PREVALENCE OF PORCINE CIRCOVIRUS 2 (PCV2)-RELATED WASTING ON BELGIAN FARMS WITH OR WITHOUT A HISTORY OF POSTWEANING MULTISYSTEMIC WASTING SYNDROME

Porcien circovirus 2 (PCV2)-gerelateerd wegkwijnen op Belgische bedrijven met of zonder een voorgeschiedenis van groeistopsyndroom

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ABSTRACT

In this study, the prevalence of PMWS affected pigs was determined in three Belgian farms with a history of PMWS and in four control farms with no suspicion of PMWS. The diagnosis of PMWS was based on the fulfillment of all four of the following criteria: the presence of wasting, gross lesions and histopathological lesions suggestive of PMWS and the presence of high PCV2 titers in the lymphoid organs. In the farms with a history of PMWS, 2% of the piglets died of PMWS during the observation period. In the control farms, 0.1% of the piglets were found to be affected with PMWS. Serological examinations showed that all piglets became infected with PCV2 on all farms and no differences in serological profiles were seen between farms or litters with or without PMWS-affected piglets. PRRSV infections were clearly demonstrated in PMWS-affected and non-affected piglets but no correlation was seen between simultaneous infections with PCV2 and PRRSV and the occurrence of PMWS.

SAMENVATTING

In deze studie werd de prevalentie van het groeistopsyndroom bepaald op drie bedrijven met een voorgeschiedenis van het groeistopsyndroom en op vier controlebedrijven waar geen vermoeden van het groeistopsyndroom aanwezig was. De diagnose van het groeistopsyndroom was gebaseerd op de gelijktijdige aanwezigheid van vier criteria: wegkwijnen, macroscopische en histopathologische letsels die wijzen op het groeistopsyndroom, en een hoge PCV2-titer in de lymfoïde organen. Tijdens de studie stierf in de bedrijven met een voorgeschiedenis van het groeistopsyndroom 2% van de biggen tengevolge van het groeistopsyndroom. In de controlebedrijven werd bij 0,1% van de opgevolgde biggen de diagnose van het groeistopsyndroom gesteld. Serologisch onderzoek toonde aan dat alle biggen op alle bedrijven geïnfecteerd werden met PCV2 en dat er geen verschillen waren tussen de serologische profielen van tomen met of zonder biggen aangetast door het groeistopsyndroom. Infecties met PRRSV werden duidelijk aangetoond bij aangetaste en niet-aangetaste biggen maar er was geen correlatie tussen gelijktijdige infecties met PCV2 en PRRSV en het optreden van het groeistopsyndroom.

INTRODUCTION

Postweaning multisystemic wasting syndrome (PMWS) was described for the first time in 1991 on a Canadian pig farm (Harding, 1996) and later worldwide (Allan and Ellis, 2000) as a disease that affects piglets between 5 and 12 weeks of age. A circovirus, designated porcine circovirus type 2 (PCV2), was associated with PMWS (Ellis *et al.*, 2000). This virus was found to be widespread on all swine farms (Segalés and Domingo, 2002). The reproduction of PMWS by experimental inoculation, however, has only been partially successful (Allan *et al.*, 1999; Bolin *et al.*, 2001; Sanchez *et al.*, 2003). Other factors appear to play a role in the induction of the disease.

The most remarkable clinical sign in PMWS affected piglets is progressive weight loss resulting in severe cachexia (referred to as wasting) at the terminal stage. Other symptoms that have been described include respiratory distress, diarrhea, anemia and jaundice. Macroscopic lesions that have been reported in PMWS cases include interstitial pneumonia, enlargement of lymph nodes, nephritis and gastric ulcers (Segalés and Domingo, 2002). Neither the clinical signs nor the gross pathological lesions can be considered pathognomonic for PMWS. A diagnosis based only on these parameters is largely unreliable. Therefore, other features need to be included. The generally observed histological features include systemic lymphocyte depletion of all lymphoid tissues and the infiltration of newly recruited monocytes in these depleted areas (Rosell *et al.*, 1999). These lesions are somewhat more typical. Since PCV2 is enzootic and all piglets become infected at an early age, the demonstration of only the virus or viral components is not sufficient for making the diagnosis of PMWS.

It is striking that in different studies on PMWS, morbidity rates vary considerably, ranging from 4 to 30% (Segalés and Domingo, 2002). While farm-related or unknown cofactors, in addition to PCV2 infection, may be responsible for this variation, it is clear that diagnoses often are made merely on the basis of one or two of the above mentioned parameters. Therefore, it has been proposed that a series of examinations should be carried out and that the combination of clinical signs, macroscopic lesions and histopathological lesions suggestive of PMWS, together with the detection of PCV2 in the affected organs, should be used for a reliable diagnosis of PMWS (Sorden, 2000). A strong correlation has already been observed between the presence of high titers of PCV2 (= $10^{4.5}$ $TCID_{50}$ / gram) and the appearance of PMWS-specific histopathological lesions in lymphoid organs (Ladekjær-Mikkelsen *et al.*, 2002; Sanchez *et al.*, 2003).

The aim of the present study was to use these four combined criteria to determine the number of PMWS affected piglets in the total population of piglets and in the group of piglets that died during the observation period, both on farms with a history of PMWS, which were defined as farms with an increased percentage of piglets showing wasting (>1% of the piglets) and with a positive diagnosis of PMWS, and on control farms without a history of PMWS and with a normal percentage of piglets showing wasting and no indications of PMWS. Further, an attempt was made to determine whether PCV2 serologic patterns were different on farms and in litters with or without PMWS affected piglets, and whether a relation could be established with porcine reproductive and respiratory syndrome virus (PRRSV) infections, since PRRSV has been suggested as a possible cofactor in the development of PMWS (Harms *et al.*, 2001; Rovira *et al.*, 2001).

MATERIALS AND METHODS

Selection of farms

Seven farms were included in this study, being classified either as farms with a history of PMWS or as farms without a history of PMWS (control farms). The criteria for including a farm with a history of PMWS were the presence of >1% of piglets showing wasting with high mortality (> 80%) and a positive diagnosis of PMWS in at least one of the piglets showing wasting. To make the diagnosis of PMWS, the following criteria were used: (1) clinical signs: presence of wasting, (2) pathological lesions: presence of pneumonia, general enlargement of lymph nodes, nephritis or gastric ulcers, (3) histopathological lesions: presence of lymphoid depletion, (4) virological examination: detection of high PCV2 titers in inguinal lymph nodes, determined by titrating suspensions of the inguinal lymph node on PCV2 negative PK-15 cells, as previously described (Sanchez *et al.*, 2001). Three farms were included as farms with a history of PMWS (P1, P2, P3).

The second group (C1, C2, C3, C4) consisted of 4 control farms without a history of PMWS, with <1% of pigs showing wasting and no established diagnosis of PMWS.

Study design: clinical and serological monitoring; PMWS examinations

On all three farms with a history of PMWS and on three of the four control farms (C1, C2 and C3), 2 to 5 litters were randomly selected for clinical and serological follow-up. All piglets in these litters were monitored clinically every two weeks, starting from 2 weeks of age up to 14 weeks of age. At the same time, blood was taken from 3 to 6 piglets per litter to establish the serological profile against PCV2. Animals that died during the period of monitoring were collected and examined for PMWS in accordance with the four criteria mentioned above. Due to the low mortality on the control farms, dead piglets originating from non-monitored litters and from a fourth control farm (C4) were also included in the examination for PMWS.

If a serologically monitored piglet was affected with PMWS, the serological profile for PRRSV was also established for all the piglets in the affected

piglet's litter in order to determine whether a co-infection of PCV2 with PRRSV had occurred in the litter.

Detection of antibodies against PCV2 and PRRSV

PCV2 antibody titers were determined using an indirect immunoperoxidase monolayer assay (IPMA), as described by Labarque *et al.* (2000a). PK-15 cells were seeded in 96-well cell culture plates and inoculated with PCV2 isolate stoon-1010 (Meehan *et al.*, 1998). After 72 hours of incubation, the cells were washed, dried and stored at –20°C until use. Just before use, the cells were thawed, fixed in 4% paraformaldehyde and washed in phosphate buffered saline (PBS). All tested sera were heat-inactivated at 56°C for 30 minutes. Four-fold dilutions of the sera were added to the fixed cells and incubated at 37°C for 1 hour. Subsequently, the cells were washed three times with PBS + 0.1% Tween (Sigma, Germany) and incubated with rabbit-anti-swine conjugated with horse-radish peroxidase (HRP) diluted 1:250 (DAKO A/S, Denmark). After one hour of incubation at 37°C, the cells were washed three times in PBS + 0.1% Tween and bound antibodies were visualized by adding a substrate solution of 3-amino-5-ethylcarbazole in 0.05 M acetate buffer and 0.05% H_2O_2. The reaction was blocked by replacing the substrate solution by acetate buffer. The results were examined by light microscopy and the IPMA titer was defined as the reciprocal of the highest serum dilution showing a positive reaction.

Antibodies against PRRSV were detected by an IPMA, as described by Labarque *et al.* (2000b). In brief, Marc-145 cells were seeded in 96-well cell culture plates and inoculated with a Belgian isolate of PRRSV (94V360). Eighteen hours after inoculation, the cells were dried and frozen. From this point on, the IPMA for PRRSV was identical to that described for PCV2.

RESULTS

Prevalence of PMWS on farms with a history of PMWS

On the three farms with a history of PMWS, 155 piglets were followed clinically. Nine of these piglets died between 2 and 14 weeks of age (4 out of 41 piglets in P1, 2/63 in P2, 3/51 in P3). These piglets were collected and examined for the four criteria of PMWS described above. The results are shown in Table 1. Four of these piglets died without showing wasting (1 piglet in P1, 1 in P2, 2 in P3). They showed no macroscopical or microscopical lesions suggestive of PMWS and were negative for PCV2. The remaining five piglets died after a period of wasting. None of these piglets had gastric ulcers or nephritis. One of the piglets (piglet 2 in P1) showed severe polyserositis. Another piglet (piglet 3 in P3) showed polyarthritis. Neither of these two piglets showed any lymphoid depletion and both were negative for PCV2 in their inguinal lymph nodes. Bacteriological examination showed the presence of *Streptococcus suis* in the lesions of the one piglet and *Arcanobacterium pyogenes* in the lesions of the other. The remaining three piglets showed pneumonia and general enlargement of the lymph nodes. Beside gross lesions suggestive of PMWS, lymphoid depletion and the presence of PCV2 were observed in the inguinal lymph nodes. The PCV2 titers in the lymph nodes of these piglets' titers ranged from $10^{4.3}$ to $10^{6.2}$ $TCID_{50}$ / gram. Thus, the examinations resulted in a positive PMWS diagnosis for only 3 out of 155 monitored piglets (2%).

Prevalence of PMWS on control farms

Thirty-two of 1000 monitored piglets on the control farms died between 2 and 14 weeks of age and were examined. The results are shown in Table 1. Twenty-six piglets died without clinical or pathological suspicion of PMWS. PCV2 was not detected in these piglets. The remaining 6 piglets showed wasting and were clinically suspected for PMWS. Autopsy showed no gastric ulcers or nephritis in any of these piglets. Enteritis was detected in three piglets and the remaining three piglets (piglet 8 in C2 and piglets 12 and 13 in C4) showed pneumonia and enlargement of lymph nodes. Only one piglet (piglet 13 in C4) showed lymphoid depletion and was positive for PCV2. The PCV2 titer in its lymph node was high ($10^{5.5}$ $TCID_{50}$ / gram). Piglet 4 in C1 showed wasting but no macroscopical or microscopical lesions suggestive of PMWS. It was positive for PCV2 but the virus was present at low titers ($10^{2.6}$ $TCID_{50}$ / gram) in the lymph nodes. The lack of lesions suggestive of PMWS indicated that the PCV2 infection was not the cause of this piglet's death. Thus, on the control farms PMWS was diagnosed in one piglet (1‰).

Serologic profiles against PCV2

The evolution of PCV2 antibody titers for all monitored litters is shown in Figure 1. All piglets received maternally derived antibodies (MDA) against PCV2. At the age of two weeks, the titers of these MDA showed high variation both between and within farms (IPMA titers ranging from 3.1 log4 to 7.7 log4). Low

Table 1. Clinical, pathological and virological findings in deceased piglets originating from farms with and farms without a history of PMWS.

	Farm	Pig	Clinical signs	Gross lesions*		Histopathology	PCV2 titer**
			Wasting	Pneumonia	Enlargement of lymph nodes	Lymphoid depletion	in inguinal lymph nodes
Farms with history of PMWS	P1	1	-	-	-	-	< 1.5
		2	+	-	-	-	< 1.5
		3	+	+	+	+	6.2
		4	+	+	+	+	4.3
	P2	1	-	-	-	-	< 1.5
		2	+	+	+	+	4.3
	P3	1	-	-	-	-	< 1.5
		2	-	-	-	-	< 1.5
		3	+	-	-	-	< 1.5
Farms without history of PMWS	C1	1-3	-	-	-	-	< 1.5
		4	+	-	-	-	2.6
	C2	1-6	-	-	-	-	< 1.5
		7	-	-	+	-	< 1.5
		8	+	+	+	-	< 1.5
		9	+	-	-	-	< 1.5
	C3	1-4	-	-	-	-	< 1.5
		5	-	+	-	-	< 1.5
		6	+	-	-	-	< 1.5
	C4	1-7	-	-	-	-	< 1.5
		8-11	-	+	-	-	< 1.5
		12	+	+	-	-	< 1.5
		13	+	+	+	+	5.5

+: present -: absent
*: non of these piglets had gastric ulcers or nephritis
**: $\log_{10}$ $TCID_{50}$ /gram
grey: piglets that meet all criteria of PMWS

MDA titers (< 4.0 log4 IPMA titer) were found in one litter in C2 and in three litters in C3.

After a variable period of decline of the MDA (2 to 12 weeks), all litters, except one in P1 and one in P3, seroconverted to PCV2. Seroconversion occurred when the MDA IPMA titer was between 3.1 log4 and 4.4 log4. PMWS in piglets was observed around the moment of PCV2 seroconversion in the litter. Only in the four litters with the low MDA titers mentioned above did the piglets became temporarily seronegative for PCV2. In C3 the piglets remained seronegative for up to 6 weeks and showed seroconversion at 12 weeks of age, 3 weeks after they had been moved to the fattening unit.

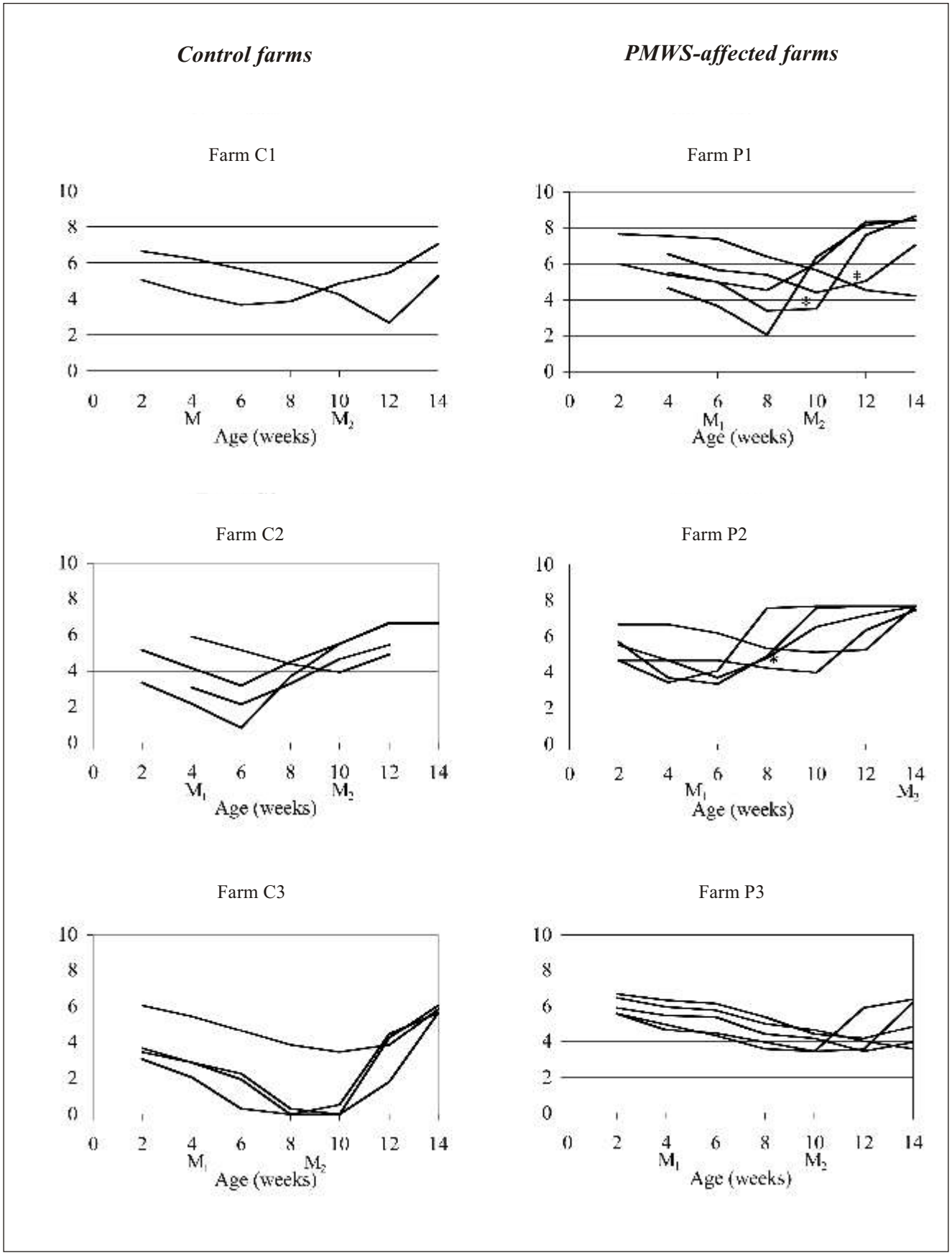

Figure 1. Evolution of IPMA-antibody titers against PCV2 per litter in farms with and farms without a history of PMWS.

M_1: movement to weaning unit

M_2: movement to fattening unit

* Death of a piglet diagnosed as PMWS in this litter

Serologic profiles against PRRSV in litters with piglets affected with PMWS

Blood for serological monitoring had been collected from only one PMWS-diagnosed piglet (piglet 4 in P1). Blood was taken from this piglet from 2 weeks of age until it died at 12 weeks of age. Since this litter contained a piglet that was affected with PMWS, the sera of all monitored piglets from this litter were also examined for antibodies against PRRSV. All piglets had maternally derived antibodies against PRRSV. The serologic profile against PRRSV showed that all five piglets became seronegative at 6 weeks of age. One piglet seroconverted at 8 weeks of age, one at 10 weeks and the remaining three, including the PMWS-affected piglet, at 12 weeks. Three piglets, including the PMWS-affected piglet, seroconverted simultaneously against PRRSV and PCV2 but only one of them developed PMWS. This indicates that there was no correlation between PRRSV/PCV2 dual infections and the occurrence of PMWS in this litter.

DISCUSSION

In the present study, it was shown that all piglets on PMWS-affected and control farms had maternally derived antibodies against PCV2 and that seroconversion to PCV2 occurred after transfer to the weaning units or the fattening unit, indicating that the piglets got infected between 4 and 12 weeks of age. Two percent of the piglets on the farms with a history of PMWS developed PMWS and one PMWS case was found in the thousand piglets followed on four control farms. PMWS was thus a rare event in this study, clearly showing that PCV2 infections are generally subclinical. PCV2 infection induced disease only under exceptional circumstances, as seen on 2 farms (P1 and P2), where piglets developed PMWS associated with PCV2 infection. The percentage of PMWS positive piglets on these affected farms was very low (average of 2% on the farms with a history of PMWS) compared to those described in other countries such as the United Kingdom (up to 22%) (Gresham *et al.*, 2000), Hungary (15%) (Kecskeméti *et al.*, 2000), the United States (up to 15%) (Kiupel *et al.*, 1998), France (11%) (Madec *et al.*, 2000) and Spain (up to 30%) (Segalés and Domingo, 2002). Limited losses caused by PMWS as described here were also reported in Denmark (Hassing *et al.*, 2002). The reason why the situation in both Belgium and Denmark is different from the situation in other major pig producing countries is not known. One of the factors could be the differences in defining a positive diagnosis of PMWS. On the farms with a history of PMWS, 3 out of 5 piglets showing wasting were diagnosed to be PMWS-affected, using the four criteria proposed by Sorden (2000). The other two piglets were negative for PCV2 and did not show lymphoid depletion. The cause of wasting and death in these piglets was polyserositis with isolation of *Streptococcus suis* and polyarthritis in which *Arcanobacterium pyogenes* was isolated. These results show that PMWS can be part of a multifactorial problem and that a diagnosis of PMWS in some pigs should not automatically be extended to other pigs that show wasting. An interesting finding in the present study was that one piglet originating from a farm without suspicion of PMWS fulfilled all the criteria for being affected by PMWS. This suggests that a single case of PMWS may occur on farms without being diagnosed. Only when the number of piglets showing wasting increases considerably will attention be drawn to this problem. This finding suggests that PMWS can occur on any farm but that the number of affected animals on a farm is very variable.

The serologic profiles against PCV2, as shown in the present study, were comparable with previously observed profiles on Belgian farms without PMWS-related problems (Labarque *et al.*, 2000a). The evolution of antibodies against PCV2 was similar in litters on farms with or without a history of PMWS. The passive immunity declined at a varying rate and was gradually replaced by active immunity. This showed that PCV2 systematically infected all piglets between 4 and 14 weeks of age on all monitored farms. All piglets that developed PMWS showed a gradual replacement of MDA, similar to what was seen in their unaffected littermates and in age-related piglets of other litters. On two farms, a litter did not show seroconversion before 14 weeks of age, although piglets in other litters in the same environment did seroconvert. This could be due to PCV2 infection occurring in the presence of rather high MDA titers. Charreyre *et al.* (2002) demonstrated that high titers of MDA interfere with the onset of active immunity upon PCV2 infection. The serologic profile in one litter of C2 and three litters of C3, both healthy control farms in the present study, was somewhat unusual. These four litters received low MDA compared to other litters, suggesting that the sows had not recently been (re)-infected with PCV2. In C3, the piglets in these litters became seronegative for PCV2 and remained seronegative throughout the whole period in the weaning units, suggesting that the circulation of PCV2 in the unit was very low at that time. These piglets seroconverted after they were moved into the fattening unit at 9 weeks of age. This farm was known to maintain strict sanitary measures. Pigs of different ages were strictly separated from one another to pre-

vent the circulation of pathogens in between groups of piglets. This suggests that good hygiene and the separation of piglets may prevent the virus from circulating intensively on a farm. Besides decreasing the circulation of PCV2, sanitary measures will also decrease the circulation of other pathogens which can serve as secondary factors in the induction of PMWS. Sanitary measures have already been shown to be successful in controlling PMWS on farms (Madec *et al.*, 2000). In the present study, simultaneous infections with PRRSV and PCV2 were seen both in PMWS-affected and healthy piglets, indicating that there was no correlation between the combined presence of the two viruses in the pigs and the occurrence of PMWS.

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