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1 **A study on the severity and relevance of porcine circovirus type 2 infections**  
2 **in Dutch fattening pigs with respiratory diseases.**

3

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15

16

17 Running head: Role PCV2 in porcine respiratory diseases

18 **ABSTRACT**

19 This study was set up to get more insights in the severity and relevance of porcine circovirus type 2  
20 (PCV2) infections in Dutch fattening farms in an endemic PCV2-situation with no clinical signs of  
21 post-weaning multisystemic wasting syndrome (PMWS). In part A of the study, in total 29  
22 commercial fattening farms with varying percentages of pneumonia and pleurisy at slaughter were  
23 examined. Blood samples were collected at random by cross-sectional sampling; 10 in the age of 10-  
24 12 weeks, 10 at the age of 16 weeks and 10 blood samples at the end of the finishing period (20 - 22  
25 weeks of age). Serum samples were examined for the presence of PCV2 IgM and IgG antibodies and  
26 for antibodies against other porcine lung pathogens. In part B, **8 “high”- and 8 “low”** herds were  
27 selected. **The 8 “high” herds were defined as herds having high percentages of lung lesions**  
28 **(pneumonia) at slaughter, and the 8 “low” herds had low percentages of pneumonia at slaughter.**  
29 For **both the “high” and “low” herds**, 3 pigs showing signs of respiratory distress were selected for  
30 necropsy (n=48). Lung tissue samples were examined post-mortem for macroscopic and  
31 histopathological lesions, and for the presence of bacteria and viruses. The results of part A showed  
32 that, pigs at 16 weeks of age with IgM antibodies against PCV2 had a lower probability of having  
33 pleuritis at slaughter (OR 0.34, P<0.000). Pigs in the age category of 20 – 22 weeks, and with IgM  
34 antibodies against PCV2, also had a lower probability of having pneumonia at slaughter (OR 0.29,  
35 P=0.032). In part B lobus apicalis pneumonia, PCV2 in macroscopically unaffected lungs, *Pasteurella*  
36 *multocida*, *M. hyopneumoniae*, and swine influenza viruses were all found significantly more often in  
37 **“high” than in “low” pigs** at autopsy. High PCV2 DNA loads (>10<sup>4</sup> PCV2 DNA copies/mg) were  
38 found in lungs of 14 (58%) **“high”, and in 7 (29%) of the “low” pigs** (P=0.13). In 11 of the 19  
39 affected lungs from **“high”** pigs, high PCV2 DNA loads were found in combination with one or more  
40 other lung pathogens, while this was found only in 5 of the 17 affected lungs from **“low” pigs**  
41 (P=0.02). This study confirms the hypothesis that PCV2 plays a role in pneumonia and pleurisy in 10 -  
42 24 weeks old fattening pigs, not only in herds with a high prevalence of PMWS, but also in herds with  
43 no clinical signs of PMWS.

44

45 Keywords: Porcine circovirus type 2, respiratory disease, lungs, swine

46 **1. INTRODUCTION**

47 Porcine circovirus type-2 associated diseases (PCVAD) are an important concern to swine producers  
48 around the world. In addition to post-weaning multisystemic wasting syndrome (PMWS), porcine  
49 circovirus type 2 (PCV2) has also been associated with porcine dermatitis and nephropathy syndrome  
50 (PDNS), porcine respiratory disease complex (PRDC) and proliferative and necrotizing pneumonia  
51 (PNP) (Allan et al., 1999; Opriessnig et al., 2004; Wellenberg et al., 2004; Opriessnig et al., 2006;  
52 Grau-Roma and Segalés, 2007; Opriessnig et al., 2007). In the US, PCV2 associated pneumonia was  
53 the most common presentation of PCV2 associated diseases (Opriessnig et al., 2007).

54 PCV2 has often been associated with lung lesions in pigs co-infected with PRRSV, swine influenza  
55 viruses (SIV) or *Mycoplasma hyopneumoniae*. These agents appeared to create a synergistic  
56 environment that resulted in increased amounts of PCV2 and unusual severe respiratory disease  
57 outbreaks. However, there have also been reports on uncomplicated PCV2 pneumonia cases in  
58 western Canada (Opriessnig et al., 2007). These lesions included necrotizing and ulcerative  
59 bronchiolitis, granulomatous inflammation in the alveolar septa and mixed inflammation and  
60 fibroplasias in the lamina propria and peribronchiolar areas.

61 More insight is warranted on the role of PCV2 as etiological agent in porcine respiratory diseases such  
62 as PRDC and PNP in endemic situations. In addition, little or no information is available about  
63 whether PCV2 can be associated with pneumonia in pigs from Dutch swine herds, as described for US  
64 and western Canada.

65 The purpose of this study was to get more insight in the severity and relevance of PCV2 infections in  
66 Dutch pigs with symptoms of respiratory distress in an endemic situation (situation with no or low  
67 prevalence of PMWS: **Wellenberg and Segalés, 2006**). In studies on the association between PCV2  
68 infections and respiratory disease, the role of other concurrent co-infections can not be excluded and  
69 therefore, this study was not only focused on the detection of PCV2 infections, but also on the  
70 presence of other infectious agents associated to respiratory diseases in swine.

71 The results of this study may be used to gather more evidence on the hypothesis that PCV2 plays a  
72 role as etiological agent in respiratory diseases such as PRDC and PNP.

73

## 74 2. MATERIALS EN METHODS

75 This study was divided in two parts: A and B. In part A, blood samples were examined for the  
76 presence of antibodies directed against pathogens associated with porcine lung lesions. In part B, pigs  
77 from 8 herds, with high percentages of lung lesions (pneumonia) at slaughter (“**high**”), and pigs from  
78 8 herds, having low percentages of pneumonia at slaughter, **were examined. These pigs, from both**  
79 **groups of herds, were selected on showings signs of respiratory distress, and further examined**  
80 **post mortem.**

81

### 82 2.1 Part A

83

#### 84 2.1.1 Herds

85 In total, 29 commercial fattening farms, with no signs of PMWS and with varying percentages of  
86 pneumonia and pleurisy at slaughter, were involved in this part of the study. **The farms were**  
87 **recruited all over The Netherlands, and not from certain districts. In the selection of herds we**  
88 **did not take into consideration management systems, herd size, hygiene, breeding lines, feed and**  
89 **medications.** For each herd, the following technical data of 2006 were recorded (mean values); the  
90 number of slaughtered pigs, growth rate (grams/day), feed conversion (feed to gain; kg/kg), mortality  
91 percentage, percentage of slaughter pigs showing macroscopic lung lesions and pleuritis at slaughter.

92 In addition, the “2006 over-all slaughterhouse percentage” on recorded pneumonia and pleuritis in  
93 slaughtered pigs were recorded for each slaughterhouse. The relative pneumonia and pleuritis scores  
94 of each herd were calculated by dividing the percentages of pneumonia and pleuritis found at slaughter  
95 with the “2006 overall slaughterhouse percentage” of the corresponding slaughterhouse. **This was**  
96 **done to correct for the “slaughterhouse effect” during our statistical calculations, as differences**  
97 **between slaughterhouses in determining and registering abnormalities in lungs and pleura were**  
98 **considerable.**

99

#### 100 2.1.2 Animals and blood samples (cross-sectional)

101 Blood samples (n=30, except for one herd n=18 and another herd n=20) were collected **cross-**  
102 **sectionally** from slaughter pigs of the 29 herds. In general, 10 blood samples were collected from pigs  
103 in the age of 10 - 12 weeks (0 - 2 weeks after entering the fattening unit), 10 blood samples from pigs  
104 at the age of 16 weeks and 10 blood samples from pigs at the end of the finishing period (between 20 -  
105 22 weeks of age). **Blood samples were taken at random, so without any prior selection based on**  
106 **clinical signs. Pigs were not vaccinated against swine influenza virus (SIV), *Mycoplasma***  
107 ***hyopneumoniae*, *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis*. Three herds used**  
108 **PRRS-MLV vaccination.**

109

110 Blood samples were sent to the laboratory and serum samples were examined using routine  
111 procedures. All sera (in total n=858) were examined for the presence of antibodies directed against  
112 PCV2 IgM and IgG antibodies (Ingezim PCV-ELISA, Madrid, Spain), to determine whether the PCV2  
113 infection was in an acute or chronic phase. Serum samples were tested also for antibodies against  
114 porcine reproductive and respiratory syndrome viruses (PRRSV; by Idexx HerdChek PRRS 2XR  
115 ELISA, Westbrook Maine, USA), *Mycoplasma hyopneumoniae* (HerdChek M hyo, Idexx, Westbrook  
116 Maine, USA), swine influenza viruses (SIV)-H1N1, -H1N2 and -H3N2 by haemagglutination  
117 inhibition (HI) tests, and *Actinobacillus pleuropneumoniae* (Chekit-App-ApxIV ELISA, Idexx,  
118 Westbrook Maine, USA). **Antibody tests were performed according to the descriptions of the**  
119 **manufacturer (Idexx, Westbrook Maine, USA). In 3 herds, using a PRRS-MLV vaccine, serum**  
120 **samples with S/P ratio <2.0 were considered to be negative for infection with field strains of PRRSV.**

121

122

## 123 **2.2 Part B**

124

### 125 **2.2.1 Herds and animals**

126 Based on the results from part A, 8 “high” and 8 “low” herds were selected. **The 8 “high” herds were**  
127 **defined as the herds, described in part A, having high percentages of lung lesions (pneumonia) at**  
128 **slaughter, and the 8 “low” herds had low percentages of pneumonia at slaughter.**

129 **For both the “high” and “low” herds**, 3 pigs showing signs of respiratory distress (e.g. dyspnoea)  
130 were selected, **and further examined after necropsy**. In situations with no clear respiratory distress,  
131 16 weeks old pigs were selected. All pigs (n=48) were selected by one and the same veterinarian.  
132 During the visits, the same veterinarian recorded age and clinical signs of each selected pig. None of  
133 the pigs was treated with antimicrobials within one week prior to necropsy.

134

### 135 **2.2.2 Necropsy and examinations for lung pathogens**

136 At necropsy, all euthanized pigs were examined for macroscopic lesions, in particular lung lesions.  
137 Lung tissue samples from “high” and “low” pigs were microscopically examined for histopathological  
138 lesions using a haematoxylin-eosin staining, and for the presence of bacteria and viruses including  
139 PCV2 viral DNA load. From each affected lung, one sample was taken from the transition area  
140 between macroscopically affected (with lesions) and unaffected tissue. A second sample, or one  
141 sample in case of absence of macroscopic lesions, was taken from each animal from a macroscopically  
142 unaffected area of the lung. For each pig, the same area of the lung was selected (the cranial ventral  
143 part of the same selected lobe).

144 Virological and bacteriological examinations were performed on all examined lungs. The following  
145 techniques were performed for viral examinations; a quantitative real-time PCR for PCV2, real-time  
146 PCR tests for EU- and US-type PRRSV, and the influenza-A viruses H1N1, H1N2 and H3N2  
147 (Wellenberg et al., 2004; Van Maanen et al., 2006). The amount of PCV2 DNA was examined in  
148 each lung at two different areas (see above). This was done to determine the difference in PCV2 viral  
149 DNA load in affected and unaffected parts of lungs within the same “high” or “low” pig.

150 Lung samples were examined for the presence of *Actinobacillus pleuropneumoniae*, *Pasteurella*  
151 *multocida*, *Haemophilus parasuis* and *Streptococcus spp* by cultivation techniques, and for  
152 *Mycoplasma hyopneumoniae* by quantitative real-time PCR (Dubosson et al., 2004).

153

### 154 **2.3 Statistical analyses**

155 **Serological data from part A** were subjected to generalized ordinal regression analysis in Stata  
156 **version 10 using the program Gologit2 (Williams, 2006)** Outcome variables are respectively the

157 percentage of affected lungs, and the percentage of affected pleura, both subdivided in three  
 158 categories. The next model is used:

$$159 \quad \log it(p(Y_i > k)) = \alpha_k + \sum_{i=1}^N X_i \cdot \beta_{ik} \quad k = 1,2$$

160 where:  $Y_i$  : outcome variable, percentage affected lungs or percentage affected pleura

161  $\alpha_k$ : boundary between category k and category k+1

162  $X_i$ : explanatory variables

163  $\beta_{ik}$ : odds-ratios for transition from category k to category k+1

164

165 The model is a combination of the ordered logistic regression model (ologit, Stata 10) and the  
 166 multinomial logistic regression model (mlogit, Stata 10). If possible ordered logistic model is  
 167 used, assuming  $\beta_{i1} = \beta_{i2}$ . If this assumption is invalid the multinomial model is used, implicating  
 168 that for some i  $\beta_{i1} \neq \beta_{i2}$

169 The explanatory variables IgM and IgG antibodies against PCV2 as well as the relative  
 170 percentage of affected lungs and affected pleura at the slaughterhouse are forced into the model.  
 171 For all other explanatory variables a backwards elimination procedure was used, with a P-value  
 172 of 0.05. The hierarchical nature of the problem is addressed by correcting the standard errors of  
 173 all estimated odds ratios for within herd correlation.

174

175 For part B first a univariate analysis was done. The proportions test (using Stata version 10, pr-  
 176 test) was used to look for significant differences between high herds and low herds concerning  
 177 the macroscopic and histopathological findings, including data from bacteriological and  
 178 virological examinations (n=48). A P-value of <0.05 was chosen to indicate a significant  
 179 difference. In a second stage a multivariate analysis was done using the logistic model

$$180 \quad \log it(p(\text{high})) = \beta_0 + \sum_{i=1}^N X_i \cdot \beta_i$$

181 where: p(high) : probability to be a high herd

182  $X_i$ : explanatory test results

183  $\beta_i$ : odds ratio

184 Again, a backwards elimination procedure was used, with a P-value of 0.05.

185

### 186 3. RESULTS

187

#### 188 3.1 Part A

189 The overall technical data are presented in Table 1. Data from pigs from 29 herds were collected but  
190 three herds were excluded in the data presented in Table 1. Their data sets were not complete (the  
191 slaughterhouse percentages of 2006 were not available).

192 The mean number of slaughtered pigs per herd in 2006 was 1651 and this number varied between  
193 herds from 669 to 7600. The recorded lung lesions and pleuritis in slaughtered pigs varied per herd  
194 between, 0.1 – 17.7% and 0.6 – 35.8%, respectively. The relative lung lesion and pleuritis cases per  
195 herd varied between 4 – 495%, and 5 – 284% (corrected for the “2006 slaughterhouses percentages”).

196

#### 197 Table 1

198

##### 199 3.1.1 Serological data part A

200 Table 2 presents the overall serological data. It shows that, in all 29 herds, antibodies were detected  
201 against PCV2, SIV-H1N1, SIV-H3N2 and *A. pleuropneumoniae*. *A. pleuropneumoniae* and SIV  
202 antibodies in 10-12 week old pigs were considered to be maternal antibodies.

203 PCV2-IgM antibodies were already detected in pigs from 4 herds around the age of 10 - 12 weeks, but  
204 more often in pigs around the age 16 weeks; namely in pigs from 23 (79%) of the 29 examined herds.

205 In 24% of the examined herds, PCV2-IgM was detected in 20 - 22 week old pigs, while PCV2-IgG  
206 was detected in 20 – 22 week old pigs from all herds. PRRSV antibodies were detected in 25 (86%) of  
207 the herds.

208

#### 209 Table 2

210

211 **3.1.2 Statistical analyses of the data from part A**

212 In total, data from 26 herds were used for statistical analyses. From 3 herds the slaughterhouse  
213 percentages of 2006 were not available. Three categories were made for both the outcome variables; a)  
214 the percentage of affected lungs at the slaughterhouse, and b) the percentage of affected pleura at the  
215 slaughterhouse. **The distribution of the three categories and the respective percentage affected**  
216 **lungs and pleura over the three categories are represented in Table 3 for affected lungs and for**  
217 **affected pleura.** The categories were defined in such a way that three categories of approximately the  
218 same number of observations were formed.

219 The dataset is furthermore subdivided in data from young finishers (10 – 12 weeks), middle aged  
220 finishers (16 weeks) and old finishers (20 - 22 weeks). Taken together, this means that for each of the  
221 3 age groups statistical analyses were performed for each of the two outcome variables, so six  
222 analysis. In every part of the analysis e.g. the variables IgG and IgM antibodies against PCV2 and the  
223 slaughterhouse reference percentages for either lungs or pleura were fixed in the statistical models.

224 The following variables were inserted in the statistical models; growth rates (in grams/day), feed  
225 conversion, percentage dead loss, IgG and IgM antibodies against PCV2, antibodies against *A.*  
226 *pleuropneumoniae*, *Mycoplasma hyopneumoniae*, PRRSV (ELISA S/P-ratios), H1N1, H1N2, and  
227 H3N2 antibody titres, and SIV seroconversion on herd level (seroconversion was defined as an  
228 increase of at least 2 titre steps; 0=no seroconversion, 1=seroconversion between 10 - 12 and 16  
229 weeks, or between 16 and 20 - 22 weeks, 2=seroconversion between 10 - 12 and 16 weeks and  
230 between 16 and 20 - 22 weeks as well).

231

232 **Table 3**

233

234 Results of the statistical analysis of Part A (Stata 10, Gologit2 procedure) showed that pigs at 16  
235 weeks of age with IgM antibodies against PCV2 had a lower probability of having pleuritis at  
236 slaughter (OR 0.34,  $P < 0.000$ ). Pigs, in the age category of 20 - 22 weeks, with IgM antibodies against  
237 PCV2 also had a lower probability of having pneumonia at slaughter (OR 0.29,  $P = 0.032$ ). Young  
238 finishers (pigs of 10 - 12 weeks) with an antibody titre against SIV-H1N1 showed a lower probability

239 of having pleuritis at slaughter (OR 0.39, P=0.033). At herd level, pigs of 16 weeks of age with a  
240 seroconversion for SIV-H1N1 showed a higher probability of pleuritis (OR 1.50, P=0.017). Old  
241 finishers (pigs of 20 – 22 weeks) with antibodies against *A. pleuropneumoniae* had a higher  
242 probability of pleuritis at slaughter (OR 5.10, P=0.015). Growth in all age categories (grams/day) was  
243 negatively correlated to pleuritis (10 - 12 weeks old pigs: OR 0.98, P=0.004; 16 week old pigs: OR  
244 0.97, P<0.000; and 20 – 22 weeks old pigs: OR 0.96, P<0.000). **Figure 1 shows the box-and-**  
245 **whisker-plot of growth per day for the three pleuritis categories. The difference in growth**  
246 **between pleuritis category 1 and 2 was 56 grams per day more for category 1 (category 1 had a**  
247 **mean of 805 and category 2 had a mean of 749 grams/day). This is a significant difference**  
248 **(Statistix for Windows, version 8.0, Two sample T Test, P=0.0054).**

249 **Pigs in the age of 10 – 12 weeks and 16 weeks with a higher percentage dead loss at herd level**  
250 **had a higher probability of having pneumonia at slaughter: OR 3.27, P=0.009 and OR 2.85,**  
251 **P=0.010, respectively. Figure 2 presents the percentage dead loss for each of the three categories**  
252 **of percentage affected lungs at slaughter. Especially the highest lung scores (category 3) were**  
253 **correlated to an increase in percentage dead loss.**

254

255 **Figure 1, Figure 2.**

256

257 **3.2 Part B**

258 The 8 “high” herds had high percentages of pigs with lung lesions (pneumonia) at slaughter (**mean of**  
259 **the relative percentages of pigs with lung lesions was 157%; range 130% – 189%), and for the 8**  
260 **“low” herds, the mean of the relative percentages of pigs with lung lesions at slaughter was 48%**  
261 **(range 14% - 74%).**

262

263 **3.2.1 Post-mortem examinations**

264 **The mean age of “high”- and “low” pigs with respiratory distress at necropsy was 18.0 and 17.3**  
265 **weeks, respectively (range between 13 and 24 weeks). Macroscopic lesions (pneumonia, catarrhal**  
266 **pneumonia) and/or histopathological lung lesions (interstitial pneumonia, chronic forms of pneumonia**

267 and pleuritis, pleuropneumoniae, necrotizing and ulcerative pneumonia) were found in pigs (n=19)  
268 from “high” herds, and in pigs (n=17) from “low” herds as well (Table 4). In Table 4, for each “high”  
269 and “low” pig, the macroscopic and histopathological lesions, PCV2 DNA loads in macroscopic  
270 affected and unaffected lungs, SIV/PRRSV, pathogenic bacteria (*Arcanobacterium pyogenes*,  
271 *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica*, *Pasteurella multocida*, *Haemophilus*  
272 *parasuis*, *M. pleuropneumoniae*), and *Streptococcus spp* / *E. coli* detection in lungs are presented.

273

#### 274 **Table 4**

275

#### 276 **3.2.2 Statistical analyses of the data from part B**

277 Results of the macroscopic and histopathological examinations, and the detection of the different lung  
278 pathogens, were analysed **statistically per animal (Table 5)**. The presented results in Table 5 are the  
279 proportions of positive animals for “high” and “low”, the P-values for the difference in proportion  
280 between those groups and whether the corresponding variable was found more or less often in pigs  
281 from “high” herds compared to pigs from “low” herds. The following variables were found  
282 significantly more often in “high” than in “low” pigs: lobus apicalis pneumonia, presence of PCV2 in  
283 macroscopically unaffected lungs, presence of SIV, presence of *Pasteurella multocida* and presence of  
284 *M. hyopneumoniae*. In addition, high PCV2 DNA loads ( $>10^4$  PCV2. DNA copies/mg) were found in  
285 lungs of 14 (58%) “high”, and in 7 (29%) of the “low” pigs (P=0.13).

286 The mean PCV2 DNA copy load in macroscopically affected lungs from “high” pigs was also higher  
287 than the mean PCV2 DNA copy load in macroscopically affected lungs from “low” pigs, **although**  
288 **this difference for macroscopically affected lungs was not significant**. The differences in the mean  
289  $\log_{10}$  PCV2 DNA copies/mg values between “high” and “low” pigs was 1.6 for the macroscopically  
290 affected parts of the lungs and 2,0 for the macroscopically unaffected parts of the lungs.

291 Interstitial pneumonia, *Streptococcus spp* and a-pathogenic bacteria were found significantly more  
292 often in “low” pigs.

293 **After the multivariate analysis on data of all 16 herds (8 “high” and 8 “low” herds) two**  
294 **variables remained, namely PCV2 from unaffected lungs with an Odds ratio of 8.7 (95%**

295 confidence interval; 1.2 - 63.3, and P-value 0.033), and interstitial pneumonia with an Odds ratio  
296 of 0.10 (95% confidence interval; 0.015 – 0.665, and P-value 0.017).

297

### 298 3.2.3 PCV2 and co-infections

299 In one “low” pig with pneumonia (no 9224), the PCV2 DNA load in affected lungs was high ( $>10^6$   
300 copies/mg) while no other viral or bacterial pathogens were detected. In all other lungs with  
301 macroscopic and/or histopathological lesions from “high” and “low” pigs where PCV2 was detected,  
302 PCV2 was found in combination with other lung pathogens, such as *Mycoplasma hyopneumoniae*, *P.*  
303 *multocida*, *A. pleuropneumoniae*, SIV or PRRSV.

304 In 11 of the 19 affected lungs (with macroscopic or histopathological lesions) from “high” pigs, high  
305 PCV2 DNA loads of  $>10^4$  DNA copies/mg were found in combination with one or more other lung  
306 pathogens, while this was found only in 5 of the 17 affected lungs from “low” pigs (P=0.02) (Table 4).

307

308 **Table 5**

309

310 **4. DISCUSSION**

311 In Europe, the economic losses in swine industry related to respiratory diseases are very high. PRRSV,  
312 SIV, *Mycoplasma hyopneumoniae*, *Pasteurella multocida* and *Actinobacillus pleuropneumoniae*, are  
313 all known contributors to this disease complex. PCV2 also has been found in association with porcine  
314 respiratory diseases, but its role as aetiological agent is still not clear (Allan et al., 1999; Opriessnig et  
315 al., 2004; Grau-Roma and Segalés, 2007; Opriessnig et al., 2007). More insights are warranted on the  
316 role of PCV2 as etiological agent in porcine respiratory diseases, especially in herds without clinical  
317 signs of PMWS. In PMWS cases the load of PCV2 in lung tissue can be very high (Segalés et al.,  
318 1997; Segalés and Domingo, 2002; Chae, 2004), but in many European countries the number of  
319 PMWS cases has been decreasing within the last 5 years (Wellenberg and Segalés, 2006).

320 In part A of this study, the PCV2 IgM data show interesting results. These data show that the detection  
321 of IgM antibodies against PCV2 in pigs of 16 and 20 – 22 weeks of age correlates with reduced  
322 percentages of pleuritis or lung lesions at slaughter. This indicates that fattening pigs older than 16  
323 weeks are probably more capable in coping with the effects of PCV2 infections than piglets in the age  
324 of 10 - 12 weeks. PCV2 infections in young fattening pigs are probably associated with an increased  
325 probability of finding lung lesions and pleuritis at slaughter.

326 The serological data on *A. pleuropneumoniae* in 20 – 22 weeks old pigs were associated with pleuritis  
327 scores at slaughter (OR 5, P=0.015). It is well known that *A. pleuropneumoniae* is able to cause  
328 pleuropneumoniae in fattening pigs.

329 Finding antibodies against SIV-type H1N1 in 10 – 12 weeks old pigs reduces the probability of  
330 pleuritis at slaughter (OR 0.39, P=0.033). These antibodies, at the age of 10 – 12 weeks, are probably  
331 maternal antibodies that protect young fattening pigs against H1N1 infections.

332 Growth, feed conversion and percentage dead loss are not explanatory variables for the percentage  
333 lung lesions or pleuritis at slaughter themselves, but they are the result of (amongst others) the disease  
334 status of a herd. As such, they may reflect the disease situation in the herds. Daily weight gain  
335 (growth), which is an important factor in the economic viability of the pig industry, was significantly  
336 reduced in herds with medium or high levels of pleuritis at slaughter. Differences in daily weight gain  
337 were up to 56 grams / animal / day between the highest and the lowest category affected pleura.

338 The fact that feed conversion was not found to be significantly related to pleuritis at slaughter can  
339 probably be explained by the fact that these sick pigs have reduced appetite, resulting in less growth  
340 but not in a significant change in feed conversion. In addition, for young and middle aged fattening  
341 pigs, the percentage dead loss was also found to increase the probability of finding lung lesions at  
342 slaughter.

343 A reduction in growth and a higher mortality rate in herds with high percentages (>10%) of pleuritis at  
344 slaughter, represent in the current Dutch situation a reduction in economic value of more than € 4.- per  
345 produced finisher. Four Euro is currently a substantial part of the net profit per raised pig

346

347 In part B of this study, we selected pigs with respiratory distress **from both the “high” and the “low”**  
348 **herds. Due to this selection, there was no statistical difference in the number of pigs with**  
349 **pathological lung lesions between “high” (n=19) and “low” herds (n=17). This indicates that the**  
350 **selection of affected pigs, based on signs of respiratory distress, in “high” and “low” herds was**  
351 **done properly.** We found lobus apicalis pneumonia, PCV2 in macroscopically unaffected lungs,  
352 *Pasteurella multocida*, *M. hyopneumoniae* and swine influenza viruses significantly more often in  
353 **lungs from pigs of herds with high percentages of pneumonia** at slaughter age than in herds with  
354 low percentages.

355 The assumption that PCV2 infected pigs are more susceptible to secondary bacterial infections (Dorr  
356 et al., 2007), is supported by the fact that in affected lungs (with macroscopic or histopathological  
357 lesions) high PCV2 DNA loads of  $>10^4$  DNA copies/mg were found often in combination with one or  
358 more other lung pathogens such as *Pasteurella multocida*, *Mycoplasma hyopneumoniae*, and swine  
359 influenza viruses. Pathogens like PRRSV, swine influenza viruses, and *Mycoplasma hyopneumoniae*  
360 have been associated with the porcine respiratory disease complex (PRDC). PRDC is a complex  
361 disease in which PCV2 was suggested to play an important role too (Kim et al., 2003; Harding, 2004;  
362 Opriessnig et al., 2007). In recent studies with pigs in Korea and the United States, co-infections of  
363 PCV2 with one or more pathogens were detected in 85% and 98.1% of the examined pigs (Kim et al.,  
364 2003; Opriessnig et al., 2007). They also concluded that co-infections with other viral and bacterial  
365 pathogens severely worsened the clinical outcome of porcine circovirus type-2 (associated) diseases.

366 The serology data of part A on *A. pleuropneumoniae* are not confirmed by the results from part B, the  
367 post-mortem examinations of pigs from “high” and “low” herds. **The difference in results between**  
368 **these two parts of this study on *A. pleuropneumoniae* can possibly be explained that for both the**  
369 **“high” and “low” herds clinically affected animals were selected for post-mortem examination,**  
370 **and the fact that an *A. pleuropneumoniae* infection gives clear clinical signs.** In the acute phase of  
371 respiratory distress, the number of *A. pleuropneumoniae* infected “high” pigs was not higher than the  
372 number of *A. pleuropneumoniae* infected “low” pigs (P=0.68).

373 In conclusion, the fact that PCV2-IgM in 16 week and 20 – 22 week old pigs was associated with a  
374 reduced probability of pneumonia and pleuritis at slaughter, and that high PCV2 loads in combination  
375 with *Pasteurella multocida*, *Mycoplasma hyopneumoniae*, and swine influenza were found more often  
376 in affected lungs from pigs of herds with high percentages of pneumonia and pleurisy at slaughter,  
377 support the hypothesis that PCV2 plays a role in lung lesions and pleuritis in fattening pigs, not only in  
378 herds with high percentages of PMWS, but also in herds with no or low percentages of PMWS cases.  
379

380 **5. REFERENCES**

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448 **Table 1:** Technical data of the farms in 2006 (n=26)

	<b>Mean value</b>	<b>Variation</b>
Number of slaughtered pigs	1651	669 - 7600
Daily weight gain	772	689 - 855
Mortality rate (%)	2.6	0.9 – 5.9
Relative lung score	124%	4 – 495%
Relative pleuritis score	96%	5 – 284%

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451 **Table 2:** Percentages of farms and pigs (between brackets) with antibodies at the three different  
 452 moments of sampling (**n= 29 herds and 818 sera**).

Agent	Age of the pigs		
	10-12 weeks	16 weeks	20-22 weeks
PCV2 IgM	14% (4%)	79% (36%)	24% (11%)
PCV2 IgG	31% (6%)	83% (55%)	100% (93%)
PRRSV	69% (29%)	72% (52%)	86% (72%)
SIV H1N1	97% (23%)	86% (34%)	93% (65%)
SIV H1N2	76% (14%)	69% (22%)	79% (43%)
SIV H3N2	100% (47%)	100% (34%)	100% (64%)
<i>M. hyopneumoniae</i>	7% (1%)	31% (7%)	52% (25%)
<i>A. pleuropneumoniae</i>	96% (44%)	59% (27%)	72% (42%)

453

454

455 **Table 3: Descriptive statistics of the three categories with the percentages of affected lungs and**  
 456 **pleura at slaughterhouse.**

	Number of observations (n = 752)	Mean (%)	Standard deviation (SD)	Minimum (%)	Maximum (%)
Category "Lung"					
1 (low)	209	2.0	1.2	0.1	3.5
2 (medium)	268	6.2	1.8	4.0	9.0
3 (high)	275	13.9	2.8	10.1	17.7
Category "Pleura"					
1 (low)	240	2.2	1.1	0.6	3.7
2 (medium)	255	13.3	6.2	4.1	20.9
3 (high)	257	27.2	4.6	21.5	35.8

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458

459 **Table 4:** Macroscopic and histopathological lesions, PCV2 DNA loads in macroscopic affected and  
 460 unaffected lungs, SIV/PRRSV pathogenic bacteria and *Streptococcus spp* / *E. coli* detection in lungs  
 461 of “high” and “low” pigs.

“high” herd	Animal ID	Macroscopic lesions	Histopathological lesions	PCV2 DNA copies/mg (log10)		SIV and/or PRRSV	Pathogenic bacteria <i>Streptococcus spp</i> and/or <i>E.coli</i>
				affected lung	unaffected lung		
3	61395		1	2,81	2,30	1	
	60178		1	0	2,30	1	
	64815			5,15	0		
7	3838	1		2,68	3,51	1	1
	3640		1	7,15	7,26		1
	29			-	5,51		
8	1312			1,73	0		
	1284			0	1,79		
	1344	1		2,76	2,34		1
13	4450	1		4,52	4,91	1	1
	1454	1		5,38	5,87	1	1
	68	1		7,34	7,41	1	1
18	2101	1		3,54	3,45		1
	2348	1		7,15	6,76		1
	2113	1		5,68	6,00	1	1
21	2818		1	6,23	6,48	1	
	2268	1		4,91	4,72	1	1
	2222	1		6	6	1	1
23	73202			4,11	4,15	1	
	71033		1	5,62	5,48	1	
	525	1		5,51	6,11	1	1
31	596		1	0	0	1	
	639	1		0	0	1	1
	682	1		0	0	1	1

462

463

464

"low" herd	Animal ID	Macroscopic lesions	Histopathological lesions	PCV2 DNA copies/mg (log10)		SIV and/or PRRSV	Pathogenic bacteria <i>Streptococcus</i> spp and/or <i>E.coli</i>
				affected lung	unaffected lung		
5	9459	1		1,89	0		1
	9194			1,77	0		
	9189		1	7,51	6,52	1	1
6	8763	1		5,92	6,73		1
	5958	1		3,15	2,52		
	8654	1		0	0		
9	2811		1	0	0		
	2142		1	1,69	0		
	58		1	0	0		
11	9155	1		0	0	1	1
	7711			0	0		
	9733		1	1,90	2,52	1	1
12	41018		1	2,30	0	1	1
	39481		1	0	0	1	
	42450		1	0	0		
16	195			0	0		
	NN	1		4,86	4,58	1	1
	24			6,45	6,79		
27	42667			0	0		
	42587			0	0		1
	44298			0	0		
29	9224	1		6,86	6,93		
	9242		1	5,52	5,15	1	
	9075		1	4,36	3,34	1	1

465

466

467 **Table 5: Results of the univariate analyses of the post-mortem examinations, PCV2 DNA, viral**  
 468 **lung pathogens, and bacteria. The proportion of positive tests in “high” herds and “low” herds**  
 469 **are presented as well as the P-value and whether the (single) variable was found more often in**  
 470 **“high” herds than in “low” herds.**

Variable	Proportion		P-value	
	“high”	“low”		
No pathological abnormalities	0.210	0.330	0.330	Ns*
Firm lungs	0.000	0.125	0.074	Ns
Pneumonia	0.080	0.125	0.637	Ns
Lobus apicalis pneumonia	0.250	0.042	<b>0.041</b>	More in “high”
Catarrhal pneumonia	0.083	0.042	0.551	Ns
Interstitial pneumonia	0.042	0.292	<b>0.020</b>	More in “low”
Necrotic pneumonia	0.000	0.042	0.312	Ns
Abscess pneumonia	0.000	0.042	0.312	Ns
Chronic pneumonia	0.042	0.000	0.312	Ns
Pleuritis and pneumonia	0.042	0.000	0.312	Ns
Chronic pleuritis	0.083	0.083	1.000	Ns
PCV2 from affected lungs	0.458	0.250	0.131	Ns
PCV2 from unaffected lungs	0.583	0.208	<b>0.008</b>	More in “high”
SIV (swine influenza viruses)	0.375	0.125	<b>0.046</b>	More in “high”
PRRSV	0.417	0.208	0.120	Ns
PRRS + <i>H. parasuis</i>	0.000	0.042	0.310	Ns
No bacteria	0.042	0.083	0.550	Ns
A-pathogenic bacteria	0.125	0.375	<b>0.046</b>	More in “low”
Mixed flora	0.250	0.167	0.480	Ns
<i>Arcanobacterium pyogenes</i>	0.000	0.042	0.312	Ns
<i>A. pleuropneumoniae</i>	0.167	0.125	0.680	Ns
<i>Bordetella bronchiseptica</i>	0.042	0.000	0.312	Ns
Streptococci ( <i>S. spp</i> )	0.000	0.167	<b>0.037</b>	More in “low”
<i>Pasteurella multocida (Pm)</i>	0.250	0.042	<b>0.041</b>	More in “high”
<i>E. coli</i>	0.000	0.042	0.312	Ns
<i>Haemophilus parasuis (Hps) + Pm</i>	0.042	0.000	0.312	Ns
<i>Mycoplasma hyopneumoniae</i>	0.417	0.042	<b>0.002</b>	More in “high”

471 Ns\*: not significant

472

473

474 **Figure 1.** Box-and-whisker-plot of growth by three categories of percentage pleuritis at slaughter

475 (n=26 herds).

476

477

478 **Figure 2.** Box-and-whisker-plot of percentage dead loss by three categories of percentage affected

479 lungs at slaughter (n=26 herds).

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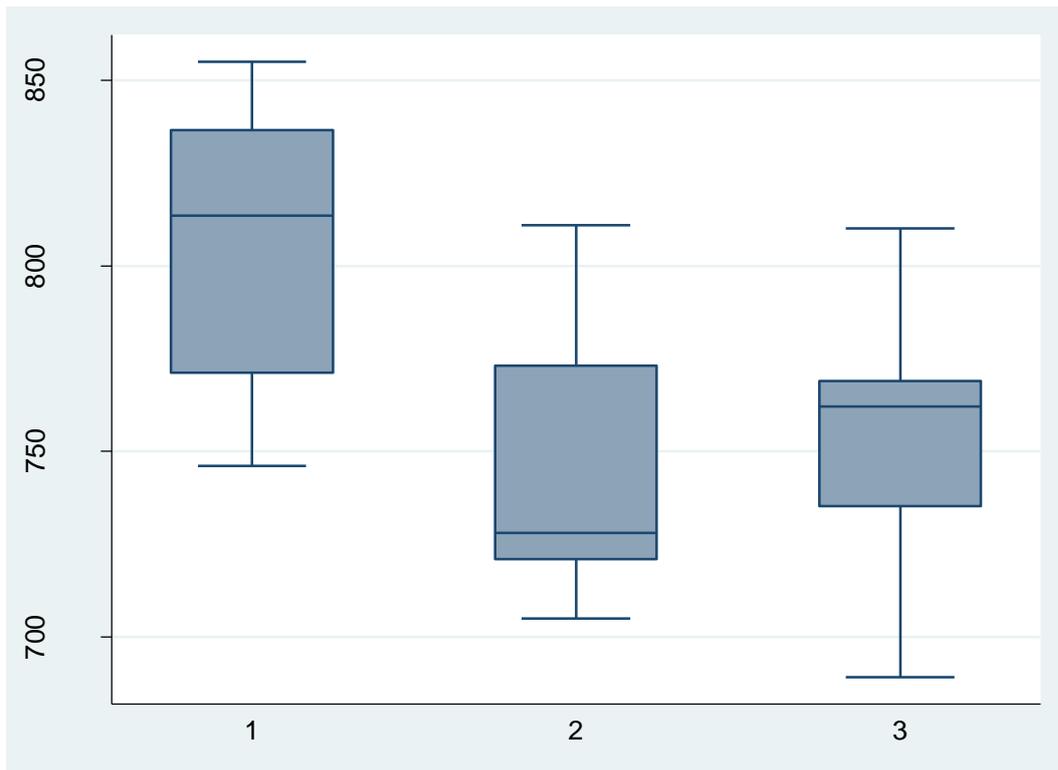


Figure 1.

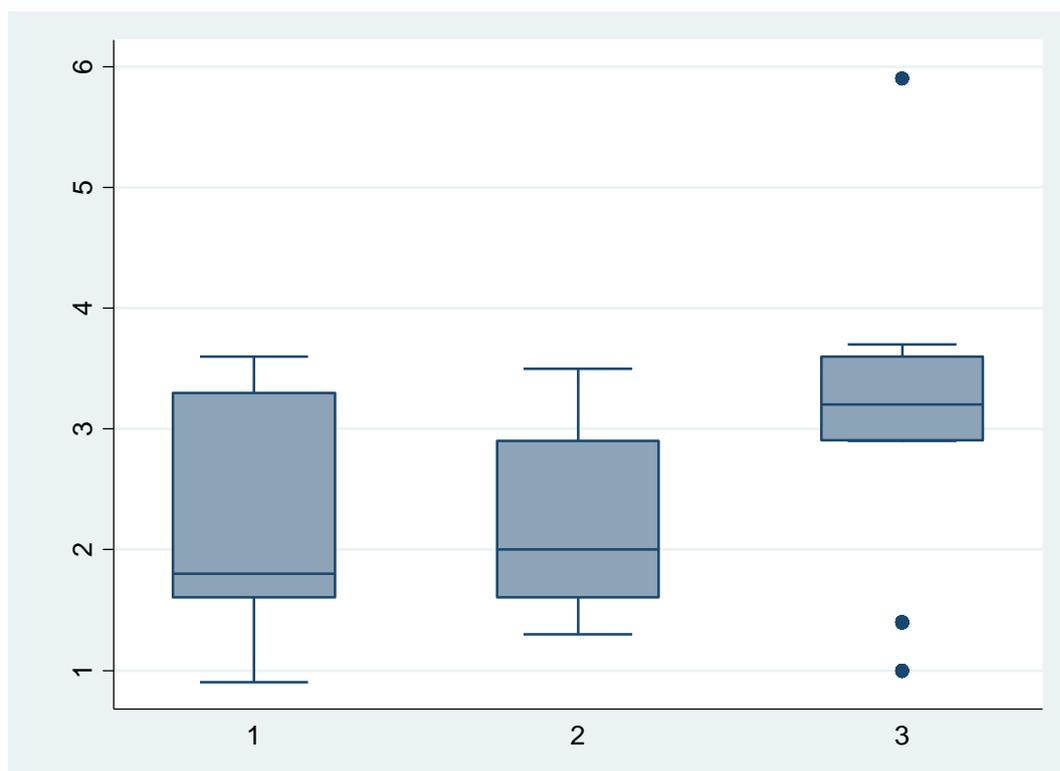


Figure 2.