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

4

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16 Tables: 4

17 Figures: 3

18

19 **Abstract**

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

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

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

37

38 *Keywords:* PMWS, PCV2, epidemiology, mingling pigs

39

40 **Introduction**

41

42 Post-weaning multisystemic wasting syndrome (PMWS) is an important disease in weaned
43 pigs worldwide. PMWS was first described in Canada in 1991 as a chronic disease with
44 progressive weight loss in pigs from 4-16 weeks of age (Harding and Clark, 1997). Since
45 then, the disease has been diagnosed in many countries in North America, Europe and Asia
46 (Allan and Ellis, 2000), and in Denmark since 2000 (Hassing et al, 2002). The clinical signs
47 of PMWS comprise unthriftiness /wasting, paleness of the skin, enlarged lymph nodes and
48 occasionally jaundice, respiratory symptoms or diarrhoea (Harding and Clark, 1997; Sorden,
49 2000; Ladekjaer-Mikkelsen et al., 2002). Affected animals have lesions in lymphoid organs
50 characterized by lymphoid depletion and the presence of giant cells and inclusion bodies
51 (Allan et al., 1998; Ellis et al., 1999; Ladekjaer-Mikkelsen et al., 2002; Segales et al., 2004).
52 PCV2 has proved to be necessary but not sufficient for development of PMWS, since the
53 virus is present in both affected and unaffected pigs and herds (Allan et al., 1999; Ladekjaer-
54 Mikkelsen et al., 2002).

55 The PCV2 virus is probably transmitted between pigs by the oro-fecal and/or respiratory
56 routes (Caprioli et al.; 2006) and vertical transmission has also been documented (West et al.,
57 1999; Ladekjaer-Mikkelsen et al., 2001). The high prevalence of PCV2 in almost all herds of
58 all pig producing countries indicates that the transmission of PCV2 is very effective (Rose et
59 al., 2003; Lopez-Soria et al., 2005). In contrast, only a few studies have been performed on
60 the “transmission” of the PCV2 associated disease complexes (PCVDs), i.e., whether PMWS
61 can be “transmitted” from affected to unaffected pigs. A study performed in New Zealand
62 demonstrated disease development in healthy pigs in direct or indirect contact with PMWS
63 affected pigs when they were mingled at 4 weeks of age but not when they were mingled at

64 12 weeks of age (Jaros et al., 2006). The purpose of the present studies was to confirm if
65 PCV2 positive pigs from PMWS unaffected herds can develop PMWS following mingling
66 with pigs from PMWS-affected herds. Control groups were included to exclude the
67 possibility that transportation or mingling by itself could induce PMWS.

68

69 **Materials and methods**

70

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

77

78 *Pigs*

79 Pigs were obtained from 12 different herds (Table 2). Serological screening showed that all
80 12 herds had antibodies against PCV2. All the herds were also seropositive for *Mycoplasma*
81 *hyopneumoniae* and porcine reproductive and respiratory syndrome virus (PRRSV) according
82 to the owner's information. Herds G, H and 4 were additionally found to be infected with
83 toxigenic *Pasteurella multocida*. Vaccine against porcine parvovirus was used in sows from
84 all 12 herds and pigs in herds G, H, and 4 were vaccinated against toxigenic *Pasteurella*
85 *multocida*. None of the herds vaccinated against PCV2. Eight of the herds were PMWS-
86 affected (A, B, C, D, E, F, G, H) and four were unaffected (1, 2, 3, 4) according to the EU
87 definition (<http://www.pcvd.org>). The unaffected herds were characterized by low morbidity

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

90 The PMWS-affected herds were visited 2-4 days before the start of the studies at which time
91 27-29 weaners (aged 8-14 weeks of age) with clinical symptoms of PMWS were selected and
92 ear tagged. The same veterinarian visited the unaffected herds one week before the study
93 started to make sure that no clinical PMWS symptoms were present. The 46 pigs from the
94 unaffected herds in study I were transferred to the research facility when they were 4-5 weeks
95 old, whereas the 80 pigs in study II were 5-6 weeks old.

96

97 *Research facilities*

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

112

113 *Experimental setup*

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

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

135

136 *Serology*

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

143 Antibodies against PCV-2 were measured by an inhouse developed ELISA. The PCV2
144 antigen was produced by serial passages of a Danish field isolate from 2002 (designation
145 D/67782) of PCV2 on PK15 cells (kindly provided by Dr. G. Allan, Queens University,
146 Belfast, N. Ireland). The cell culture was treated with glucosamine (Sigma) for 20 min, as
147 previously described (Tischer et al., 1987). Virus was purified from infected cell cultures
148 following freeze-thawing and centrifugation. Precipitated virus was collected by
149 centrifugation and the pellet was resuspended by stirring for 1 h at RT with 1% Triton X-100
150 (Serva, Bie&Berntsen, Rødovre, Denmark) in PBS. After centrifugation at 14,000 rpm at
151 5°C, the supernatant was pelleted through a 25% sucrose (in PBS) cushion by
152 ultracentrifugation at 40,000 rpm 5°C o.n. The virus pellet was resuspended in 50 mM
153 Tris/HCl buffer pH 7.6. The antigen was stored at -40°C until use.

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

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

177 *Necropsy*

178 Pigs demonstrating severe clinical disease including severe wasting were euthanized during
179 the studies. At the termination of the studies, all unthrifty pigs were euthanized. All pigs from
180 PMWS unaffected herds in study I and all pigs in study II that were euthanized or died
181 spontaneously were necropsied. At necropsy, tissue samples of *lnn. inguinales*, *lnn.*
182 *mesenterica* and spleen were immediately fixed by immersion into 4% paraformaldehyde at
183 22°C for histopathological examination. Twin samples were frozen at -20°C for cryostat

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

192

193 *Statistics*

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

203

204 **Results**

205

206 *Clinical signs and pathology*

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

221

222 *Serology*

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

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

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

242

243 **Discussion**

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

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

289

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295

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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

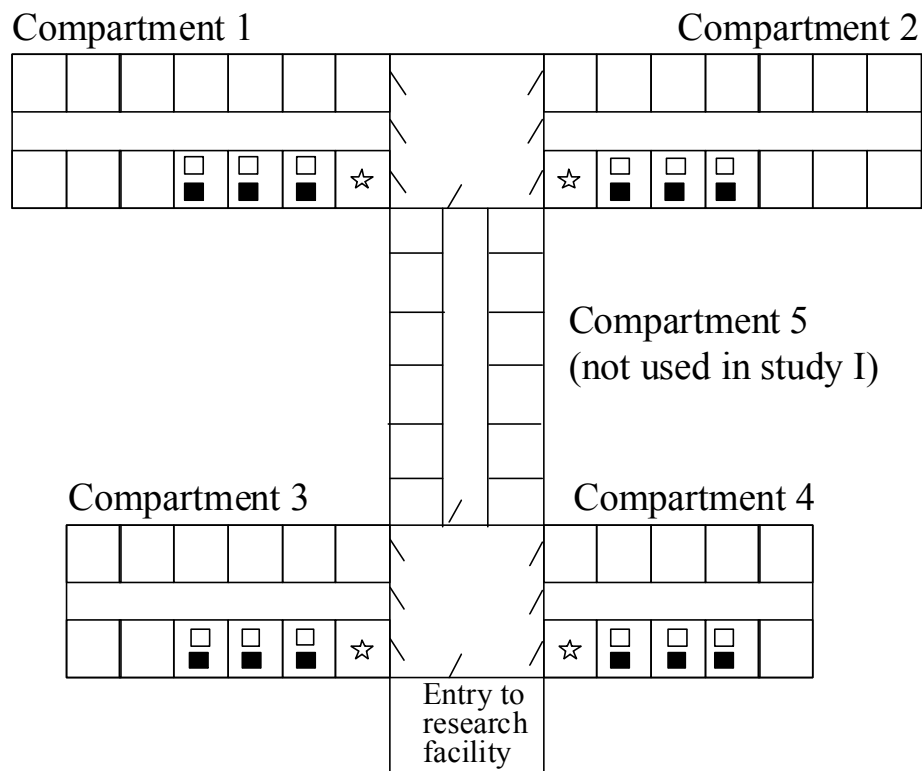
- 1 Table 4. PRRS status of PMWS-affected herds and of pigs from PMWS-nonaffected herds
 2 during study II at location I.
 3

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.



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Figure 2. The research facility, study II. Entry to compartments and change of boots and clothes (★). The pigs from the PMWS affected herds were 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 were 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 were mingled.

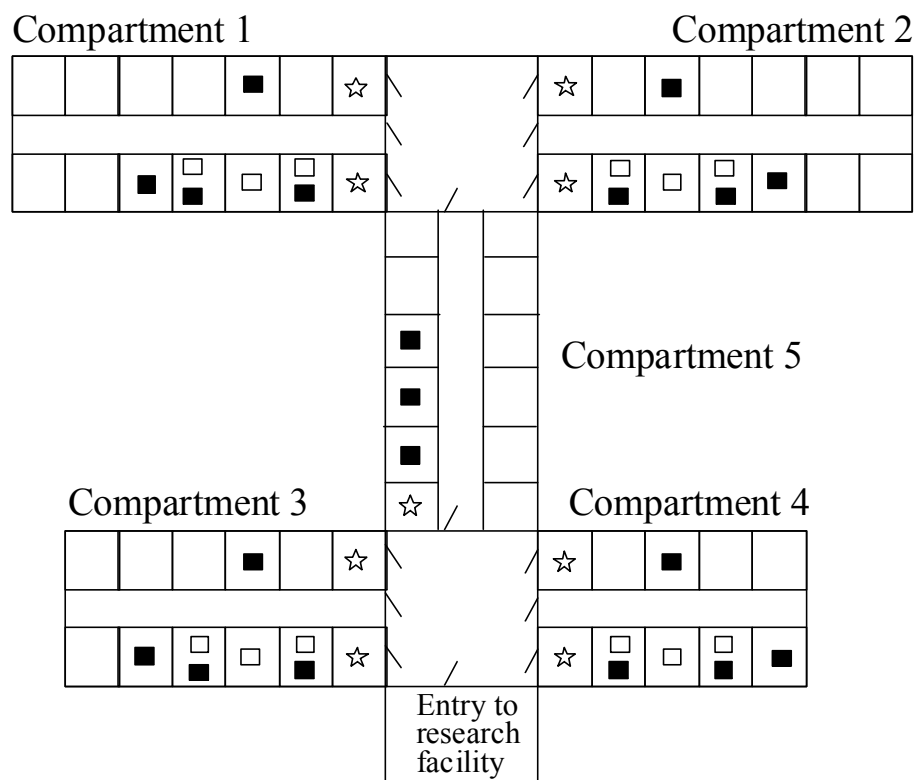


Figure 3.

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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