain lesions are of very occasional occurrence in PCV2-SD (Rosell et al., 1999). However, two unrelated publications described cerebellar lymphohistiocytic vasculitis combined with hemorrhages and/or lymphohistiocytic meningitis in pigs naturally affected with PCV2-SD (Correa et al., 2007; Seeliger et al., 2007). Moreover, degeneration and necrosis of the gray and white matter associated to the necrotizing vasculitis was shown in one of these studies (Seeliger et al., 2007). In both cases, macrophages and endothelial cells were found to be positive to PCV2. In a more recent report, non-suppurative polioencephalomyelitis was found in a co-infection with porcine teschovirus and PCV2 within a PCV-SD background (Takahashi et al., 2008). The former virus was the only one found in brain lesions and considered the cause of them; it was speculated that immunosuppression driven by the PCV2-SD facilitated the infection by porcine teschovirus (Takahashi et al., 2008). Whether certain PCV2 strains may have some tropism for endothelial cells from the brain or epithelial cells of meninges is unknown. PCV2 sequencing from several strains of one of the mentioned studies revealed 89–100% identity to previous isolates that were not related with neurological lesions (Seeliger et al., 2007).

Kidney lesions have been widely described in PCVDs (Segalés et al., 2004). Pigs affected by PCV2-SD usually display interstitial nephritis, which has been described as tubulointerstitial lymphoplasmacytic nephritis, interstitial granulomatous nephritis, and mixed types (Sarli et al., 2008). In all these lesion types, PCV2 is relatively abundant, not only within inflammatory cells but also renal epithelial cells (Sarli et al., 2008). On the other hand, kidney lesions can be very striking in PDNS cases, with severe fibrino-necrotizing glomerulitis with non-suppurative interstitial nephritis and necrotizing vasculitis in renal pelvis (Drolet et al., 1999; Segalés et al., 1998). PCV2 has been detected within the interstitial inflammatory infiltrates in the kidney as well as in renal tubular cells in PDNS cases (Rosell et al., 2000a). More recently, renal tubular necrosis and interstitial hemorrhage ("turkey-egg kidney") with high amounts of PCV2 in renal tubular cells were described in a Yorkshire cross pig (Imai et al., 2006). These authors claimed that the renal lesions in this pig were not typical of either "classical" PCV2-SD or PDNS (exudative glomerulonephritis was not a feature of the studied animal). However, this animal had necrotizing vasculitis in kidney and spleen (Imai et al., 2006), which suggests a systemic lesion of blood vessels. Therefore, the likelihood that this animal was an atypical case of PDNS (Segalés et al., 2004) is very high.

The abovementioned condition named APE displayed gross lesions characterized by accumulation of clear fluid in the thoracic cavity and lack of pulmonary collapse, with moderate to severe interstitial edema (Cino-Ozuna et al., 2011). Microscopic examination of the lungs revealed interstitial edema, with diffuse interstitial infiltration of macrophages and lymphocytes. A common finding was fibrinoid necrosis of the blood vessel walls, with surrounding regions showing evidence of alveolar edema. In most of the affected pigs, there was diffuse lymphoid depletion, and a few pigs had rhinitis. This apparent novel condition seems to join lymphoid lesions with vascular damage, which would be inclusive into the scope of PCV2-SD (Langohr et al., 2010; Opriessnig et al., 2006b), although with a more acute clinical course.

4. Diagnostic tips for PCVDs

PCV2-SD is accepted to be a multifactorial pig disease in which PCV2 is the essential infectious agent (Segalés et al., 2005a). Due to the ubiquitous spread of the virus, it is very likely that most if not all PCVDs displaying overt clinical signs should be considered of multifactorial, complex causality as well, being PCV2 the strictly needed factor. Among all PCVDs, PCV2-SD has been traditionally considered the most economically significant one (Armstrong and Bishop, 2004). However, the widespread use of vaccines is nowadays demonstrating that the PCV2-SI might be even more economically important worldwide, since most if not all farms are infected with the virus. In any case, the precise cost of the subclinical infection (Young et al., 2011) is still to be elucidated in a clear-cut form.

The first step in diagnosis always involves clinical sign assessment when overt disease is perceived. Table 1 summarizes dominating clinical signs in each of the PCVDs considered in this review, even it is important to note that most of these symptoms are not specific of each of the PCVDs. Therefore, a differential diagnostic list must be established for every condition, as given elsewhere (Harding and Clark, 1997; Segalés, 2002; Segalés et al., 2005a). Interestingly, the use of PCV2 vaccines seems to represent the obligated step to assess the effects of PCV2-SI (Young et al., 2011).

Nowadays is possible to give diagnostic recommendations for all recognized PCVDs (Segalés, 2002; Segalés et al., 2005a; Sorden, 2000), as indicated in Table 1. These diagnostic criteria are considered very stringent since PCV2 is a ubiquitous virus and diagnosis of PCVDs cannot simply rely on detection of the virus or antibodies against it (McNeilly et al., 2002; Opriessnig et al., 2007; Segalés and Domingo, 2002). There are a number of PCVDs with a relatively easy laboratory diagnostic approach on an individual basis (PCV2-SD, PCV2-LD, PCV2-ED and the late term form of PCV2-RD), even some overlapping may occur among them, as mentioned before. The laboratory assessment of a PCV2-SI would be relatively easy as well, but the widespread nature of the virus usually prevents field veterinarians from establishing such diagnosis. However, the link of PCV2 with return-to-estrus has been so far only established by means of an experimental setup (Mateusen et al., 2007). Therefore, these observations should be contrasted by field data and, consequently, the proposed diagnostic criteria for this condition (Table 1) await further investigations.

Taking into account the strong correlation observed between the amount of PCV2 antigen and/or nucleic acid and the severity of PCV2-SD histopathological lesions, alternative diagnostic methods avoiding the euthanasia of the pig have been suggested. Specifically, several studies have proposed real time quantitative PCR (qPCR) thresholds in serum as indicative of PCV2-SD diagnosis: $10^{4.7}$ (Harding et al., 2008), $10^{6.21}$ (Grau-Roma et al., 2009), $10^{6.91}$ (Fort et al., 2007), 10^{7} (Brunborg et al., 2004; Olvera et al., 2004; Segalés et al., 2005b) and $10^{7.43}$ (Grau-Roma et al., 2009) viral copies/mL. Marked variation in qPCR detection limits has been shown among laboratories (Harding et al., 2009; Hjulsager et al., 2009). In consequence, the potential diagnostic PCV2 load threshold is strongly dependent on the laboratory and particular technique used. Importantly, sensitivity and/or specificity values observed from qPCR tests (used separately or combined with serology) were not able to substitute histopathology plus detection of PCV2 in tissues for the individual PCV2-SD diagnosis (Grau-Roma et al., 2009). Lately, an attempt to optimize sensitivity and specificity of qPCR on a herd basis was performed by means of pooled serum samples (Cortey et al., 2011). Although these technical parameters did not improve, serum pools seemed to be an alternative at a low economic cost for the quantification of PCV2 loads in PCV2-SD suspected herds.

Diagnosis of PCVDs other than PCV2-SD by qPCR has been poorly explored, although quantification values have been determined in cases of PCV2-SI (Brunborg et al., 2010; Cortey et al., 2011; Grau-Roma et al., 2009), PCV2-RD (Brunborg et al., 2007; Hansen et al., 2010a) and PDNS (Aramouni et al., 2011; Olvera et al., 2004). Interesting data on PCV2-RD diagnosis came from a repopulated Danish pig herd that experienced an increase in numbers of stillborn and mummies (Hansen et al., 2010a). Based

on the availability of production records and samples over time, the diagnostic value of different techniques was evaluated. The immunohistochemical detection of PCV2 in fetal heart was mainly useful to diagnose acute stages of reproductive failure, whereas qPCR was found as a sensitive diagnostic method within a wider time span (Hansen et al., 2010a). Specifically, values of qPCR higher than 10^7 PCV2 DNA copies/500 ng of fetal tissues have been considered a strong indication of PCV2-RD (Brunborg et al., 2007; Hansen et al., 2010a), and values higher than 10^5 PCV2 DNA copies/500 ng of fetal tissues were found in fetuses with myocarditis (Brunborg et al., 2007). In contrast, IgG measurements in fetal fluids gave unpredictable results as indication of intrauterine infection with PCV2 (Hansen et al., 2010a).

Individual case definition is the key to define when a given animal suffers from a particular disease. However, prevention and control measures should rely on the establishment of a herd diagnosis, rather than to base them in a single pig diagnosis. A formal proposal to establish PCV2-SD diagnosis at farm level has been given (www.pcvd.eu). Such herd diagnosis should be based on two elements: (1) a significant increase in postweaning mortality associated to the presentation of clinical signs compatible with PCV2-SD compared to the historical background in the herd, and (2) individual diagnosis of PCV2-SD (Table 1) in at least 1 out of 3–5 necropsied pigs performed contemporaneously to the mentioned increase in mortality (www.pcvd.eu). Ruling out any other potential cause of increase in mortality would be also necessary in such scenario. So far, no herd case diagnostic definitions have been proposed for PCVDs other than PCV2-SD.

The marked PCV2 vaccine efficacy caused a lower interest on PMWS diagnoses. At least a proportion of field veterinarians prefer a "trial and error" system instead of establishing a laboratory diagnosis. The major reason for such preference is the difficulty of establishing an unequivocal diagnosis of disease that fits into the internationally accepted PCVD case definitions.

Nowadays, the worldwide tendency in pig health programs is to massively vaccinate piglets against PCV2 (in both clinically affected and subclinically infected farms). In such scenario, the laboratory diagnosis (mainly of PCV2-SD) previous to vaccination might not make sense. In contrast, the interest of establishing a laboratory confirmed diagnosis might be increased in other contexts: (1) farms with PCVD-like clinical signs in pigs already vaccinated against PCV2, and (2) farms in which results obtained by PCV2 vaccination are under realistic expectations. Those scenarios are and will be relatively new. Veterinarians must be aware that a PCV2 vaccine "might not work as expected" or "may fail". To reach such conclusions should be the product of a complete diagnostic study, not only on PCV2 but also on other causes of growth retardation and mortality.

5. Discussion

The ubiquitous nature of PCV2 infection and the difficulties of consistent PCV2-SD experimental reproduction posed difficulties to understand how this virus was able to cause overt disease (Segalés et al., 2005a). More than 15 years after the initial descriptions of PCV2-SD (Harding, 1996), the precise mechanisms by which a PCV2 infected pig develops a PCV2-SI or a clinical PCVD are still to be elucidated (Kekarainen et al., 2010). Therefore, clinical and pathological studies have been essential in the description and characterization of overt PCVD and allowed speculating on the pathogenesis of these conditions (Harding, 2004; Segalés et al., 2004). In addition, the clinico-pathological scope of PCV2 infections has been expanded since late 90s and conditions nowadays grouped within the terminology PCVD follow strict diagnostic criteria (Table 1). PDNS is probably the biggest question mark regarding PCV2 causality. Case definition of this syndrome does not include the detection of PCV2 and the likelihood of experimental reproduction of PDNS is low due to its assumed pathogenesis, an immunocomplex disease (Helie et al., 1995; Segalés et al., 1998; Thibault et al., 1998). Therefore, and even the clinico-pathological scope may get wider in the future (Cino-Ozuna et al., 2011), there is still a number of issues that deserves further investigation in the field of PCV2 infection. Lymphocyte depletion and granulomatous inflammation of lymphoid tissues (Kim and Chae, 2004; Nielsen et al., 2003; Tsai et al., 2010), necrotizing lymphadenitis (Galindo-Cardiel et al., 2011; Kim and Chae, 2005), cardiovascular injury (Langohr et al., 2010; Opriessnig et al., 2006b) and liver lesions (Krakowka et al., 2004; Resendes et al., 2011; Sinha et al., 2011) are just some examples on well pathologically described features, but with a poor knowledge on their fine pathogenic mechanisms. Moreover, in spite of some PCV2-RD experimental studies (Mateusen et al., 2007; Pensaert et al., 2004; Sánchez et al., 2001, 2004) little is known on the pathogenesis of PCV2 infection in the reproductive tract, embryos and fetuses.

The success of PCV2 vaccination is paralleled with the control of clinical disease as well as prevention of tissue lesions (Desrosiers et al., 2009; Horlen et al., 2008; Kixmoller et al., 2008; Pejsak et al., 2010; Segalés et al., 2009). In consequence, the widespread use of vaccination in farms with PCVD clinical and PCV2-SI outcomes will probably shift the general pig health from a period of worldwide severe clinical outbreaks (1997–2007) to self-limiting subclinical infections with occasional outbreaks. Therefore, it is important for both field veterinarians and pathologists to be updated regarding PCV2 infection outcomes as well as on diagnostic possibilities and their correct interpretation.

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