Monitoring of Porcine Circovirus Type 2 infection through air and surface samples in vaccinated and unvaccinated fattening farms.

Monitoring of PCV2 infection through air and surface samples

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14 **Summary**

15 Air and surfaces of swine farms are two alternative samples to obtain information about the health
16 status of the herd. The aim of this study was to assess air and surface sampling for the detection of
17 Porcine Circovirus Type 2 (PCV2) in vaccinated and unvaccinated fattening farms, studying the
18 relationship between the viral load in these samples with the viremia at herd level. Three swine
19 fattening batches (one unvaccinated; two vaccinated) were monitored at 10, 12, 14, 16 and 18
20 weeks old; at each stage, blood, air and different surfaces were sampled and analysed by qPCR. In
21 all herds, PCV2 was detected in all types of samples. Whenever viremia was detected, PCV2 was
22 also detected in air and surface samples, even in those cases with a low estimated prevalence (1.6
23 %); moreover, in two out of the three herds, PCV2 was detected in air and surface samples earlier
24 than in the blood of the sampled population. In addition, a good correlation between the viremia of
25 pig population and the PCV2 load in air and surface samples was found in both cases ($\tau = 0.672$ and
26 0.746 respectively; $p < 0.05$). These results show that air and surface samples could be useful tools
27 to monitor PCV2 infection, being suitable for detecting the virus in cases of low prevalence and
28 even before pigs develop viremia; therefore, these sampling techniques would speed up the
29 implementation of the required measures to prevent productive and economic losses due to PCV2
30 infection.

31

32 **Keywords**: air sampling, environmental samples, monitoring, Porcine circovirus type 2 (PCV2),
33 qPCR

34 **INTRODUCTION**

35 Surveillance in domestic pigs is important to detect diseases and establish the health status of herds.
36 Failing to detect diseases may have important sanitary and economic consequences for farms,
37 leading to endemic and persistent problems and hindering the success of control programmes
38 (Arruda & Gauger, 2019). This is especially challenging in diseases that can be maintained in a herd
39 by a small proportion of infected animals. In these cases, detecting the disease can be very laborious
40 and expensive since it usually requires elevated sample sizes (Kittawornrat et al., 2014; Ramirez et
41 al., 2012), which may represent several limitations in field conditions. Thus, improving protocols
42 and techniques that can help farmers to detect diseases more efficiently are still needed.
43 In the last few years, the swine sector has introduced different sampling methods in order to provide
44 accurate information of a high number of animals while minimising the investment in time and
45 money. The detection of pathogens in oral and processing fluids, umbilical cords, skin wipes, air
46 and farm surfaces are some examples (Garrido-Mantilla et al., 2019; Lopez, Angulo, Zimmerman,
47 & L Linhares, 2018; Martín-Valls, Hidalgo, Cano, & Mateu, 2018; Neira et al., 2016; Prickett, Kim,
48 Simer, Yoon, & Zimmerman, 2008). In addition, these methods present the advantage of being non-
49 invasive and some of them, such as oral fluids or processing fluids, have shown a good agreement
50 with the detection of Influenza A virus, PCV2 and PRRS virus in nasal or serum samples (Nielsen
51 et al., 2018; Romagosa, Gramer, Joo, & Torremorell, 2012; Vilalta et al., 2018). However, they still
52 have certain limitations, for example, umbilical cords and processing fluids can only be employed
53 during the first few days of life; detection in oral fluids, which is based on the natural curiosity of
54 the pigs to interact with cords (Romagosa et al., 2012), can underestimate the infection level since
55 sick animals can refuse to interact with their surroundings (Hart, 1988) and, therefore, may not be
56 included in the sample.
57 In this context, the detection of pathogens in air samples collected inside farm facilities has been
58 pointed out as a promising tool for the disease surveillance of swine pathogens such as MRSA,
59 Influenza A virus and *M. hyopneumoniae* (Corzo, Culhane, Dee, Morrison, & Torremorell, 2013;
60 Damte et al., 2014; Friese et al., 2012). Regarding PCV2 detection, only three studies have analysed

61 air from commercial swine farms (Anderson et al., 2020; Evgrafov et al., 2013; Verreault et al.,
62 2010). Two of them were carried out before vaccines against this virus were available, thus a high
63 number of infected pigs was to be expected; however, neither of them estimated the prevalence of
64 the infection. Similarly, in recent years, Anderson et al. (2020) detected PCV2 in air samples from
65 one fattening farm, although they did not provide any information on the infection level in that herd.
66 Currently, vaccination is a widespread measure that has led to an important reduction of the
67 prevalence of PCV2 (Dvorak, Yang, Haley, Sharma, & Murtaugh, 2016), so that a high number of
68 serum samples are usually necessary to detect the infection. In this new epidemiological context, air
69 sampling could be a valuable alternative, but it is still necessary to assess the performance of this
70 technique regarding the infection level within the herd.
71 On another note, surface sampling has also been used to study pathogens like MRSA or Influenza A
72 (Espinosa-Gongora et al., 2012; Neira et al., 2016), and has proved useful for monitoring some of
73 them without compromising animal welfare (Bangerter, Sidler, Perreten, & Overesch, 2016).
74 Regarding PCV2, the number of cross-sectional studies that have employed surface sampling to
75 detect it is very reduced (Díaz-Cao et al., 2018; Dvorak, Lilla, Baker, & Murtaugh, 2013; López-
76 Lorenzo et al., 2019), and none of them have monitored the infection over time.
77 Against this background, the objective of this study was to assess the suitability of air and surface
78 sampling to monitor the evolution of PCV2 load in vaccinated and unvaccinated fattening farms,
79 estimating the correlation of viral load in these samples with the PCV2 viremia at herd level.

80 **MATERIALS AND METHODS**

81 **Characteristics of the farms and origin of the animals**

82 This study was performed in three commercial swine fattening farms with an all-in/all-out system
83 (AI-AO), with a complete washing, disinfection and depopulation of at least one week between
84 batches. All the farms had the same structure, with a central alley and pens to each side. Each pen
85 housed approximately 15 pigs and consisted of a partially slated floor, a totally solid wall, one pig
86 hopper and one nipple drinker. The buildings were ventilated by automated lateral windows which

87 were opened or closed depending on the indoor temperature of the farm, and food and water were
88 available *ad libitum*.
89 Approximately five months before starting this study, an increase of mortality (up to 8 % in the
90 mortality rate) and an increase of uneven weight at the slaughter age had been observed in the
91 fattening batches. At the beginning of the trial, samples of inguinal lymph nodes and spleen were
92 taken from dead pigs and PCV2 compatible lesions (moderate to severe lymphocyte depletion) were
93 confirmed, also verifying a severe PCV2 amount by immunohistochemical methods. Thus,
94 according to the established criteria, PCV2-Systemic Disease had been diagnosed at herd level in all
95 the included farms (Grau-Roma, Fraile, & Segalés, 2011). In addition, it must be pointed out that all
96 the farms were supplied with nine-week-old piglets by the same farrow to wean farm every two
97 weeks. This origin herd was negative to PRRS virus and the piglets were only vaccinated against *M.*
98 *hyopneumoniae* at one week of age.

99 **Study design**

100 Three batches (one from each fattening farm) were monitored in this study: one batch of pigs
101 unvaccinated against PCV2 (Group 0, control; n=360) and two batches vaccinated at 4 weeks of age
102 with different commercial vaccines (Groups 1 and 2; n=380 and n=490, respectively). Samples of
103 blood, air and surfaces were taken in these fattening farms when the pigs were 10, 12, 14, 16 and 18
104 weeks of age.

105 Blood samples

106 Blood samples were taken from the jugular vein. The sample size was calculated assuming an
107 estimated prevalence of 50% and a precision of 11.5% with the package epiDisplay in R (Virasakdi
108 Chongsuvivatwong, 2018; R Core Team, 2018); as a result, 60 animals in Groups 0 and 1 (5
109 pigs/pen; 12 different pens) and 65 pigs in Group 2 (5 pigs/pen; 13 different pens) were sampled in
110 the first visit. In the following visits, the same number of pigs was randomly sampled from the same
111 pens.

112 Air samples

113 In each visit, six air samples (50 L/min for 30 minutes each one) were taken using the air sampler
114 MD8 Airport (Sartorius AG, Göttingen, Germany) with sterile gelatin filters of 80 mm in diameter
115 and a pore size of 3 μm (Sartorius Stedim Biotech GmbH, Göttingen, Germany). The air sampler
116 was placed in the central alley at 1/3 ("a" point) and 2/3 ("b" point) of the length of the building. At
117 each point, samples were taken at three different heights: at the ground level, at the pen railing
118 height and at a height of two meters. After finishing the sampling, each filter was kept in an
119 individual package at room temperature until processed.

120 Surface samples

121 In each visit, surface samples were taken from five locations: the central alley of the farm, the pen
122 railing, the pig hopper, the pen wall and the pen floor (the samples from the hopper, the pen wall
123 and the pen floor were taken from the pens where pigs were sampled). A previously described
124 swabbing method was used as it has proved useful to detect virus from the environment of livestock
125 productions (Prieto et al., 2014). The sampling protocol for each surface was performed as indicated
126 in previous studies (López-Lorenzo et al., 2019) and is briefly described in Table 1. After sample
127 collection, each swab was kept in an individual tube at room temperature until processed.

128 **Laboratory analysis**

129 All samples (blood, air and surfaces) were processed at the laboratory in the first 24 hours.
130 Blood samples from the same pen were pooled together (five samples/pool). DNA extraction was
131 carried out from 200 μl of each pool using a commercial DNA extraction kit (High Pure PCR
132 Template Preparation Kit, Roche Diagnostics GmbH, Mannheim, Germany) following the
133 manufacturer's instructions. The obtained DNA was collected in 100 μl of elution buffer and kept at
134 -30 ºC until qPCR analysis.
135 The filters used for air sampling were transferred from their packages to Stomacher bags and 5 ml
136 of sterile phosphate-buffered saline with 0,05% Tween 20 (PBST, all reagents supplied by Sigma-
137 Aldrich, Missouri, United States) was added to each one to dissolve them. The obtained solution
138 was homogenized for one minute and left to settle down for 15 minutes. After that, 1 ml of

139 supernatant from each solution was transferred to a sterile Eppendorf tube and kept at -30ºC until
140 the DNA extraction was performed.
141 Surface samples were processed by adding 5 ml of PBST to each tube containing the swab. They
142 were vortexed for one minute and subsequently left to settle down for 15 minutes. After that, 1 ml
143 of supernatant from each sample was placed in a sterile Eppendorf tube and kept at -30ºC until the
144 DNA extraction was performed.
145 Due to the nature of air and surface samples, a previously recommended serial qPCR analysis which
146 involves two different DNA extraction protocols was performed in order to avoid PCR inhibition
147 (Prieto et al., 2017). For the first qPCR, a commercial DNA extraction kit (High Pure PCR
148 Template Preparation Kit, Roche Diagnostics GmbH, Mannheim, Germany) was used.
149 Subsequently, qPCR analysis was performed using a commercial kit which targets the ORF2 gene
150 (EXOone PCV2 oneMIX, EXOPOL S. L., Zaragoza, Spain), following the manufacturer's
151 instructions. Negative samples to the first qPCR were re-extracted with a second commercial DNA
152 extraction kit (Nucleospin® Soil, Macherey-Nagel GmbH & Co KG, Düren, Germany) and
153 analysed with the same qPCR protocol. The manufacturer's instructions were followed for both
154 extraction procedures, using 200 µl of each sample as starting material and collecting the extracted
155 DNA in 100 µl of elution buffer; in addition, an exogenous internal control (EXOone EXIC,
156 EXOPOL S.L., Zaragoza, Spain) was added to each sample to identify possible qPCR inhibition.
157 qPCR positive and negative controls were supplied by the manufacturer and were used in each run.
158 A sample was considered positive when $Ct \leq 42$ for the PCV2 detection channel. In addition, the
159 positive control was used to calculate the standard curve by preparing serial ten-fold dilutions (5
160 $x10^5$ - $5x10^1$ copies/µl). All qPCR reactions were run in duplicate on an Applied Biosystems ABI
161 Prism 7500 thermocycler (Thermo Fisher Scientific, Waltham, MA, USA). The amplification
162 protocol was the one indicated by the manufacturer.
163 The amount of PCV2 DNA quantified for each sample was expressed as number of copies/m^3 of air
164 and number of copies/swab for air and surface samples respectively.
165 **Data analysis**

166 The individual prevalence of PCV2 was estimated for each stage using a frequentist approach from
167 the pooled results with the web resource EpiTools (Sergeant, 2018). A method assuming a fixed
168 pool size and a perfect test was chosen (Cowling, Gardner, & Johnson, 1999).
169 The proportion of positive blood pools, air samples and surface samples for each group at each
170 sampling stage was calculated. Subsequently, Kendall's-tau (τ) was used to determine the
171 association between the PCV2 load detected by qPCR and the proportion of positives from each
172 type of sample (blood, air and surfaces), as well as to test the correlation between the PCV2 load
173 detected in blood pools and the PCV2 load detected in air and surface samples. For the latter case,
174 the PCV2 load was considered as 0 copies in the negative samples and the correlation was
175 performed using the mean number of copies of PCV2 in blood pools, air samples and surface
176 samples for each group and sampling moment. In addition, the correlation was also assessed using
177 an estimator of τ for zero-inflated data (Pimentel, 2009) (Supporting Information). All statistical
178 analyses were conducted with the software R (R core Team, 2018). The *p* value was considered
179 significant when < 0.05.

180 **RESULTS**

181 **Descriptive results**

182 Viremia

183 The individual estimated prevalence of PCV2 for each group at each age is summarized in Table 2;
184 briefly, the prevalence throughout the study ranged from 30.11 to 100 % in the Control Group and
185 from 1.59 to 1.73 % in the vaccinated groups. In the Control Group, all blood pools tested negative
186 to PCV2 at 10 weeks of age; since then, viremia was detected until the end of the study. The peak of
187 viremia appeared at 12 weeks of age (11/12 positive blood pools) with the PCV2 load ranging from
188 7.65×10^4 to 2.34×10^8 PCV2 copies/ml; from that moment, viremia decreased gradually (Figure
189 1.A). In Group 1, viremia was firstly detected at 14 weeks of age in 1/12 blood pools (Figure 1.B);
190 from that moment until the end of the study only 1/12 blood pools was positive in each sampling,
191 with the PCV2 load ranging from 1.54×10^3 to 9.49×10^4 PCV2 copies/ml. In Group 2, viremia was

192 only detected when pigs were 18 weeks old (Figure 1.C.), only 1/13 blood pools tested positive
193 (4.11 x10^3 PCV2 copies/ml).
194 Therefore, PCV2 viremia was detected in the Control Group earlier than in both vaccinated groups
195 (1 and 2), and the number of positive blood pools was always higher in the Control Group than in
196 groups 1 and 2. Similarly, at the same age, a higher PCV2 load was found in samples from
197 unvaccinated pigs than in those from vaccinated animals, with the only exception of some blood
198 pools at 18 weeks of age.
199 Air samples
200 The results of air samples for each group and age are shown in Table 3. Both in the Control Group
201 and in Group 1, PCV2 DNA was detected in air samples earlier than in blood ones. In the Control
202 Group, PCV2 was detected in the air at 10 weeks of age (1/6 samples positive). As with blood
203 samples, the peak of PCV2 load in air also appeared at 12 weeks of age and decreased gradually
204 since then in this group (Figure 1.A). In Group 1, the first detection was at 12 weeks old, 3/6 air
205 samples tested positive (one at each height), also concurring with the highest PCV2 load in air
206 which decreased gradually since then (Figure 1.B). In Group 2, PCV2 was not detected until pigs
207 were 18 weeks of age, with three positive air samples (one at each height).
208 Thus, as it occurred with viremia, PCV2 was detected in air samples from the Control Group earlier
209 than in both vaccinated groups. Moreover, the number of positive air samples was always higher in
210 the Control Group than in Groups 1 and 2 for all sampling stages, and the samples from the Control
211 Group showed a higher PCV2 load than those from the vaccinated groups, with the exception of
212 some samples from Group 2 at 18 weeks of age (Table 3).
213 Surface samples
214 The results of surface samples for each group and age are shown in Table 4. In all groups, PCV2
215 DNA was detected in surface samples earlier than in blood pools. Concretely, in the Control Group,
216 PCV2 was detected at 10 weeks of age in the central alley and in the pen floor (Figure 1.A). Since
217 then, all environmental samples tested positive at every sampling stage. In Group 1 the first
218 detection was at 12 weeks of age in the sample from the central alley; in the following samplings,

219 PCV2 was detected in all types of surfaces except for the pen railing. Similarly, in Group 2 the virus
220 was firstly detected at 16 weeks of age in samples from the central alley and the pig hopper; in the
221 following visit, PCV2 was still detected in the central alley.
222 Therefore, PCV2 was detected in surface samples from the Control Group earlier than in those from
223 both vaccinated groups. In addition, the virus was detected in all types of samples from the Control
224 Group, whereas in Groups 1 and 2, all the samples from the pen railing were negative. Moreover, at
225 the same age, all the samples from the unvaccinated group contained a higher PCV2 load than those
226 from the vaccinated groups (Table 4).

227 **Correlation between viremia and PCV2 load in air and surfaces**

228 A positive association between the proportion of positive samples and the PCV2 load was observed
229 for each type of sample, which means that the detection of more positive samples was associated
230 with a higher load of virus in them: blood pools ($\tau = 0.903$), air samples ($\tau = 0.803$) and surface
231 samples ($\tau = 0.836$) with $p < 0.001$ in all cases.
232 The mean of the PCV2 load in air samples showed a good correlation with the mean of PCV2
233 copies in blood pools ($\tau = 0.672$, $p = 0.001$). In particular, the PCV2 load in air samples taken at the
234 ground level showed the highest correlation with the mean of viremia ($\tau = 0.786$, $p < 0.001$),
235 followed by those taken at the height of the pen railing ($\tau = 0.762$, $p < 0.001$) and at a height of two
236 meters ($\tau = 0.631$, $p = 0.003$). Similarly, the mean of PCV2 copies calculated from all the surface
237 samples correlated strongly with the mean of PCV2 copies in blood samples ($\tau = 0.746$, $p < 0.001$).
238 This correlation was also significant for each type of surface sample with the following τ: central
239 alley, 0.724; pen railing, 0.710; hopper, 0.810; pen wall, 0.801; and pen floor, 0.659 ($p < 0.003$ in
240 all cases). The significance of all the aforementioned associations found with Kendall's tau was
241 confirmed by the zero-inflated estimator ($p < 0.05$).

242 **DISCUSSION**

243 The main finding of this longitudinal study was the earlier detection of PCV2 DNA in air and/or
244 surface samples than in the conventional method of viremia detection by collecting blood from a
245 representative number of pigs. Furthermore,whenever viremia was detected, PCV2 DNA was also

246 found in air and/or surface samples, which suggests that these environmental sampling methods
247 could be suitable for diagnosis and monitoring of PCV2 infection in swine production. It must be
248 mentioned that the sampling techniques employed in this study were able to detect PCV2 DNA
249 when the herd infection prevalence was estimated at 1.59 % (CI 95% 0.00 – 8.55 %), thus
250 suggesting that these methods could be suitable for farms with low prevalence.
251 Our results are consistent with those obtained by Garrido-Mantilla et al., (2019), who have
252 suggested that environmental samples can outperform blood samples from a representative number
253 of animals as a surveillance tool. In cases of low infection prevalence, the traditional method of
254 detecting viremia is hindered by the need for a high number of animals, which increases the
255 workload and the costs, as well as it reduces animal welfare. In the case of PCV2, the primary
256 replication of the virus in the tonsils may cause a certain level of excretion prior to the development
257 of viremia (Rosell et al., 1999), a fact that could explain the results of our study, which
258 demonstrates an earlier detection of the virus in air and surface samples than in conventional
259 methods.
260 The good correlation between the viremia at herd level and the PCV2 load in air and surface
261 samples obtained in this study has also been observed for other viruses such as Influenza A (Neira
262 et al., 2016). Our results suggest that air and surface samples could also be a useful tool to monitor
263 PCV2 infection in AI-AO management systems, especially in rearing gilt farms, which have a
264 similar management to fattening farms. Vaccinating gilts against PCV2 at the appropriate moment
265 is important to avoid the infection and, as a consequence, the risk of reproductive failure (Oropeza-
266 Moe, Delgado, & Framstad, 2017). This vaccination is a common strategy during the quarantine
267 period (Segalés, 2015), although in some cases it is performed before the gilts leave the rearing
268 farm. In fact, checking the gilt's status regarding PCV2 and bringing forward the vaccination have
269 already been recommended (Eddicks et al., 2018). Thus, environmental samples could be used to
270 monitor PCV2 infection in this type of farms and identify the optimal moment for the vaccination in
271 a simple, cheaper, and non-stressful way.

272 As it could be expected, both vaccines modified the infection dynamics, delaying the age of PCV2
273 detection both in blood and environmental samples. However, our results also indicate that the
274 infection prevalence could increase from the 18th week of age in pigs vaccinated at 4 weeks of age.
275 This fact has been observed in other studies which detected a stable viremia in vaccinated pigs from
276 the 18th to the 25th week of life (Feng, Segalés, Fraile, López-Soria, & Sibila, 2016; Haake et al.,
277 2014). This may not have a significant impact in slaughter pigs but may be relevant in gilts, hence
278 the importance of detecting the infection in this group of animals even when the prevalence is low.
279 Traditionally, the effectiveness of vaccination has been evaluated regarding the improvement in
280 productive indexes and the reduction of viremia (Jeong, Park, Choi, & Chae, 2015; Park, Seo, Han,
281 & Chae, 2014). Environmental samples can also allow this evaluation by measuring the reduction of
282 viral excretion from vaccinated animals (Prieto et al., 2018). By reducing PCV2 excretion,
283 vaccination also decreases the spread of the infection in the herd. This fact is supported by our
284 results, since a lower number of positive samples and lower PCV2 loads were detected in the
285 vaccinated groups regarding the control group. Accordingly, the time lag of 2-6 weeks in the PCV2
286 environmental detection in both vaccinated groups regarding the control group is the result not only
287 of the protection conferred by the vaccines, but also of the decrease in viral excretion that they
288 cause.
289 However, several aspects must be considered in the assessment of environmental samples. In the
290 case of air sampling, it must be mentioned that the PCV2 load depends on the viremia of the herd,
291 but also on the ventilation of the farm; thus, a higher ventilation could reduce the viral load present
292 in the air (Corzo et al., 2013). In the three studied farms, ventilation was automatically controlled
293 depending on the interior temperature; as pigs grow, the ventilation is increased. The peak of PCV2
294 load in air samples was detected in the Control Group and Group 1 at 12 weeks of age, a stage of
295 low ventilation which coincides with the peak of viremia in the Control Group. As pigs carried on
296 growing, the number of positive samples as well as the viremia and the PCV2 load in air samples
297 decreased, coinciding with the reduction of the infection level but also with the typical increase of
298 ventilation in the last stages. On the other hand, a decreasing tendency in the viral load was also

299 observed in surface samples, but this effect was much less pronounced compared to air samples;
300 this fact seems reasonable, since the decrease of PCV2 in surfaces mainly depends on the reduction
301 of the viremia and not so much on the ventilation.
302 As indicated by other authors, the environmental load of PCV2 can be used as an indicator of herd
303 health (Beach & Meng, 2012). Thereby, air sampling seems a very interesting method since the
304 whole pig population contributes to the results in a similar way. This implies an advantage of air
305 sampling over other types of herd samples like oral fluids, which would only represent the pigs of
306 the sampled pen (Oliver-Ferrando et al., 2016). Taking into account the obtained results, it seems
307 essential to sample at least two points across the farm on similar size herds; however, on larger
308 farms more sampling points would probably be necessary. Regarding the height of the sampling
309 point, this factor did not seem to have an influence on the PCV2 detection in this study, which is
310 consistent with what has been observed for *Salmonella* spp. on poultry farms (Adell et al., 2014).
311 With respect to surface sampling, our results suggest that samples from hoppers, walls and floors of
312 the pens seem to be the most suitable as surveillance tools, possibly due to their direct contact with
313 pigs. Nevertheless, more studies are necessary to refine these diagnostic methods since other
314 variables could be influencing the results.
315 Finally, the advantages and drawbacks of air and surface sampling must be mentioned. The main
316 advantage of both methods is that they provide information on the sanitary status of a great number
317 of animals with only a few samples, which represents a saving in time and costs; moreover, they do
318 not require specifically trained personnel. In addition, these techniques are also respectful with
319 animal welfare since both methods are non-invasive. Regarding the disadvantages, these
320 environmental techniques could be influenced by some farm factors, like the effect of ventilation in
321 the case of air sampling or that the provided information is limited to particular pens in the case of
322 surface sampling, as it has been previously commented; for these reasons, further studies that
323 contribute to identify and to assess these factors are needed. Another issue is that qPCR does not
324 indicate the viability of viral particles; however PCV2 is a highly resistant virus (Martin, Le Potier,

& Maris, 2008; O’Dea et al., 2008), so its detection by these methods should be interpreted as a risk of infection.

CONCLUSION

Air and surface sampling are useful tools to monitor infections by PCV2. In this study, a good correlation between viremia and PCV2 load in air and surface samples has been observed. Whenever viremia was detected, PCV2 was also detected in air and surface samples. Furthermore, these sampling techniques enable the detection of PCV2 infection earlier than the conventional method of sampling a representative number of pigs, which would allow to establish preventive measures prior to the development of the disease. Therefore, the knowledge generated by this study may be useful to improve control programmes through the detection of PCV2 by environmental sampling methods, allowing an improvement in the current diagnostic schemes in terms of efficiency and animal welfare.

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CONFLICT OF INTEREST

Víctor Rodríguez-Vega is an employee of Boehringer Ingelheim Animal Health Spain.

ETHICAL STATEMENT

The protocols and procedures which involve animal sampling (blood samples) were approved by the bioethics committee of the University of Santiago de Compostela.

DATA AVAILABILITY STATEMENT

351 The data of this study are available from the corresponding author upon reasonable request.

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521

Table 1. Environmental samples and sampling protocol.

Central alley	100 steps were taken wearing polyethylene boot covers. Afterwards, both boot covers were swabbed as previously indicated in Dee et al. (2002): in zigzag from the toe region to the heel.
Pen railing	1 m of the pen railing was swabbed in zigzag (the same point in all visits).
Hopper	Eight different hoppers (the same ones in all visits) were selected and an area of 25x25 cm was swabbed in each one (Espinosa-Gongora et al., 2012). The hoppers were located in the same pens used for sampling the pen wall and the pen floor.
Pen wall	Eight different pens (the same ones in all visits) were selected and an area of 25x25cm, located at the height of the snout in the resting area, was swabbed. The same pens were used for sampling the hopper and the pen floor.
Pen floor	100 steps were taken wearing polyethylene boot covers. Afterwards, both boot covers were swabbed in the same way as for the central alley. The same pens were used for sampling the hopper and the pen wall.

522

Table 2. Results of blood pools and individual estimated prevalence of PCV2.

		Weeks of age				
		10	12	14	16	18
Group 0 (control, n=364)	n. pos / n. tot	0 / 12	11 / 12	12 / 12	12 / 12	10 / 12
	Estimated prevalence (CI 95%)	- (-)	39.16% (17.38 – 70.84)	100% (-)	100% (-)	30.11% (13.50 – 53.88)
Group 1 (vaccinated, n=381)	n. pos./n. tot	0 / 12	0 / 12	1 / 12	1 / 12	1 / 12
	Estimated prevalence (CI 95%)	- (-)	- (-)	1.73% (0.00 – 9.26%)	1.73% (0.00 – 9.26%)	1.73% (0.00 – 9.26%)
Group 2 (vaccinated, n=490)	n. pos./n. tot	0 / 13	0 / 13	0 / 13	0 / 13	1 / 13
	Estimated prevalence (CI 95%)	- (-)	- (-)	- (-)	- (-)	1.59% (0.00 – 8.55%)

523 -: not calculated

Table 3. Results of individual air samples at each moment (PCV2 copies/m^3 air).

	Height	Sampling Point	Weeks of age				
			10	12	14	16	18
Group 0 (control, n=364)	Ground level	a	-	9.25×10^6	1.10×10^5	2.25×10^4	-
		b	-	2.42×10^7	9.43×10^5	3.73×10^5	3.75×10^4
	Pen railing	a	-	3.98×10^6	8.38×10^4	2.33×10^5	1.19×10^3
		b	-	2.96×10^6	3.36×10^6	1.60×10^6	2.53×10^4
	Two meters	a	1.35×10^6	1.33×10^6	5.34×10^4	3.68×10^4	-
		b	-	1.74×10^6	4.81×10^6	7.80×10^5	5.20×10^4
Group 1 (vaccinated, n=381)	Ground level	a		1.19×10^4	-	-	-
		b	-	-	6.13×10^2	-	5.39×10^2
	Pen railing	a	-	2.63×10^4	6.28×10^3	2.04×10^3	-
		b	-	-	-	-	-
	Two meters	a	-	-	1.96×10^3	-	-
		b	-	5.70×10^2	1.81×10^3	-	-
Group 2 (vaccinated, n=490)	Ground level	a	-	-	-	-	-
		b	-	-	-		2.55×10^4
	Pen railing	a	-	-	-	-	-
		b	-	-	-	-	5.93×10^4
	Two meters	a	-	-	-	-	-
		b	-	-	-	-	5.00×10^4

-: indicates a negative result

a: 1/3 of the building length

b: 2/3 of the building length

Table 4. Results of individual surface samples at each moment (PCV2 copies/swab).

	Surface	Week age				
		10	12	14	16	18
Group 0 (control, n=364)	Central alley	$7.45\ x10^{5}$	$2.03\ x10^{7}$	$7.45\ x10^{7}$	$3.75\ x10^{7}$	$1.17\ x10^{7}$
	Pen railing	-	$2.05\ x10^{7}$	$2.81\ x10^{7}$	$7.36\ x10^{5}$	$1.14\ x10^{7}$
	Hopper	-	$1.94\ x10^{7}$	$5.23\ x10^{6}$	$5.97\ x10^{5}$	$1.35\ x10^{5}$
	Pen wall	-	$3.65\ x10^{6}$	$4.53\ x10^{7}$	$7.96\ x10^{4}$	$3.42\ x10^{5}$
	Pen floor	$1.77\ x10^{4}$	$1.84\ x10^{8}$	$2.57\ x10^{8}$	$7.70\ x10^{6}$	$1.20\ x10^{7}$
Group 1 (vaccinated, n=381)	Central alley	-	$2.22\ x10^{5}$	$1.36\ x10^{6}$	$4.08\ x10^{5}$	$1.29\ x10^{5}$
	Pen railing	-	-	-	-	-
	Hopper	-	-	$3.82\ x10^{4}$	$1.12\ x10^{5}$	$6.04\ x10^{3}$
	Pen wall	-	-	$1.38\ x10^{5}$	$1.38\ x10^{3}$	-
	Pen floor	-	-	$3.98\ x10^{3}$	$5.13\ x10^{3}$	-
Group 2 (vaccinated, n=490)	Central alley	-	-	-	$2.53\ x10^{3}$	$1.18\ x10^{5}$
	Pen railing	-	-	-	-	-
	Hopper	-	-	-	$1.39\ x10^{4}$	-
	Pen wall	-	-	-	-	-
	Pen floor	-	-	-	-	-

-: indicates a negative result

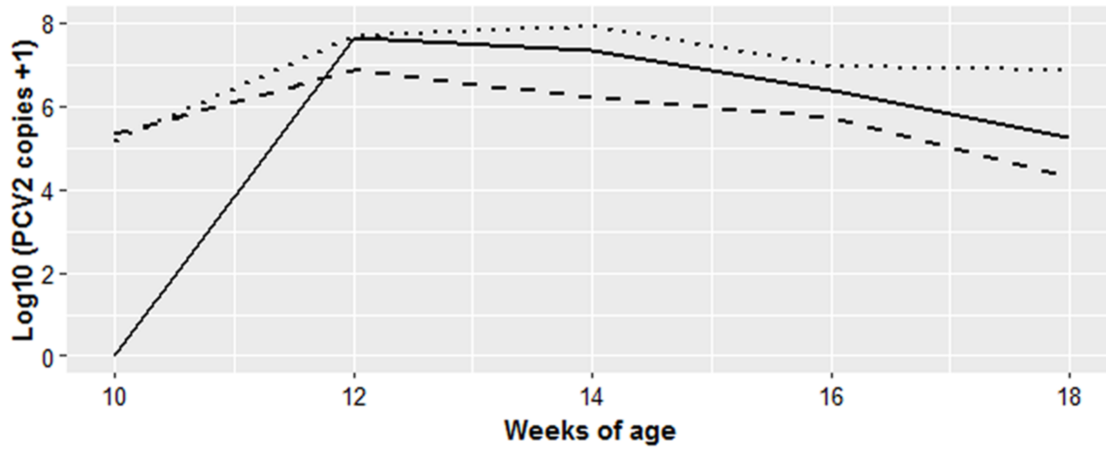
525

526 **Figure legends**

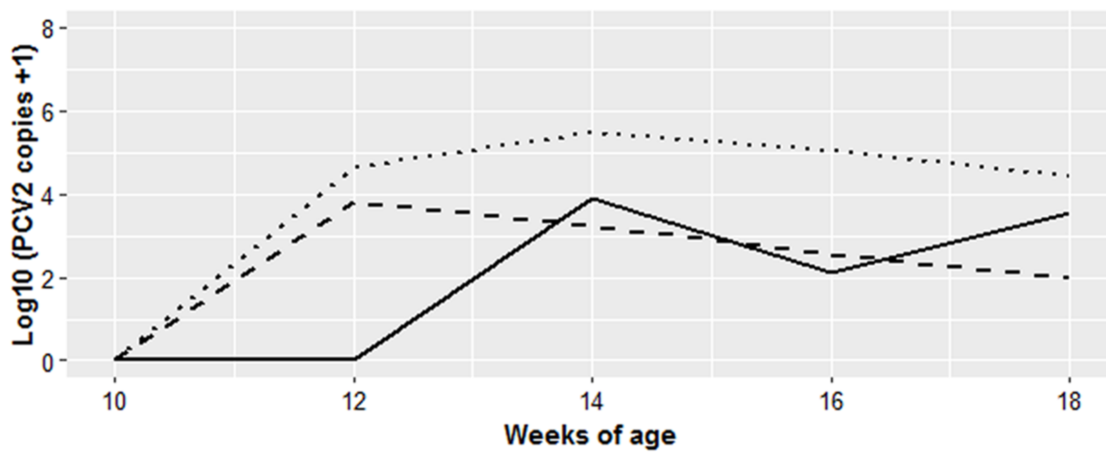
527 **Figure 1. Monitoring of PCV2 infection in Group 0 (1.A, Control group), Groups 1 and 2 (1.B
528 and 1.C, vaccinated groups respectively)**. For each week, PCV2 loads are shown as Log10 (x + 1)
529 where x is the mean of PCV2 copies/ml, the mean of PCV2 copies/m^3 of air and the mean of PCV2
530 copies/ swab for blood, air and surface samples respectively. Negative samples computed as 0 in the
531 number of PCV2 copies.

532

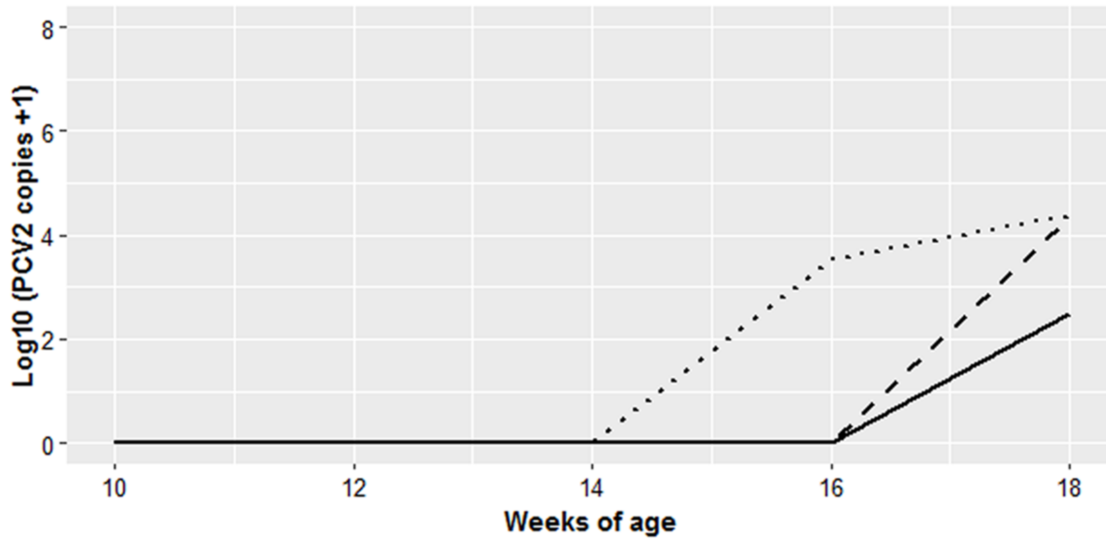
A)



B)



C)



533

Sample Blood Air Surfaces