Comparative serologic and virologic study of commercial swine herds with and without postweaning multisystemic wasting syndrome

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Abstract

A comparative serologic and virologic study was performed in pigs from 5 herds with postweaning multisystemic wasting syndrome (PMWS) and 2 herds without PMWS in Quebec. In each herd, 60 blood samples were collected at 4-wk intervals from pigs from 3 to 23 wk of age. The serum was evaluated for the presence of antibodies to porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV), as well as for the presence of nucleic acid of PCV2, PRRSV, and porcine parvovirus (PPV), by means of the polymerase chain reaction (PCR). Serologic profiles for PCV2 were very similar in 6 of the 7 herds, including the 2 without PMWS, and were characterized by a gradual decrease in antibody titres from 3 until 11 wk of age, followed by seroconversion at 15 wk, and high PCV2 antibody titres thereafter in all pigs. Only starting at 11 to 15 wk of age could PCV2 viremia be detected, except in 1 herd, in which clinical signs were observed at 6 to 7 wk of age. A PCV2 viremia could be detected within the same pigs for a minimum of 8 wk, and the virus could still be detected in 41% of the serum samples obtained at 23 wk of age. The antibody level did not appear to influence the occurrence of disease, since titres were similar in pigs in the herds with or without PMWS. Infection with PRRSV, as demonstrated by PCR and seroconversion, preceded that of PCV2 by at least 1 mo in both types of herd. Both PRRSV and PCV2 were detected in some pigs in 5 of the 7 herds, including 1 herd without PMWS. Porcine parvovirus could be detected in serum by PCR in 2 herds with PMWS after the onset of clinical signs and also in 1 herd without PMWS. Genomic analysis of PCV2 strains identified in the herds without PMWS indicated complete or very high homology (99.4% to 100%) with the PCV2 strains identified in 4 herds with PMWS. In our field study, the triggering of PMWS in the herds could not be linked to coinfection with either PRRSV or PPV or to the use of a specific immunostimulant, such as vaccines, or to particular genomic differences between the PCV2 strains identified.

Résumé

*Une étude sérologique et virologique comparative de porcs de 7 troupeaux du Québec avec (5 troupeaux) et sans (2 troupeaux) syndrome de dépérissement post-sevrage (SDPS) a été réalisée. Pour chaque troupeau, des sérums (*n = *60) ont été prélevés à 4 semaines d'intervalle de porcs âgés de 3 à 23 semaines. Tous les sérums récoltés ont été éprouvés pour la présence d'anticorps contre le circovirus porcin type 2 (CVP2) et le virus du syndrome reproducteur et respiratoire du porc (SRRP). La recherche par PCR d'acide nucléique du CVP2, du virus SRRP, et du parvovirus porcin (PVP) a également été réalisée. Les profils sérologiques de CVP2 étaient très similaires dans 6 des 7 troupeaux incluant les 2 troupeaux sans SDPS et étaient caractérisés par une baisse progressive des anticorps de 3 à 11 semaines d'âge, suivie d'une séroconversion à 15 semaines et de hauts titres en anticorps anti-CVP2 par la suite chez tous les porcs. La virémie pour le CVP2 ne pouvait être détectée qu'à partir de 11 à 15 semaines d'âge, sauf dans 1 troupeau où les signes cliniques avaient été observés à 6 à 7 semaines d'âge. La virémie pour le CVP2 a pu être détectée chez un même porc pendant au moins 8 semaines, et le CVP2 a pu encore être démontré dans 41 % des sérums collectés à l'âge de 23 semaines. Le niveau d'anticorps ne semble pas avoir influencé l'apparition de la maladie puisque les titres mesurés étaient similaires chez les porcs d'un troupeau avec et sans SDPS. Le moment d'infection par le virus SRRP, tel que démontré par PCR et par séroconversion, précédait celui du CVP2 d'environ 1 mois dans les troupeaux avec ou sans SDPS. Une virémie pour les 2 virus, CVP2 et virus SRRP, a pu être démontrée chez certains porcs dans 5 des 7 toupeaux incluant 1 troupeau non-SDPS. Le PVP a été démontré par PCR dans le sérum de porcs de 2 troupeaux avec SDPS après le début des signes cliniques mais également dans celui de porcs d'un troupeau sans SDPS. L'analyse génomique des souches CVP2 identifiées dans 2 troupeaux sans SDPS a indiqué une homologie totale ou très élevée (99,4 % à 100 %) avec les souches identifiées dans 4 troupeaux avec SDPS. Dans notre étude de terrain, le déclenchement du SDPS dans les troupeaux n'a pu être relié aux co-infections avec le virus SRRP ou le PVP ni à l'utilisation d'immunostimulants spécifiques tels des vaccins ou encore à des différences génomiques entre les souches CVP2 identifiées.*

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Introduction

Postweaning multisytemic wasting syndrome (PMWS) was identified as a new condition in 1997 in Western Canada. It is characterized by wasting, dyspnea, lymph node hypertrophy, and sometimes diarrhea and jaundice (1). This new syndrome has been associated with porcine circovirus type 2 (PCV2), a virus antigenically and genetically different from PCV1 (2–6). However, PMWS is sporadic, whereas PCV2 infection is widespread in swine and has been present in the swine population since at least 1985 in Canada and Belgium and since 1973 in Ireland (7–9). In addition, field studies have demonstrated that PCV2 is not always associated with clinical signs and lesions of PMWS (10,11).

Although generally only mild to moderate lesions have been reproduced experimentally in young pigs by inoculation with PCV2 (12–15), severe disease has been demonstrated following coinfection with porcine parvovirus (PPV) (12,14,16) and porcine reproductive and respiratory syndrome virus (PRRSV) (17–19), as well as after immunostimulation (20). Cofactors such as coinfecting pathogens and immunostimulation have been suggested by experimental studies; however, the exact mechanisms triggering PMWS in the field are unresolved, and very few field studies in herds with and without PMWS have been published.

The present study was undertaken to determine whether there were differences in the kinetics of PCV2 infection and in the genomic sequences of PCV2 strains identified in herds with and without PMWS in Quebec. We also investigated other factors possibly related to the triggering of PMWS; namely, the presence of PRRSV and PPV, as well as the use of immunomodulating agents, such as vaccines.

Materials and methods

Herds and blood collection

We studied 7 herds from Quebec, 5 with clinical signs of PMWS (herds A to E) and 2 without (herds N and P). The herds with PMWS were selected according to Harding's description (21). Sixty blood samples were collected from pigs in each herd at approximately 4-wk intervals from 3 to 23 wk of age. The samples from herd A were collected from the same 10 pigs over time (cohort study), whereas the samples from the other herds were collected from 10 pigs in the various age groups at 1 time (cross-sectional study). In addition, 240 blood samples were collected from pigs approximately 6 mo of age at 2 geographically different slaughterhouses in 2001 and 2002. Serum was separated from whole blood by centrifugation and then stored at −20°C until tested.

Serologic analyses

Detection of antibodies to PCV2 and titrations were performed by indirect immunofluorescence as previously described (7,15). The serum was tested at a 1/20 dilution. Antibodies to PCV2 were titrated in serum from 2 herds with PMWS (herds A and E) and 1 herd without PMWS (herd P). The level of antibodies to PRRSV was determined by a commercial enzyme-linked immunosorbent assay (ELISA; IDEXX Laboratories, Inc., Westbrook, Maine, USA).

Polymerase chain reaction (PCR)

We extracted DNA from 200 μL of serum with the use of a commercial kit (DNeasy Tissue Kit; Qiagen Inc., Mississauga, Ontario) and determined the presence of PCV2 nucleic acid with the use of PCV2-specific primers (CF8-CR8), as previously described (10). For detection of PRRSV, we extracted RNA from 250 μL of serum with the use of TRIZOL LS reagent (Canadian Life Technologies, Burlington, Ontario) and carried out PCR with the following pairs of primers amplifying, respectively, ORF5 and ORF7 (5FN: 5'-ATGTTGGGGAAATGCTTGACC-3' with 5DN: 5'-GTTCCGCTGAAACTCTGGTTA-3'; UNI7F: 5'-ATG GCCAGCCAGTCAATC-3' with UNI7R: 5'-GGATCAGGCG CACAGTATG-3'). For the PCR, 2 μL of cDNA was added to a PCR mixture with final concentrations of 1.50 mM $MgCl_2$, 1X PCR buffer, 0.2 mM of each dNTP, 1 μM of each primer, and 2.5 U of *Taq* DNA polymerase (Canadian Life Technologies) per 50 μL. Amplification was achieved by 35 cycles of denaturing at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. The PCR was ended with a final extension step of 10 min at 72°C. The presence of PPV DNA was detected by PCR with specific primers amplifying a region coding for the nonstructural protein gene (protocol and primers provided by Dr. P. Tijssen, Institut Armand-Frappier/INRS, Laval, Quebec).

Sequencing and genomic comparison of PCV2 strains

The PCV2 strains detected in each herd were sequenced over the entire genome. Several pairs of primers were designed to generate overlapping PCR-amplified fragments (22). The PCR reactions were performed with 5 μL of DNA added to 45 μL of reaction mixture, containing final concentrations of 1.25 mM $MgCl_2$, 1X PCR buffer, 0.2 mM of each dNTP, 1 μM of each primer, and 2.5 U of *Taq* DNA polymerase (Canadian Life Technologies). The DNA was amplified by 35 cycles of denaturing at 95°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min.

The PCR products were purified with a commercial kit (QIAquick PCR purification kit, Qiagen) according to the manufacturer's instructions. Purified PCR products were sequenced in both directions by standard automated-sequencing methods at a commercial facility (University Core DNA Sequencing Laboratory, University of Calgary, Calgary, Alberta). The nucleotide sequences were aligned by means of the CLUSTAL W, version 1.7, multiple sequence alignment program.

Statistical analysis

Titres for the pigs from different herds at various periods were compared with a general linear model and Tukey's post-hoc tests (SAS, version 8.2, SAS Institute, Cary, North Carolina, USA). The level for statistical significance was set at 0.05. Mean titres were expressed as geometric means.

Table I. Characteristics of each herd

Herd	PMWS status	PRRSV status	Vaccination in piglets	PRRSV vaccination in sows	Age (wk) PMWS clinical signs	PCV2 sero-conversion	PCV2 in serum (PCR)	PRRSV sero-conversion	PRRSV in serum (PCR)	PPV in serum (PCR)
A	+	+	None	+	12–13	15	15–23	11	11–15	19
B	+	+	None	+	15–16	15	11–19	11	7–11	–
C	+	+	Atophic rhinitis	+	9–11	15	11–23	11	3–11	–
D	+	+	None	+	12–14	15	15–23	11	11–19	15
E	+	−	None	−	6–7	7	7–19	–	–	–
N	−	+	*Haemophilus parasuis*	+	None	15	15–19	11	11–15	19–23
P	−	−	None	−	None	15	15–23	–	–	–

PMWS — postweaning multisystemic wasting syndrome; PRRSV — porcine reproductive and respiratory syndrome virus; PCV2 — porcine circovirus type 2; PCR — polymerase chain reaction; PPV — porcine parvovirus

Results

Clinical characteristics of herds

All of the herds were farrow-to-finish operations with only 1 source of piglets. In 4 of the 5 herds with PMWS (A to D), clinical signs were first observed between 9 and 15 wk of age, whereas in 1 herd (E) they were observed at 6 to 7 wk of age (Table I). Clinical signs were observed in either late-nursery stage (C and E) or early-grower stage (A, B, and D), usually between 3 and 6 wk following transfer of the animals in the respective stages. In addition, 4 of the 5 herds with PMWS were PRRSV-positive. Aside from vaccination against atrophic rhinitis in herd C, no vaccination was performed in the piglets of the herds with PMWS. Vaccination against PRRS was practised in sows of herds A, B, C, and D. Among the herds without clinical signs of PMWS, PRRSV was present in herd N but not herd P. In herd N, sows were vaccinated against PRRS and piglets against *Haemophilus parasuis* at 6 and 9 wk of age.

Serologic profiles

The serologic profiles of each herd for PCV2 and PRRSV are shown in Figure 1. For the 10 pigs in herd A, all the blood samples collected at 3 wk of age showed the presence of PCV2 antibodies. The number of PCV2-antibody-positive pigs decreased until 11 wk, then increased at 15 wk, all pigs being positive by 19 and 23 wk of age. A serologic profile similar to that in herd A was obtained for 5 of the other 6 herds; 3 of the herds with PMWS in which clinical signs occurred at 9 to 15 wk of age (B, C, and D) and the 2 herds without PMWS (N and P) (Figure 1). In these 5 herds the number of pigs with PCV2 antibodies decreased from 3 to 11 wk and increased from 15 to 23 wk, in spite of the fact that 2 herds did not have PMWS (Table I). When titrations were performed on serum from herd A, a decrease in the level of antibodies followed by seroconversion was clearly demonstrated (Figure 2). Initially the low to moderate titres (1/80 to 1/640) observed at 3 wk decreased, and 9 of 10 pigs became seronegative by 11 wk. Then seroconversion to PCV2 was observed in 6 pigs at 15 wk (titres 1/160 to 1/1280). By 19 wk all pigs had seroconverted and the antibody titres were high (1/5120 to 1/20 480); the titres were still high (1/1280 to 1/20 480) in all the pigs at 23 wk of age. The general serologic profile obtained in a herd without PMWS (P) was similar to that obtained in herd A except at week 15, when the titres were greater (Figure 2).

In herd E, in which clinical signs were observed at 6 to 7 wk of age, a different pattern of PCV2 infection occurred. At 3 wk of age, 8 of 10 pigs demonstrated low to moderate antibody titres (1/40 to 1/320), similar to those obtained for herds A and P at this time point ($P > 0.05$). By 7 wk, 9 of 10 serum samples were positive, and an increase in titres (1/40 to 1/15 120) suggested seroconversion in this herd at this time point. All 10 pigs sampled at 11, 15, 19, and 23 wk demonstrated a strong PCV2 antibody reaction by indirect immunofluorescence.

The 240 blood samples collected at slaughterhouses were all PCV2-antibody-positive. Antibody kinetics for PRRSV in the 5 herds positive for this virus (herds A, B, C, D, and N) had the same general profiles as for PCV2 except that seroconversion to PRRSV preceded that to PCV2 by 1 mo (Table I, Figure 1). Most pigs at 3 wk of age demonstrated PRRSV antibodies, whereas at 7 wk of age few pigs were seropositive and the ELISA S/P ratios were lower. At 11 wk most pigs were seropositive, with mean S/P ratios greater than 0.9. Thereafter, all pigs remained seropositive, with S/P ratios greater than 1.2.

Polymerase chain reaction detection

In all the herds, PCV2 could be identified by PCR in serum samples obtained only at certain ages (Figure 1, Table I). In herds with PMWS, the highest rate of PCV2 detection was generally first observed at the time of PCV2 seroconversion; that is, at 7 wk of age for herd E and at 15 wk of age for the other herds (A, B, C, and D). PCV2 could still be detected in many samples (24 of 50) collected at 23 wk of age. In both of the herds without PMWS (N and P), PCV2 nucleic acid was detected mainly at 15 to 19 wk of age, and at 23 wk of age 5 of the 20 samples were still PCV2-positive. The total number of PCV2-positive serum samples was greater in the herds with PMWS (43%) than in the herds without PMWS (25%); furthermore, the PCR signal was generally stronger in the herds with PMWS.

In all 5 PRRSV-positive herds, the time of the highest rate of virus detection in serum varied between 7 and 11 wk of age, 4 wk before the

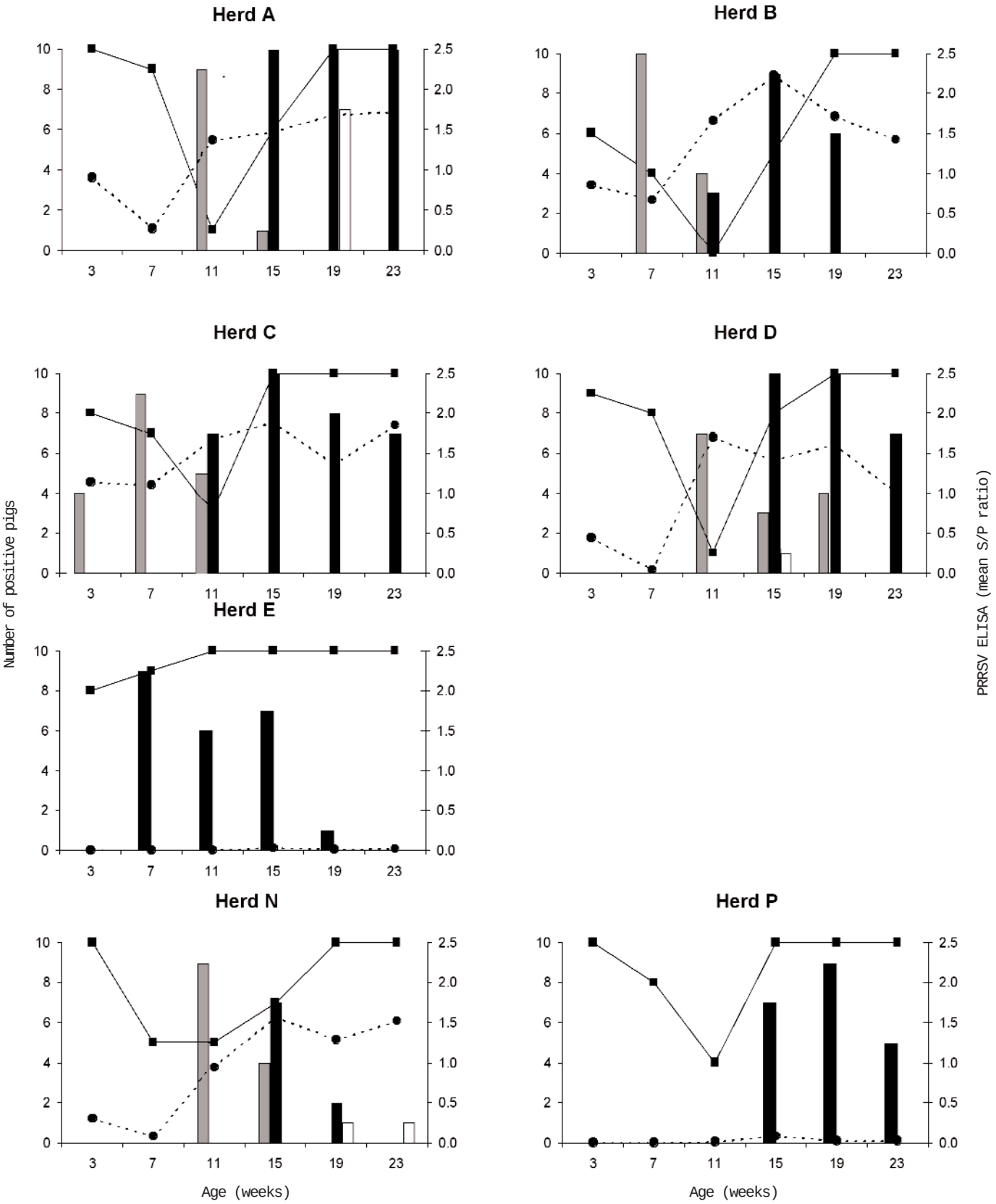

Figure 1. Serologic and virologic profiles of herds with postweaning multisystemic wasting syndrome (PMWS) (A to E) or without PMWS (N and P). Blood was collected from 10 pigs per age group from 3 to 23 wk of age. The number of pigs positive for antibody to porcine circovirus type 2 (PCV2) (squares) was determined by indirect immunofluorescence. The level of antibodies to porcine reproductive and respiratory syndrome virus (PRRSV) was determined by enzyme-linked immunosorbent assay and expressed as mean S/P ratios (circles). The numbers of serum samples positive for PCV2 (black), PRRSV (grey), or porcine parvovirus (PPV; white) were determined by the polymerase chain reaction (PCR).

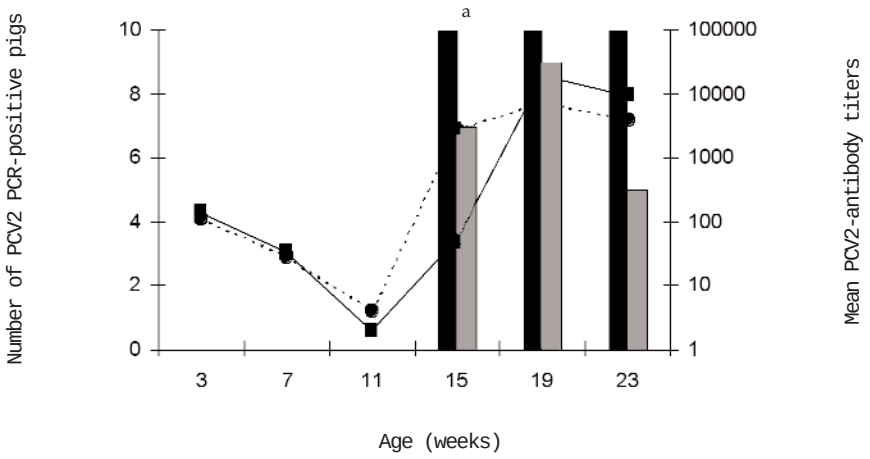

Figure 2. Comparative PCV2 serologic profiles and PCV2 PCR detection for 10 pigs per age group in herd A (with PMWS) and herd P (without PMWS). Anti-PCV2 antibody titres for herd A (squares) and herd P (circles) were determined by indirect immunofluorescence. Geometric means are reported.
[a] Significant difference between herds A and P at $P < 0.05$. The numbers of PCV2-positive pigs from herd A (black) and herd P (grey) were determined by PCR.

first detection of PCV2 in these samples. In contrast with PCV2, PRRSV was detected in pigs 3 wk of age in 1 herd. Both PCV2 and PRRSV could be detected by PCR in the serum of a few pigs in some herds with PMWS, as well as in 1 herd without PMWS.

Nucleic acid of PPV was detected in a few serum samples from only 3 of the 7 herds, including a herd without PMWS (N). Detection of PPV occurred mainly at 19 wk of age, which was after the onset of PMWS in affected herds.

Sequence analysis

Sequence analysis of each of the 7 PCV2 strains showed that the complete genome of each strain was 1768 nt long. The PCV2 strains identified in all the herds except D were closely related, displaying 99.4% to 100% nucleotide-sequence identity. The PCV2 strains of herds B, C (with PMWS), and N (without PMWS) demonstrated 100% nucleotide identity. The complete homology between these strains could not be related to a common geographic area or to a common source of piglets or of breeding animals. The PCV2 strains from herds P (without PMWS) and E (with PMWS) displayed 99.8% homology with those of herds B, C, and N. The PCV2 strain identified in herd D was less related to the 6 other strains, displaying 97.4% to 97.6% nucleotide identity with them.

Comparison of the ORF2 gene, which is believed to code for the capsid protein, revealed that the 7 PCV2 isolates shared 94.9% to 100% nucleotide-sequence identity and 94% to 100% amino-acid sequence identity. The ORF2 amino-acid sequence of the strain identified in herd D shared less identity with the other strains, which were very similar (different in only 1 to 3 amino acids).

Discussion

Since PCV2 infection appears to be widespread in swine herds compared with PMWS and is not necessarily associated with clinical signs or even lesions attributed to PMWS, it was of interest to follow the infection pattern of PCV2 and some potential coinfecting viruses, such as PRRSV and PPV, in herds with or without clinical PMWS. Very similar PCV2 serologic profiles were obtained in pigs from herds with or without PMWS, as previously reported from a serologic investigation of western Canadian herds of a common genetic source (23). In our study, in herds with or without PMWS, low levels of antibodies were present at 3 wk of age and then gradually decreased, reaching a very low level or vanishing around 11 wk of age. At this time; the pigs were exposed to infection by the virus, as demonstrated by the detection by PCR of viral nucleic acid in pigs' serum by 11 and 15 wk of age and a sharp rise in PCV2 antibody titres as well as in the number of PCV2-seropositive pigs. At 19 and 23 wk of age, all pigs remained seropositive, which is in agreement with the 100% seroprevalence of PCV2 antibodies in the tested slaughterhouse pigs. Previous experimental studies have demonstrated that PCV2 could be detected by PCR in serum of weaned pigs and infected boars 4 and 8 wk postinoculation, respectively (15,24). In the present study, PCV2 viremia could be detected within the same animals for at least 8 wk, which confirms the prolonged persistence of PCV2 in pigs after natural infection.

The kinetics of the decrease in antibody titres or in the number of positive animals suggest that the antibodies present at 3 wk of age are most likely of maternal origin (passive immunity). Indeed, it would be unlikely that if the piglets were infected before or around birth and had an active response by 3 wk of age they would show such a rapid decline in antibody titre. From experimental infections it has been reported that antibodies can be detected from 11 to 90 d following infection (24). Passive immunity may confer a certain protection against PCV2 infection and clinical disease, as suggested by Rodriguez-Arrioja et al (25), who showed a relationship between mortality associated with PMWS and low serum titres at 7 wk of age. However, in the present study the pigs of herd E showed clinical signs of PMWS at 6 to 7 wk of age in spite of levels of maternal antibodies similar to those of herds A

and P. Also, the occurrence of PMWS cannot be explained by a lower level of humoral immunity, since titres were comparable in herd A (with PMWS) and herd P (without PMWS). However, these results were obtained at the herd level, whereas passive immunity and the protective role of PCV2 maternal antibodies is preferably evaluated at an individual level, considering that PMWS develops in a low percentage of pigs in a herd.

The viral load in the environment should also be investigated as a potential factor in the triggering of PMWS. In the 2 herds without PMWS, the number of serum samples positive for PCV2 by PCR was less than the number in herds with PMWS; the detection signal was also weaker, suggesting a lower viral load, in the pigs in the herds without PMWS. Our observations at the herd level are in agreement with those of a previous study in which levels of PCV2 DNA in serum from PMWS-affected pigs were significantly higher than those in clinically healthy pigs, as demonstrated by quantitative PCR assay (26).

Lesions or clinical signs of PMWS have been reported to be more severe after experimental coinfection with PPV or PRRSV (12,14,16–19), and it has been suggested that the production of PMWS in swine may require PCV2 and an additional infectious agent, such as PPV or PRRSV, for full expression of the disease. The role of coinfecting pathogens in the triggering of clinical signs could not be clearly demonstrated in the present field study. Although PRRSV was present in 5 of the 7 herds, serologic curves and PCR detection indicated that PRRSV infection occurred several weeks earlier than PCV2 infection in the herds with PMWS as well as in the herds without PMWS. PRRSV cannot be totally excluded as a cofactor in the triggering of PMWS since circulation of both PRRSV and PCV2 was observed in herds A, B, C, and D, and infection of a few pigs with both viruses was also demonstrated. However, cocirculation and coinfection were also observed in a herd without PMWS (N). Moreover, PMWS-positive herd E was negative for PRRSV. As for PPV, it could be detected in only 2 PMWS-affected herds after the onset of clinical signs of PMWS and in 1 herd without PMWS as well. In a field study of 12 severely affected farrow-to-finish farms, the severity of PMWS did not differ between PRRSV-positive and PRRSV-negative herds (27).

Following an experimental model, immune activation during the early phase of PCV2 infection has been suggested as the key component of the pathogenesis of PMWS (20). Recently, nonspecific stimulation of the immune system by a vaccine or an immunomodulator drug potentiated viral replication and increased the severity of clinical signs during an outbreak of PMWS (28). In the present study, no links to the use of immunostimulants, such as the administration of vaccines, could be identified. In only 1 herd with PMWS (C) was a vaccine for atrophic rhinitis administered to piglets at least 6 wk before the onset of PMWS.

Previous studies have reported genomic sequences of PCV2 strains identified from pigs showing clinical signs of PMWS (5,29,30), but few studies have reported PCV2 sequences from animals without PMWS. In the present study, no relationship could be established between the genomic sequence of PCV2 strains identified in herds with or without PMWS. Indeed, the PCV2 strains identified in 6 of the 7 herds (4 with and 2 without PMWS) were closely related, sharing at least 99.4% of their nucleotide-sequence identity and more than 98.7% of their amino-acid sequence identity for the capsid protein coded by ORF2. Moreover, 1 PCV2 strain from a herd without PMWS displayed 100% homology with 2 PCV2 strains from herds with PMWS. In a recent phylogenetic study of PCV2 strains from pigs with various clinical conditions, including PMWS, no association was found between clusters or groupings of strains and clinical signs (22).

In the present study, the kinetics of PCV2 infection in herds with or without PMWS were similar in most cases. The triggering of PMWS in the herds could not be linked to coinfections with PRRSV or PPV or to the use of a specific immunostimulant, such as vaccines, or to particular genomic differences between the PCV2 strains identified. Additional field studies are needed to better understand risk factors associated with PMWS and its triggering mechanisms.

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References

1. Harding JCS, Clark EG. Recognizing and diagnosing postweaning multisystemic wasting syndrome (PMWS). Swine Health Prod 1997;5:201–203.
2. Allan GM, McNeilly F, Kennedy S, et al. Isolation of porcine circovirus-like viruses from pigs with a wasting disease in the USA and Europe. J Vet Diagn Invest 1998;10:3–10.
3. Ellis J, Hassard L, Clark E, et al. Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. Can Vet J 1998;39:44–51.
4. Hamel AL, Lin LL, Nayar GPS. Nucleotide sequence of porcine circovirus associated with postweaning multisystemic wasting syndrome in pigs. J Virol 1998;72:5262–5267.
5. Meehan BM, McNeilly F, Todd D, et al. Characterization of novel circovirus DNAs associated with wasting syndromes in pigs. J Gen Virol 1998;79:2171–2179.
6. Morozov I, Sirinarumitr T, Sorden SD, et al. Detection of a novel strain of porcine circovirus in pigs with postweaning multisystemic wasting syndrome. J Clin Microbiol 1998;36:2535–2541.
7. Magar R, Müller P, Larochelle R. Retrospective serologic a survey of antibodies to porcine circovirus type 1 and type 2. Can J Vet Res 2000;64:184–186.
8. Mesu AP, Labarque GG, Nauwynck HJ, Pensaert MB. Seroprevalence of porcine circovirus types 1 and 2 in the Belgian pig population. Vet Q 2000;22:234–236.
9. Walker IW, Konoby CA, Jewhurst VA, et al. Development and application of a competitive enzyme-linked immunosorbent assay for the detection of serum antibodies to porcine circovirus type 2. J Vet Diagn Invest 2000;12:400–405.

10. Larochelle R, Antaya M, Morin M, Magar R. Typing of porcine circovirus in clinical specimens by multiplex PCR. J Virol Methods 1999;80:69–75.
11. Larochelle R, Morin M, Antaya M, Magar R. Identification and incidence of porcine circovirus in routine field cases in Québec as determined by PCR. Vet Rec 1999;145:140–142.
12. Allan GM, Kennedy S, McNeilly F, et al. Experimental reproduction of severe wasting disease by co-infection of pigs with porcine circovirus and porcine parvovirus. J Comp Pathol 1999;121:1–11.
13. Ellis J, Krakowka S, Lairmore M, et al. Reproduction of lesions of postweaning multisystemic wasting syndrome in gnotobiotic piglets. J Vet Diagn Invest 1999;11:3–14.
14. Kennedy S, Moffett D, McNeilly F, et al. Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. J Comp Pathol 2000;122:9–24.
15. Magar R, Larochelle R, Thibault S, Lamontagne L. Experimental transmission of porcine circovirus type 2 (PCV2) in weaned pigs: a sequential study. J Comp Pathol 2000;123:258–269.
16. Krakowka S, Ellis JA, Meehan B, et al. Viral wasting syndrome of swine: experimental reproduction of postweaning multisystemic wasting syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine parvovirus. Vet Pathol 2000;37:254–263.
17. Allan GM, McNeilly F, Ellis J, et al. Experimental infection of colostrum deprived piglets with porcine circovirus 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) potentiates PCV2 replication. Arch Virol 2000;145:2421–2429.
18. Harms PA, Sorden SD, Halbur PG, et al. Experimental reproduction of severe disease in CD/CD pigs concurrently infected with type 2 porcine circovirus and porcine reproductive and respiratory syndrome virus. Vet Pathol 2001;38:528–539.
19. Rovira A, Balasch M, Segalés J, et al. Experimental inoculation of conventional pigs with porcine reproductive and respiratory syndrome virus and porcine circovirus 2. J Virol 2002;76:3232–3239.
20. Krakowka S, Ellis JA, McNeilly F, et al. Activation of the immune system is the pivotal event in the production of wasting disease in pigs infected with porcine circovirus-2 (PCV2). Vet Pathol 2001;38:31–42.
21. Harding JC. Post-weaning multisystemic wasting syndrome (PMWS): preliminary epidemiology and clinical presentation. Proc Am Assoc Swine Pract 1997:503.
22. Larochelle R, Magar R, D'Allaire S. Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) strains from cases presenting various clinical conditions. Virus Res 2002;90:101–112.
23. Harding JCS, Clark EG, Ellis JA. The clinical expression of porcine circovirus. Allen D, ed. Leman Swine Conf 1999:252–254.
24. Larochelle R, Bielanski A, Müller P, Magar R. PCR detection and evidence of shedding of porcine circovirus type 2 in boar semen. J Clin Microbiol 2000;38:4629–4632.
25. Rodriguez-Arrioja GM, Segalés J, Calsamiglia M, et al. Dynamics of porcine circovirus type 2 infection in a herd of pigs with postweaning multisystemic wasting syndrome. Am J Vet Res 2002;63:354–357.
26. Liu Q, Wang L, Willson P, Babiuk LA. Quantitative, competitive PCR analysis of porcine circovirus DNA in serum from pigs with postweaning multisystemic wasting syndrome. J Clin Microbiol 2000;38:3474–3477.
27. Madec F, Eveno E, Morvan P, et al. Post-weaning multisystemic wasting syndrome (PMWS) in pigs in France: clinical observations from follow-up studies on affected farms. Livestock Prod Sci 2000;63:223–233.
28. Kyriakis SC, Saoulidis K, Lekkas S, et al. The effects of immunomodulation on the clinical and pathological expression of postweaning multisystemic wasting syndrome. J Comp Pathol 2002;126:38–46.
29. Fenaux M, Halbur PG, Gill M, et al. Genetic characterization of type 2 porcine circovirus (PCV-2) from pigs with postweaning multisystemic wasting syndrome in different geographic regions of North America and development of a differential PCR-restriction fragment length polymorphism assay to detect and differentiate between infections with PCV-1 and PCV-2. J Clin Microbiol 2000;38:2494–2503.
30. Mankertz A, Domingo M, Folch JM, et al. Characterization of PCV-2 isolates from Spain, Germany and France. Virus Res 2000;66:65–77.