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1 **Impact of porcine circovirus type 2 (PCV2) infection on hepatitis E** 2 **virus (HEV) infection and transmission under experimental conditions**

3

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16

17 **Abstract**

18 Hepatitis E virus is a zoonotic pathogen for which pigs have been identified as the main reservoir in
19 industrialised countries. HEV infection dynamics in pig herds and pigs are influenced by several
20 factors, including herd practices and possibly co-infection with immunomodulating viruses. This
21 study therefore investigates the impact of porcine circovirus type 2 (PCV2) on HEV infection and
22 transmission through experimental HEV/PCV2 co-infection of specific-pathogen-free pigs. No
23 statistical difference between HEV-only and HEV/PCV2-infected animals was found for either the
24 infectious period or the quantity of HEV shed in faeces. The HEV latency period was shorter for
25 HEV/PCV2 co-infected pigs than for HEV-only infected pigs (11.6 versus 12.3 days). Its direct

26 transmission rate was three times higher in cases of HEV/PCV2 co-infection than in cases of HEV-
27 only infection (0.12 versus 0.04). On the other hand, the HEV transmission rate through
28 environmental accumulation was lower in cases of HEV/PCV2 co-infection ($4.3 \cdot 10^{-6}$ versus
29 $1.5 \cdot 10^{-5}$ g/RNA copies/day for HEV-only infected pigs). The time prior to HEV seroconversion
30 was 1.9 times longer in HEV/PCV2 co-infected pigs (49.4 versus 25.6 days for HEV-only infected
31 pigs). In conclusion, our study shows that PCV2 affects HEV infection and transmission in pigs
32 under experimental conditions.

33

34 **Keywords**

35 Co-infection; PCV2; HEV; infection kinetic; transmission experiment

36

37 **1. Introduction**

38

39 Hepatitis E virus is a non-enveloped single-stranded RNA virus that can cause acute hepatitis in
40 humans. Chronic cases have also been described, mainly in immunocompromised patients
41 (Lhomme *et al.*, 2016). Genotypes 3 and 4 affect both humans and other animal species, and are
42 responsible for sporadic autochthonous cases of hepatitis in humans in industrialised countries
43 (Doceul *et al.*, 2016). In particular, genotype 3 is widespread in pig populations (Salines *et al.*,
44 2017) and a number of autochthonous cases have been linked to the consumption of undercooked
45 pork meat, especially liver products (Colson *et al.*, 2012; Guillois *et al.*, 2016). In order to limit the
46 risk of contaminated products entering the food chain, it is crucial to understand the factors
47 influencing HEV transmission and persistence in pig herds. High variability in HEV infection
48 dynamics has previously been described (Salines *et al.*, 2017) and may be related to husbandry
49 practices in terms of hygiene, biosecurity and rearing conditions (Walachowski *et al.*, 2014; Lopez-
50 Lopez *et al.*, 2018) or to individual characteristics such as protection conferred by maternally-
51 derived antibodies (Andraud *et al.*, 2014). Various factors affecting swine immune response may

52 also influence the course of HEV infection. Notably, in a previous study, we have shown that pigs
53 experimentally co-infected with porcine reproductive and respiratory syndrome virus (PRRSV)
54 exhibited chronic HEV infection with extended latency and infectious periods, increased faecal
55 shedding and transmission, as well as an increased risk of HEV-positive livers at slaughter (Salines
56 *et al.*, 2015). Porcine circovirus type 2 (PCV2) also has immunomodulating characteristics for
57 instance by inhibiting IFN- α production and by increasing the expression of IL-10, an anti-
58 inflammatory cytokine (Darwich et Mateu, 2012). PCV2 may therefore impact HEV infection
59 dynamics. Moreover, as the primary causative agent of post-weaning multisystemic wasting
60 syndrome (PMWS) and other porcine circovirus-associated diseases (PCVADs), it can sometimes
61 induce hepatitis in pigs (Rosell *et al.*, 2000). However, to date, only few data report on HEV/PCV2
62 co-infection (Martin *et al.*, 2007; Hosmillo *et al.*, 2010; Savic *et al.*, 2010; Yang *et al.*, 2015; Jackel
63 *et al.*, 2018). In these studies, PCV2 and HEV were simultaneously detected in pigs but the impact
64 of co-infections on HEV dynamics was not investigated.

65 Given the lack of data on this specific issue, the present study was designed to investigate how
66 PCV2 infection impacts HEV infection dynamics (in terms of viral shedding duration and quantity,
67 transmission and humoral immune response). A transmission experiment was therefore carried out,
68 with specific-pathogen-free (SPF) pigs infected with HEV or co-infected with HEV and PCV2 at
69 the same time.

70

71 **2. Material and methods**

72

73 2.1. Experimental design

74 The trial was conducted at ANSES's air-filtered level-3 biosecurity facilities. The 44 five-week-old
75 SPF Large White piglets included in the study were HEV- and PCV2-free and with no maternal
76 antibodies against these two viruses at the beginning of the study. These piglets were randomly
77 allocated into eight groups, housed in six rooms (Figure 1). Two negative control pigs were housed

78 in Room 1. The four piglets housed in Room 2 were only orally inoculated with a PCV2-b
79 genogroup suspension (GenBank accession number AF201311), titrating 10^5 TCID₅₀/mL in a
80 volume of 5 mL. In Rooms 5 and 6 (groups 4, 5, 6), three piglets per group were orally inoculated
81 with 10^7 HEV RNA copies of a genotype 3 HEV suspension (strain FR-SHEV3f, GenBank
82 accession number JQ953666) in a volume of 10 mL. In Rooms 3 and 4 (groups 1, 2, 3), three
83 piglets per group were orally inoculated with both HEV and PCV2, following the same inoculation
84 protocols as for the other groups. In each of the six groups (HEV-only and HEV/PCV2), the three
85 inoculated piglets were in contact with three pen mates (contact piglets) from day 1. Individual
86 faecal samples were collected three days before inoculation and three times a week until the end of
87 the experiment at 49 days post inoculation (dpi). Blood samples were collected before inoculation
88 and once a week until the end of the experiment. Clinical examination was also performed (clinical
89 signs, rectal temperature, faeces consistence, weight, food consumption and trough cleanliness were
90 recorded daily). After euthanasia, necropsies were performed and organ and fluid samples collected,
91 among them liver and bile samples. The experiment was performed in accordance with EU and
92 French regulations on animal welfare in experiments. The protocol was approved (referral 17-022)
93 by the ANSES/ENVA/UPEC ethical committee registered under number #16.

94

95 2.2. Sample analyses

96 After performing manual total RNA extraction, HEV RNA in faecal samples was quantified using
97 real-time quantitative RT-PCR as described in Barnaud *et al.* (2012). Results were expressed in
98 HEV RNA copy number per gram of faeces (RNA copies/g). Since HEV shedding in faeces and
99 presence in serum have been shown to be correlated (Salines *et al.*, 2018), HEV RT-PCR was
100 performed on serum samples of 49 day-old pigs only if their faeces were positive at 46 and/or 49
101 dpi. Similarly, and as bile is considered as a relevant proxy of liver status (de Deus *et al.*, 2008;
102 Bouwknecht *et al.*, 2009), bile samples of 49-day old pigs having positive faecal samples at 46
103 and/or 49 dpi were analysed. Anti-HEV antibodies were detected using the HEV ELISA 4.0V kit

104 (MP Diagnostics, Illkirch, France) according to the manufacturer's instructions, apart from the
105 serum quantity used (10 μ L instead of the recommended 20 μ L). Samples were considered to be
106 positive when their optical density (OD) at a wavelength of 450 nm was higher than the threshold,
107 which was defined as the mean optical density of negative control pig samples +0.3. PCV2 DNA
108 was extracted and quantified from the serum using real-time PCR based on TaqMan technology as
109 described in Grasland *et al.* (2005). Results were expressed in genomic equivalent DNA copies/mL
110 of serum. PCV2-antibodies were detected by PCV2 specific ELISA as already described with a
111 positive cut-off for OD ratios higher than 1.5 (Fablet *et al.*, 2017).

112

113 2.3. Statistical analyses

114 The infectious period and time prior to HEV seroconversion were estimated using survival analyses.
115 Two parametric models were tested (lognormal and Weibull survival time distributions) and
116 compared using the Akaike Information Criterion (AIC). Cox-proportional hazard models were
117 used to assess the effect of PCV2 co-infection on the lengths of the infectious period and the time
118 prior to HEV seroconversion. The distributions of individual HEV viral loads in faeces were
119 analysed according to time since inoculation (with and without co-infection). A linear mixed model
120 taking into account repeated measurements over time was used for this investigation in order to
121 assess the different quantities of HEV particles shed by co-infected as opposed to HEV-only
122 infected pigs.

123

124 The HEV infection dynamics in each group were modelled using a SEIR (Susceptible – Exposed –
125 Infectious – Recovered) model as per the estimation process described in Gallien *et al.* (2018).
126 Briefly, pigs were considered as “susceptible” during the time window from exposure (day 0 = day
127 of inoculation) to the point at which they actually became infected (t_{mf}), progressing to the
128 “exposed” state. The time at which individuals were considered to be “infectious” (i.e. began
129 shedding), denoted t_{sh} , was considered to lie between the times of the last HEV-negative PCR

130 sample (t_{neg}) and the first HEV-positive PCR faecal sample (t_{pos}) for each animal ($t_{neg} < t_{sh} <$
131 t_{pos}). The latency period δ_E therefore corresponds to the delay between infection and shedding
132 ($\delta_E = t_{sh} - t_{inf}$). Pigs were considered “recovered” as soon as they no longer produced HEV-
133 positive PCR samples. Two transmission routes were considered to be involved in this infection
134 process: transmission by direct contact between pen mates and oro-faecal transmission via the
135 environmental compartment. Environmental viral load E_t represents the accumulation of viral
136 particles in the environment through faecal shedding by infected animals. E_t is partially offset by its
137 clearance rate ($\delta = 0.3 \text{ day}^{-1}$) and was calculated as described in Andraud *et al.* (2013) and
138 Salines *et al.* (2015). Let β_{DC} and β_{Env} denote direct contact and environmental transmission rates,
139 respectively. The force of infection exerted on a typical susceptible individual i located in pen k at
140 time t is defined by:

$$\lambda_k(t) = \beta_{DC} \frac{I_k(t)}{n-1} + \beta_{Env} \frac{E_k(t)}{n},$$

141 where I and E respectively represent the number of infectious animals and the viral load in pen k at
142 time t , n being the total number of pigs in each pen. With these notations, the probability p_i of
143 individual i getting infected at time $T_{inf}^{(i)}$ is given by

$$p_i = 1 - \exp\left(-\lambda_k\left(T_{inf}^{(i)}\right)\right)$$

144 while the probability of having escaped infection in time interval $[0, t_{inf}^{(i)}]$ is given by

$$145 \quad q_i = \exp\left(-\int_0^{T_{inf}^{(i)}} \lambda_k(\tau) d\tau\right).$$

146 An informative gamma prior was used to analyse the duration of the latency period δ_E . Its
147 parameters were fixed using data from previous experiments and from observations of inoculated
148 pigs ($\alpha = 4, \kappa = 3$). Very wide normal distributions were initially used as prior for the log-
149 transformed transmission rates ($\log(\beta_{DC}) \sim N(-2, 4)$ and $\log(\beta_{Env}) \sim N(-8, 4)$). The global
150 likelihood can be written as:

$$\begin{aligned}
& L(T_{Neg}, T_{Pos}, I, E | \beta_{DC}, \beta_{Env}, \delta_E, T_{inf}, \alpha, \kappa) \\
= & \prod_{i \in \text{contact-infected}} e^{-\int_0^{T_{inf}^{(i)}} \lambda(\tau) d\tau} \times \left(1 - e^{-\lambda(T_{inf}^{(i)})}\right) * \gamma(\delta_E^{(i)}, \alpha, \kappa) \\
& \times \prod_{i \in \text{contact-non infected}} e^{-\int_0^{\max(t_{obs})} \lambda(t) dt} \\
& \times \prod_{i \in \text{inoculated}} \gamma(\delta_E^{(i)}, \alpha, \kappa)
\end{aligned}$$

151 The first term of the likelihood denotes the probability of detected infections occurring for an
152 individual i at time $T_{inf}^{(i)}$; the second term represents the probability of observed infection failure
153 whenever some individual would remain susceptible throughout the experiment; and the third term
154 gives the distribution of the latency period in seeder pigs. Bayesian inference was performed using
155 the Metropolis-Hastings algorithm: ten independent chains of 50,000 iterations were run with a
156 burn-in period of 10%. Initial values were randomly drawn from prior distributions. Convergence
157 was assessed by inspecting parameter outputs visually as well as through conventional diagnostic
158 tests (Heidelberger, Geweke and Gelman-Rubin diagnostics). The impact of PCV2 infection on the
159 HEV latency period and the transmission parameters' distribution were then assessed using a
160 Kruskal-Wallis test. All the analyses were performed using R software (R 3.5.1).

161

162 **3. Results**

163

164 **3.1. Infection data**

165 No clinical sign related to PCV2 or HEV infection was observed in any infected pig. All PCV2
166 inoculated pigs and pigs in contact were seropositive at 28 dpi except one that was found
167 seropositive at 45 dpi (Supplementary File 1). Control pigs and HEV-only inoculated pigs remained
168 PCV2 seronegative throughout the study. PCV2 viraemia in contact pigs started between 10 and 28
169 dpi and lasted until 28 to 49 dpi. Viral loads ranged between 1.10^3 and 8.10^6 genomic equivalent
170 DNA copies/mL of serum with a viraemia peak around 17 days post-inoculation (Supplementary

171 File 1). HEV infection data are presented in Figures 2 and 3 for quantitative RT-PCR on faecal
172 samples and serological results respectively. All but two animals (one HEV/PCV2-inoculated pig
173 and one HEV contact pig) shed HEV during the experiment. Inoculated animals started to shed
174 HEV between 11 and 25 dpi, and contact animals between 23 and 46 dpi. Sporadic or intermittent
175 shedding was observed in a few animals (Figure 2). Of the 36 pigs, 20 produced anti-HEV
176 antibodies: 14 of the 18 HEV-only infected pigs versus just six of the 18 HEV/PCV2 co-infected
177 pigs. Seroconversion occurred between 24 and 49 dpi for inoculated animals, and between 38 and
178 45 dpi for contact animals (Figure 3). At the end of the experiment, four out of the 17 analysed pigs
179 (23%) had HEV RNA in their bile and one of them was viraemic (6%), with a viral load of $4.7 \cdot 10^3$
180 RNA copies/mL (Figure 2). These positive pigs were HEV/PCV2 co-infected (both inoculated and
181 contact pigs).

182

183 3.2. Estimated durations related to HEV infection dynamics

184 Latency periods were estimated at 12.3 days [4.4-25.5] in HEV-only pigs and 11.6 days [2-21.6] in
185 HEV/PCV2 co-infected pigs. The latency period was significantly shorter in HEV/PCV2 co-
186 infected pigs than in HEV-only infected pigs ($p < 0.05$).

187 Survival analysis of the infectious period (lognormal distribution) gave a mean duration of 11.8
188 days [8.3-16.7] for HEV-only infected animals and 16.6 days [10.7-25.9] for HEV/PCV2 co-
189 infected animals. No statistical difference was found between HEV-only and HEV/PCV2-infected
190 pigs (HR = 0.6 [0.3-1.4], $p > 0.05$).

191 Survival analysis of the time prior to HEV seroconversion (using the Weibull distribution) gave a
192 mean duration of 25.6 days [19.3-33.8] for HEV-only infection and 49.4 days [40.4-60.4] for
193 HEV/PCV2 co-infection. The time prior to HEV seroconversion was statistically longer in
194 HEV/PCV2- than in HEV-only infected pigs (HR = 0.3 [0.1-0.8], $p < 0.05$).

195

196 3.3. HEV shedding and environmental accumulation

197 The distribution of the shed HEV viral load against time (with and without co-infection) is shown in
198 Figure 4. The linear mixed model accounting for repeated measurements did not show the PCV2
199 infection to have any impact on the quantity of HEV particles shed by inoculated or contact animals
200 ($p > 0.05$). The viral load accumulated in the environment was modelled for each experimental pen.
201 The environment was HEV-free until 15 to 20 dpi, when the environmental load increased and
202 reached $4 \cdot 10^5$ to $2 \cdot 10^6$ before dropping at the end of the trial (data not shown) when there were no
203 remaining shedders in the pen.

204

205 3.4. HEV transmission parameters

206 In our experimental settings, a single HEV-only infected pig was able to infect 0.04 pigs per day by
207 direct contact ($\beta_{DC} = 0.04 [2 \cdot 10^{-5} - 0.24]$), whereas the direct transmission rate for HEV/PCV2 co-
208 infected pigs was estimated to be significantly higher, with a three-fold difference ($0.12 [5 \cdot 10^{-4} -$
209 $0.4]$; Figures 5 and 6). The environmental transmission rate β_{Env} can be considered as the average
210 number of animals that a single genome equivalent is able to infect when present in the pen
211 environment. β_{Env} was estimated at $1.5 \cdot 10^{-5}$ g/RNA copies/day [$2 \cdot 10^{-6}$; $4 \cdot 10^{-5}$] when pigs were
212 HEV-only infected versus $4.3 \cdot 10^{-6}$ g/RNA copies/day [$7 \cdot 10^{-8}$; $1.3 \cdot 10^{-5}$] when pigs were
213 HEV/PCV2 co-infected (Figures 5 and 6). It was statistically lower in cases of HEV/PCV2 co-
214 infection than for HEV-only infected pigs ($p < 0.05$).

215

216 4. Discussion

217

218 Understanding factors likely to influence HEV infection dynamics on pig farms is a pivotal step in
219 the design of HEV surveillance and control programmes aiming to mitigate the risk of human
220 exposure to HEV. Of those factors, immunomodulating pathogens are suspected to play a key role
221 and PRRSV has previously been shown to strongly influence HEV infection dynamics (Salines *et*

222 *al.*, 2015). The main aim of the present study was to investigate the potential impact of PCV2 co-
223 infection on HEV infection dynamics under experimental conditions.

224 PCV2 infection dynamics in our experimental settings did not differ from data in the available
225 literature (Andraud *et al.*, 2008), suggesting that HEV did not impact PCV2 dynamics. Animal
226 follow-up showed high inter-individual variability of HEV infection dynamics, both in HEV-only
227 and HEV/PCV2-infected pigs, with average latency periods of 12.3 and 11.6 days, and infectious
228 periods of 11.8 and 16.6 days respectively. This high variability was already highlighted in
229 previously-published studies on the topic, especially in cases of natural infection by the oral route
230 (Bouwknegt *et al.*, 2009; Andraud *et al.*, 2013; Salines *et al.*, 2015). This variability was taken into
231 account for the parameter estimation by taking uninformative or little informative prior
232 distributions; algorithm convergence therefore allows to gain confidence in the obtained results. For
233 the HEV-only infected group, the infection kinetics slightly vary from those described in Andraud
234 *et al.* (2013), who reported a latency period of 6.9 days [5.8-7.9] and an infectious period of 9.7
235 days [8.2-11.2]. This gap may be related to the different HEV strain used for inoculation (strain FR-
236 SHEV3e in Andraud *et al.* (2013), versus strain FR-SHEV3f in the present trial) as well as to the
237 lower inoculation dose (10^7 genomic equivalent in the present experiment versus 10^8 in the
238 HEV/PRRSV experiment). In the trial described by Bouwknegt *et al.* (2009), the infectious period
239 was estimated at between 13 and 49 days, depending on the replicate block, but their pigs were
240 intravenously inoculated (versus oral inoculation in the present experiment).

241 From our analyses, no statistical difference was found between HEV-only and HEV/PCV2 groups,
242 either in the infectious period, or in the quantity of HEV shed in faeces. The latency period was
243 found to be less than one day shorter in HEV/PCV2 co-infected pigs than in HEV-only infected
244 pigs which, although statistically significant, is likely to have a limited biological impact on HEV
245 infection dynamics. The direct transmission rate of HEV was found to be three times higher in cases
246 of HEV/PCV2 co-infection than in cases of HEV-only infection (0.12 versus 0.04), meaning that
247 one co-infected pig is likely to infect three times more pigs than a pig infected only with HEV. The

248 environmental transmission rate of HEV was found to be lower in cases of HEV/PCV2 co-infection
249 ($4.3 \cdot 10^{-6}$ versus $1.5 \cdot 10^{-5}$ g/RNA copies/day for HEV-only infected pigs), meaning that three times
250 more HEV particles in the environment are needed in order to infect a pig already carrying PCV2.
251 The lower environmental force of infection in cases of PCV2 infection may delay HEV infections.
252 Short time to slaughter after HEV infection seems to be a key point of liver contamination. Thus,
253 delaying HEV infection is likely to increase the risk of pig livers containing HEV at slaughter time.
254 Regarding immune response, fewer HEV/PCV2-infected pigs than HEV-only infected pigs
255 presented a humoral immune response (6/18 versus 14/18 pigs, respectively). Moreover, the time
256 prior to HEV seroconversion was 1.9 times longer in HEV/PCV2 co-infected pigs than in HEV-
257 only infected pigs (49.4 versus 25.6 days). This could be especially problematic if pig HEV status is
258 screened using serological method: this long time prior to HEV seroconversion would lead to many
259 false negative animals. Although PCV2 did not affect HEV infection dynamics as much as PRRSV
260 did in the trial that we previously conducted (Salines *et al.*, 2015), it cannot be excluded that in
261 combination with other factors, as for PMWS, it may influence HEV infection. This is consistent
262 with the immunomodulating effect of both PCV2 and PRRSV described in literature, where innate
263 immunity is somewhat suppressed due to a reduction in the IFN α response, delaying the onset of the
264 adaptive response (Darwich et Mateu, 2012; Butler *et al.*, 2014). Four out of the 17 tested pigs had
265 HEV RNA in the bile at the end of the experiment, which can be considered as a reliable proxy of
266 the liver contamination. This late-stage positivity illustrates the increased risk of having HEV
267 positive livers entering the food chain when animals were co-infected. Moreover, the detection of
268 one HEV/PCV2 co-infected pig being HEV viraemic at the end of the experiment also raises the
269 question of a potential risk linked to other pork products that is still debated in the literature (Salines
270 *et al.*, 2018). Further analyses would be necessary to assess the level of contamination of pig
271 muscles in cases of PCV2 infection, especially as correlations between HEV RNA levels in
272 muscles, liver and faeces have been shown (Salines *et al.*, 2018). Such analyses could inform on the
273 risk for public health linked to the consumption of undercooked or raw pig meat or other pork

274 products that do not contain liver. Our present results could also be used to feed dynamic models
275 representing HEV spread and persistence on farms in which PCV2 may circulate. Our data,
276 obtained under controlled conditions, can also add supplementary explanations to the previously
277 published field studies in which HEV and PCV2 were detected simultaneously in pigs and in which
278 causal relationship was suspected but not demonstrated (Martin *et al.*, 2007; Hosmillo *et al.*, 2010;
279 Savic *et al.*, 2010; Yang *et al.*, 2015; Jackel *et al.*, 2018). Further work is needed to investigate
280 whether there are other underlying immune mechanisms specific to co-infecting viruses. Moreover,
281 it should be noted that the pigs in the present experiment were simultaneously inoculated with HEV
282 and PCV2; the same kind of study could be reproduced with different inoculation time sequences
283 (e.g. pigs inoculated with PCV2 a week before HEV) and probably with more pigs included to
284 reduce the impact of inter-individual variability in infection dynamics.

285

286 To our knowledge, this study is the first to focus on the impact of HEV/PCV2 experimental co-
287 infection on HEV infection and transmission in pigs. Our results show that, in experimental
288 settings, PCV2 co-infection increases the direct transmission of HEV and impairs the humoral
289 immune response towards it. The effect observed in this PCV2/HEV co-infection trial was less
290 marked than previously observed when PRRSV was involved, however, and failed to explain the
291 long-term HEV shedding that has been observed in the field at an individual level. A combination
292 of PCV2 co-infection with other factors may lead to chronic HEV infection. Additional studies (e.g.
293 on-farm intervention studies, other co-infection trials, dynamic modelling approaches) should
294 therefore be conducted to explore the potential synergistic effects of multiple co-infections and
295 devise effective control strategies that would include measures targeting intercurrent pathogens
296 (vaccination, eradication programme).

297

298 **Figures**

299 **Figure 1.** Experimental design of the HEV/PCV2 co-infection trial.

300 **Figure 2.** HEV RNA quantification in faecal, bile and serum samples from HEV-only and
301 HEV/PCV2-infected pigs (inoculated and contact animals, n=36). In yellow: Quantitative HEV RT-
302 PCR results for individual faecal samples (HEV RNA copies/g of faeces) at each sampling time.
303 Shaded zones correspond to periods during which infected individuals were considered as
304 “infectious”, corresponding to the time between the first and final HEV-positive faecal samples for
305 each animal. In blue and red: Quantitative HEV RT-PCR for bile and serum samples respectively
306 (HEV RNA copies/mL) of 49 day-old pigs for which faecal samples were positive at 46 and/or 49
307 dpi. dpi: days post inoculation; nd: not detected, na: not analysed.

308 **Figure 3.** Kinetic of HEV seroconversion. Results for individual sera samples (in different colours
309 and shape) from HEV/PCV2-infected pigs (upper panel) and HEV-only (lower panel) (inoculated
310 and contact animals, n=36). OD: optical density; cut off value = 0.3.

311 **Figure 4.** Distribution of the number of HEV genome equivalents (log RNA copies/g faeces) shed
312 by individual pigs, versus time, in HEV inoculated and contact animals with or without PCV2 co-
313 infection (n=36).

314 **Figure 5.** Running average of transmission parameter estimates from ten independent Monte-Carlo
315 Markov chains for (a) HEV-only and (b) HEV/PCV2-infected groups.

316 **Figure 6.** Distribution of direct and environmental HEV transmission parameters estimated from
317 ten independent Monte-Carlo Markov chains.

318

319 **Supplementary Files**

320 **Supplementary File 1.** PCV2 DNA quantification in serum (a) and PCV2 antibodies detection (b)
321 from HEV-only and HEV/PCV2 infected pigs (inoculated and contact animals, n=36).

322

323 **Authors’ contributions and acknowledgements**

324 MS and MA developed the mathematical model, analysed the data and drafted the manuscript. MP
325 and CB analysed the HEV and PCV2 samples respectively, and interpreted the results. NP and BG

326 supervised the HEV- and PCV2-related laboratory work respectively, and helped coordinate the
327 study. NR conceived and coordinated the study, in addition to participating in the animal
328 experiment and data analyses. All the co-authors revised the manuscript and approved the final
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331 experiment.

332

333 **Competing interests and funding**

334 The authors declare that they have no competing interests. The trial was funded by INAPORC.
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336

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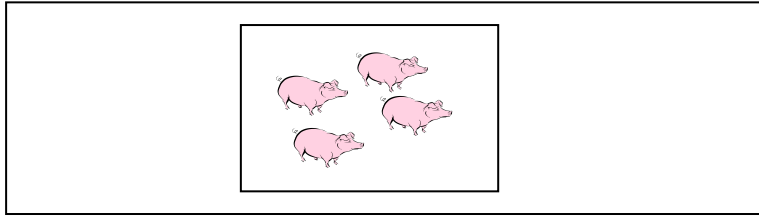
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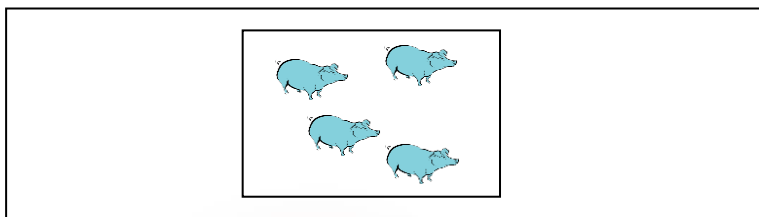
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Room 1



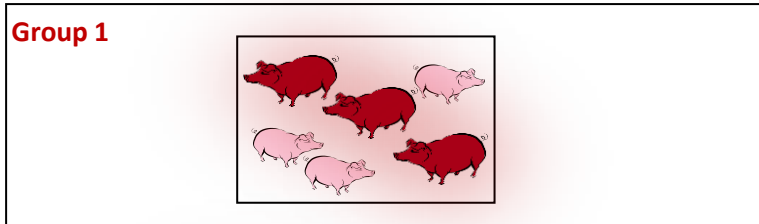
Contact pigs or negative controls

Room 2



PCV2 inoculated pigs

Room 3

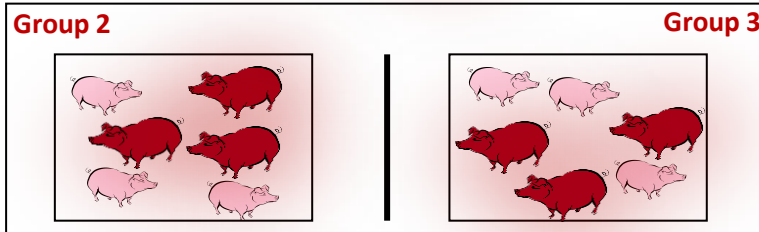


HEV/PCV2 inoculated pigs



HEV-only inoculated pigs

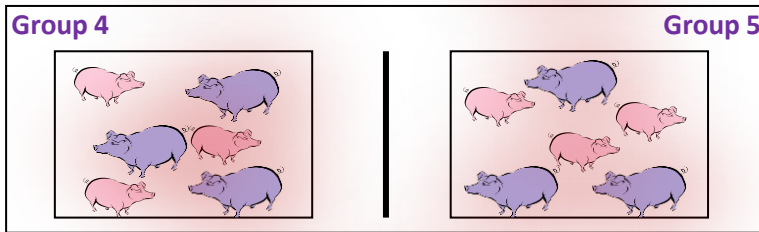
Room 4



Group 2

Group 3

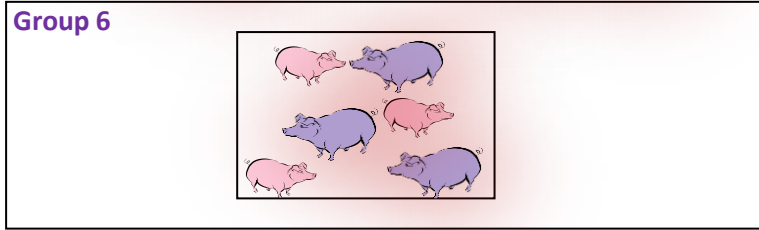
Room 5



Group 4

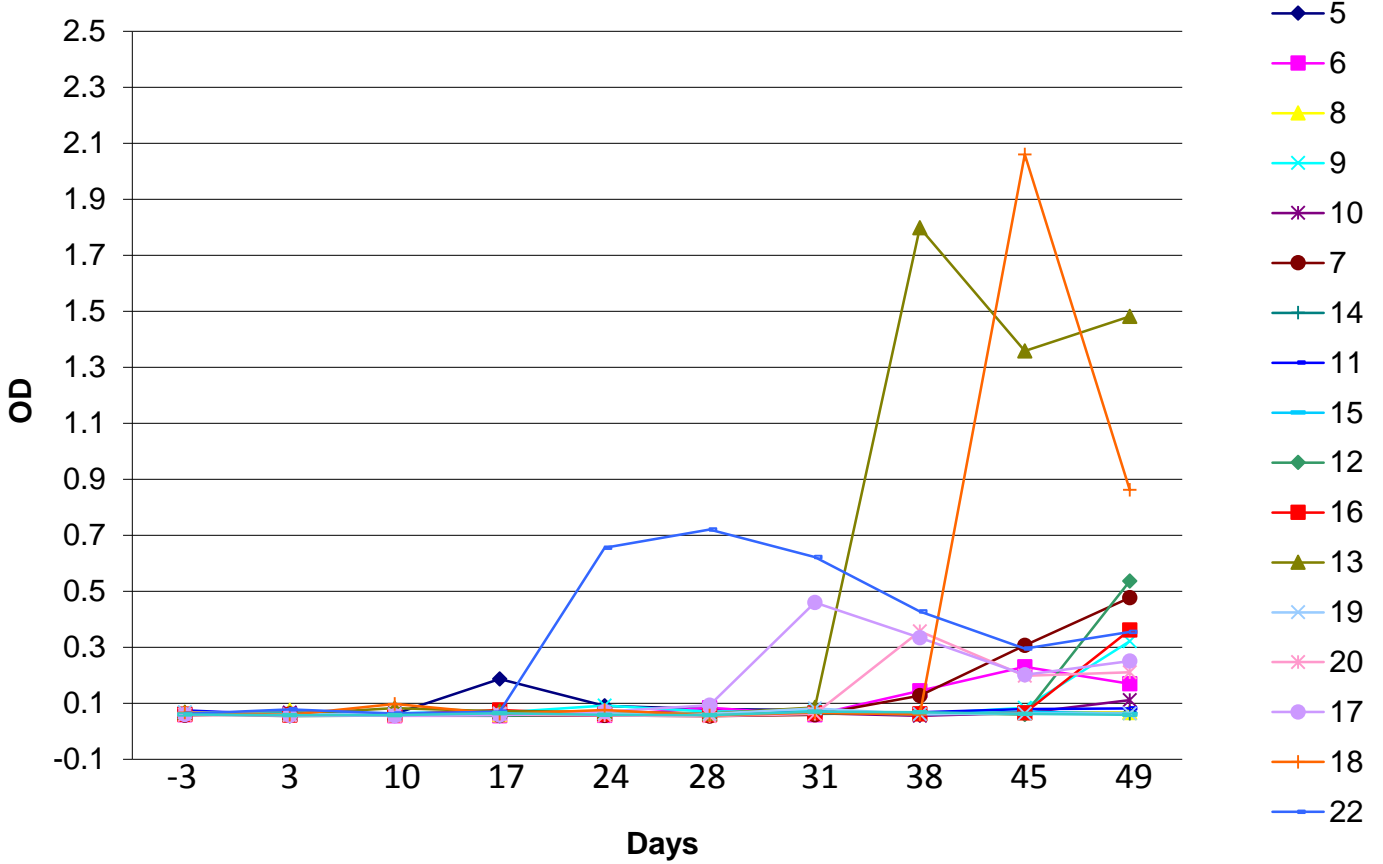
Group 5

Room 6

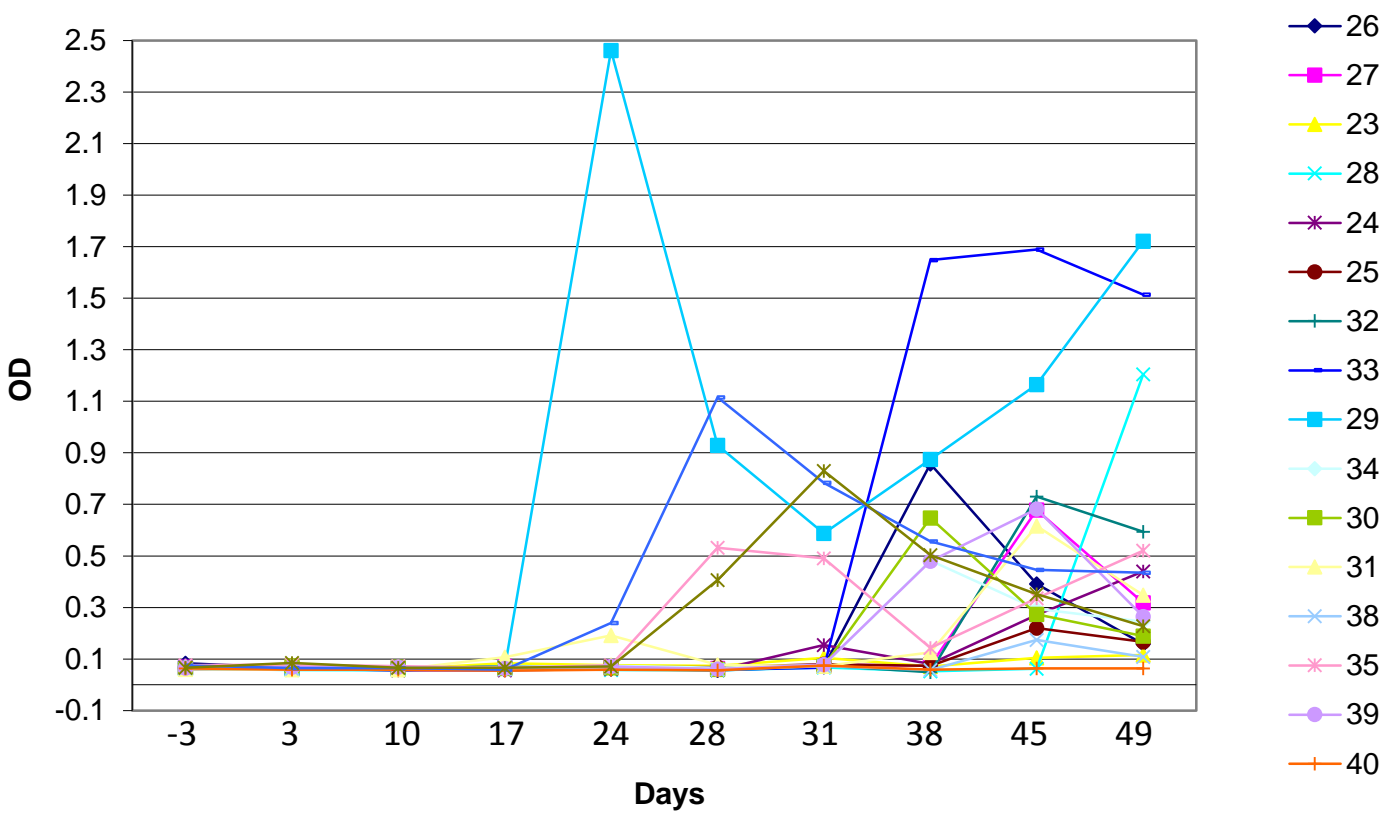


Group 6

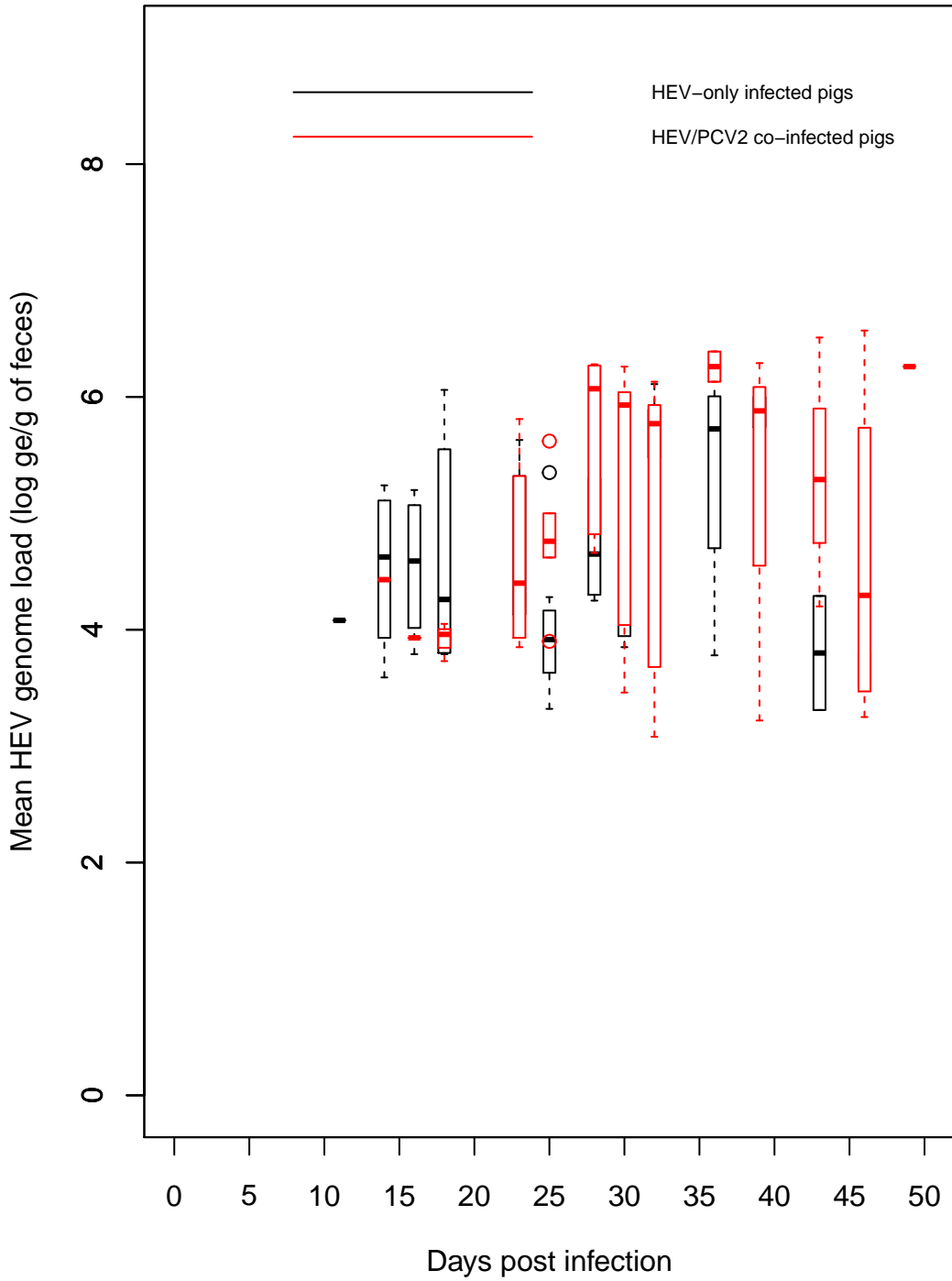
HEV/PCV2 co-infected pigs



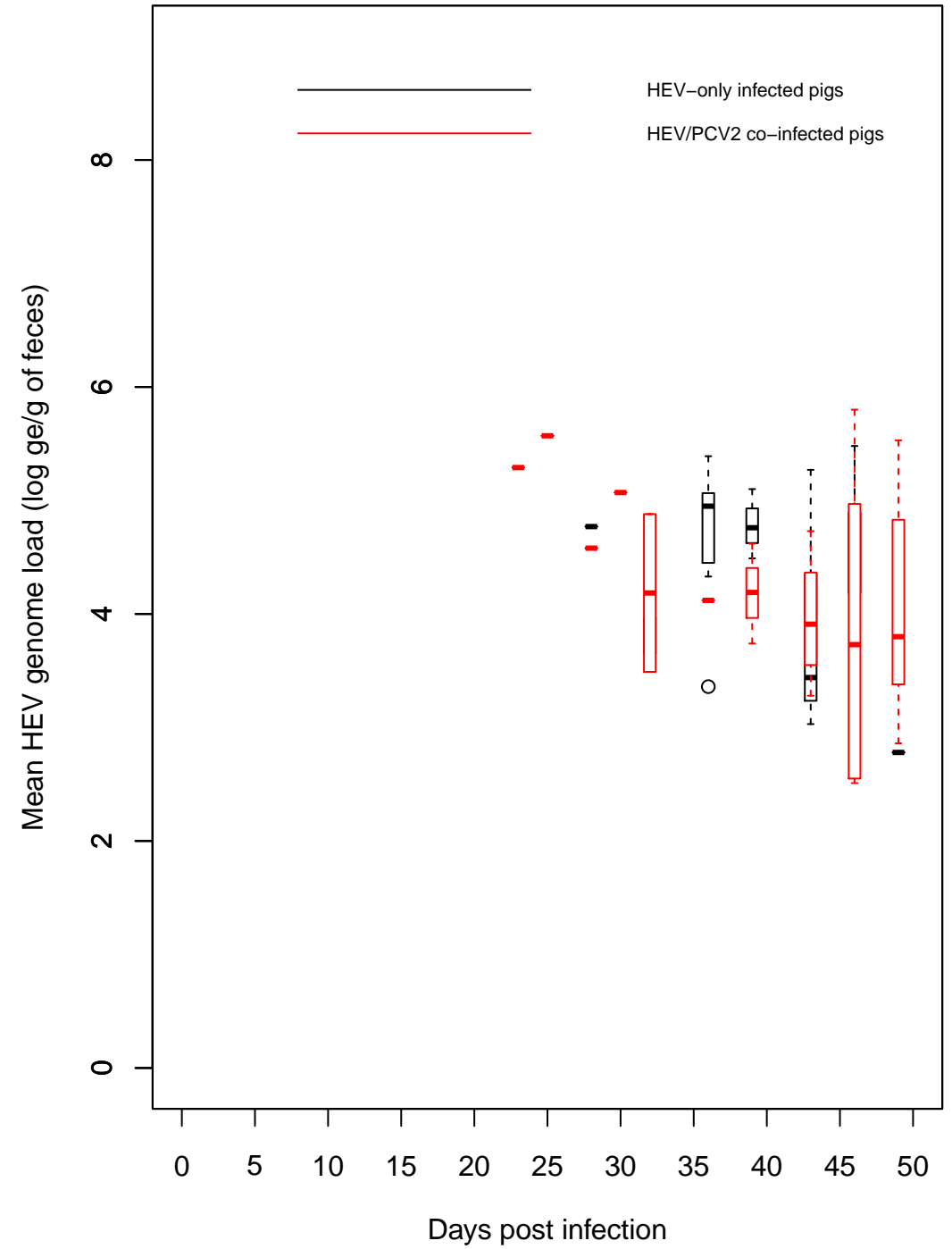
HEV-only infected pigs



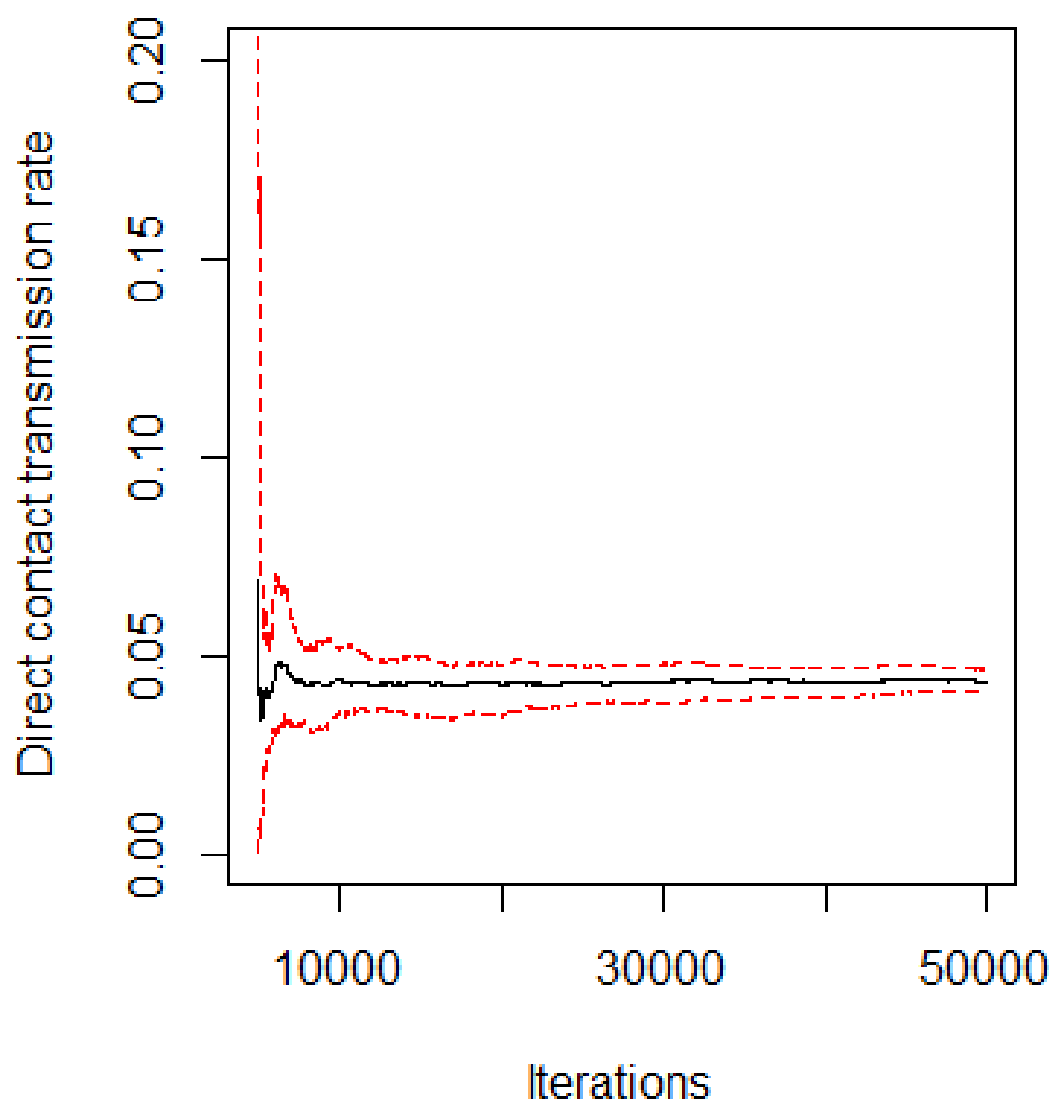
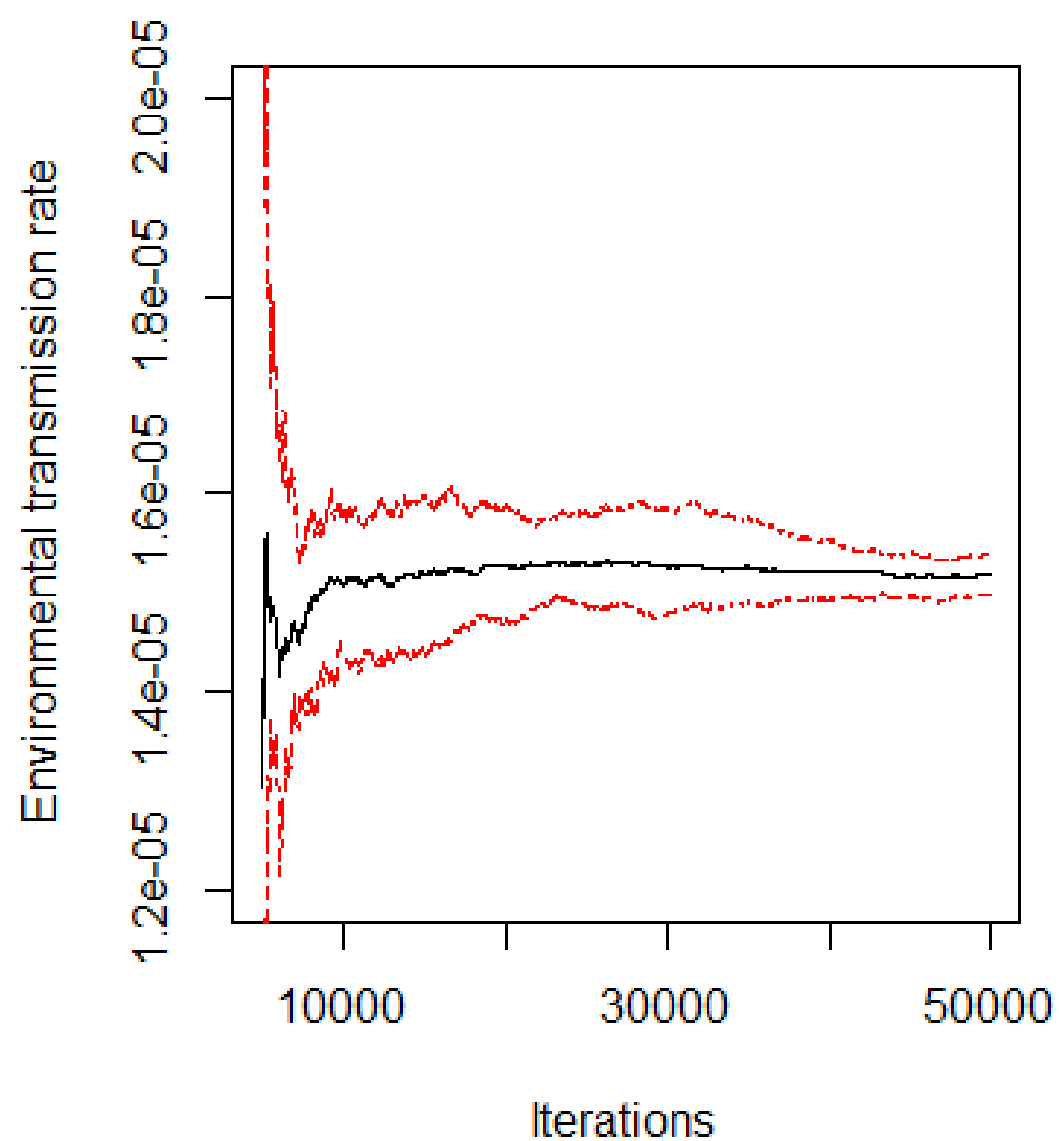
Inoculated pigs



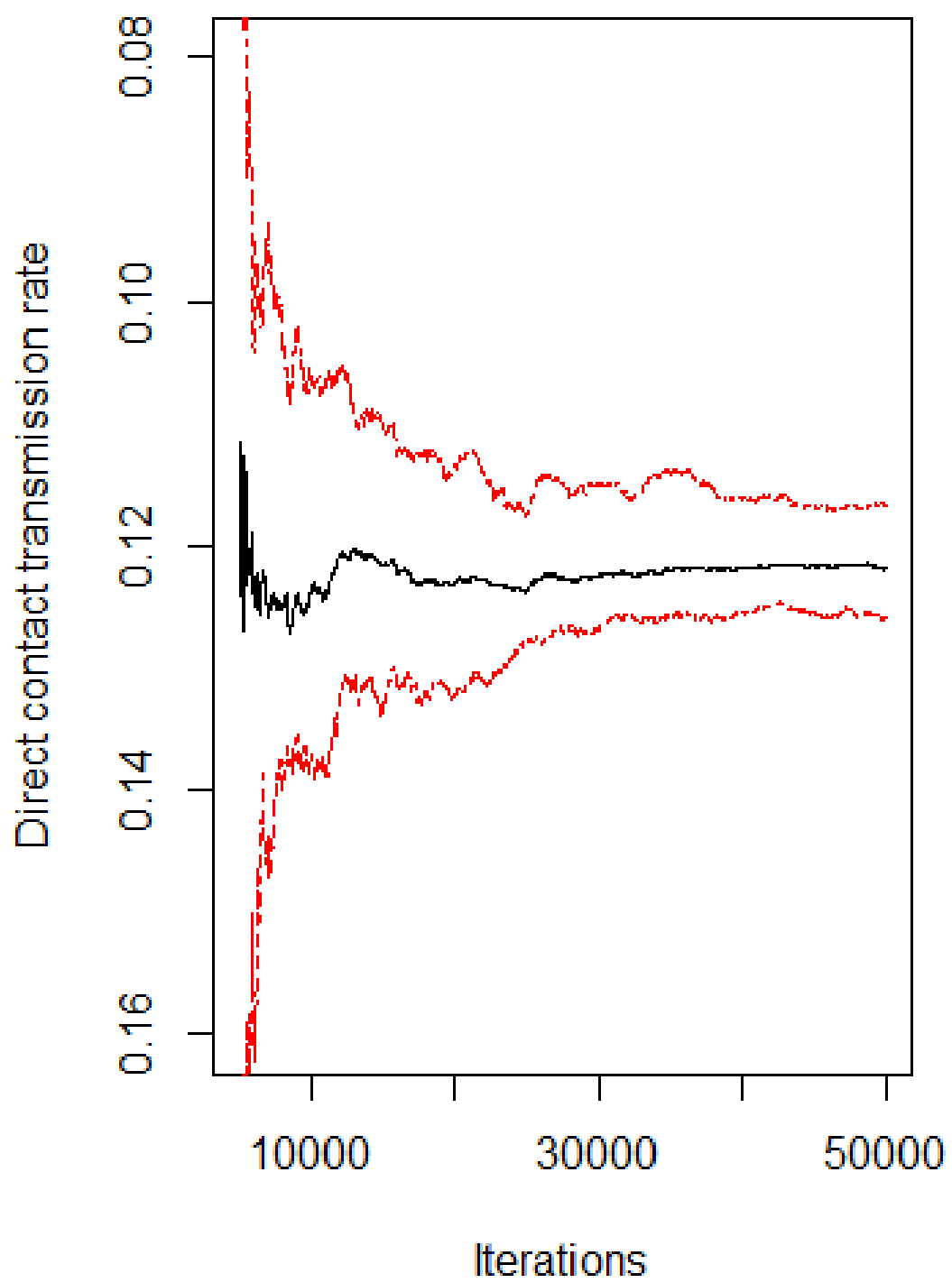
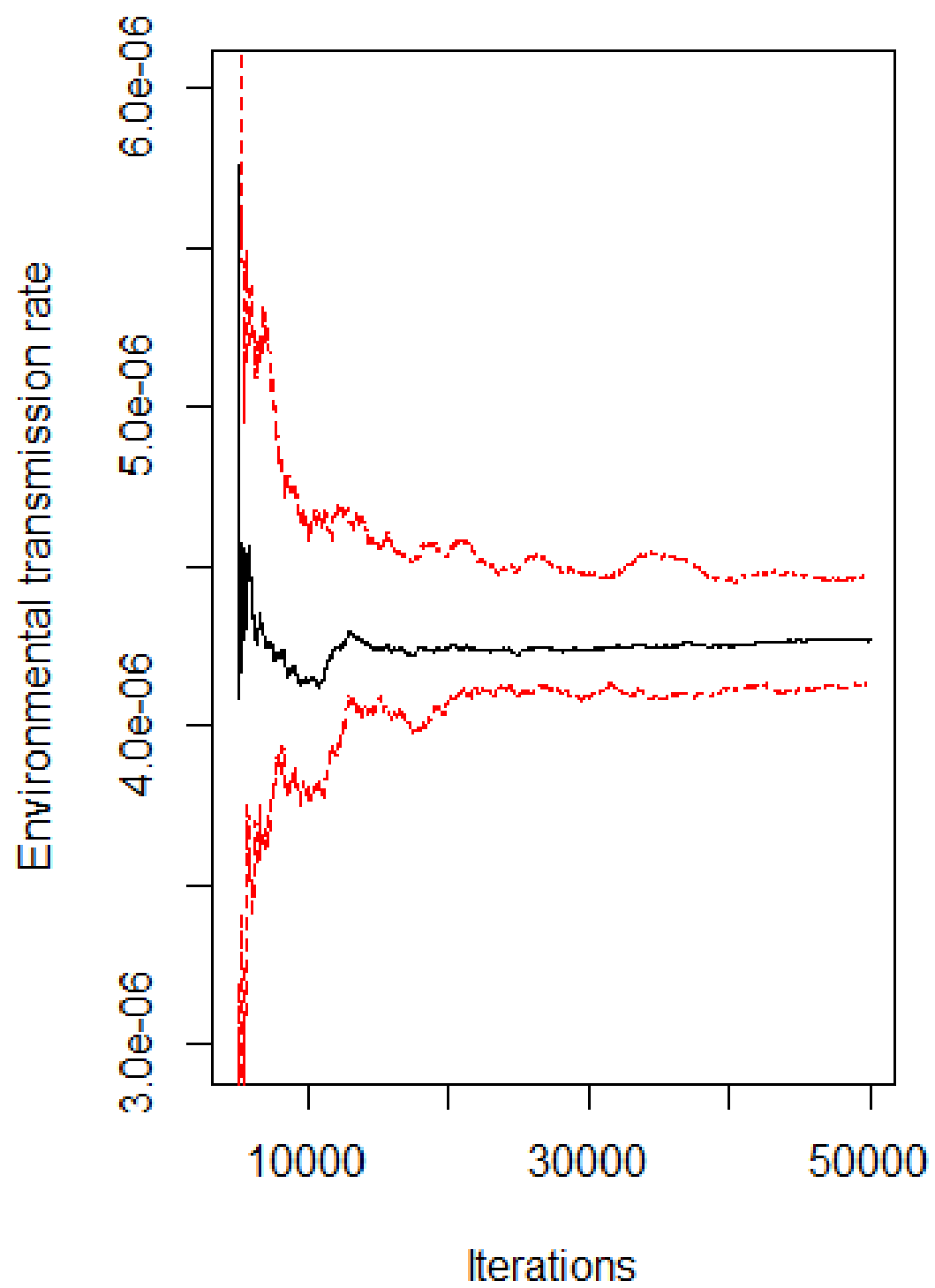
Contact pigs



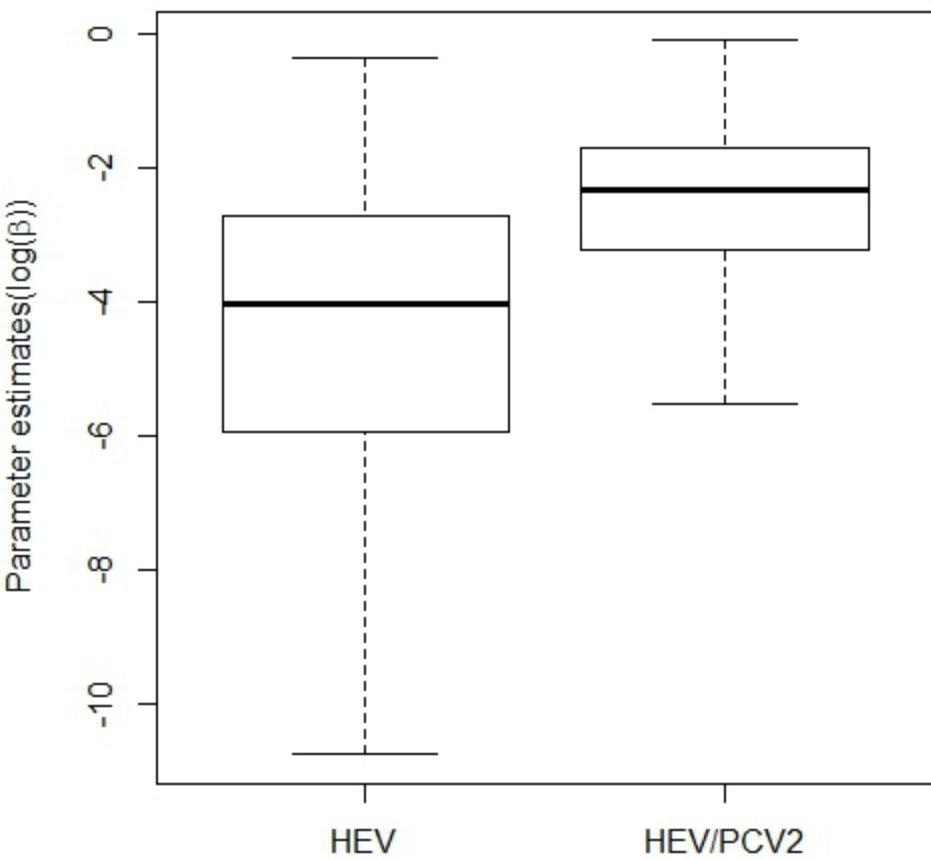
a. HEV-only infected groups



b. HEV/PCV2 co-infected groups



Direct transmission rate distribution



Environmental transmission rate distribution

