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1 **Transmission of different variants of PCV2 and viral dynamics in a research**
2 **facility with pigs mingled from PMWS-affected herds and non-affected herds**

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10
11 *Keywords:* PCV2, PMWS, horizontal transmission, sequencing, real-time PCR, quantification.

12
13 **Abstract**

14 Postweaning Multisystemic Wasting Syndrome (PMWS) has been identified in most swine-
15 producing countries worldwide. The disease has resulted in significant health challenges and
16 economic damage to the swine industry. The aim of this study was to determine horizontal
17 transmission of porcine circovirus type 2 (PCV2) and to examine viral dynamics in pigs in a
18 controlled PMWS transmission study. In the study pigs from PMWS-affected herds and non-
19 affected herds were permitted to have close contact (same pen), nose-to-nose contact (to pigs in
20 neighbouring pens) or no physical contact (pen across the aisle and pens in other compartments).
21 By DNA sequence analysis, eight variants of genotype PCV-2b were identified in the research
22 facility. From the spread of these PCV2-variants it was concluded that PCV2 primarily infects
23 through direct contact and nose-to-nose contact. PCV2 genome sequences were obtained from
24 selected pigs at arrival to the research facility and again when the same pigs developed PMWS.

1 This analysis showed that pigs from PMWS-affected herds developed PMWS caused by the same
2 variant of PCV2 as they carried when entering the research facility. In contrast, pigs from non-
3 affected herds developed PMWS with PCV2-variants identified in pigs from PMWS-affected herds.
4 This was probably connected to at least 10^3 higher mean serum-titer of PCV2 in pigs from PMWS-
5 affected herds as compared to pigs from non-affected herds at the beginning of the transmission
6 study. The study further showed that pigs able to control the PCV2-infection, as measured by the
7 PCV2-titer in serum, recovered clinically (pigs from PMWS-affected herds) or stayed healthy (pigs
8 from non-affected herds). Like this, pigs with a PCV2 titer below 5×10^8 copies/ml serum during
9 the study period had a chance of recover from the PCV2 infection whereas pigs with PCV2 titers
10 above 5×10^8 copies/ml serum at any time point generally died from PMWS.

11

12 **1. Introduction**

13

14 Post-weaning Multisystemic Wasting Syndrome (PMWS) was first observed in Canada in 1991
15 (Harding and Clark, 1997). It has today a worldwide distribution and is considered one of the most
16 important swine diseases in Europe. Usually PMWS appears in pigs aged two to four months.
17 Affected pigs show wasting or unthriftiness, enlarged lymph nodes, respiratory distress and
18 occasionally jaundice and diarrhea (Segalés et al., 2005a). The most distinct microscopic lesions in
19 lymphoid organs are lymphoid cell depletion as well as formation of multinucleated giant cells and
20 basophilic intracytoplasmic inclusion bodies (Segalés and Domingo, 2002). Infection with PCV2 is
21 needed for full expression of PMWS. However, not all pigs infected with PCV2 will develop the
22 disease (Segalés et al., 2005a).

23 Infection with PCV2 is systemic (Bolin et al., 2001; Brunborg et al., 2004) with a higher viral load
24 in serum and tissues of pigs suffering from PMWS as compared to healthy pigs (Brunborg et al.,

1 2004; Liu et al., 2000; Olvera et al., 2004; Rovira et al., 2002; Segalés et al., 2005b). The
2 respiratory system may be the route of entry, as suggested by the capacity of the virus to infect
3 bronchial and bronchiolar epithelial cells (Magar et al., 2000). Alternatively or complementary,
4 PCV2 may infect the nasopharynx and tonsils and spread via the blood or lymph (Magar et al.,
5 2000). PCV2 has also been detected in bronchial, nasal, tonsillar, salivary, ocular, faecal and
6 urinary swabs (Bolin et al., 2001; Krakowka et al., 2000; Segalés et al., 2005b; Shibata et al., 2003;
7 Grau-Roma et al., 2008b), again with a significant higher viral load in secretions from PMWS-
8 affected pigs (Segalés et al., 2005b; Grau-Roma et al., 2008b). This suggests that oro-nasal
9 secretions as well as urine and faeces are potential routes of viral shedding.

10 The incubation period for development of PMWS is approximately two weeks (Albina et al., 2001).
11 Interestingly, when PCV2-inoculated piglets were placed in contact with specific pathogen-free
12 (SPF) piglets, disease in the contact-exposed SPF piglets was recorded 16 days after appearance of
13 clinical signs in the inoculated pigs. This observation suggests that the contact-exposed SPF piglets
14 became infected during the clinical phase of the inoculated piglets (Albina et al., 2001) where the
15 viral load in secretions is expected to be high. PCV2 is able to persist in the pigs for a long period.
16 Thus, infectious PCV2 viruses were detected in tissues of a single pig sacrificed at day 125 post-
17 infection (Bolin et al., 2001). In blood and secretions, virus has been detected until post-infection
18 day 70, the day of necropsy (Bolin et al., 2001). The long persistence of virus in secretions enables
19 transmission of virus for a long period. Hence, direct contact with pigs carrying a 42-days old PCV2
20 infection resulted in transmission of virus to 3 of 3 control pigs (Bolin et al., 2001).

21 PMWS commonly affects pigs 2-3 weeks after weaning (Harding and Clark, 1997) suggesting that
22 natural infection occurs at about the time of weaning and mingling with other pigs. In order to
23 establish disease control strategies, it is important to understand the horizontal transmission of
24 PMWS and PCV2 in starter barns. In a transmission study, we have previously shown that pigs

1 from PMWS-free herds developed PMWS following mingling with pigs with PMWS. PMWS
2 developed in the PMWS-free pigs after close contact (same pen), nose-to-nose contact (to pigs in
3 neighbouring pens) or no direct contact (pen across the aisle) (Kristensen et al., 2009). In the
4 present study, we specifically determined horizontal transmission of PCV2 and examined viral
5 dynamics in pigs from this transmission study to elucidate if PCV2, in addition to PMWS, was
6 transmitted from PMWS-affected pigs to non-affected pigs. This is important knowledge as it is
7 unclear if transmission of the disease PMWS coincide with transmission of PCV2. Horizontal
8 transmission of PCV2 was determined by studying the spread of different variants of PCV2 in the
9 research facility, while viral dynamics was examined by quantifying the viral load in serum at
10 different time points by real-time PCR.

11

12 **2. Materials and methods**

13

14 *2.1. Experimental design and sampling*

15

16 The PCV2 transmission study was performed with pigs collected from 6 different herds in Denmark
17 as described elsewhere (Kristensen et al., 2009; study II). Four of the herds showed problems with
18 PMWS and had mortalities from 5-15%. All four herds were seropositive for PRRS (except herd E
19 (figure 1)), PCV2 and mycoplasma (Kristensen et al., 2009). Two of the herds were free from
20 PMWS and had mortalities of approx. 2% (Kristensen et al., 2009). These two herds were
21 seropositive for PCV2 and mycoplasma (Kristensen et al., 2009). From the four herds with PMWS,
22 27 pigs (6-11 weeks old) with clinical signs of PMWS (wasting and unthriftiness) were collected
23 from each herd. From the two healthy herds, 144 pigs (4-5 weeks old) were collected from each
24 herd. All the selected pigs from PMWS-affected herds (108 pigs) and 216 pigs from the non-

1 affected herds were mingled in 4 compartments (Comp. 1-4) at the same farm (Figure 1). As on-site
2 controls, 18 of the selected pigs from the healthy herds were placed in a separate compartment
3 (Comp. 5, Figure 1). The pens in the compartments were separated by bars permitting the pigs to
4 have nose-to-nose contact. As off-site controls, 54 of the selected pigs from the healthy herds were
5 kept at a separate farm. The duration of the infection study was 46-48 days.

6 Blood samples were collected from all the pigs at arrival. In addition samples were collected three
7 weeks later and at the termination of the study. The blood samples were centrifuged and serum
8 stored at -80°C . Clinical signs were recorded continuously during the study period. Pigs showing
9 severe clinical signs referable to PMWS (wasting, unthriftiness, dyspnoea and diarrhoea) were
10 euthanized and mesenteric lymph nodes collected at necropsy. The lymph nodes were frozen at $-$
11 20°C . Pigs, from whom mesenteric lymph nodes were collected, achieved the full PMWS-
12 diagnosis if they together with the clinical signs showed characteristic histopathological lesions in
13 lymphoid tissue (lymphocyte depletion together with histiocytic infiltration and giant cell or
14 inclusion bodies) and detection of moderate to massive amount of PCV2 antigen by
15 immunofluorescence microscopy (Kristensen et al., 2009).

16

17 *2.2. PCV2 PCR amplification and sequencing*

18

19 DNA was extracted from mesenteric lymph nodes and serum using QIAamp DNA Mini Kit
20 (QIAGEN, Ballerup, Denmark) according to the manufacturer's instructions. The PCV2 genome
21 was PCR amplified in three overlapping reactions with the primer pairs listed in Table 1. The PCR
22 reactions were performed as previously described (Dupont et al., 2007). Occasionally the resulting
23 PCR products, as visualised by gel electrophoresis, showed unspecific amplification of genomic pig
24 DNA together with genomic PCV2 DNA. In these cases the PCR products were purified with a

1 QIAquick PCR Purification Kit (QIAGEN) and PCR amplified once more with the nested primer
2 pairs listed in Table 1. The PCR products were sequenced on both strands with the sequencing
3 primers listed in Table 1. The sequence reactions were performed by Agowa (Germany).

4 5 *2.3. Sequence analysis*

6
7 Complete PCV2 genomes were assembled using ChromasPro version 1.33
8 (<http://www.technelysium.com.au/ChromasPro.html>) and aligned using ClustalX version 1.8.2
9 (<http://www.pasteur.fr>).

10 11 *2.4. Real-time PCR*

12
13 PCV2 was quantified by real-time PCR on serum samples obtained at arrival and at death or
14 termination of the experiment. Real-time PCR with PriProET chemistry was performed as
15 previously described (Hjulsager et al., 2008).

16 17 **3. Results**

18 19 *3.1. Spread of PCV2*

20
21 To investigate transmission of PMWS and PCV2, pigs with clinical symptoms of PMWS
22 originating from PMWS-affected herds were mingled with clinically healthy pigs originating from
23 non-affected herds on a research facility as shown in Figure 1. During the study period, 44 pigs out
24 of 324 (pigs in compartment 1-4) were diagnosed with PMWS as shown by clinical signs together

1 with characteristic histopathological lesions in lymphoid tissue and PCV2 antigen detection by
2 immunofluorescence microscopy (data not shown). Thirty of the pigs came from PMWS-affected
3 herds, whereas, 14 came from non-affected herds. None of the healthy control pigs in compartment
4 5 and at the separate control farm were diagnosed with PMWS.

5 To obtain a more precise picture of PCV2-transmission in the compartments, full PCV2 genome
6 sequences were obtained from all 44 pigs that developed PMWS. PCV2 sequencing showed the
7 presence of 8 major variants of a PCV2 sequence (GenBank acc. nr. EF565344) belonging to
8 genotype PCV-2b (Segalés et al., 2008) corresponding to genotype 1 (Olvera et al., 2007). Single
9 base differences separating the 8 variants served as specific markers, which could be used to track
10 the spread of each PCV2 variant among pigs in the experiment. The 8 PCV2-variants were
11 distributed in the compartments so that 2-3 different variants were characteristic to each
12 compartment (Table 2).

13 The placement of each pig that developed PMWS generally showed that pigs carrying the same
14 variant of PCV2 were placed in the same pen or in neighbouring pens (Fig. 2). Of the 14 pigs from
15 healthy herds that developed PMWS, 8 were placed in close contact (same pen) with pigs from
16 PMWS-affected herds, which developed PMWS with the same variant of PCV2. Four of the pigs
17 developed PMWS with the same variant of PCV2 as pigs from PMWS-affected herds placed in
18 neighbouring pens. In two cases, pigs from healthy herds developed PMWS with a PCV2 variant
19 characteristic to another compartment (PCV2 variant 3 and 4).

20 Development of PMWS in the pigs during this experiment may either have been caused by the
21 variant of PCV2, which the animals carried when they entered the research facility, or the animals
22 may have been infected with PCV2 from other pigs in the research facility. To distinguish between
23 these two possibilities, PCV2 was sequenced from the pigs shown in table 3 at arrival to the
24 research facility (serum samples) and compared to PCV2 sequences obtained when they died or

1 were euthanized (lymph nodes). As shown in Table 3, pigs from PMWS affected herds developed
2 PMWS with the same variant of PCV2 that they carried at arrival, except for pig number 17. All
3 pigs from non-affected herds developed PMWS caused by other variants of PCV2 than they carried
4 when entering the research facility (Table 3). Interestingly, these PCV2-variants were identical to
5 those found in pigs from PMWS-affected herds placed in the same pen or neighbouring pens (Table
6 2 and Fig. 2).

7 8 *3.2. Study of PCV2 dynamics by real-time PCR*

9
10 To study the relationship between PMWS status and PCV2 titer in serum, real-time PCR was used
11 to quantify PCV2 in serum samples obtained at arrival to the research facility and again at death or
12 at the termination of the study (Fig. 3).

13 Pigs from PMWS-affected herds that recovered clinically during the study period (Fig. 3, A) had a
14 mean PCV2 titer below 10^8 copies/ml serum at arrival declining by two log units during the study
15 period. In contrast, pigs from PMWS-affected herds that died from PMWS during the study period
16 (Fig. 3, B) had a mean PCV2 titer above 10^9 copies/ml serum at arrival. This high titer was
17 maintained until death.

18 Healthy pigs from non-affected herds had a mean PCV2 titer below 10^5 copies/ml serum at arrival.
19 Pigs that remained healthy during the study (Fig. 3, C) had a mean PCV2 titer below 10^7 copies/ml
20 serum at the end of the study, whereas pigs that were diagnosed with PMWS (Fig. 3, D) obtained a
21 mean PCV2 titer in serum at 10^{10} copies/ml.

22 None of the healthy pigs from non-affected herds placed as on-site controls in compartment 5 (Fig.
23 1) and as off-site controls on a separate control farm developed PMWS. At arrival the mean PCV2
24 titer in these pigs were below 10^4 copies/ml serum. Interestingly, at the end of the study, the mean

1 PCV2 titer in the on-site control pigs increased to 10^9 copies/ml serum (Fig. 3, E), whereas the
2 mean PCV2 titer in the off-site control pigs declined to below the detection limit (Fig. 3, F).

3

4 **4. Discussion**

5

6 To our knowledge this is the first study that has examined horizontal transmission of distinct
7 variants of PCV2 in a research facility where pigs with clinical signs of PMWS were mingled with
8 healthy pigs. The closest is an observation by Grau-Roma et al. (2008a) where two sows located on
9 the same farm and in the same room were shown to contain identical PCV2 sequences, indicating
10 horizontal transmission of PCV2.

11 In the present study 13.6 % of the pigs in compartment 1-4 were diagnosed with PMWS. Of the
12 pigs from PMWS-affected herds, 28 % acquired PMWS, whereas only 6.5 % of the pigs from non-
13 affected herds acquired PMWS. The significantly higher rate of PMWS in pigs from PMWS-
14 affected herds is most likely due to the fact that these pigs were selected based on clinical signs of
15 PMWS.

16 By comparison of PCV2 sequences obtained from selected pigs at arrival to the research facility and
17 again at death caused by PMWS, it was concluded that pigs from non-affected herds developed
18 PMWS with a PCV2 variant coming from pigs belonging to PMWS-affected herds. In contrast, pigs
19 from PMWS-affected herds generally developed PMWS with the same variant of PCV2 as they
20 carried when entering the research facility. Pig number 17 (Table 3) developed PMWS with a PCV2
21 variant slightly different from the variant carried at entrance to the research facility. This variant
22 was not detected in other pigs and possible was a result of mutation during the study period. The
23 reason why pigs from non-affected herds were infected with PCV2 from pigs from PMWS-affected
24 herds is probably connected to an at least 10^3 times higher mean titer of PCV2 in serum from pigs

1 from PMWS-affected herds as compared to pigs from non-affected herds at the start of the
2 experiment. Like this, there has been found a positive and significant correlation between PCV2
3 viral loads present in serum, and in nasal and rectal swabs (Grau-Roma et al., 2008b).

4 By studying the spread of different variants of PCV2 in the research facility, we found that PCV2
5 primarily was transmitted to pigs from non-affected herds through direct contact (8 out of 14 pigs)
6 and nose-to-nose contact (4 out of 14 pigs). More seldom PCV2 was transmitted without physical
7 contact of the pigs (2 out of 14 pigs). We speculate that PCV2 in these two cases was transmitted by
8 air or by personnel. Transmission by air was minimized by never open doors into two or more
9 compartments at the same time, whereas transmission of PCV2 by personnel was minimized by
10 changing clothes and boots before entering the pens and by using disposable gloves. In conclusion,
11 our results support the notion that direct contact with faeces and urine, and frequent nose contact are
12 the most important factors for spreading of PCV2. This is in agreement with detection of PCV2 in
13 nasal, faecal and urinary swabs (Segalés et al., 2005b; Shibata et al., 2003).

14 Pigs from PMWS-affected herds that recovered clinically and survived throughout the study period
15 had at arrival a mean titer of PCV2 100 times lower than pigs from PMWS-affected herds that got a
16 confirmed diagnosis of PMWS. At termination of the study, the PCV2 titer in these pigs was
17 declined even further indicating that these pigs were recovering from the PCV2 infection. In
18 contrast, pigs diagnosed with PMWS maintained a high mean PCV2 titer in serum until death
19 supporting that these pigs did not have the ability to recover from the PCV2 infection.

20 The mean PCV2 titer in pigs from non-affected herds that remained healthy during the transmission
21 study increased 65 times during the study period, whereas the PCV2 titer increased 10^6 times in
22 pigs that were diagnosed with PMWS. This strongly indicates that all pigs were affected by PCV2
23 infection but pigs able to control the PCV2-infection stayed healthy.

1 Pigs from healthy herds kept at the off-site control farm had a mean PCV2 titer around the detection
2 limit at arrival and we did not see any increase in the PCV2 titer in these pigs during the study
3 period. In contrast, we observed a 10^5 time increase in the mean PCV2 titer in the on-site control
4 pigs. This considerable increase in mean PCV2 titer was coupled to 2 out of 14 pigs with a PCV2
5 titer of 10^9 - 10^{10} copies pr. ml serum (Data not shown) at the end of the study. The rest of the on-site
6 control pigs had a PCV2-titer below 10^8 copies pr. ml serum. We speculate that these two pigs
7 would have developed PMWS if the study had continued. These results strongly indicate that the
8 on-site control pigs were affected by the PMWS-status of the research facility; despite they were
9 kept isolated in compartment 5. We sequenced PCV2 from 3 on-site control pigs with a high PCV2
10 titer to clarify if PCV2 was transmitted to them from the other compartments (data not shown). At
11 the start of the transmission study, PCV2 was undetectable in these pigs, but at the end of the study
12 all pigs harboured variant 3. PCV2 variant 3 was characteristic to PMWS-affected pigs in
13 compartment 2 (Fig. 2) supporting transmission of PCV2 by air or by personnel. However, the
14 mean PCV2-titer in the on-site control pigs increased more slowly than in the healthy pigs mingled
15 with pigs from PMWS-affected herds as determined by quantification of PCV2 in serum samples
16 collected after three weeks (data not shown).

17 Pigs from both PMWS-affected herds and non-affected herds that were diagnosed with PMWS had
18 a very variable PCV2 titer in serum at death ranging from 10^6 - 10^{11} copies/ml (data not shown).
19 Most often, however, the PCV2 titer in serum was above 10^7 copies/ml, the previously suggested
20 threshold for the PMWS diagnosis (Brunborg et al., 2004; Olvera et al., 2004; Segalés et al., 2005b;
21 Grau-Roma et al., 2008b). Interestingly, all pigs in this study from both PMWS-affected herds and
22 non-affected herds (except the two on-site control pigs) that were not diagnosed with PMWS had a
23 PCV2 titer below 5×10^8 copies/ml serum at all time points (data not shown). The window between
24 10^7 and 5×10^8 copies of PCV2 pr ml serum is therefore particular interesting, as it seems like the

1 pigs are wobbling between getting control over the PCV2-infection in this window or die from
2 PMWS.

3 In conclusion, this study shows that transmission of PMWS from PMWS-affected pigs to healthy
4 pigs coincide with transmission of PCV2 variants hosted by the PMWS-affected pigs. Furthermore,
5 the study confirms that PCV2/PMWS are readily transmitted from diseased to non-diseased pigs by
6 direct and indirect contact. In addition the results indicate that PCV2 is transmissible to healthy pigs
7 isolated on a farm harbouring PMWS-affected pigs. These findings may have important implications
8 for herds that experience outbreak of PMWS since very strong bio-security barriers between
9 sections are needed to avoid spread of the virus/disease.

10

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12

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16

17 **References**

18

19 Albina, E., Truong, C., Hutet, E., Blanchard, P., Cariolet, R., L'Hospitalier, R., Mahe, D., Allee, C.,
20 Morvan, H., Amenna, N., Le, D.M., Madec, F., Jestin, A., 2001. An experimental model for
21 post-weaning multisystemic wasting syndrome (PMWS) in growing piglets. *J. Comp Pathol.*
22 125, 292-303.

- 1 Bolin, S.R., Stoffregen, W.C., Nayar, G.P., Hamel, A.L., 2001. Postweaning multisystemic wasting
2 syndrome induced after experimental inoculation of cesarean-derived, colostrum-deprived
3 piglets with type 2 porcine circovirus. *J. Vet. Diagn. Invest* 13, 185-194.
- 4 Brunborg, I.M., Moldal, T., Jonassen, C.M., 2004. Quantitation of porcine circovirus type 2 isolated
5 from serum/plasma and tissue samples of healthy pigs and pigs with postweaning
6 multisystemic wasting syndrome using a TaqMan-based real-time PCR. *J. Virol. Methods*
7 122, 171-178.
- 8 Chae, C., 2004. Postweaning multisystemic wasting syndrome: a review of aetiology, diagnosis and
9 pathology. *Vet. J.* 168, 41-49.
- 10 Dupont, K., Nielsen, E.O., Bækbo P., Larsen L.E., 2007. Genomic analysis of PCV2 isolates from
11 Danish archives and a current PMWS case-control study supports a shift in genotypes with
12 time. *Vet. Microbiol.* 128, 56-64.
- 13 Grau-Roma, L., Crisci, E., Sibila, M., López-Soria, S., Nofrarias, M., Cortey, M., Fraile, L., Olvera,
14 A., Segalés, J., 2008a. A proposal on porcine circovirus type 2 (PCV2) genotype definition
15 and their relation with postweaning multisystemic wasting syndrome (PMWS) occurrence.
16 *Vet Microbiol.* 128, 23-35.
- 17 Grau-Roma, L., Hjulsgager, C.K., Sibila, M., Kristensen, C.S., López-Soria, S., Enøe, C., Casal, J.,
18 Bøtner, A., Nofrarias, M., Bille-Hansen, V., Fraile, L., Bækbo, P., Segalés, J., Larsen, L.E.,
19 2008b. Infection, excretion and seroconversion dynamics of porcine circovirus type 2
20 (PCV2) in pigs from postweaning multisystemic wasting syndrome (PMWS) affected farms
21 in Spain and Denmark. Doi:10.1016/j.vetmic.2008.10.007

- 1 Harding, J.C.S., Clark, E.G., 1997. Recognizing and diagnosing postweaning multisystemic wasting
2 syndrome (PMWS). *Swine Health and Prod.* 5, 201-203.
- 3 Hjulsager, C., Grau-Roma, L., Sibila, M., Enøe, C., Larsen, L.E., Segalés, J., 2008. Inter-laboratory
4 and inter-assay comparison on two real-time PCR techniques for quantification of PCV2
5 nucleic acid extracted from field samples. Doi:10.1016/j.vetmic.2008.06.014
- 6 Krakowka, S., Ellis, J.A., Meehan, B., Kennedy, S., McNeilly, F., Allan, G., 2000. Viral wasting
7 syndrome of swine: experimental reproduction of postweaning multisystemic wasting
8 syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine
9 parvovirus. *Vet. Pathol.* 37, 254-263.
- 10 Kristensen, C.S., Bækbo, P., Bille-Hansen, V., Bøtner, A., Vigre, H., Enøe, C., and Larsen, L.E.,
11 2009. Induction of Porcine Postweaning Multisystemic Wasting Syndrome (PMWS) in pigs
12 from PMWS unaffected herds following mingling with pigs from PMWS affected herds.
13 Submitted to *Vet. Microbiol.* Doi: 10.1016/j.vetmic.2009.04.004
- 14 Liu, Q., Wang, L., Willson, P., Babiuk, L.A., 2000. Quantitative, competitive PCR analysis of
15 porcine circovirus DNA in serum from pigs with postweaning multisystemic wasting
16 syndrome. *J. Clin. Microbiol.* 38, 3474-3477.
- 17 Magar, R., Larochele, R., Thibault, S., Lamontagne, L., 2000. Experimental transmission of
18 porcine circovirus type 2 (PCV2) in weaned pigs: a sequential study. *J. Comp Pathol.* 123,
19 258-269.
- 20 Olvera, A., Cortey, M., Segalés, J., 2007. Molecular evolution of porcine circovirus type 2
21 genomes: phylogeny and clonality. *Virology* 357, 175-185.

- 1 Olvera, A., Sibila, M., Calsamiglia, M., Segalés, J., Domingo, M., 2004. Comparison of porcine
2 circovirus type 2 load in serum quantified by a real time PCR in postweaning multisystemic
3 wasting syndrome and porcine dermatitis and nephropathy syndrome naturally affected pigs.
4 *J. Virol. Methods* 117, 75-80.
- 5 Rovira, A., Balasch, M., Segalés, J., Garcia, L., Plana-Duran, J., Rosell, C., Ellerbrok, H.,
6 Mankertz, A., Domingo, M., 2002. Experimental inoculation of conventional pigs with
7 porcine reproductive and respiratory syndrome virus and porcine circovirus 2. *J. Virol.* 76,
8 3232-3239.
- 9 Segalés J., Olvera A., Grau-Roma L., Charreyre C., Nauwynck H., Larsen L., Dupont K.,
10 McCullough K., Ellis J., Krakowka S., Mankertz A., Fredholm M., Fossum C., Timmusk S.,
11 Stockhofe-Zurwieden N., Beattie V., Armstrong D., Grassland B., Baekbo P., Allan, G.,
12 2008. PCV-2 genotype definition and nomenclature. *Vet Rec.* 162, 867-868.
- 13
- 14 Segalés, J., Allan, G.M., Domingo, M., 2005a. Porcine circovirus diseases. *Anim Health Res. Rev.*
15 6, 119-142.
- 16 Segalés, J., Calsamiglia, M., Olvera, A., Sibila, M., Badiella, L., Domingo, M., 2005b.
17 Quantification of porcine circovirus type 2 (PCV2) DNA in serum and tonsillar, nasal,
18 tracheo-bronchial, urinary and faecal swabs of pigs with and without postweaning
19 multisystemic wasting syndrome (PMWS). *Vet. Microbiol.* 111, 223-229.
- 20 Segalés, J., Domingo, M., 2002. Postweaning multisystemic wasting syndrome (PMWS) in pigs. A
21 review. *Vet. Q.* 24, 109-124.
- 22 Shibata, I., Okuda, Y., Yazawa, S., Ono, M., Sasaki, T., Itagaki, M., Nakajima, N., Okabe, Y.,
23 Hidejima, I., 2003. PCR detection of porcine circovirus type 2 DNA in whole blood, serum,

1 oropharyngeal swab, nasal swab, and feces from experimentally infected pigs and field
2 cases. J. Vet. Med. Sci. 65, 405-408.

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1 Table 1

2 Sequences of PCR and sequencing primers. Primer positions are indicated relatively to a reference
3 strain with the GenBank accession number EF565344.

4

5 Tabel 2

6 Position of base differences identified in PCV2 genomes isolated from pigs that developed PMWS
7 as compared to a reference strain with GenBank accession number EF565344. Pigs originating from
8 herds with PMWS were from herd hdE-H, whereas, pigs originating from non-affected herds were
9 from herd hd3 and hd4.

10 ¹Compartment, see Fig. 1.

11

12 Table 3

13 Position of base differences identified in PCV2 genomes isolated in this study as compared to a
14 reference strain with GenBank accession number EF565344. The PCV2 genomes were isolated
15 from serum collected before mingling of the pigs (arrival) and from lymph nodes from the same
16 pigs after they developed PMWS (termination).

17

18 Fig. 1. The research facility used in the PCV2 transmission study. The pigs used in the study were
19 collected from four PMWS affected herds (herd E-H) and two non-affected herds (herd 3 and 4).

20 Pigs from the PMWS-affected herds (+) were mingled with pigs from the non-affected herds (–) in
21 pens as shown in the figure. The number of pigs in each pen is indicated. Bars, permitting the pigs
22 to have nose-to-nose contact, separated the pens.

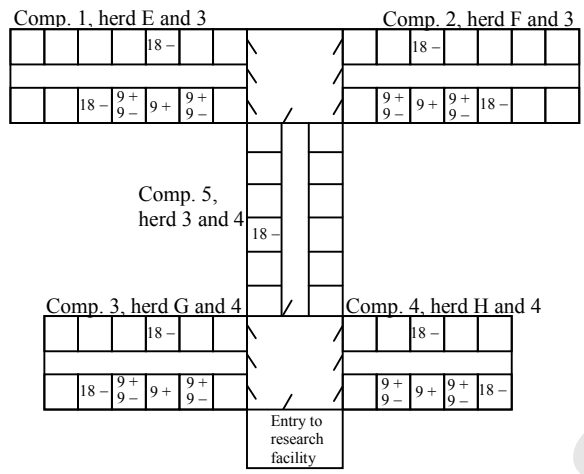
23

1 Fig. 2. Placement of pigs that developed PMWS. Closed circles indicate pigs from PMWS affected
2 herds (herd E-H). Open circles indicate pigs from non-affected herds. Each figure of the research
3 facility shows the placement of pigs containing a distinct PCV2 variant (1-8).

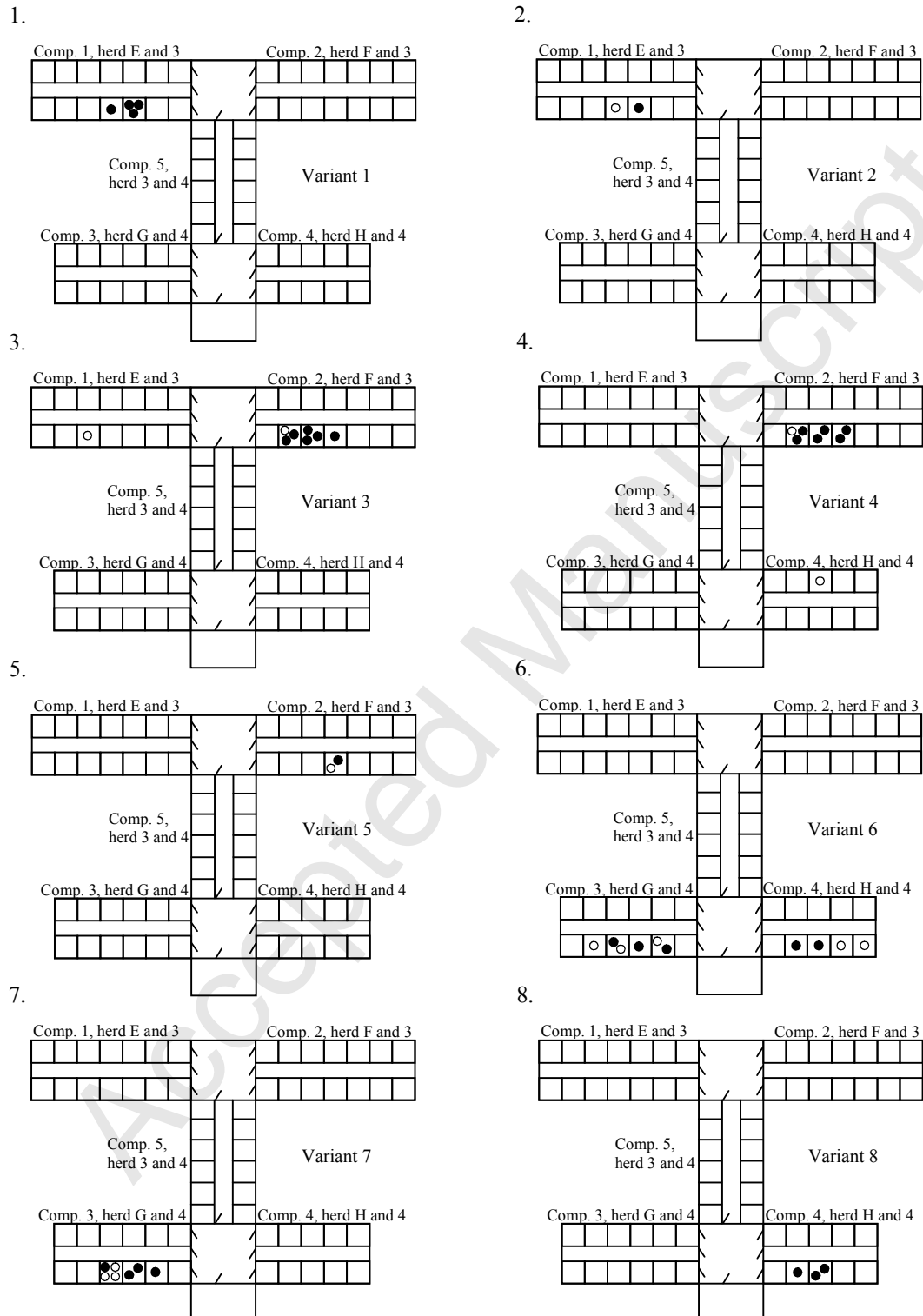
4
5 Fig. 3. PCV2 load mean and confidence interval in serum of pigs from PMWS-affected herds and
6 non-affected herds at arrival (white bars) and at death or end of study (black bars). n: number of
7 animals. The detection limit in the real-time PCR was 10^3 PCV2 DNA copies/ml serum. **A:** Pigs
8 showing clinical signs of PMWS at arrival. At the end of the study all the pigs were recovered
9 clinically. **B:** Pigs showing clinical signs of PMWS at arrival. All the pigs died early in the study
10 and were diagnosed with PMWS clinically as well as histopathologically. **C:** Healthy pigs without
11 clinical signs of PMWS at arrival. At the end of the study they still showed no clinical signs of
12 PMWS. **D:** Healthy pigs without clinical signs of PMWS at arrival. All pigs died late in the study
13 and were diagnosed with PMWS clinically as well as histopathologically. **E:** Healthy pigs without
14 clinical signs of PMWS placed as on-site controls in compartment 5 (Fig. 1). All pigs stayed
15 clinical PMWS-free during the study. **F:** Healthy pigs without clinical signs of PMWS placed as
16 off-site controls on a separate control farm. All pigs stayed clinical PMWS-free during the study.

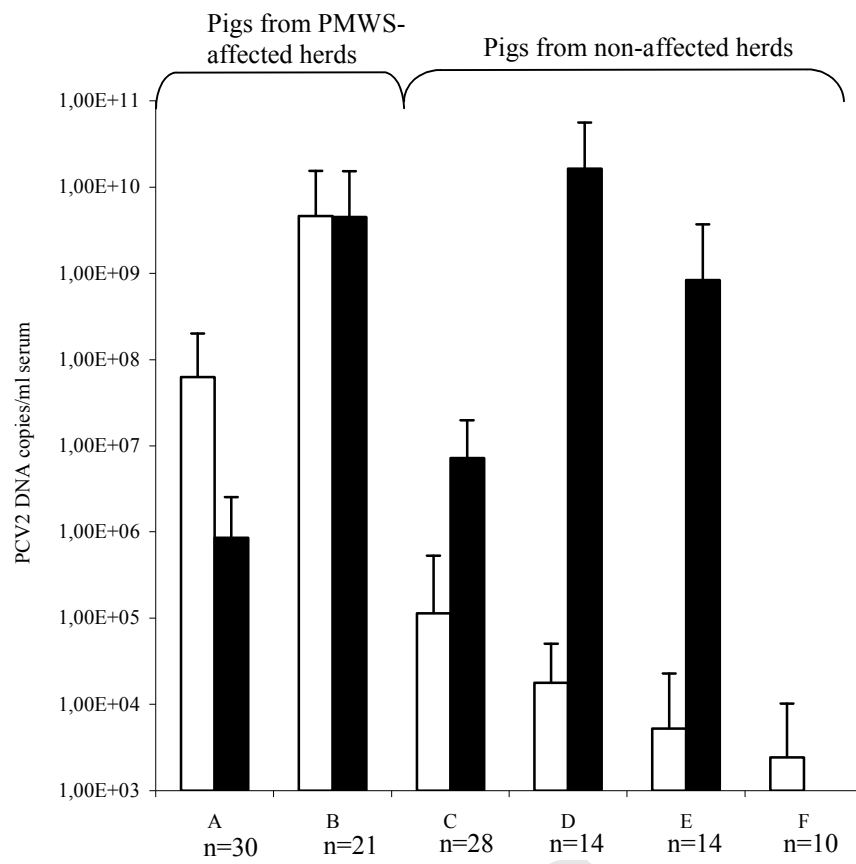
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	Position (nt)	Sequence (5'→3')	Fragment length (bp)	Reference
PCR				
PCV2-1for	121-137	CTCCGAAGACGAGCGC	791	Dupont et al., 2007
PCV2-1rev	912-890	TAGCATTCTTCCAAAATACCAAG		Dupont et al., 2007
PCV2-2for	1308-1327	GTTTACATAGGGGTCATAGG	713	Dupont et al., 2007
PCV2-2rev	253-233	TGCTTCTTCACAAAATTAGCG		Dupont et al., 2007
PCV2-3for	760-782	TAGAGACTAAAGGTGGAAGTGA	781	Dupont et al., 2007
PCV2-3rev	1541-1524	TCCTGGGCGGTGGACATG		Dupont et al., 2007
Nested PCR				
NestPCV2-1for	131-150	CGAGCGCAAGAAAATACGGG	758	This study
NestPCV2-1rev	889-867	GAAGTAATCCTCCGATAGAGAGC		This study
NestPCV2-2for	1348-1372	ACAAAGTTATCATCTAGAATAACAG	659	This study
NestPCV2-2rev	239-222	ATTAGCGAACCCCTGGAG		This study
NestPCV2-3for	780-799	GTACCTTTTTTGCCCGCAG	688	This study
NestPCV2-3rev	1468-1446	CTGTACCCTTTGAATACTACAGA		This study
Sequencing				
SeqPCV2-1for	149-171	GGATCTTCCAATATCCCTATTTG		Dupont et al., 2007
SeqPCV2-1rev	871-854	AGAGCTTCTACAGCTGGG		Dupont et al., 2007
SeqPCV2-2for	1370-1387	CAGCACTGGAGCCCACTC		Dupont et al., 2007
SeqPCV2-2rev	221-203	GTGAGGTGTTTCGTCCTTCC		Dupont et al., 2007
SeqPCV2-3for	799-819	GTATTCTGATTACCAGCAATC		Dupont et al., 2007
SeqPCV2-3rev	1451-1430	TACAGAATAAGAAAGGTTAAGG		Dupont et al., 2007

Base position	87C	104G	296C	413T	551T	919A	925C	944C	1483C	1531C	1617T
Comp1											
13hdE										T	
15hdE										T	
16hdE										T	
23hdE										T	
17hdE										T	
214hd3					C				T	T	
539hd3								T			
Comp2											
35hdF								T			
38hdF								T			
43hdF								T			
45hdF		C						T			
48hdF								T			
51hdF								T			
247hd3								T			
36hdF	T							T			
39hdF	T							T			
40hdF	T							T			
41hdF	T							T			
52hdF	T							T			
53hdF	T							T			
244hd3	T							T			
49hdF	T		T					T			
253hd3	T		T					T			
Comp3											
66hdG											
78hdG											
82hdG											
301hd4											
325hd4											
583hd4											
62hdG											
72hdG											G
73hdG											G
87hdG											G
322hd4											G
324hd4											G
327hd4	G										
Comp4											
92hdH											
119hdH											
368hd4											
595hd4											
91hdH			G					G			
100hdH			G								
106hdH			G								
381hd4	T							T			

Base position	87C	424C	436G	919A	925C	1483C	1531C
Pigs from herds with PMWS							
17arrival						T	T
17termination						T	T
35arrival				T			
35termination				T			
36arrival	T				T		
36termination	T				T		
66arrival							
66termination							
119arrival							
119termination							
Pigs from non-affected herds							
214arrival		T	C				T
214termination				C		T	T
244arrival					T		
244termination	T				T		
583arrival							T
583termination							