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Review

A review of porcine circovirus 2-associated syndromes and diseases

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Abstract

Clinical expression of porcine circovirus 2 (PCV2) infection in swine may result in several distinct syndromes and diseases including post-weaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure, porcine respiratory disease complex, granulomatous enteritis, necrotizing lymphadenitis, and possibly exudative epidermitis. Association of PCV2 with congenital tremor in piglets is still controversial. The extent of the involvement of PCV2 in swine disease other than PMWS is currently poorly understood. This review concentrates on PCV-2-associated syndromes and diseases other than PMWS.

Keywords: Porcine circovirus; Porcine dermatitis and nephropathy syndrome; Porcine respiratory disease complex; Post-weaning multisystemic wasting syndrome; Review

1. Introduction

Currently, porcine circovirus 2 (PCV2) is considered to be an important emerging pathogen associated with a number of different syndromes and diseases in pigs. PCV2 was first recognized in 1996, when infection was identified in specific-pathogen-free swine herds in western Canada. It was reported as a new syndrome, termed post-weaning multisystemic wasting syndrome (PMWS) (Clark, 1997; Harding and Clark, 1997).

Since the identification of PCV2 and its association with PMWS, PCV2 has been increasingly isolated from pigs affected with various other clinical manifestations. Overall, clinical syndromes and diseases associated with PCV2 infections are divided into pre- and post-natal manifestations. In the former, PCV2 infection is linked to reproductive failures (Josephson and Charbonneau, 2001; Ladekjaer-Mikkelsen et al., 2001; Kim et al., 2004; O'Connor et al., 2001; West et al., 1999), whereas the post-natal manifestations of the disease in Europe, Asia and North America, are predominantly PMWS and porcine respiratory disease complex (PRDC) (Allan and Ellis, 2000; Harms et al., 2002; Kim et al., 2003b). In parts of England, Ireland and elsewhere, a syndrome known as porcine dermatitis and nephropathy syndrome (PDNS) is more prevalent than PMWS (Allan and Ellis, 2000; Gresham et al., 2001; Meehan et al., 2001; Thomson et al., 2001a). In addition, PCV2 also has been associated with granulomatous enteritis, necrotizing lymphadenitis, and possibly exudative epidermitis. However, the extent of the involvement of PCV2 in swine disease other than PMWS is not clear. Recently, PMWS was reviewed in detail (Chae, 2004) and this review concentrates therefore on PCV-2-associated syndromes and diseases other than PMWS.

2. Characterization of PCV2

The porcine circoviruses (PCV) are members of the genus *Circovirus*, family Circoviridae, which are the smallest non-enveloped, single-stranded, circular DNA viruses that replicate autonomously in mammalian cells (Mankertz et al., 1997; Studdert, 1993; Todd et al., 1991). The virion DNA, encapsulated by a single viral protein, is a single-stranded negative sense circularized molecule of roughly 1,800 bases that has six open

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reading frames (ORFs) (Hamel et al., 1998; Lukert et al., 1995; Mankertz et al., 1997; Meehan et al., 1998). Two types of PCV have been characterized and were subsequently named PCV1 and PCV2 (Meehan et al., 1998). PCV1 is a persistent contaminant of the porcine kidney cell lines, PK-15 (Tischer et al., 1974). It has never been associated with naturally occurring disease and experimental inoculation of pigs did not result in clinical disease (Allan et al., 1995; Krakowka et al., 2000; Tischer et al., 1986). PCV1 is therefore considered to be avirulent. In contrast, PCV2 is identified as virulent porcine pathogen.

PCV2 has been recovered from PMWS tissues in Europe, Asia, and North America (Allan et al., 1998; Ellis et al., 1998; Fenaux et al., 2000), sequenced (Fenaux et al., 2000; Hamel et al., 2000; Larochelle et al., 2002; Mankertz et al., 2000; Meehan et al., 1998) and shown to differ significantly from avirulent PCV1 (Hamel et al., 1998; Tischer et al., 1974, 1986) in the virion nucleotide sequence for the single nucleocapsid protein (Hamel et al., 1998; Meehan et al., 1998). PCV2 isolates from all over the world have since been isolated and sequenced and all are highly homologous (>96%) to each other but not to PCV1 (roughly 62%) suggesting that PCV2 isolates are all members of a single pathogenic virus genotype. Furthermore, all of the characterized isolates of PCV2 associated with PMWS are antigenically similar to each other using monoclonal and polyclonal antibodies (Allan et al., 1999b).

It has recently been hypothesized that different types of PCV2 may be responsible for different disease presentations. Two studies have suggested that PCV2 isolated from reproductive failure and PDNS may be phenotypically or genetically different from PCV2 associated with PMWS (Meehan et al., 2001; O'Connor et al., 2001). However, PCV2 isolates from different clinical disease manifestations have been sequenced and all are highly homologous (overall >90–96%). Most of these studies have found minor differences in the respective PCV genomes (Choi et al., 2002; Farnham et al., 2003; Meehan et al., 2001; O'Connor et al., 2001) but at this time it remains unclear what significance these minor differences may have. Sequence analysis of ORF1 and ORF2 genes has revealed that the extent of nucleotide variation is greater for the ORF2 than ORF1 (Fenaux et al., 2000; Hamel et al., 2000; Mankertz et al., 2000). The alterations in ORF2, which encodes for the major structural capsid protein (Nawagitgul et al., 2000), suggest there may be a link between capsid protein variation and pathogenicity of PCV2. Modification of the major viral capsid may alter determinants involved in tissue tropism or virus-host interactions. One study has suggested that the minor variation in the ORF2 of PCV2 may account for different in tropism with respect to the host organism (Mankertz et al., 2000). In addition, other host factors such as age, route of infection, etc. may affect the pathogenicity and clinical manifestations of PCV2 infection.

3. Post-weaning multisystemic wasting syndrome

PMWS is now well established as a wasting disease associated with PCV2 and is a major economic concern in all pig-producing areas of the world. In Asia and Europe, PMWS occurs in both endemic and epidemic forms. In North America, the sporadic form of the disease predominates. The disease has been reproduced in piglets by either inoculation with PCV2 alone or in PCV2-infected swine co-infected with porcine parvovirus (PPV) or porcine reproductive and respiratory syndrome virus (PRRSV). It also occurs when PCV-2-infected piglets are immuno-stimulated by injections of an immunogen emulsified in an oil-based macrophage-targeted adjuvant (Allan et al., 1999a, 2000; Choi and Chae, 2000; Ellis et al., 1999b; Harms et al., 2001; Kennedy et al., 2000; Kim et al., 2003a; Krakowka et al., 2000, 2001; Kyriakis et al., 2002). PMWS has been comprehensively reviewed by several authors (Allan and Ellis, 2000; Chae, 2004; Segales and Domingo, 2002).

4. Porcine dermatitis and nephropathy syndrome

PDNS is a relatively new and often fatal disease that primarily affects recently weaned and feeder pigs from 1.5 to 4 months of age (Smith et al., 1993; Thibault et al., 1998). The syndrome was first recognized in the UK in 1993 (Smith et al., 1993). Since then, it has been reported in several countries including Korea and North America (Choi and Chae, 2001; Duran et al., 1997; Ramos-Vara et al., 1997; Rosell et al., 2000). PDNS is generally sporadic. In fatal cases, cutaneous lesions consist of severe necrotizing vasculitis affecting the dermis and subcutis, characterized by leukocytoclastic (the presence of neutrophils with nuclear fragments) inflammation involving capillaries, small and medium-sized venules, and arterioles, accompanied by epidermal necrosis and ulceration and dermal haemorrhage (Choi and Chae, 2001; Duran et al., 1997; Smith et al., 1993; Thibault et al., 1998).

Pigs affected with PDNS are older pigs, particularly animals ranging from 12 to 14 weeks of age, but PDNS has also been described in finishing pigs and replacement gilts. The first signs were skin lesions that were multifocal, well circumscribed, slight raised, dark red, circular to irregular and 1–20 mm in diameter. Shortly after the appearance of the skin lesions, pigs may become pyrexic, with rectal temperatures $\geqslant 41$ °C, and display clinical signs of anorexia, severe weight loss, and depression. Pigs with these signs usually die rapidly and mortality in

affected pigs is approximately 20% (Done et al., 2001; Duran et al., 1997).

Significant gross lesions are present consistently in the skin and kidneys of swine with PDNS, although other organs may also be affected (Choi and Chae, 2001; Duran et al., 1997; Thibault et al., 1998). Gross skin lesions consist of several round to irregularly shaped red to purple macules and papules that coalesce over the perineum and distal limbs to form large irregular patches. The skin lesions are usually first noted over the hind-quarters, limbs and abdomen but may progress to involve the thorax, flank or ears. The kidneys are enlarged and have pale cortices with multiple red circular haemorrhagic cortical foci measuring 2–4 mm in diameter. Renal and inguinal lymph nodes are usually enlarged and red (Ramos-Vara et al., 1997).

The characteristic microscopic lesions of PDNS are generalized severe necrotizing vasculitis and fibrinonecrotic glomerulonephritis (Choi and Chae, 2001; Choi et al., 2002; Duran et al., 1997; Ramos-Vara et al., 1997). Microscopically, the most significant lesion is the presence of severe, fibrinoid, necrotizing vasculitis in the dermis, subcutis, kidney (Fig. 1), lymph nodes, stomach, spleen, and liver. Other renal lesions consist of exudative glomerulonephritis and interstitial nephritis. In lymph nodes, there is lymphoid depletion and occasional necrosis of lymphocytes in both the cortex and paracortex. Numerous multinucleated giant cells are often scattered in the cortex and paracortex. Positive hybridization signals for PCV2 can be detected in renal tubular epithelial cells, fusiform interstitial cells, and macrophage-like cells. These cells usually localized around vessels of the renal pelvis and among infiltrating non-positive mononuclear cells in the interstitium of the renal cortex and medulla (Choi and Chae, 2001). Distinct positive labelling has been found scattered throughout the cortex and paracortex of the lymph node (Choi and Chae, 2001; Choi et al., 2002).

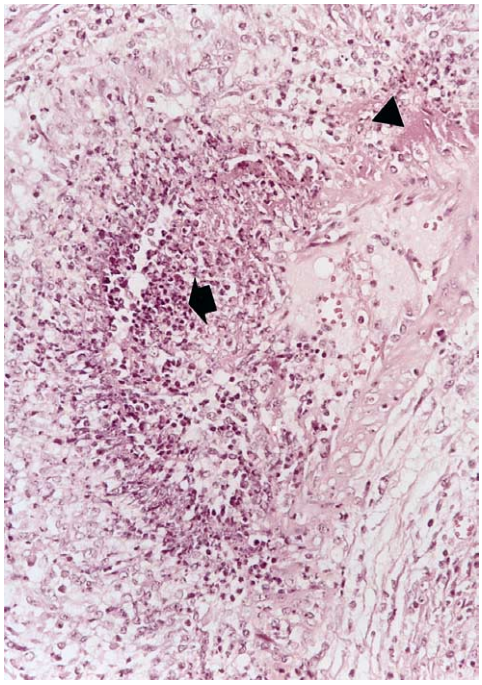

Fig. 1. Pig kidney with PDNS. Arteriole has a severe necrotizing vasculitis characterized by a mixed inflammatory infiltrates (arrow), hyalinosis of the vessel wall (arrow head). Haematoxylin and eosin.

The vasculitis associated with PDNS is thought to involve an immune-mediated mechanism (Rosell et al., 2000; Thibault et al., 1998). However, to date, its pathogenesis remains undetermined. The putative mechanisms of viral infection-related vascular diseases are many and varied, and more than one mechanism may be operational in this type of syndrome. The two major mechanisms for viral infection-associated vasculitis are direct viral invasion and damage, and humoral-mediated injury and/or cell-mediated injury to the vessel wall (Lie, 1996). One possible mechanism for the development of leukocytoclastic vasculitis in hepatitis C virus-infected humans is vascular deposition of high molecular weight immune complexes which initiate activation of endothelial cells and inflammatory cell infiltration, leading to altered vascular permeability and vessel wall damage (Agnello and Abel, 1997). Since PCV2 was not detected in endothelial cells in cases of PDNS (Choi and Chae, 2001; Choi et al., 2002; Rosell et al., 2000), a direct effect of these two viruses on endothelial cells subsequent to infection is an unlikely mechanism of vasculitis in PDNS. These observations therefore suggest that PCV2 is likely to induce vascular lesions indirectly, possibly via formation of immune complexes.

Several studies have reported single outbreaks of either PMWS or PDNS in pig herds (Choi and Chae, 2001; Choi et al., 2000). Recently, however, concurrent outbreaks of PMWS and PDNS have been reported in the same herd (Choi et al., 2002). Many herds in both Europe and Korea have reported PMWS and PDNS outbreaks occurring almost simultaneously or in close chronology (Choi et al., 2002; Gresham et al., 2001). Although PCV2 is associated with both PMWS and PDNS, there appears to be no direct relationship between PMWS and PDNS in the affected herds. The pigs with PMWS never progress into PDNS and the pigs with PDNS also never progress into PMWS. Furthermore, PCV2 nucleic acids have been demonstrated in more abundance in lymph nodes from pigs with PMWS than in those from pigs with PDNS. In contrast, PCV2 nucleic acids were demonstrated in more abundance in the kidney from pigs with PDNS than in those with PMWS (Choi et al., 2002). It is possible that the difference in distribution of PCV2 between PMWS and PDNS could be due to different tissue tropisim of different PCV2 strains. Thus PCV2, which has a high tropism for the kidney, may induce PDNS whereas

PCV2, which has high tropism for lymph nodes, may induce PMWS.

The demonstration of PCV2 antigens and nucleic acids, closely associated with skin and renal lesions, has led to speculation that PCV2 is an aetiologic agent of PDNS (Choi and Chae, 2001; Rosell et al., 2000). However, evidence suggests that other pathogens such as *Pasteurella multocida* (Lainson et al., 2002; Thomson et al., 2001b) or combinations of pathogens such as PCV2 and PRRSV (Choi and Chae, 2001; Rosell et al., 2000; Thibault et al., 1998) may also induce PDNS. Although PCV2 has been consistently detected in PNDS, lesions consistent with this syndrome have yet to be reproduced experimentally. It is likely that many factors such as overcrowding, poor ventilation, co-mingling of different age groups, co-infection of viruses and bacteria, and other stressors may act in a synergistic fashion to trigger as yet unidentified mechanisms to induce PDNS. Further studies are therefore needed to define the pathogenic role of these factors.

Diagnosticians and swine practitioners should now consider PDNS as an increasingly common cause of skin lesions and differentiate it from other diseases that it may mimic such as erysipelas, *Actinobacillus suis*, exudative epidermitis and swine pox. In addition, the renal lesions in PDNS should be differentiated from those seen in salmonellosis. Moreover, PDNS must also be considered in the differential diagnosis of classical swine fever virus (CSFV) and African swine fever virus infection. Clinical signs of PDNS may in some instances be similar to CSFV, especially low virulence strains, e.g. during the CSFV outbreak in 2000 in UK (Moenning et al., 2003). Diagnostic differentiation from African swine fever virus (low virulence strains) is also of concern, due to similar clinical signs (King et al., 2003).

5. Porcine respiratory disease complex

Porcine respiratory disease complex is a serious health problem in growing and finishing pigs aged around 16–22 weeks. It is characterized by slow growth, decreased feed efficiency, lethargy, anorexia, fever, cough, and dyspnoea (Halbur, 1998; Thacker, 2001). Pneumonia in pigs with PRDC is due to a combination of both viral and bacterial agents, such as PCV2, PRRSV, swine influenza virus (SIV), *Mycoplasma hyopneumoniae*, *A. pleuropneumoniae*, and *P. multocida* (Halbur, 1998; Thacker, 2001). The identification of PCV2 in proliferative necrotizing pneumonia cases has led some authors to suggest that PCV2 could be an important contributor to PRDC (Ellis et al., 1999a). The consistent detection of PCV2, and lower prevalence of other viral and bacterial pathogens in pigs examined with PRDC in Korea, has led us to strongly support this view (Kim et al., 2003b).

The role PCV2 plays in PRDC always involves interaction or synergism with other respiratory pathogens. In our study, over 55% of cases diagnosed as PRDC had evidence of concurrent infections of both PCV2 and PRRSV (Kim et al., 2003b). This is supported by experimental evidence indicating that synergism occurs between these two viruses (Allan et al., 2000; Harms et al., 2001). Although PCV2 does not increase the severity of PRRS lesions, PRRSV certainly potentiates the action of PCV2 (Allan et al., 2000). Experimental co-infection with PCV2 and PRRSV also induces more severe respiratory signs and pulmonary lesions (Harms et al., 2001). The bronchointerstitial pneumonia produced by co-infection of PCV2 and PRRSV is compatible with the typical lesions seen in field cases of PRDC submitted to our diagnostic laboratory as well as those reported elsewhere (Drolet et al., 2003).

The clinical signs seen in field cases are non-specific and variable. In growing and finishing pigs PCV2-associated PRDC is characterized by slow growth, prolonged cough, and dyspnoea that is refractory to antibiotic therapy. There is also a marked increase in mortality from single and multiple concurrent bacterial infections (Done, 2002; Harms et al., 2002; Kim et al., 2003b; Thacker, 2001). Co-infection of PCV2 with additional bacterial or mycoplasmal pathogens is frequently diagnosed in PRDC. In a recent survey in Korea, the combination of PCV2 and *P. multocida* (38 cases) was most prevalent followed by PCV2 and *M. hyopneumoniae* (33 cases) (Kim et al., 2003b).

A hallmark of the microscopic lesions of PCV2-associated PRDC is bronchointerstitial pneumonia with peribronchial and peribronchiolar fibrosis. Moderate to marked multifocal peribronchial and peribronchiolar fibrosis were present and this often extended into the airway lamina propria (Fig. 2). Alveolar septa were markedly thickened by infiltrates of macrophages and lesser numbers of lymphocytes and plasma cells. Many alveolar septa were entirely lined by hypertrophied type 2 pneumocytes, and alveolar spaces contained abundant necrotic debris. Positive hybridization signals for PCV2 can be detected in interstitial macrophages (Fig. 3) (Kim et al., 2003b).

Because of considerable diagnostic overlap between PCV2-associated PRDC and PMWS, the diagnosis of PCV2-associated PRDC must meet four criteria: (i) the presence of respiratory signs such as prolonged dyspnoea that are refractory to antibiotic therapy, (ii) the presence of characteristic pulmonary microscopic lesions, (iii) the presence of PCV2 within these lesions, and (iv) the absence of the characteristic microscopic lesions of PMWS in lymphoid tissues. These four criteria separately are non-diagnostic of PCV2-associated PRDC. Diagnosis of PMWS is made if the pigs have the characteristic microscopic lesions of PRDC in the lung and PMWS in lymphoid tissues. Since the clinical

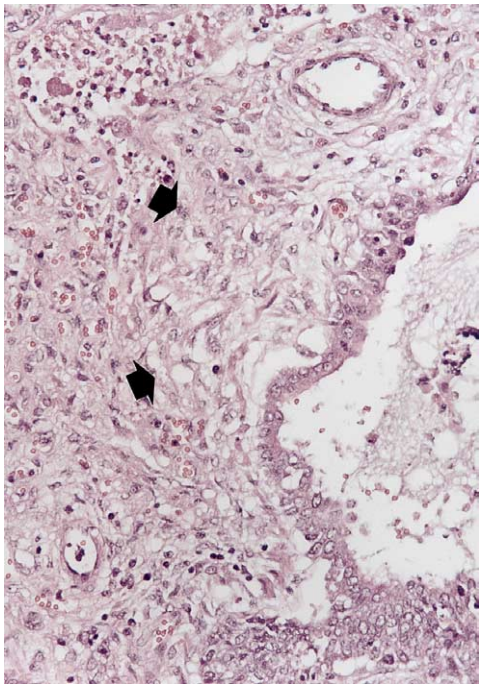

Fig. 2. Pig lung with PRDC. Moderate to marked multifocal peribronchial and peribronchiolar fibrosis (arrows) are present and often extended into the airway lamina propria. Haematoxylin and eosin.

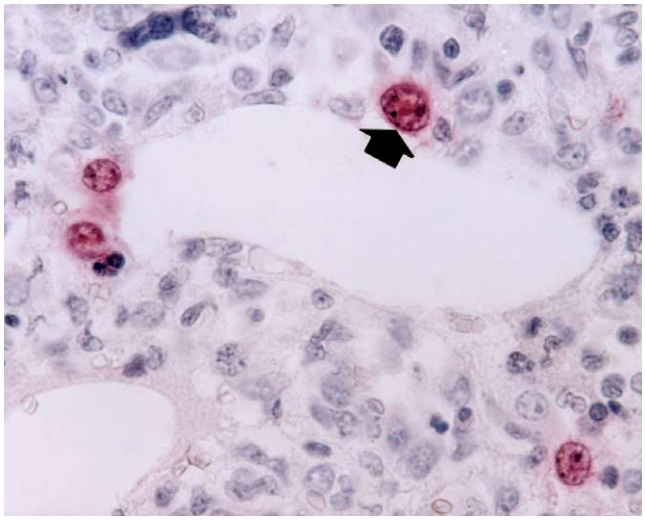

Fig. 3. Pig lung with PRDC. PCV2 antigen (red reaction) was detected in macrophages (arrow). Immunohistochemistry; alkaline phosphatase, red substrate, haematoxylin counterstain.

signs of PCV2-associated PRDC are non-specific and variable, the presence of PCV2 DNA or antigen in lung tissues, demonstrated by in situ hybridization and immunohistochemistry (Kim et al., 2003b), together with a bronchointerstitial pneumonia including peribronchial and peribronchiolar fibrosis are used as the main important criteria for the diagnosis of PCV2-associated PRDC.

PCV2-associated PRDC should be differentiated from PMWS clinically and histopathologically. PMWS is characterized by cachexia, dyspnoea, and occasionally jaundice or pallor in pigs of a younger age group, typically between 8 and 16 weeks (Allan and Ellis, 2000; Harding and Clark, 1997; Kim and Chae, 2002b). Histologically, the most striking and consistent lesions of PCV2-associated PRDC are bronchointerstitial pneumonia with peribronchial and peribronchiolar fibrosis and without evidence of characteristic PMWS lesions in non-respiratory tissues (Harms et al., 2002; Kim et al., 2003b). In contrast, PMWS is characterized by widespread granulomatous inflammation, multinucleated giant cells, and variable numbers of intracytoplasmic basophilic viral inclusion bodies within infiltrating histiocytes and macrophages (Allan et al., 1999a,b; Choi and Chae, 1999; Choi et al., 2000; Ellis et al., 1999a,b; Kennedy et al., 2000; Kim et al., 2002; Krakowka et al., 2000).

6. Reproductive failure

There have been several reports of PCV2-associated reproductive failure. Consistent clinical signs on affected farms include elevated abortion, stillbirths and fetal mummification (Josephson and Charbonneau, 2001; Kim et al., 2004; Ladekjaer-Mikkelsen et al., 2001; O'Connor et al., 2001; West et al., 1999). This reproductive failure is characterized by late-term abortion and delivery of stillborn near-term fetuses or premature piglets. Midgestation abortion, mummified fetuses, early embryonic death was also observed in a recent Korean study (Kim et al., 2004). In an experimental study, fetal inoculation with PCV2 resulted in reproductive failure manifested as stillborn, partial mummies, and weak-born piglets along with clinically normal piglets (Johnson et al., 2002). Detection of PCV2 antigen and nucleic acid in the stillborn piglets suggested that PCV2 can be present in large amounts within fetuses infected in utero indicating that vertical transmission may be an important means of transmission (Kim et al., 2004; Meehan et al., 2001; West et al., 1999). Transplacental transmission of PCV2 has also been reported in gilts (Ladekjaer-Mikkelsen et al., 2001).

Histopathologically, there are no characteristic lesions in PCV2-associated aborted fetuses. Microscopic lung lesions were present in 4/12 aborted fetuses from late gestation and 2/6 stillborn piglets in our study. When present the lesions were multifocal, and of mild to moderate severity. The pneumonia was characterized by infiltration of mononuclear cells into alveolar spaces (Kim et al., 2004). There were extensive areas of myocardial degeneration or necrosis with oedema and mild fibrosis as well as a diffuse moderate infiltration of lymphocytes and macrophages (West et al., 1999).

Significant histological lesions were not detected in the other organs.

Detection of PCV2 from aborted fetuses in all stages of gestation suggests that there was an association between reproductive failure and the presence of the virus in a proportion of cases and that a causal role for PCV2 in reproductive failure is therefore possible (Kim et al., 2004). It has been confirmed that PCV2 is associated with reproductive failure at all stages of gestation (Sanchez et al., 2001). The pathogenesis of PCV2 infection in pregnant sows appears to differ from that of PRRSV infection, in which virus may cross the placenta at late gestation only (Christianson et al., 1993). The consequences of maternal infection at various stages of gestation may reflect the relative or absolute ability of the conceptus to support PCV2 replication, rather than the effectiveness of the so-called placental barrier. Therefore, it seems that the placental barrier may not play an important role in determining the reproductive consequences of maternal infection at different times during gestation.

Diagnostic tests used to determine if PCV2 is a cause of abortion and reproductive failure are virus isolation, PCR, immunohistochemistry and in situ hybridization. PCR can be used to detect PCV2 in PMWS cases or in aborted and stillborn fetuses. However, since PCV2 is a common infection of clinically normal pigs, its presence cannot be used to confirm PMWS. Whether or not the same is true for aborted and stillborn fetuses remains to be discovered. On the basis of our study, spleen and lymph nodes appeared to be the most suitable organs for detection of PCV2 in fetuses (Kim et al., 2004).

PCV2 may also infect the boar. The shedding of PCV2 in semen from infected boars has been demonstrated (Kim et al., 2001, 2003c; Larochelle et al., 2000). Most PCV2 DNA was detected in the seminal fluid and non-sperm cell fractions of the boar semen samples tested (Kim et al., 2001). A high prevalence of PCV2 in pig semen was also found using the PCR assay (Kim et al., 2001, 2003c). This latter finding suggests that semen may be a significant vehicle for the transmission of PCV2. Efficient production of high quality semen is of major importance to the swine industry, especially to artificial insemination (AI) organizations. Semen used for AI has great potential as a source for the transmission of infectious disease. The exclusion of semen contaminated with emerging porcine pathogens such as PCV2 from commercial semen stocks is a priority for the AI industry. Further studies are needed to determine the infectivity of PCV2 in porcine semen.

7. Granulomatous enteritis

Granulomatous enteritis is another clinical manifestation of PCV2 infection. Ten cases have been diagnosed so far in eight pig herds, giving a morbidity of 10–20% but a mortality of 50–60%. The disease occurs in pigs between 40 and 70 days of age. The clinical signs are diarrhoea, which is initially yellowish but progress to black with retardation of growth. Antibiotic therapy was ineffective in the all cases treated.

The most consistent and predominant histopathological feature seen in these cases were granulomatous inflammation and lymphoid depletion in the Peyer's patches of both small and large intestines. The granulomatous inflammation was characterized by infiltrates of epithelioid cells and multinucleated giant cells (Fig. 4). Another typical histopathological feature was the presence of intracytoplasmic inclusion bodies. Large, multiple, basophilic or amphophilic grape-like intracytoplasmic inclusion bodies were often seen in the cytoplasm of histiocytic cells and multinucleated giant cells. No microscopic lesions were seen in other organs. No organisms were observed within any granulomatous lesions by Ziehl-Neelsen and Grocott's methenamine silver stains. A strong hybridization signal for PCV2 was detected in the cytoplasm of histiocytes and multinucleated giant cells in Peyer's patches in all 10 pigs tested (Fig. 5).

The diagnosis of PCV2-associated granulomatous enteritis was made on the basis of three criteria: (i) the presence of diarrhoea, (ii) the presence of characteristic microscopic lesions in the Peyer's patches but not in the lymph node, and (iii) presence of the PCV2 within these lesions. It may be difficult to differentiate PCV2-associ-

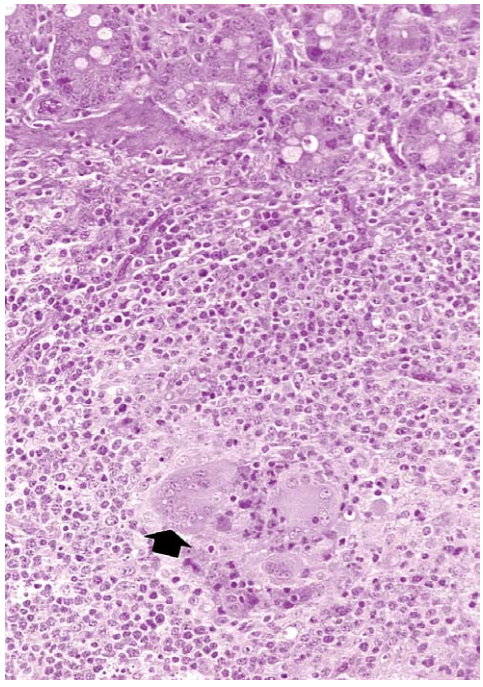

Fig. 4. Pig small intestine with granulomatous enteritis. A cluster of histiocytes and multinucleated giant cells (arrow) are seen in Peyer's patches. Haematoxylin and eosin.

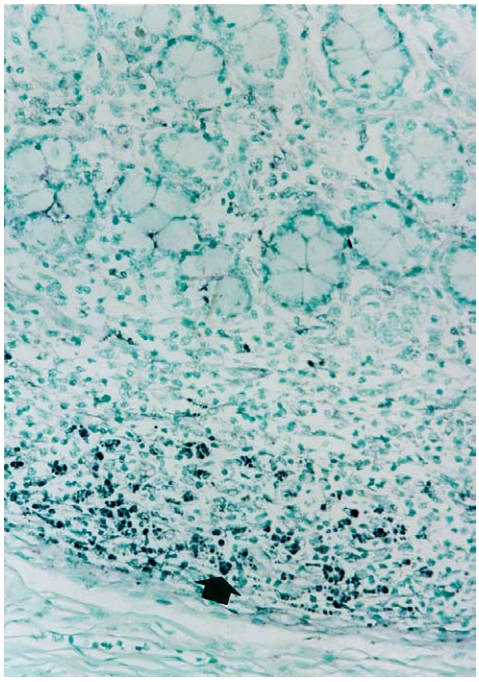

Fig. 5. Pig small intestine with granulomatous enteritis. PCV2 DNA is detected in macrophages (arrow). In situ hybridization; alkaline phosphatase, nitroblue tetrazolium/5-bromocresyl-3-indolylphosphate, methyl green counterstain.

ated enteritis from PMWS clinically and histopathologically because of considerable diagnostic overlap between the two conditions. If characteristic microscopic lesions of PMWS are seen in lymph nodes, the case should be diagnosed as PMWS. Further studies are necessary to be sure that PCV2-associated granulomatous enteritis is distinct clinical entity, nevertheless, the disease should be considered as a differential diagnosis for growing and finishing pigs with antibiotic non-responsive diarrhoea.

8. Exudative epidermitis

Exudative epidermitis is an acute, rapidly progressive, often fatal, superficial pyoderma. *Staphylococcus hyicus* is the casual agent of exudative epidermitis, but predisposing factors are probably necessary for the disease to appear (Aarestrup and Wegener, 1997; Anderesen et al., 1993; Tanabe et al., 1996). The skin of affected pigs is covered with an odoriferous exudate of serum and sebum, imparting a dirty, moist and greasy appearance (Jones, 1956; Mebus et al., 1968). The disease chiefly affects piglets from five to 35 days of age, although mild cases occur in older pigs. The morbidity varies from 10% to 100%, and the mortality, from 5% to 90%, with an average of 25%.

The results of our survey indicate that PCV2 and PPV are highly prevalent in pigs with exudative epidermitis (Kim and Chae, 2004). One may argue that pigs with exudative epidermitis had concomitant PCV2 and/or PPV infection because both viruses are highly prevalent in pig populations. Although no PMWS occurred in any of the pigs studied, those with exudative epidermitis showed a high number of PCV2 and PPV infected cells in skin lesions but low numbers of PCV2-positive cells in lymphoid tissues. PCV2 and PPV have been demonstrated as cofactors in the full experimental reproduction of exudative epidermitis (Wattrang et al., 2002; Whitaker et al., 1990), and it is likely that these viruses are involved in lesion development and/or progression.

9. Necrotizing lymphadenitis

This is a new clinical manifestation of PCV2 infection. Five cases of necrotizing lymphadenitis have now been diagnosed from five different herds in our laboratory. Two pigs were submitted because of a retardation of growth that was accompanied by diarrhoea. Two pigs were presented with a slight incoordination and pyrexia (40.5–41.7 °C) and one pig died suddenly without clinical signs. At necropsy, the main findings were in the inguinal lymph nodes, which were usually homogeneous white on the cut surface.

The predominant microscopic lesions in the lymph nodes were follicular necrosis in the centre of prominent lymphoid follicles. Necrotic foci of 1–10 cells showing pyknosis and karryorrhexis were commonly observed in the centre of prominent follicles and less often in the surrounding lymphoreticular tissues (Fig. 6). Necrotic cells were characterized by cell shrinkage, cytoplasm and chromatin condensation, and some of them contained nuclear fragments. Granulomatous inflammation and intracytoplasmic inclusion bodies, which were

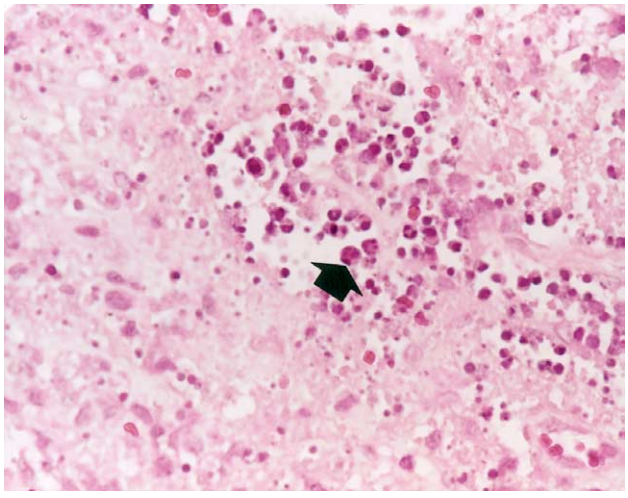

Fig. 6. Pig lymph node with necrotizing lymphadenitis. Apoptotic lymphocytes in characterized shrunken cells with acidophilic cytoplasm and condensed nuclei (arrow). Haematoxylin and eosin.

characteristic histopathological lesions in PMWS, were not seen in the lymph nodes. Application of the TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labelling) reaction on sections of affected lymph nodes revealed intense, specific staining in nuclei and nuclear fragments of lymphocytes (Fig. 7). Apoptotic bodies of various sizes exhibited distinct staining and the cytoplasm of apoptotic cells was also often stained. PCV2 DNA and antigen (Fig. 8) was consistently detected in the necrotic cells and neighboring normal macrophages by in situ hybridization and immunohistochemistry, respectively. Apoptotic cells were more abundant than PCV2-infected cells in all sections of lymph nodes examined. Other viruses such as CSFV and PRRSV were not detected in the lesions.

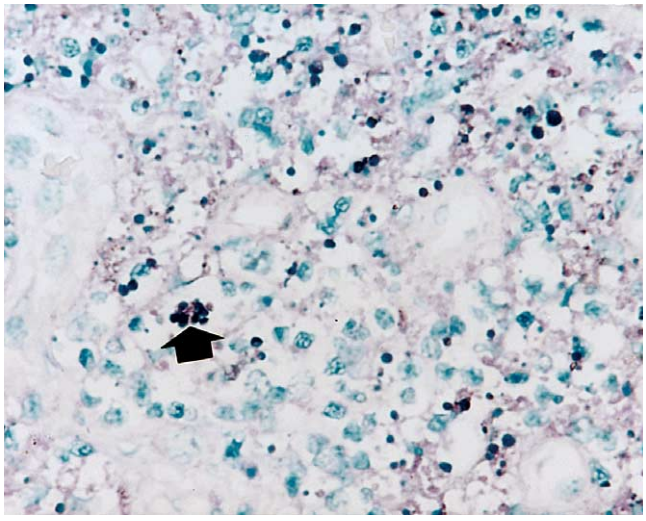

Fig. 7. Pig lymph node with necrotizing lymphadenitis. Apoptotic cells are positive in the nuclei for TUNEL assay (arrow). TUNEL, methyl green counterstain.

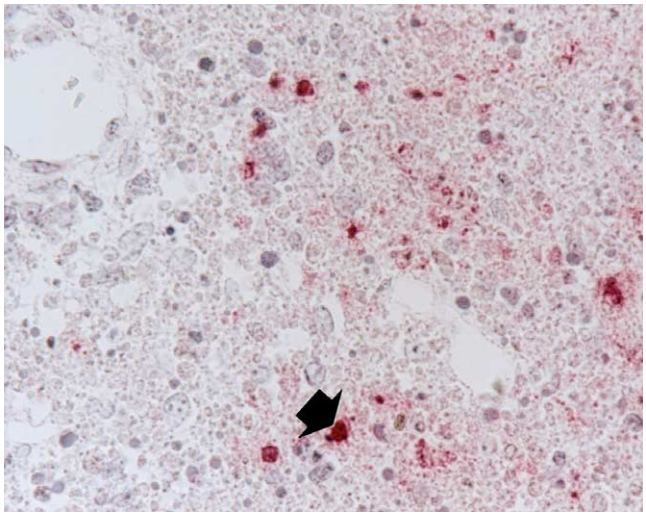

Fig. 8. Pig lymph node with necrotizing lymphadenitis. PCV2 antigen (red reaction) is detected in apoptotic cells (arrow). Immunohistochemistry; alkaline phosphatase, red substrate, haematoxylin counterstain.

The mechanism of apoptosis in these lesions has not been determined. Apoptosis has been proposed to account for loss of B lymphocytes and disruption of cytokine signalling for loss of T lymphocytes in PMWS-affected pigs (Shibahara et al., 2000). Pigs subclinically infected with PCV2 also have minimal amount of PCV2 genome or antigen in lymphoid tissues (Allan and Ellis, 2000; Calsamiglia et al., 2002). However, it cannot be ruled out that PCV2 was incidentally present in the necrotizing lymphoid lesions. The PCV2 antigens were always present in all necrotic lesions, suggesting PCV2 antigen is associated with the necrotizing lymphadenitis. To our knowledge, this is the first report of necrotizing lymphadenitis associated with PCV2.

10. Congenital tremor

Congenital tremor in pigs is associated with demyelination of the brain and spinal cord. Cases of congenital tremor in which histological lesions are visible are classified as type A, whereas those in which no lesions are apparent are referred to as type B (Done and Harding, 1967). Possible causes include CSFV (type A1), pseudorabies virus or unknown viral infection (type A2), and sex-linked (type A3) or autosomal-linked (type A4) recessive genes, and trichlorfon toxicosis (type A5). Type A2 form is the most common form of congenital tremor and is characterized by clonic contractions of varying severity that decrease with time and usually resolve by four weeks of age. Mortality in affected pigs may be as high as 50% and is the result of inability to suckle. The association of PCV2 with congenital tremor is controversial. Hines and Lukert (1994) were first to report a potential association, and later PCV2 nucleic acid and antigen were demonstrated in the brain and spinal cords of affected pigs using in situ hybridization, indirect fluorescent assay, and polymerase chain reaction (Stevenson et al., 2001). However, a recent study does not support the hypothesis that PCV1 and PCV2 are linked to porcine congenital tremor (Kennedy et al., 2003) and further studies will be required to resolve the situation.

11. Conclusions

Clinical expression of PCV2 infection in swine results in several distinct syndromes and diseases. The infection presents a major challenge for veterinary pathologists, virologists and epidemiologists who are involved in developing strategies for its diagnosis and prevention. The reason for the variety of clinical manifestations associated with PCV2 is not fully understood. It may be argued that the identification of PCV2 in such a wide

spectrum of clinical manifestations may in fact be the result of its ubiquity rather than its pathogenicity. Further studies are therefore needed to determine the pathogenesis of this emerging viral infection.

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