

Original Article

Porcine cytomegalovirus infection is not associated to the occurrence of post-weaning multisystemic wasting syndrome

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Abstract

Porcine cytomegalovirus (PCMV) is a *Betaherpesvirus* that causes lifelong latent infections in swine; occasionally, it may be associated with inclusion body rhinitis in piglets and reproductive disorders in pregnant sows. Post-weaning multisystemic wasting syndrome (PMWS) a condition where porcine circovirus type 2 (PCV2) infection is necessary – though not sufficient – to trigger disease, has become one of the major health problems to the porcine productive chain. Despite the high expected prevalence of both PCMV and PCV2 in swine-raising farms, no links between PCMV and PMWS have been investigated so far. In view of that, the present study was conducted to search for relations between PCMV infections and the occurrence of PMWS. Spleen and sera of PMWS-affected and non-PMWS-affected animals were examined. In PMWS-affected animals, PCMV DNA was detected in 88.4% of the spleen samples and 7.6% of the sera, whereas in non-PMWS-affected pigs, PCMV DNA was detected in 72.7% of the spleens and 10% of sera. Such differences were not statistically significant. These findings showed despite the high prevalence of PCMV infections in the swine population examined, no positive or negative association could be inferred from the presence of PCMV DNA and the occurrence of PMWS.

Keywords: PCMV, PCVAD, PMWS, porcine cytomegalovirus, SuHV-2.

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Introduction

Post-weaning multisystemic wasting syndrome (PMWS) is a porcine disease which has become a major burden to the swine productive chain (Gillespie *et al.* 2009). PMWS is one of the more often reported conditions presently known as ‘porcine circovirus-associated diseases’ (PCVAD). The virus involved, porcine circovirus type 2 (PCV2), is a member of the genus *Circovirus*, family *Circoviridae* (International Committee on Taxonomy of Viruses – ICTV, 2014). PMWS is characterized clinically by

pallor of the skin, respiratory distress, occasional diarrhea, jaundice and, most prominently, wasting in post-weaning pigs of about 2–4 months of age (Ellis *et al.* 1998; Allan & Ellis 2000). PCV2 is necessary, though not sufficient, to induce PMWS (Krakowka *et al.* 2000; Harms *et al.* 2001). Variables affecting the virus, the host – including co-infections – and herd management conditions have been shown to influence in development of disease (Opriessnig *et al.* 2007). Moreover, one or more agents may be involved. Several studies have been conducted in search for links between PCVAD and other swine

pathogens (Ellis *et al.* 1999, 2000; Allan & Ellis 2000; Allan *et al.* 2000; Krakowka *et al.* 2000; Pogranichniy *et al.* 2002; Rose *et al.* 2003; McMahon *et al.* 2006; Segales *et al.* 2009; Blomstrom *et al.* 2010).

Porcine cytomegalovirus (PCMV, also known as swine herpesvirus 2 - SuHV-2) is a *Betaherpesvirus* that causes lifelong latent infections in swine; occasionally, it may be associated with inclusion body rhinitis in piglets and reproductive disorders in pregnant sows (Edington *et al.* 1976). The virus is ubiquitous in swine, where it induces latent infections in the host, a common feature between members of the *Herpesviridae* family (Tucker *et al.* 1999; Garkavenko *et al.* 2004; Davison *et al.* 2009). Infection can be reactivated and viral replication enhanced by immunosuppressive conditions (Fishman & Rubin 1998; Mueller *et al.* 2002, 2003; Gollackner *et al.* 2003). Despite the high prevalence PCMV in swine worldwide (Rondhuis *et al.* 1980; Assaf *et al.* 1982; Tajima *et al.* 1993; Hamel *et al.* 1999), associations between PCMV and PMWS have not been investigated so far. As PCV2 is an immunosuppressing virus, it became of interest to examine whether PCMV would play any role in the development of PMWS.

Evidence pointing out the immunosuppressive effect of PCV2 is abundant. PMWS-affected pigs show increased incidence of diseases associated with opportunistic pathogens or unexpected pathogens (Carrasco *et al.* 2000; Nunez *et al.* 2003; Cavallini Sanchez *et al.* 2006; Zlotowski *et al.* 2006; Szeredi & Szentirmai 2008). However, the mechanisms by which immunosuppression occurs are not fully understood. Immune function studies in PMWS-affected pigs revealed extensive depletion of lymphocytes in lymphoid tissues (Chianini *et al.* 2003) and reduction in circulating B and T cells (Nielsen *et al.* 2003). Moreover, increased interleukin 10 (IL-10) expression was observed in PCV2 infection suggesting that IL-10-mediated immunosuppression may play an important role in the pathogenesis of PMWS (Crisci *et al.* 2010; Doster *et al.* 2010). These findings support the involvement of the immune system in PMWS pathogenesis. The aim of this study was to search for some associations between the detection of PCMV DNA in pigs and the occurrence of PMWS.

Materials and methods

Spleen and serum samples

Spleen and serum samples were received from pig farms in Rio Grande do Sul State, Brazil. The case group (PMWS-affected pigs) consisted of 77 spleens from 1 to 4 months old piglets and 92 serum samples from 1 to 4 months old piglets. In the case group, the pigs displayed dyspnoea, enlargement of superficial inguinal lymph nodes, pallor, jaundice and diarrhoea. The diagnosis of PMWS was confirmed by typical macroscopic lesions at necropsy, histopathology and demonstration of PCV2 DNA in tissues by PCR.

The control group (non-PMWS-affected pigs) consisted of 11 samples of spleen tissue from healthy pigs at slaughtering age and serum samples of 119 pigs >6 months old. Additionally, 24 serum samples from healthy from 1 to 4 months old piglets were included as controls.

DNA extraction

DNA extraction from spleens was performed with sodium iodide as described previously (Teixeira *et al.* 2013). DNA of sera was extracted from 500 μ L volumes using a phenol-chloroform method. The extracted DNA was resuspended with 50 μ L of TE buffer. The quantity and quality of the extracts were analysed with the aid of a spectrophotometer (Nanodrop[®] 1000 DE, USA).

PCR for PCMV

Primers for PCMV detection were designed by Hamel and collaborators (Hamel *et al.* 1999). The primer sequences correspond to nucleotides 37 to 64 and 449 to 420 on the PCMV DNA polymerase gene. The expected size of the amplification product is 413 bp. The PCR was carried out in 25 μ L volumes containing 2 μ L of DNA (100 ng for spleen samples and 2 μ L of DNA extracted from serum samples), 5 pmol of each primer, 0.8 mmol/L dNTPs, 1.5 mmol/L MgCl₂ and 1 U Taq DNA polymerase (Invitrogen Carlsbad, USA). The PCR program consisted of an initial reaction at 94°C for 3 min,

followed by 35 cycles at 94°C (30 s), 61°C (30 s) and 72°C (30 s), with a final extension of 5 min at 72°C. PCR products were subjected to electrophoresis on a 1% agarose gel, stained with ethidium bromide and visualized on UV light.

To avoid contamination, separate rooms were used to prepare reaction buffers and to perform the PCR reactions, to extract DNA, and to examine PCR products. Disposable filter tips were used throughout. Additional controls with ultra-pure water instead of sample DNA were included in every 10 PCR tubes. Possible inhibitory effects of serum DNA on PCR reaction were evaluated by amplification of an unrelated amplicon in a separate PCR performed with each serum sample (data not shown).

Sensitivity assay

In order to determine the PCMV PCR sensitivity, an amplicon from a spleen containing PCMV DNA (as later confirmed by sequencing) was cloned in plasmid pCR 2.1 following the manufacturer’s protocol (pCR 2.1 TOPO TA Cloning Kit; Invitrogen Carlsbad, USA). The sensitivity of the PCR was determined by amplification of 10-fold dilutions of known amounts of plasmid DNA. These experiments were repeated three times. The plasmid containing PCMV genome fragment was used as positive control in the PCR throughout the study.

Statistical analysis

Statistical analysis was performed applying the chi-square test available in DagStat (Mackinnon 2000) to compare the proportion of PCMV detected in the case and control groups. The level of significance was set to $P \leq 0.05$.

Results

The PCR employed to amplify PCMV genome fragments in spleen and sera of PMWS-affected and non-PMWS-affected pigs was shown to amplify a minimum number of genome copies equivalent to 100 molecules/reaction of PCMV DNA, as extracted from the PCMV DNA-containing plasmid (Fig. 1).

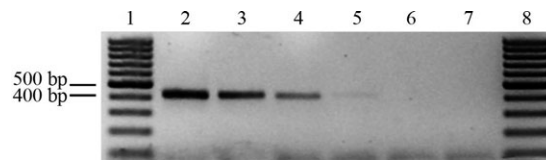


Fig. 1 Determination of PCR sensitivity for detection of PCMV. Known copy numbers of the plasmid containing the PCR- targeted region of the PCMV DPOL gene (lanes 2–6: 10^5 – 10^1 molecules per reaction, respectively) were submitted to PCR amplification (for details refer to methods). The detection limit of the PCR was 100 molecules/reaction. Lanes 1 and 8, molecular size marker; lane 7: negative control. DPOL, DNA polymerase; PCMV, porcine cytomegalovirus.

Table 1. PCMV DNA detection in spleen and sera from PMWS-affected and non-PMWS-affected pigs

Groups		No. of samples	Samples with PCMV DNA tested
Spleen	PMWS-affected pigs (1–4 months old)	77	68 (88.4%)
	Non-PMWS-affected pigs (>6 months old)	11	8 (72.7%)
	Total	88	76 (86.4%)
Serum	PMWS-affected pigs (1–4 months old)	92	7 (7.6%)
	Non-PMWS-affected pigs (>6 months old)	119	14 (11.8%)
	Non-PMWS-affected pigs (1–4 months old)	24	2 (8.3%)
	Total	235	24 (10.2%)

PCMV, porcine cytomegalovirus; PMWS, post-weaning multisystemic wasting syndrome.

In spleen tissues, PCMV DNA was detected in 88.3% (68/77) of samples from PMWS-affected pigs and in 72.7% (8/11) from non-PMWS-affected pigs (Table 1). Such differences were not statistically significant ($P \geq 0.05$).

In sera, PCMV DNA was identified in 7.6% (7/92) of PMWS-affected pigs (Table 1), whereas in non-PMWS-affected pigs PCMV DNA was detected in 8.3% (2/24) in 1–4 months old pigs 11.8% (14/119) of >6 months old pigs (Table 1). Statistical analysis of the frequencies of PCMV DNAemia in PMWS-affected and non-PMWS-affected groups revealed that such frequencies were not significantly different ($P \geq 0.05$).

Discussion and conclusions

Associations of PCV2 with other swine pathogens have been shown to play some role in the development of PCVAD. Co-infections with PCV2 and other pig pathogens may lead to the development of PMWS (Allan & Ellis 2000). Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) can act synergistically with PCV2 and lead to PMWS development (Pogranichniy *et al.* 2002; Rose *et al.* 2003). Porcine parvovirus (PPV) has also been found to play a role in PMWS: co-infections with PCV2 and PPV were detected in approximately 15% of PMWS cases (Ellis *et al.* 2000). In addition, experimental co-infections with PCV2 and PPV resulted in PMWS reproduction in gnotobiotic, colostrum-deprived pigs (Ellis *et al.* 1999). Other pathogens which have been reported to be capable of participating in the establishment of PMWS include *Mycoplasma hyopneumoniae* (Pallares *et al.* 2002), *Cryptosporidium parvum* (Nunez *et al.* 2003), Aujeszky's disease virus (Quintana *et al.* 2001; Maldonado *et al.* 2005), influenza virus and bacterial pneumonias (Kim *et al.* 2002; Pogranichniy *et al.* 2002; Dorr *et al.* 2007; Wei *et al.* 2010). Against such a background, a search for a role for PCMV in PMWS, particular in view of the immunosuppressive potential of PCV2, would be expected to reveal some sort of interaction. PCV2 is known to induce immunosuppressive effects on the host, predisposing to viral, bacterial and mycotic infections (Segales *et al.* 2004). Opportunistic infections have often been observed in herds with PMWS (Carrasco *et al.* 2000; Kim *et al.* 2002; Pallares *et al.* 2002; Nunez *et al.* 2003; Cavallini Sanches *et al.* 2006; Zlotowski *et al.* 2006). Whether the immunosuppressing effect of PCV2 facilitates co-infection or whether a co-infecting pathogen would induce immunosuppression that could trigger PMWS still remains a matter of controversy.

In the present study, a search was made for PCMV in spleens and sera of PMWS-affected and non-PMWS-affected pigs. The presence of PCMV DNA in spleen is indicative of previous infection. This is important in view of the biology of herpesviruses, where latent infections are produced and active virus

replication may not be directly linked to any particular disease.

In humans, human cytomegalovirus-HCMV-DNA in serum was considered a sign of active viral replication, and was associated to clinical disease (Spector *et al.* 1992; Wolf & Spector 1993). On the other hand, HCMV DNA in leukocytes was considered indicative of latent infections (Ishigaki *et al.* 1991). Here, PCMV DNA was detected in serum in proportions that did not differ significantly between groups of PMWS-affected and non-PMWS-affected groups of pigs. A slightly larger proportion of PCMV DNA-containing samples was found in older, non-PMWS-affected pigs, (Table 1). However, this finding was probably more related to exposure related to aging than to other conditions, since these pigs had no signs of disease. The proportions of PCMV DNA-bearing animals were not significantly different between the two groups, revealing that the presence of PCMV DNA in serum is not associated to the occurrence of PMWS in the sampled population. More likely, PCMV DNA in sera of both PMWS-affected and non-affected pigs at equivalent proportions suggests that serum PCMV DNA is indicative of latent, rather than active PCMV infections.

The results presented here show that PCMV DNA was detected in spleens of a high proportion of the sampled animals – either with or without PMWS. The frequency of detection of PCMV DNA in the sampled population is similar to those reported in previous studies (Rondhuis *et al.* 1980; Assaf *et al.* 1982; Tajima *et al.* 1993; Hamel *et al.* 1999; Goltz *et al.* 2000). Despite the high frequency of PCMV and PCV2 infections in both groups of animals, no association between the presence of PCMV DNA and the occurrence of PMWS could be inferred. Therefore, despite the high prevalence of PCMV in the examined population of pigs, PCMV does not seem to play any significant role in the occurrence of PMWS.

In summary, results reported here show that the prevalence of PCMV infections in the population examined is high in both, PMWS-affected and non-PMWS-affected animals. Therefore, PCMV does not seem to play any significant role in the development of PMWS.

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Conflicts of interest

The authors have declared that no competing interests exist.

Contributions

Conceived and designed the experiments: SPC & PMR. Performed the experiments: SPC, GP, TFT, CLH & APMV. Analyzed the data: SPC, DD, ACF. Contributed reagents/materials/analysis tools: PMR ACF. Wrote the paper: SPC & PMR.

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