PORCINE CIRCOVIRUS TYPE 2 INFECTION: CLINICAL AND EPIDEMIOLOGICAL PRESENTATION IN BRAZILIAN SWINE HERDS

ABSTRACT

The post-weaning multisystemic wasting syndrome (PMWS) is an emerging disease with a variable morbidity and high lethality and it is caused by a porcine circovirus (PCV), a viral agent that is well distributed among swine herds worldwide. Two PCV types have been identified so far, PCV1 and PCV2. PCV1 is a normal contaminant of cultured cells in the laboratory, and does not cause clinical symptoms in pigs. PCV2 is associated with the occurrence of PMWS, porcine dermatitis and nephropathy syndrome (PDNS), among other diseases. PMWS is the most studied of those PCV2-associated diseases and is characterized clinically by dyspnea, emaciation, jaundice and enlargement of lymph nodes. Pathological lesions include lymphadenopathy, interstitial pneumonia, hepatitis and interstitial nephritis. The objective of this review is to present the studies of Brazilian isolates of PCV2 to understand the presentation of the disease and the viral behavior in Brazilian swineherds. The first characterization of PCV2 isolates from pigs with PMWS in Brazil was done at EMBRAPA (Brazilian Agricultural Research Corporation). PCV2 associated to PMWS was identified by histopathology and nested PCR (polymerase chain reaction). Reproduction of the disease and viral re-isolation was also done in susceptible piglets inoculated with PCV2 strains isolated in the Southern region of Brazil. Furthermore, the interaction of PCV2 with other porcine pathogenic agents such as porcine parvovirus (PPV) aggravates the symptoms and losses by PMWS. Nucleotide sequence comparisons revealed that the open reading frame 2 (ORF2) of Brazilian PCV-2 isolates is closely related to each other, displaying 97% identities. PCV2 DNA was amplified by nested PCR from porcine semen of boars from artificial insemination centers in Brazil, suggesting that the boar may play an important role in the transmission and pathogenesis of PCV2 to uninfected sows.

Key words:
porcine circovirus infection, swine, Brazil
INTRODUCTION

Porcine circovirus type 2 infection is an economical important disease caused by porcine circovirus type 2 (PCV2) that is well distributed among swine herds worldwide (Allan et al. 1998, Allan and Ellis 2000). The disease was first identified in 2000 in Brazil at EMBRAPA (Brazilian Agricultural Research Corporation) and has been causing losses and death in growing to finishing pigs (Zanella & Mores 2000, 2003). Although the disease was reported for the first time in 2000, porcine circovirus type 2 infection was diagnosed in archived materials since 1988 following the amplification of DNA of PCV2 by nested-polimerase chain reaction (PCR), identification of viral antigens and histopathological lesions (Zanella et al. 2004b). These results indicate that the infection by PCV2 is present in Brazil, at least since 1988.

The porcine post weaning multisystemic wasting syndrome (PMWS) is the most important syndrome caused by the PCV2 infection, but the virus is also related with several other illnesses or syndromes (Allan 2004, Ellis 2004). PMWS in pigs was first identified in Canada in 1991 and nowadays has a worldwide distribution (Allan et al. 1998, Ellis et al. 1998, Morozov et al. 1998). It is characterized by a high mortality 4-12 weeks after weaning. The most important clinical symptoms are unthriftness, wasting, dyspnea, paleness and enlargement of lymph nodes. Infection with PCV2 is essential for developing PMWS (Segalés 2002). However, other factor, such as stress and poor hygiene may contribute to the pathogenesis of the disease, since all herds have antibodies against PCV2 (Madec & Wadillove 2002).

The main problem of the PMWS is the duration of the clinical case that can persist for two years or more, if adequate control measures are not employed and maintained. Mortality rates are variable. Rates as elevated as 60% have been reported on farms with post-weaning mortality and figures of 15-25% are common (Madec et al 2000, Segales & Domingo 2002). However, not all of the herds with outbreaks of PMWS where the PCV2 was diagnosed present those mortality rates. Some of them only present an increase between 1 to 2% in those rates, the pigs can recover and the numbers return to the normality after several months. Therefore, a three-fold increase of the normal mortality rate of the herd is expected (Chae 2004). The cost of this syndrome, beside the deaths, is the sum of the poor overall growth rates and feed conversion on the farm due to the number of debilitated pigs present (Richardson 2005).

Etiology.

The occurrence of typical lesions of PMWS in pigs inoculated experimentally, the presence of elevated concentrations of antigen and the detection of the DNA of PCV2 in the tissues, the isolation of PCV2 in infected animals and the development of specific antibodies for PCV2 indicated to the researchers that PCV2 is the agent of PMWS (Allan et al. 1998, Ellis et al. 1998, Ellis & Allan 2000, Hamel et al. 1998).

Circoviruses are small, non-enveloped, icosahedral viruses and whose genome is composed of circular, single stranded DNA (Tischer et al. 1974, 1982). Moreover, the genome of circoviruses is one of the smallest among animal viruses, with 1.76 kilobases (Todd 2000). Members of the family Circoviridae include the chicken anemia virus, the psittacine beak and feather disease virus and the porcine circovirus (Todd 2000). Two types of PCV have been identified so far, PCV1, a persistent contaminant of the continuous tissue culture cells (PK-15, porcine kidney cells) which does not cause clinical symptoms in swine and PCV2, which has been associated with PMWS (Allan & Ellis 2000). A common feature of the infection by this family of viruses in animals is the association with illnesses that cause injuries in lymphoid tissues and immunosuppression (Todd 2000).

Replication of PCV in vitro is achieved by inoculation of semiconfluent monolayers of PCV-free cell lines and further treatment with D-glucosamine, a potent cell cycle inducer, which may favor viral replication (Stevenson et al. 1999). A study of three different cell lines was performed in order to analyze their susceptibility to PCV2 infection and its sensibility to D-glucosamine (Fernandes et al. 2003c). Tissue culture cells free of endogenous PCV such as ST (swine testicle cells), SK6 (swine kidney cells) and Vero cells (Green monkey kidney cells) were inoculated with PCV2 and treated with 300 mM of D-glucosamine for 30 minutes. All cell lines were susceptible to viral replication, which was demonstrated by immunofluorescence and PCR. However, ST cells were very sensitive to D-glucosamine.
treatment, which caused toxicity and cell death, decreasing viral load and replication (Fernandes et al. 2003c).

The genomic sequence of PCV2 and PCV1 show a homology of less than 80%. PCV2 genome is very conserved, contains 1768 base-pairs and presents eleven ORFs (open reading frames). Among them, seven encode proteins with molecular weight above 5 kilodaltons (Mankertz et al. 2004). Brazilian isolates of PCV2 from pigs that presented characteristic symptoms of PCV2 infection were sequenced (Fernandes et al. 2003a). PCR amplifications of PCV2 ORF2 gene from PMWS affected pigs from different swine herds of the Southern region of Brazil were performed to confirm the presence of PCV2 in the DNA extracted from those tissues. Sequence comparisons revealed that the ORF2 genes of all PCV-2 isolates are closely related to each other, displaying 97% nucleotide sequence identities. The only changes were single point mutations, no deletion, insertion or extended areas of nucleotide substitutions were observed. All four Brazilian PCV2 strains were compared with other PCV2 sequences available in GenBank and, as demonstrated previously, the PCV2 strains showed great genetic homogeneity, regardless of the origin of the isolate (Fernandes et al. 2003a).

**Epidemiology.**

Several stress causing risk factors such as elevated population density, poor air quality, mixtures of litters with different ages can exacerbate the symptoms and the gravity of the illness (Harding 1996, Harding et al. 1998, Guilmino & Wessel-Robert 2000, Madec et al. 2000). In the United States, in the majority of the swine herds where the PCV2 was diagnosed causing PMWS, there was a co-infection with the virus of the porcine reproductive and respiratory syndrome (PRRSV), where it was demonstrated that the PMWS was more severe (Sorden et al. 1998). There are no reports of PRRSV in Brazil and therefore, this is not an important association in Brazilian herds (Zanella & Vargas 2003, Zanella et al. 2004a). The two types of PCV can be transmitted from infected to non-infected pigs by vertical and horizontal form, and the vertical transmission has been shown experimentally (Balsach et al. 1999, Ellis et al. 1999a, Krakowka et al. 2000). The horizontal transmission by oral-nasal route is the most frequent. A tentative study of horizontal transmission of PCV2 was performed, in which 7 days old piglets serum negative for PCV2 were grouped in concrete walls individual pens. One group was inoculated with a PCV2 suspension, another one was a sentinels and the last one was uninfected control, which was placed in a different room. PCV2 was horizontally transmitted to sentinel, uninfected pigs, following inoculation of PCV2 to neighboring piglets. Horizontal transmission of PCV2 occurred probably by aerosol into pigs lodged in different pens but at the same room (Fernandes et al. 2003d).

The contact with infected pigs, contaminated facilities, equipment, or personal are possible factors for the horizontal transmission of the virus (Allan & Ellis 2000). However, PCV2 can be found in the semen of infected males, representing a potential source of dissemination of the infection (Kim et al. 2001, Larochele et al. 2000). Recently, PCV2 DNA was amplified by nested PCR from porcine semen of 03 boars from artificial insemination centers (AIC) in Brazil (Zanella et al. 2003b). This study analyzed semen samples of 133 boars from 07 different AIC. This finding suggests that the boar may play an important role in the transmission and pathogenesis of PCV2 to uninfected sows. The association of PCV-1 and PCV-2 with abortions and stillbirths indicates that the placental transmission can also be an important factor, if serum-negative sows get infected during the pregnancy (Allan et al. 1995, West et al. 1999).

A retrospective investigation was performed on paraffin-embedded organs from swine submitted to histopathological diagnosis between 1985 and 1998. Results indicated that PCV2 is present in Brazil for some time, at least since 1988 (Zanella et al. 2004b). It is still unknown why PCV2 associated disease suddenly became of major economic importance to the pig industry worldwide since the first isolation and identification of the virus in 1997 (Clark 1997). PMWS may be a consequence of changes in management practices, a change in the genetics of the host, or emergence of other agents that enhance disease severity during mixed infections (Staebler et al. 2005).

**The disease.**

The accepted case definition of PMWS is based on three major features: a) clinical disease, b) gross and histological lesions, c) and most important, the association of PCV2 with these lesions (Segalés 2002).
PMWS caused by PCV2 is observed in pigs between 8 to 12 weeks of age, although, the period of transmission occurs between 5 to 16 weeks (Allan & Ellis 2000). The disease continues to progress despite the use of antibiotics. Clinically, PMWS is characterized by apathy, dyspnea, progressive weight loss, enlargement of lymph nodes and with the evolution of the illness, jaundice, anemia, diarrhea and other symptoms related with secondary infections may also appear (Allan et al. 1998) (Figures 1, 2). Pathological lesions include inflammations in several organs, such as lymphadenopathy, interstitial pneumonia, hepatitis, interstitial nephritis and pancreatitis. (Ellis et al. 1998, Harding et al. 1998). PCV2 antigens and nucleic acids have been demonstrated in tissues of pigs with PMWS (Alan et al. 1998, Ellis et al. 1998, Kuipel et al. 1998, Kennedy et al. 2000, Larochelle et al. 2000). Others signs, many of them are often related to secondary infections including Enzootic pneumonia, bacterial pneumonias, Glasser’s disease, Salmonella and E. coli infections, Staphylococcal skin infections and many others (Allan 2004). Other infections caused by swine virus such as the porcine parvovirus (PPV) and PRRSV can exacerbate the infection caused by the PCV2, and aggravate the symptoms and the mortality rate (Allan et al. 1999, 2000).

Gross lesions involve several organs and include enlargement and paleness of lymph nodes (mainly inguinal, submandibular, mesenteric and mediastinal) (Figure 4) thymus atrophy, non-collapsed, tan-mottled lungs, and kidneys with multiple pale foci of variable diameter (Harding 1996, Ellis et al. 1998). Many animals with signs of wasting also present gastric ulceration of the pars oesophagea. Microscopic lesions attributable to PMWS are found in lymphoid organs (including lymph nodes, tonsil, Peyer’s patches and spleen), liver, kidney and lungs (Allan et al. 1998, Ellis et al. 1998, Rosell et al. 1999). Variable degrees of lymphocellular depletion, affecting both lymphoid follicles and parafollicular zones, and progressive multifocal to diffuse infiltration of lymphoid tissue by large histiocytic cells are the characteristic lesions. Syncytial cells are seen frequently, especially in the lymph nodes and Peyer’s patches. Cytoplasmic inclusions in histiocytic cells are also observed in Peyer’s patches and tonsil as well as necrotic lymphoid cells. Lung lesions include multifocal interstitial pneumonia with variable dissemination over the organ. Other changes include infiltration of inflammatory cells (histiocytes) and severe bronchiitis. The most common liver lesion is a lymphohistiocytic infiltration of portal zones, with variable degrees of intensity. Multifocal necrosis of single hepatocytes is also observed. In the kidney, a mild multifocal interstitial nephritis with discrete vasculitis, especially on the renal cortex area, is present. Variation in intensity and distribution of lesions in target organs in cases of PMWS probably depends on the stage of the disease in the affected pig (Rosell et al. 1999).

PCV2 can be identified in swine affected with the epidemic form of porcine dermatitis and nephropathy syndrome (PDNS). The clinical detection of PDNS is relatively easy because of the presence of cutaneous hemorrhaged infarcts, located mainly on the hind limbs and perinea area (Figure 3). At necropsy kidneys are swollen, pale and may have generalized cortical petechiae. Major histopathological findings include necrotising and fibrinous glomerulonephritis and systemic necrotising vasculitis. These lesions, associated with the presence of immunoglobulin and complement components in the damaged vessels and glomeruli, suggest a type III hypersensibility reaction as the pathogenic mechanism for the disease. In addition, interstitial pneumonia and lymphocyte depletion in lymph nodes and granulomatous inflammatory infiltrate with histiocytes and/or multinucleated giant cells are seen in affected pigs (Rosell et al. 2000).

Other diseases or syndromes are also associated with PCV2 infection. PCV2 was detected as the agent of reproductive disorders, as the co-factor for the porcine respiratory disease complex and proliferative, necrotising pneumonia (Segalés 2002). There is still some controversy about the association of PCV2 with congenital tremors and further work is needed in this area (Segalés et al. 2004).

PCV2 and other infectious agents may co-infect pigs and aggravate symptoms and losses. PPV and PRRSV are co-infectious agents that may be an important factor in the pathogenesis of PMWS (Allan et al. 1999, 2000). Although PRRSV has not been diagnosed in Brazilian swineherds yet (Zanella & Vargas 2003, Zanella et al. 2004a). PPV is a common virus. Recently it was investigated the virulence of PCV2 isolated in Santa Catarina State by coinfection with PPV (Fernandez et al. 2003b, 2005). In this work a total of 24 five day-old SPF pigs were distributed in four groups.
housed in separate rooms and inoculated by intranasal route: group 1 (G1) - control (n = 4); G2 - inoculated with PCV2 (n = 7); G3 - inoculated with PPV (n = 6); G4 - inoculated with PCV2 and PPV (n = 7). During the experiment the animals were monitored daily for clinical evaluation. Pigs were examined with 53 day-old and tissue samples analyzed for macroscopic lesions. The pathological lesions seen in G2 and G4 pigs were: enlargement of lymph nodes, mild to moderate lymph cellular depletion affecting lymphoid follicles in lymphoid organs and presence of infiltration by eosinophils in lymph nodes. PCV2 DNA was detected by nested-PCR in all pigs of G2 and G4. These findings indicate that PCV2 was transmitted to pigs by the nasal route, and the presence of PCV2 DNA found in tissue samples of pigs infected with PCV2 and PPV was more evident than in organs from pigs infected with PCV2 alone. These results support the hypothesis that PPV infection enhances PCV2 replication (Fernandes et al. 2003b, 2005).

**Diagnosis.**

Laboratory and differential diagnosis are needed to diagnose porcine circovirus type 2 infections due to similarities between the diseases associated to PCV2 and diseases caused by other viruses and bacteria (Chae 2004). The diagnosis of PMWS should be carried out based on the combinations between the observed clinical signs, pathological lesions (macro and microscopic) and on the detection of viral antigen or nucleic acid (DNA) of the PCV2 in tissues of affected pigs (Segalés 2002). In situ hibridization (ISH), immunohistochemistry (IHC) techniques and PCR have been used for PCV2 detection (Choi & Chae 1999, Larchelle et al. 2000, McNeilly et al. 1999, Rosell et al. 1999, Hamel et al. 2000).

Serological tests, like Elisa test, may be used to indicate animal exposure to the virus. Immunofluorescence antibody and immunoperoxidase monolayers (IPMA) can be also employed but are not used in a commercial basis anymore (Allan & Ellis 2000). The fact that PCV2 is ubiquitous and the serum conversion pattern is relatively similar between PMWS affected and non-affected farms, makes the use of serological techniques inappropriate to diagnose the disease.

Virological tests are available to determine the presence of viral particles such as viral isolation, IHC or immunofluorescence (Ellis et al. 1998). PCV2 nucleic acids can be detected by PCR or ISH (Choi & Chae 1999, Larchelle et al. 2000, McNeilly et al. 1999, Rosell et al. 1999, Hamel et al. 2000).

PCR is one of the most used and the most sensitive technique to detect PCV2 (Allan & Ellis 2000). Therefore, since PCV2 is widespread on farms with and without PMWS, the use of PCR solely to diagnose the syndrome is not sufficient. Recent study has shown that PCR and histopathology should be used in combination to diagnose PMWS (Zanella et al. 2003a). A total of 82 samples of porcine tissues suspected of PMWS were analyzed by histopathology and by nested-PCR to detect PV2. Among them, 51 samples (62.2%) were positive in both tests, 17 (20.7%) were positive only by histopathology, 6 (7.3%) were positive only by nested-PCR and 8 (9.8%) were negative in both tests. The fact that some samples were positive by histopathology and negative by nested-PCR may be explained by the absence of viral DNA in those samples, due to the evolutive stage of the disease. Positive samples by the nested-PCR test but negative by histopathology indicate the presence of PCV2 DNA in those samples without the occurrence of lesions or disease (Zanella et al. 2003a). Real time PCR and quantitative PCR methods may help the diagnose of the disease in vivo, since the amount of PCV2 is very high in PMWS affected pigs when compared to pigs infected subclinically (Liu et al 2000).

The differential diagnosis should be carried out for some pathogens that also cause similar clinical signs to PMWS, mainly the wasting, as the diarrhea caused by Lawsonia and Brachyspira (Guilmoto & Wessel-Robert 2000). Due to the co-infection of PCV2 and PRRSV, many lesions attributed to the PRRSV can be in fact caused by PCV2, therefore in many cases the antigen of PRRSV was not detected in those lesions or the severity of the lesion observed exceeds the quantity of PRRSV antigen present in the tissue (myocardium, lung, endothelium) (Ellis et al. 1999b).

Since this syndrome presents varied symptoms and affects the immune system, opening the road for the occurrence of other illnesses, the diagnosis is not easy. Therefore, the correct diagnosis of the PMWS is important since the symptoms can be masked by other illnesses, and it should be based on: (Sorden 2000, Segalés 2002).
Clinical signs: progressive emaciation with no response to antibiotic treatment (Figure 1).

Macroscopic lesions: enlargement of lymph nodes (Figure 4), atrophy of thymus and pulmonary consolidation with non-collapsed lungs.

Microscopic lesions: lymphocyte depletion at lymphoid tissues (lymph nodes, spleen), hystiocytic inflammatory infiltration, presence of intracytoplasmic spherical, basophilic inclusion bodies in hystiocytic cells, syncytial cells interstitial pneumonia, interstitial nephritis.

PCV2 antigen or DNA detection associated to the lesions by laboratorial techniques (ICH or ISH and PCR).

Control.

It is very difficult to control this syndrome. The virus is extremely resistant to disinfectants, there is no effective treatment of affected pigs and commercial vaccines are still unavailable. The most successful control plan is obtained with changes in management, based on a risk factors correction plan and reduction of stress factors (Guilmioto & Wessel-Robert 2000, Madec & Waddilove 2002):

- Reduction of the stress – especially environmental (temperature variations, draughts and noxious gasses) and in the animal density.

- Limit pig to pig contact– avoid mixtures of lots (age, origin). Indirect contact by needle, surgical instruments, manure or people is also important.

- Good hygiene – adopt the system “all in- all out” of rigorous form, use of efficient disinfectants for PCV2, exercise biosecurity measures.

- Good nutrition – it is important for growth and also for developing the immune system (use of antioxidants for example). Colostrum provides protection against other agents present on the farm.

Adequate hygiene measures as cleaning and disinfection with "all in-all out" must be followed. PCV2 are very resistant to common use disinfectants, mainly by being protected in the organic matter. In this way it is important a general cleaning with use of detergents before the disinfectant. A preliminary risk factors study (Zanella et al. 2003c) was accomplished in an attempt to minimize the losses due to PMWS. The losses started 10 months before the clinical and laboratorial diagnostics of PMWS caused by PCV2. At the first visit, several control measures were recommended and the performance of the pigs were evaluated for a period of 5 months afterwards. Based on the field observations, some management measures were recommended such as a good cleaning and disinfection with “all in all out” system, decrease the replacement rate below the 30% employed in the herd, and the removal of the PMWS affected pigs to a hospital pen (Figure 5). These initial measures helped to decrease the impact of the PCV2 in mortality and morbidity in this herd (Zanella et al. 2003c).

CONCLUSIONS

Porcine circovirus type 2 infection or PMWS has been considered an emerging disease. The prevalence of the virus in Brazil is still unknown but it may be disseminated in Brazilian swineherds. PCV2 is not a new virus, but a recently identified virus. A recent study shows that the porcine circovirus type 2 infection is present in Brazil since 1988, despite the PCV2 associated to the illness has been first identified in 2000. However, it is still unknown why PCV2 associated disease suddenly became of major economic importance to the pig industry worldwide since the first isolation and identification of the virus in 1997. PMWS may be a consequence of changes in management practices, change in the genetics of the host or emergence of other agents that enhance disease severity during mixed infections. There are no available vaccines or effective treatment. Just control measures associated with the improvement of sanitary and management practices are recommended presently.

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