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Induction of Porcine Postweaning Multisystemic Wasting Syndrome (PMWS) in pigs from PMWS unaffected herds following mingling with pigs from PMWS affected herds

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Tables: 4

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Abstract

In this paper we present the results from two experimental studies (I and II) investigating whether PMWS can be induced in pigs from PMWS unaffected herds by mingling with pigs from PMWS affected herds and to observe whether transportation and/or mingling of healthy pigs from unaffected herds could induce PMWS.

The studies comprised pigs from 12 different herds. Eight herds had PMWS while four were unaffected. All 12 herds were found to be infected with PCV2. Pigs from PMWS-affected herds were mingled with pigs from unaffected herds in four separate compartments in both study I and II. In addition, in study II, four groups of pigs from unaffected herds were included. Two groups with pigs transported and mingled from unaffected herds and two groups with pigs which were only transported. The PMWS diagnoses on the individual pigs were based on lymphoid depletion, histiocytic proliferation and the presence of giant cells or inclusion bodies together with the demonstration of PCV2 in lymphoid tissue.

Healthy pigs, in both studies, developed PMWS 4-5 weeks after mingling with pigs clinically affected with PMWS. None of the pigs from unaffected herds which had no contact with pigs from PMWS-affected herds developed clinical signs of PMWS. Transportation and mingling of pigs from PMWS unaffected herds in combination or alone was insufficient to provoke PMWS.

Keywords: PMWS, PCV2, epidemiology, mingling pigs

Introduction

Post-weaning multisystemic wasting syndrome (PMWS) is an important disease in weaned pigs worldwide. PMWS was first described in Canada in 1991 as a chronic disease with progressive weight loss in pigs from 4-16 weeks of age (Harding and Clark, 1997). Since then, the disease has been diagnosed in many countries in North America, Europe and Asia (Allan and Ellis, 2000), and in Denmark since 2000 (Hassing et al, 2002). The clinical signs of PMWS comprise unthriftiness /wasting, paleness of the skin, enlarged lymph nodes and occasionally jaundice, respiratory symptoms or diarrhoea (Harding and Clark, 1997; Sorden, 2000; Ladekjaer-Mikkelsen et al., 2002). Affected animals have lesions in lymphoid organs characterized by lymphoid depletion and the presence of giant cells and inclusion bodies (Allan et al., 1998; Ellis et al., 1999; Ladekjaer-Mikkelsen et al., 2002; Segales et al., 2004). PCV2 has proved to be necessary but not sufficient for development of PMWS, since the virus is present in both affected and unaffected pigs and herds (Allan et al., 1999; Ladekjaer-Mikkelsen et al., 2002).

The PCV2 virus is probably transmitted between pigs by the oro-fecal and/or respiratory routes (Caprioli et al.; 2006) and vertical transmission has also been documented (West et al., 1999; Ladekjaer-Mikkelsen et al., 2001). The high prevalence of PCV2 in almost all herds of all pig producing countries indicates that the transmission of PCV2 is very effective (Rose et al., 2003; Lopez-Soria et al., 2005). In contrast, only a few studies have been performed on the “transmission” of the PCV2 associated disease complexes (PCVDs), i.e., whether PMWS can be “transmitted” from affected to unaffected pigs. A study performed in New Zealand demonstrated disease development in healthy pigs in direct or indirect contact with PMWS affected pigs when they were mingled at 4 weeks of age but not when they were mingled at

12 weeks of age (Jaros et al., 2006). The purpose of the present studies was to confirm if PCV2 positive pigs from PMWS unaffected herds can develop PMWS following mingling with pigs from PMWS-affected herds. Control groups were included to exclude the possibility that transportation or mingling by itself could induce PMWS.

Materials and methods

Two very similar studies were performed as detailed in Table 1. Study I was performed by mingling pigs from PMWS-affected and unaffected herds in four different compartments in research facility I. Study II was performed with the same basic experimental setup as in study I. However, in addition, it included four groups of pigs from unaffected farms that were either transported or both transported and mingled but remained free from contact with pigs from PMWS-affected herds.

Pigs

Pigs were obtained from 12 different herds (Table 2). Serological screening showed that all 12 herds had antibodies against PCV2. All the herds were also seropositive for *Mycoplasma hyopneumoniae* and porcine reproductive and respiratory syndrome virus (PRRSV) according to the owner's information. Herds G, H and 4 were additionally found to be infected with toxigenic *Pasteurella multocida*. Vaccine against porcine parvovirus was used in sows from all 12 herds and pigs in herds G, H, and 4 were vaccinated against toxigenic *Pasteurella multocida*. None of the herds vaccinated against PCV2. Eight of the herds were PMWS-affected (A, B, C, D, E, F, G, H) and four were unaffected (1, 2, 3, 4) according to the EU definition (<http://www.pcvd.org>). The unaffected herds were characterized by low morbidity

and mortality among weaners (Table 2). This status persisted during the study period and until three months after.

The PMWS-affected herds were visited 2-4 days before the start of the studies at which time 27-29 weaners (aged 8-14 weeks of age) with clinical symptoms of PMWS were selected and ear tagged. The same veterinarian visited the unaffected herds one week before the study started to make sure that no clinical PMWS symptoms were present. The 46 pigs from the unaffected herds in study I were transferred to the research facility when they were 4-5 weeks old, whereas the 80 pigs in study II were 5-6 weeks old.

Research facilities

The research facility I consisted of five separate compartments (no. 1, 2, 3, 4, 5) (Figures 1 and 2), only four were used in study 1. The compartments were placed between 3 to 6 meters apart connected with common passage. Each compartment consisted of 10-14 pens. The pen size was 6.7 m² and each pen was equipped with six feeding places, two water nipples and had a concrete floor. The pens had a two-climate system with coverings and straw bedding, and each compartment was equipped with separate mechanical ventilation systems (under pressure) and included wall inlets and exhausts through the roof. The partitions between the pens were open allowing nose to nose contact and movement of feces between pens. Before onset of the study, the compartments were cleaned and disinfected with formaldehyde and had a two-week down time without pigs. The research facility II consisted of three pens, placed in three separate compartments, with more than 20 meters between compartments. The pen size was between 6 and 11,1 m² and all pens were equipped with six feeding places, two water nipples and had a concrete floor. Each pen had a two-climate system with coverings and straw bedding but had only a passive ventilation system.

Experimental setup

On the day of arrival at research facility I, the pigs were distributed to the different compartments as shown in Table 1.

In study I, the pigs were housed in three pens in each of the four compartments used (Figure 1). In each pen, nine pigs from a PMWS-affected herd were mingled with nine pigs from an unaffected herd. In study II, nine pigs from PMWS-affected herds were housed in each pen. The pigs from unaffected herds were, in compartment no. 1-4 either mingled with pigs from PMWS-affected herds (nine pigs per pen), or placed in a neighbouring pen to pigs from PMWS-affected herds (18 pigs) or across the aisle from pigs from PMWS-affected herds (18 pigs) (Figure 2). Seventy two (72) pigs from herds 3 and 4 were kept as controls in study II (Table 2) with no contact to PMWS affected pigs. At research facility I, nine pigs from herd 3 and nine pigs from herd 4 were mingled in one pen in compartment no. 5. At research facility II, nine pigs from herd 3 and nine pigs from herd 4 were mingled in a pen in one compartment. In two other compartments, 18 pigs from either herd 3 or 4 were placed in two separate pens (Table 1). On the day of arrival (day 1), pigs were ear tagged with unique numbers. In study II, blood samples for serology were taken at arrival, after 3 weeks and at termination of the study. Serum was separated by centrifugation and kept at -20°C until test. Water and feed without addition of antibiotics were offered *ad libitum* throughout the study period. The personnel changed clothes and boots before entering the pens and used disposable gloves. The same veterinarian recorded clinical signs twice a week. Observations were made daily during the study by the stockman. The duration of the study was 42 days in study I and up to 48 days in study II.

Serology

All samples were tested for antibodies against PRRSV using immunoperoxidase monolayer assay (IPMA) as previously described (Bøtner et al., 1994). The IPMA was carried out as a double test (Sørensen et al., 1998) using MARC-145 cells infected with a Danish field strain of PRRSV and with an American vaccine strain (“Ingelvac” PRRS MLV, Boehringer Ingelheim), respectively. The specificity of the IPMA was 100% and the sensitivity 71% (Sørensen et al., 1997).

Antibodies against PCV-2 were measured by an inhouse developed ELISA. The PCV2 antigen was produced by serial passages of a Danish field isolate from 2002 (designation D/67782) of PCV2 on PK15 cells (kindly provided by Dr. G. Allan, Queens University, Belfast, N. Ireland). The cell culture was treated with glucosamine (Sigma) for 20 min, as previously described (Tischer et al., 1987). Virus was purified from infected cell cultures following freeze-thawing and centrifugation. Precipitated virus was collected by centrifugation and the pellet was resuspended by stirring for 1 h at RT with 1% Triton X-100 (Serva, Bie&Berntsen, Rødovre, Denmark) in PBS. After centrifugation at 14,000 rpm at 5°C, the supernatant was pelleted through a 25% sucrose (in PBS) cushion by ultracentrifugation at 40,000 rpm 5°C o.n. The virus pellet was resuspended in 50 mM Tris/HCl buffer pH 7.6. The antigen was stored at -40°C until use.

Maxisorp ELISA plates (NUNC A/S, Roskilde, Denmark) were coated by adding 100 µl antigen/well of antigen overnight at 4°C. Test samples were diluted in a 2-fold dilution starting 1:10 in ELISA-buffer and incubated 45 minutes at 37°C on an ELISA plate shaker. A positive and a negative PCV2 swine serum were included as controls. Each dilution and ELISA buffer (serving as non-inhibiting reference - NIR) were added to 4 wells in the amount of 50 µl/well. In the following, unless otherwise stated, washes were done with

ELISA buffer. After washing, 50 µl/well of a PCV2 specific monoclonal antibody F217B6 (McNeilly et al., 2000) diluted in ELISA buffer was added to 2 wells previously incubated with each serum dilution or NIR (test wells). ELISA buffer was added to the other 2 of the 4 wells per dilution (background wells). The plates were incubated 30 minutes at 37°C on an ELISA plate shaker. After washing, ELISA plates were incubated with 100 µl/well of horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (Zymed Laboratories, cat. No. 65-6420), diluted 1:5000 in ELISA buffer with 10% normal goat serum (Zymed Laboratories, cat. No. 01-6201). Following a final wash, plates were developed for approximately 10 min at room temperature with 100 ul/well tetramethylbenzidine substrate and stopped with 100 ul/well of 1M sulphuric acid. Absorbance at 450/620 nm was determined using a standard ELISA plate reader. The ODp value was calculated according to the formula: $ODp = (\text{Serum OD} \times 100) / \text{NIR OD}$. Serum OD (for each serum) = mean serum test wells - mean serum background wells. NIR OD = mean NIRtest wells – mean NIR background wells. The result on each sample was expressed as the end point titre of the reciprocal value of the dilution giving an ODp value of 55 or lower. The sensitivity and specificity has not been calculated due to lack of negative field samples, but the results were found to be significantly correlated to results obtained by IPT (Grau-Roma et al., 2008).

Necropsy

Pigs demonstrating severe clinical disease including severe wasting were euthanized during the studies. At the termination of the studies, all unthrifty pigs were euthanized. All pigs from PMWS unaffected herds in study I and all pigs in study II that were euthanized or died spontaneously were necropsied. At necropsy, tissue samples of *lnn. inguinales*, *lnn. mesenterica* and spleen were immediately fixed by immersion into 4% paraformaldehyde at 22°C for histopathological examination. Twin samples were frozen at -20°C for cryostat

section. Sections of paraffin-embedded paraformaldehyde-fixed tissue were stained with hematoxylin and eosin for histomorphological evaluation. Cryostat sections (5µm) were fixed with acetone and stained for PCV2 antigens by using a PCV2 specific monoclonal antibody as described by Ladekjaer-Mikkelsen et al. (2002). The individual pigs were diagnosed PMWS positive according to the EU definition; i.e., when they showed clinical signs together with characteristic histopathological lesions in lymphoid tissue (lymphocyte depletion together with histiocytic infiltration and/or giant cells and/or inclusion bodies) together with detection of moderate or massive amounts of PCV2 antigen (Segales et al., 2004).

Statistics

The level of PCV-2 antibody titers measured in the pigs were utilised to test two different hypotheses: i) there was no difference in the amount of antibodies in pigs originating from affected and unaffected herds in each of the four experimental compartments and; ii) there was no difference in the level of antibodies in pigs originating from unaffected herds in experimental compartments and the control compartment. The hypotheses were tested using the nonparametric Wilcoxon rank sum test (which does not require any strict distributional assumption) and exact *p*-values. All analyses were performed in a stratified manner by sampling time (arrival, after three weeks and end of study). The statistical analyses were performed using the software SAS® version 9.

Results

Clinical signs and pathology

Pigs from PMWS affected herds weighed between 11-15 kg on arrival. Pigs from unaffected herds had an average weight of 8 kg. Pigs from the unaffected herds started to show clinical signs of PMWS 3-4 weeks after mingling with pigs from the PMWS-affected herds. The most prominent signs were depression, unthriftiness and wasting. Some pigs had dyspnoea or diarrhoea. In all compartments where pigs from nonaffected and PMWS-affected herds were mingled, 2-6 pigs from unaffected herds were diagnosed with PMWS following mingling (Table 3). The remaining euthanized pigs from unaffected herds did not show characteristic histopathological lesions although clinical signs were present. In study II, ten pigs with direct contact (same pen), three pigs with close indirect contact (neighbouring pen) and one pig placed across the aisle were diagnosed with PMWS. None of the pigs from unaffected farms, that were housed in research facility 2 and had no contact to pigs from PMWS-affected herds, showed clinical signs of PMWS although they were transported and mingled. One pig from compartment 6 was euthanized on day 2 due to lameness. From the PMWS-affected herds in both studies, 50-70% of the pigs recovered clinically during the 6-week-study period

Serology

In study II, pigs from three of the four PMWS-affected herds had antibodies against PRRSV US subtype (herd F), PRRSV EU subtype (herd G) or both subtypes (herd H) on arrival and showed increasing titers in the samples taken 3 weeks later (Table 4). Pigs from herd E remained free of antibodies to PRRSV. Pigs from all the PMWS unaffected herds were free of antibodies against PRRSV on arrival, however, these pigs seroconverted to the PRRSV subtypes harboured by the pigs from PMWS-affected herds with which they were mingled. The pigs from herd 5 remained free of antibodies against PRRSV when mingled with pigs

from herd E (Table 4). All animals in compartment 5 and at research facility II remained free of antibodies against PRRSV.

The PCV2 antibody titers (medians plus 25th and 75th percentiles) for the different groups of pigs are shown in Figure 3. In all experimental compartments the titers of antibodies against PCV2 in pigs from PMWS affected herds were, at all sampling times, significantly higher than the titers in pigs from PMWS unaffected herds. The pigs from PMWS unaffected herds in the control compartment had at all sampling times significantly lower titers of antibodies against PCV2 when compared to the pigs from PMWS unaffected herds in the experimental compartments. In general, the titer of PCV2 antibodies in PMWS affected pigs increased during the study. In contrast, the level of antibodies in pigs from PMWS unaffected herds decreased from arrival until the sampling three weeks later followed by a marked increase at the final sampling.

Discussion

To our knowledge this is the first controlled trial that show that PMWS can be induced in pigs from PMWS free herds following contact to pigs with clinical PMWS. Previously, PMWS has only been demonstrated after mingling of pigs inoculated with PCV2 and naïve pigs under experimental conditions (Okuda, et al., 2003). None of the pigs from PMWS unaffected herds developed clinical signs of PMWS after transportation and mingling with other pigs from PMWS unaffected herds in study II. Therefore, since all these pigs were PRRSV negative, it seems unlikely that stress due to transportation and mingling with other pigs from PMWS unaffected herds induced PMWS in either of the two studies. The finding that the PMWS unaffected herds remained unaffected three months after the study further sustained that it was the contact with pigs from PMWS-affected herds that induced PMWS.

254 The fact that the pigs from the PMWS unaffected herds had an increase in antibodies against
 255 PCV2 that coincided with the subsequent development of clinical signs typical of PMWS
 256 further supported the view that the pigs indeed developed PMWS, albeit it is difficult to use
 257 serology for prediction of PMWS disease status on single animals under field conditions. The
 258 PMWS diagnosis could not be confirmed by laboratory investigations in approximately 40%
 259 of the 52 pigs that were killed due to severe clinical signs of PMWS. This has previously
 260 been reported from field cases and probably represents end-stage pigs in which the virus level
 261 in tissues is low because of massive destruction of cells (Segales et al., 2004). The pigs were
 262 housed and fed under optimal conditions during the study: low stocking density, two-climate
 263 system, plenty of straw bedding and access to feed and water at all times. These conditions
 264 are different from the conditions regarded as potential triggers of PMWS. Despite these
 265 optimal conditions, the pigs developed PMWS and therefore the management factors might
 266 have a lower impact on the development of PMWS than previously suggested. PRRSV is a
 267 well-known infectious trigger of clinical PMWS (Rondriguez-Arrioja et al 1999; Allan et al.,
 268 2000; Harms et al., 2001; Pogranichniy et al 2002; Rovira et al., 2002; Rose et al., 2003) and
 269 the finding that most of the pigs that developed PMWS also had increasing antibody titers
 270 against PRRSV suggest that PRRSV may have participated or even been a necessary cofactor
 271 for the clinical manifestations seen in this study. The clinical signs were, however, more in
 272 accordance with typical findings in PMWS affected pigs rather than what is typically seen in
 273 pigs acutely infected with PRRSV and the pathological findings at necropsies indeed
 274 confirmed that the pigs had developed PMWS. Furthermore, in study II, all pigs in
 275 compartment 1 remained free of antibodies against PRRSV and despite this, PMWS was
 276 induced in two pigs originating from a PMWS unaffected herd which were mingled with
 277 PMWS affected pigs. Thus, it is clear from this study that a significant number of pigs from a

PRRSV-free and PMWS unaffected herd developed PMWS after mingling with pigs from PMWS-affected herds. In addition to the role of PRRSV, factors such as differences in PCV2 virus strain, the dose of PCV2 virus excreted by the “donor” pigs or even transmission of other unidentified infectious agents from PMWS affected pigs to PMWS unaffected pigs may have played a role. Detailed studies on PCV2 dynamics and comparisons of the viral DNA sequences found in the different groups of pigs indicate that the PCV2 virus was transmitted from PMWS affected pigs to the pigs from the PMWS unaffected herds (Dupont, submitted for publication). In conclusion, the present study showed that PMWS can be induced in pigs from PMWS unaffected herds by mingling with pigs from PMWS positive herds. This finding may have implications on trade and export of living pigs from areas where PMWS are present.

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Table 1. Distribution of pigs at the research facilities

Study	Research facility	Compartment	Pigs from PMWS-affected herds		Pigs from nonaffected herds	
			Herd	Number	Herd	Number
I	I	1	A	28	1	27
I	I	2	B	27	1	29
I	I	3	C	27	2	27
I	I	4	D	29	2	29
II	I	1	E	27	3	54
II	I	2	F	27	3	54
II	I	3	G	27	4	54
II	I	4	H	27	4	54
II	I	5			3 and 4	9+9
II	II	6			3	18
II	II	7			3 and 4	9+9
II	II	8			4	18

Table 2. Characteristics of the PMWS-affected herds (A, B, C, D) and the nonaffected herds (1, 2) delivering pigs to the study.

Herd	Number of sows	Pigs sold at	Post weaning mortality in the herds at onset of the study	Post weaning mortality in the herds during the study and three months later
A	185	Slaughter	30%	10-15%
B	200	30 kg	10-15%	10-15%
C	330	30 kg	8-10%	8-10%
D	386	30 kg	18%	13%
E	720	30 kg	6-7%	2,3%
F	340	Slaughter	5-6%	5-6%
G	910	Slaughter	10-12%	10-12%
H	420	Slaughter	15%	15%
1	150	30 kg	3%	3%
2	225	30 kg	Below 1%	Below 1%
3	1050	30 kg	2,6%	2%
4	400	30 kg	1,7%	1,2%

Table 3. Number of pigs diagnosed with PMWS during the studies.

Study	Facility	Compartment	Pigs from PMWS-affected herds			Pigs from nonaffected herds		
			Herd	Died	PMWS ¹	Herd	Died	PMWS ¹
I	I	1	A	9	nd ²	1	7	3
I	I	2	B	12	nd	1	6	3
I	I	3	C	8	nd	2	6	5
I	I	4	D	8	nd	2	8	5
II	I	1	E	9	3	3	2	2
II	I	2	F	19	15	3	8	3
II	I	3	G	11	7	4	8	6
II	I	4	H	16	5	4	7	3
II	I	5		np ³	np	3 and 4	0	0
II	II	6		np	np	3	1 ⁴	0
II	II	7		np	np	3 and 4	0	0
II	II	8		np	np	4	0	0

¹Pigs diagnosed with PMWS based on clinical signs together with characteristic histopathological lesions in lymphoid tissue (lymphocyte depletion together with histiocytic infiltration and giant cells or inclusion bodies) together with detection of PCV2 antigen by immunofluorescence.

²Pigs from PMWS-affected herds were not investigated for PMWS.

³No pigs from PMWS-affected herds in these compartments.

⁴Euthanised due to lameness

1 Table 4. PRRS status of PMWS-affected herds and of pigs from PMWS-nonaffected herds
 2 during study II at location I.

3

Compartment	PRRS status PMWS- affected herd	PRRS US ¹ status of pigs from PMWS nonaffected herds			PRRS EU ¹ status of pigs from PMWS nonaffected herds		
		Arrival	3 weeks	end	Arrival	3 weeks	end
1	Negative	-	-	-	-	-	-
2	US positive	-	+	+	-	-	-
3	EU positive	-	-	-	-	+	+
4	US and EU positive	-	+	+	-	+	+
5	None ²	-	-	-	-	-	-

4 ¹If at least one pig was positive, the compartment was considered positive.

5 ²No pigs from PMWS-affected herds present in compartment

Figure 1. The research facility, study I. Entry to compartments and change of boots and clothes (★). The pigs from the PMWS affected herds (□) and the pigs from the PMWS non-affected herds (■) where mingled in three pens in each of the compartments 1-4 with nine pigs from PMWS affected herds and nine pigs from PMWS non-affected herds in each pen.

Figure 2. The research facility, study II. Entry to compartments and change of boots and clothes (★). The pigs from the PMWS affected herds where placed in three pens in each of the compartments 1-4 (□) with nine pigs in each pen. The Pigs from the PMWS non-affected herds where placed in four pens in compartment 1-4 and three pens in compartment 5 (■) with nine pigs in each pen. Where □ and ■ are placed in the same pen, the pigs where mingled.

Figure 3.

Box and whiskers plot showing the log PCV-2 ELISA titer in pigs from PMWS-affected (thick grey line) and nonaffected herds (thin black line) in the four separate compartments in study II, research facility I. The bottom and top edges of the boxes are located at the sample 25th and 75th percentiles. The center horizontal line is drawn at the median. Whiskers show high/low extremes.

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