



Short communication

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



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ABSTRACT

Hepatitis E virus is a zoonotic pathogen for which pigs have been identified as the main reservoir in industrialised countries. HEV infection dynamics in pig herds and pigs are influenced by several factors, including herd practices and possibly co-infection with immunomodulating viruses. This study therefore investigates the impact of porcine circovirus type 2 (PCV2) on HEV infection and transmission through experimental HEV/PCV2 co-infection of specific-pathogen-free pigs. No statistical difference between HEV-only and HEV/PCV2-infected animals was found for either the infectious period or the quantity of HEV shed in faeces. The HEV latency period was shorter for HEV/PCV2 co-infected pigs than for HEV-only infected pigs (11.6 versus 12.3 days). Its direct transmission rate was three times higher in cases of HEV/PCV2 co-infection than in cases of HEV-only infection (0.12 versus 0.04). On the other hand, the HEV transmission rate through environmental accumulation was lower in cases of HEV/PCV2 co-infection ($4.3 \cdot 10^{-6}$ versus $1.5 \cdot 10^{-5}$ g/RNA copies/day for HEV-only infected pigs). The time prior to HEV seroconversion was 1.9 times longer in HEV/PCV2 co-infected pigs (49.4 versus 25.6 days for HEV-only infected pigs). In conclusion, our study shows that PCV2 affects HEV infection and transmission in pigs under experimental conditions.

1. Introduction

Hepatitis E virus is a non-enveloped single-stranded RNA virus that can cause acute hepatitis in humans. Chronic cases have also been described, mainly in immunocompromised patients (Lhomme et al., 2016). Genotypes 3 and 4 affect both humans and other animal species, and are responsible for sporadic autochthonous cases of hepatitis in humans in industrialised countries (Doceul et al., 2016). In particular, genotype 3 is widespread in pig populations (Salines et al., 2017) and a number of autochthonous cases have been linked to the consumption of undercooked pork meat, especially liver products (Colson et al., 2012; Guillois et al., 2016). In order to limit the risk of contaminated products entering the food chain, it is crucial to understand the factors influencing HEV transmission and persistence in pig herds. High variability in HEV infection dynamics has previously been described (Salines et al.,

2017) and may be related to husbandry practices in terms of hygiene, biosecurity and rearing conditions (Walachowski et al., 2014; Lopez-Lopez et al., 2018) or to individual characteristics such as protection conferred by maternally-derived antibodies (Andraud et al., 2014). Various factors affecting swine immune response may also influence the course of HEV infection. Notably, in a previous study, we have shown that pigs experimentally co-infected with porcine reproductive and respiratory syndrome virus (PRRSV) exhibited chronic HEV infection with extended latency and infectious periods, increased faecal shedding and transmission, as well as an increased risk of HEV-positive livers at slaughter (Salines et al., 2015). Porcine circovirus type 2 (PCV2) also has immunomodulating characteristics for instance by inhibiting IFN- α production and by increasing the expression of IL-10, an anti-inflammatory cytokine (Darwich et Mateu, 2012). PCV2 may therefore impact HEV infection dynamics. Moreover, as the primary causative

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agent of post-weaning multisystemic wasting syndrome (PMWS) and other porcine circovirus-associated diseases (PCVADs), it can sometimes induce hepatitis in pigs (Rosell et al., 2000). However, to date, only few data report on HEV/PCV2 co-infection (Martin et al., 2007; Hosmillo et al., 2010; Savic et al., 2010; Yang et al., 2015; Jackel et al., 2018). In these studies, PCV2 and HEV were simultaneously detected in pigs but the impact of co-infections on HEV dynamics was not investigated.

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

2. Material and methods

2.1. Experimental design

The trial was conducted at ANSES's air-filtered level-3 biosecurity facilities. The 44 five-week-old SPF Large White piglets included in the study were HEV- and PCV2-free and with no maternal antibodies against these two viruses at the beginning of the study. These piglets were randomly allocated into eight groups, housed in six rooms (Fig. 1). Two negative control pigs were housed in Room 1. The four piglets housed in Room 2 were only orally inoculated with a PCV2-b genogroup suspension (GenBank accession number AF201311), titrating 10^5 TCID₅₀/mL in a volume of 5 mL. In Rooms 5 and 6 (groups 4, 5, 6), three piglets per group were orally inoculated with 10^7 HEV RNA copies of a genotype 3 HEV suspension (strain FR-SHEV3f, GenBank accession number JQ953666) in a volume of 10 mL. In Rooms 3 and 4 (groups 1, 2, 3), three piglets per group were orally inoculated with both HEV and PCV2, following the same inoculation protocols as for the other groups. In each of the six groups (HEV-only and HEV/PCV2), the three inoculated piglets were in contact with three pen mates (contact piglets) from day 1. Individual faecal samples were collected three days

before inoculation and three times a week until the end of the experiment at 49 days post inoculation (dpi). Blood samples were collected before inoculation and once a week until the end of the experiment. Clinical examination was also performed (clinical signs, rectal temperature, faeces consistence, weight, food consumption and trough cleanliness were recorded daily). After euthanasia, necropsies were performed and organ and fluid samples collected, among them liver and bile samples. The experiment was performed in accordance with EU and French regulations on animal welfare in experiments. The protocol was approved (referral 17-022) by the ANSES/ENVA/UPEC ethical committee registered under number #16.

2.2. Sample analyses

After performing manual total RNA extraction, HEV RNA in faecal samples was quantified using real-time quantitative RT-PCR as described in Barnaud et al. (2012). Results were expressed in HEV RNA copy number per gram of faeces (RNA copies/g). Since HEV shedding in faeces and presence in serum have been shown to be correlated (Salines et al., 2018), HEV RT-PCR was performed on serum samples of 49-day-old pigs only if their faeces were positive at 46 and/or 49 dpi. Similarly, and as bile is considered as a relevant proxy of liver status (de Deus et al., 2008; Bouwknegt et al., 2009), bile samples of 49-day old pigs having positive faecal samples at 46 and/or 49 dpi were analysed. Anti-HEV antibodies were detected using the HEV ELISA 4.0 V kit (MP Diagnostics, Illkirch, France) according to the manufacturer's instructions, apart from the serum quantity used (10 µL instead of the recommended 20 µL). Samples were considered to be positive when their optical density (OD) at a wavelength of 450 nm was higher than the threshold, which was defined as the mean optical density of negative control pig samples + 0.3. PCV2 DNA was extracted and quantified from the serum using real-time PCR based on TaqMan technology as described in Grasland et al. (2005). Results were expressed in genomic equivalent DNA copies/mL of serum. PCV2-antibodies were detected by PCV2 specific ELISA as already described with a positive cut-off for OD ratios higher than 1.5 (Fablet et al., 2017).

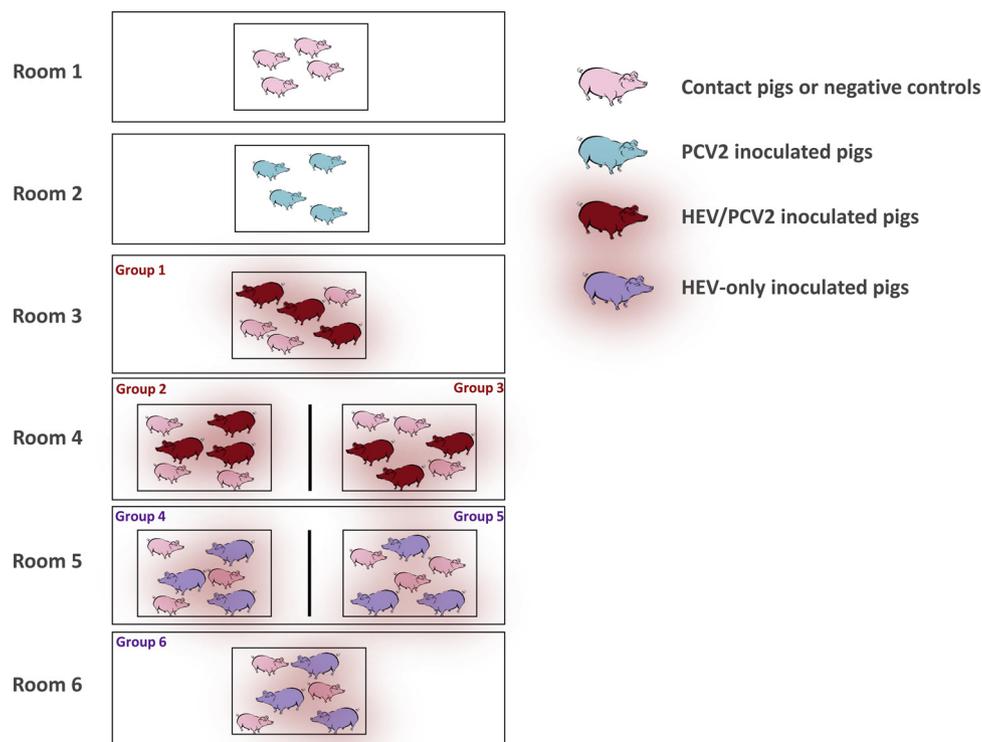


Fig. 1. Experimental design of the HEV/PCV2 co-infection trial.

2.3. Statistical analyses

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

The HEV infection dynamics in each group were modelled using a SEIR (Susceptible – Exposed – Infectious – Recovered) model as per the estimation process described in Gallien et al. (2018). Briefly, pigs were considered as “susceptible” during the time window from exposure (day 0 = day of inoculation) to the point at which they actually became infected (t_{inf}), progressing to the “exposed” state. The time at which individuals were considered to be “infectious” (i.e. began shedding), denoted t_{sh} , was considered to lie between the times of the last HEV-negative PCR sample (t_{neg}) and the first HEV-positive PCR faecal sample (t_{pos}) for each animal ($t_{neg} < t_{sh} < t_{pos}$). The latency period δ_E therefore corresponds to the delay between infection and shedding ($\delta_E = t_{sh} - t_{inf}$). Pigs were considered “recovered” as soon as they no longer produced HEV-positive PCR samples. Two transmission routes were considered to be involved in this infection process: transmission by direct contact between pen mates and oro-faecal transmission via the environmental compartment. Environmental viral load E_t represents the accumulation of viral particles in the environment through faecal shedding by infected animals. E_t is partially offset by its clearance rate ($\delta = 0.3 \text{ day}^{-1}$) and was calculated as described in Andraud et al. (2013) and Salines et al. (2015). Let β_{DC} and β_{Env} denote direct contact and environmental transmission rates, respectively. The force of infection exerted on a typical susceptible individual i located in pen k at time t is defined by:

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

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

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

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

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

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

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

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

3. Results

3.1. Infection data

No clinical sign related to PCV2 or HEV infection was observed in any infected pig. All PCV2 inoculated pigs and pigs in contact were seropositive at 28 dpi except one that was found seropositive at 45 dpi (Supplementary File 1). Control pigs and HEV-only inoculated pigs remained PCV2 seronegative throughout the study. PCV2 viraemia in contact pigs started between 10 and 28 dpi and lasted until 28–49 dpi. Viral loads ranged between 1.10^3 and 8.10^6 genomic equivalent DNA copies/mL of serum with a viraemia peak around 17 days post-inoculation (Supplementary File 1). HEV infection data are presented in Figs. 2 and 3 for quantitative RT-PCR on faecal samples and serological results respectively. All but two animals (one HEV/PCV2-inoculated pig and one HEV contact pig) shed HEV during the experiment. Inoculated animals started to shed HEV between 11 and 25 dpi, and contact animals between 23 and 46 dpi. Sporadic or intermittent shedding was observed in a few animals (Fig. 2). Of the 36 pigs, 20 produced anti-HEV antibodies: 14 of the 18 HEV-only infected pigs versus just six of the 18 HEV/PCV2 co-infected pigs. Seroconversion occurred between 24 and 49 dpi for inoculated animals, and between 38 and 45 dpi for contact animals (Fig. 3). At the end of the experiment, four out of the 17 analysed pigs (23%) had HEV RNA in their bile and one of them was viraemic (6%), with a viral load of $4.7.10^3$ RNA copies/mL (Fig. 2). These positive pigs were HEV/PCV2 co-infected (both inoculated and contact pigs).

3.2. Estimated durations related to HEV infection dynamics

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

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

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

3.3. HEV shedding and environmental accumulation

The distribution of the shed HEV viral load against time (with and without co-infection) is shown in Fig. 4. The linear mixed model accounting for repeated measurements did not show the PCV2 infection to

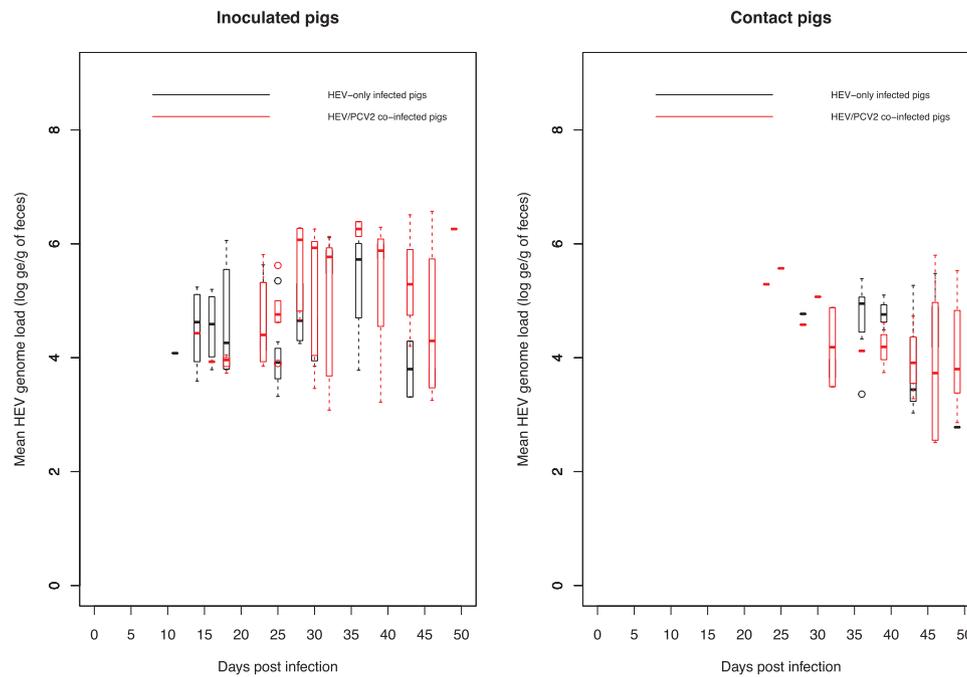


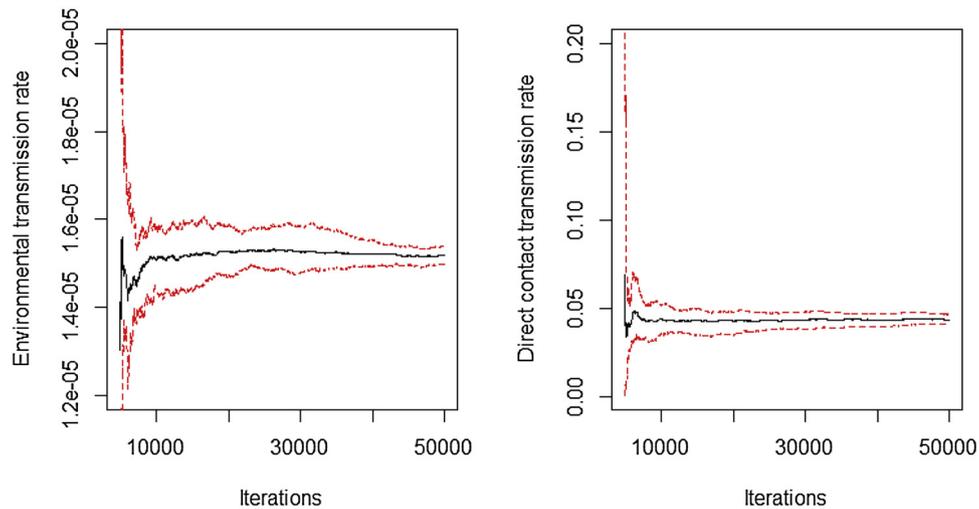
Fig. 4. Distribution of the number of HEV genome equivalents (log RNA copies/g faeces) shed by individual pigs, versus time, in HEV inoculated and contact animals with or without PCV2 co-infection ($n = 36$).

published studies on the topic, especially in cases of natural infection by the oral route (Bouwknegt et al., 2009; Andraud et al., 2013; Salines et al., 2015). This variability was taken into account for the parameter estimation by taking uninformative or little informative prior distributions; algorithm convergence therefore allows to gain confidence in the obtained results. For the HEV-only infected group, the infection kinetics slightly vary from those described in Andraud et al. (2013), who reported a latency period of 6.9 days [5.8–7.9] and an infectious period of 9.7 days [8.2–11.2]. This gap may be related to the different HEV strain used for inoculation (strain FR-SHEV3e in Andraud et al. (2013), versus strain FR-SHEV3f in the present trial) as well as to the lower inoculation dose (10^7 genomic equivalent in the present experiment versus 10^8 in the HEV/PRRSV experiment). In the trial described by Bouwknegt et al. (2009), the infectious period was estimated at between 13 and 49 days, depending on the replicate block, but their pigs were intravenously inoculated (versus oral inoculation in the present experiment).

From our analyses, no statistical difference was found between HEV-only and HEV/PCV2 groups, either in the infectious period, or in the quantity of HEV shed in faeces. The latency period was found to be less than one day shorter in HEV/PCV2 co-infected pigs than in HEV-only infected pigs which, although statistically significant, is likely to have a limited biological impact on HEV infection dynamics. The direct transmission rate of HEV was found to be three times higher in cases of HEV/PCV2 co-infection than in cases of HEV-only infection (0.12 versus 0.04), meaning that one co-infected pig is likely to infect three times more pigs than a pig infected only with HEV. The environmental transmission rate of HEV was found to be lower in cases of HEV/PCV2 co-infection ($4.3 \cdot 10^{-6}$ versus $1.5 \cdot 10^{-5}$ g/RNA copies/day for HEV-only infected pigs), meaning that three times more HEV particles in the environment are needed in order to infect a pig already carrying PCV2. The lower environmental force of infection in cases of PCV2 infection may delay HEV infections. Short time to slaughter after HEV infection seems to be a key point of liver contamination. Thus, delaying HEV infection is likely to increase the risk of pig livers containing HEV at slaughter time. Regarding immune response, fewer HEV/PCV2-infected pigs than HEV-only infected pigs presented a humoral immune response (6/18 versus 14/18 pigs, respectively). Moreover, the time prior to HEV

seroconversion was 1.9 times longer in HEV/PCV2 co-infected pigs than in HEV-only infected pigs (49.4 versus 25.6 days). This could be especially problematic if pig HEV status is screened using serological method: this long time prior to HEV seroconversion would lead to many false negative animals. Although PCV2 did not affect HEV infection dynamics as much as PRRSV did in the trial that we previously conducted (Salines et al., 2015), it cannot be excluded that in combination with other factors, as for PMWS, it may influence HEV infection. This is consistent with the immunomodulating effect of both PCV2 and PRRSV described in literature, where innate immunity is somewhat suppressed due to a reduction in the IFN α response, delaying the onset of the adaptive response (Darwich et Mateu, 2012; Butler et al., 2014). Four out of the 17 tested pigs had HEV RNA in the bile at the end of the experiment, which can be considered as a reliable proxy of the liver contamination. This late-stage positivity illustrates the increased risk of having HEV positive livers entering the food chain when animals were co-infected. Moreover, the detection of one HEV/PCV2 co-infected pig being HEV viraemic at the end of the experiment also raises the question of a potential risk linked to other pork products that is still debated in the literature (Salines et al., 2018). Further analyses would be necessary to assess the level of contamination of pig muscles in cases of PCV2 infection, especially as correlations between HEV RNA levels in muscles, liver and faeces have been shown (Salines et al., 2018). Such analyses could inform on the risk for public health linked to the consumption of undercooked or raw pig meat or other pork products that do not contain liver. Our present results could also be used to feed dynamic models representing HEV spread and persistence on farms in which PCV2 may circulate. Our data, obtained under controlled conditions, can also add supplementary explanations to the previously published field studies in which HEV and PCV2 were detected simultaneously in pigs and in which causal relationship was suspected but not demonstrated (Martin et al., 2007; Hosmillo et al., 2010; Savic et al., 2010; Yang et al., 2015; Jackel et al., 2018). Further work is needed to investigate whether there are other underlying immune mechanisms specific to co-infecting viruses. Moreover, it should be noted that the pigs in the present experiment were simultaneously inoculated with HEV and PCV2; the same kind of study could be reproduced with different inoculation time sequences (e.g. pigs

a. HEV-only infected groups



b. HEV/PCV2 co-infected groups

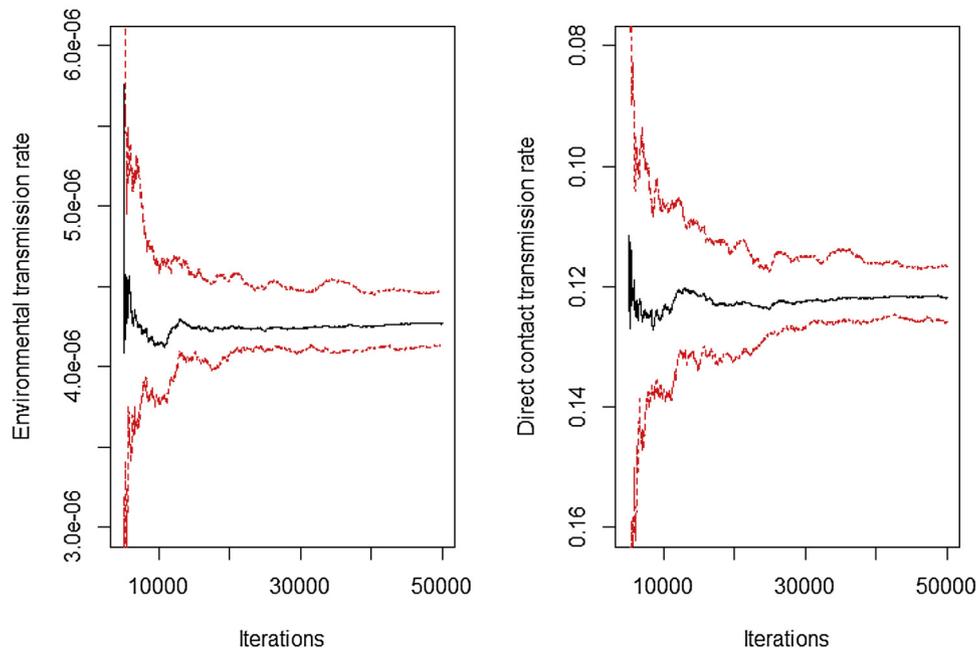


Fig. 5. Running average of transmission parameter estimates from ten independent Monte-Carlo Markov chains for (a) HEV-only and (b) HEV/PCV2-infected groups.

inoculated with PCV2 a week before HEV) and probably with more pigs included to reduce the impact of inter-individual variability in infection dynamics.

To our knowledge, this study is the first to focus on the impact of HEV/PCV2 experimental co-infection on HEV infection and transmission in pigs. Our results show that, in experimental settings, PCV2 co-infection increases the direct transmission of HEV and impairs the humoral immune response towards it. The effect observed in this PCV2/HEV co-infection trial was less marked than previously observed when PRRSV was involved, however, and failed to explain the long-term HEV shedding that has been observed in the field at an individual level. A combination of PCV2 co-infection with other factors may lead to

chronic HEV infection. Additional studies (e.g. on-farm intervention studies, other co-infection trials, dynamic modelling approaches) should therefore be conducted to explore the potential synergistic effects of multiple co-infections and devise effective control strategies that would include measures targeting intercurrent pathogens (vaccination, eradication programme).

Authors' contributions

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

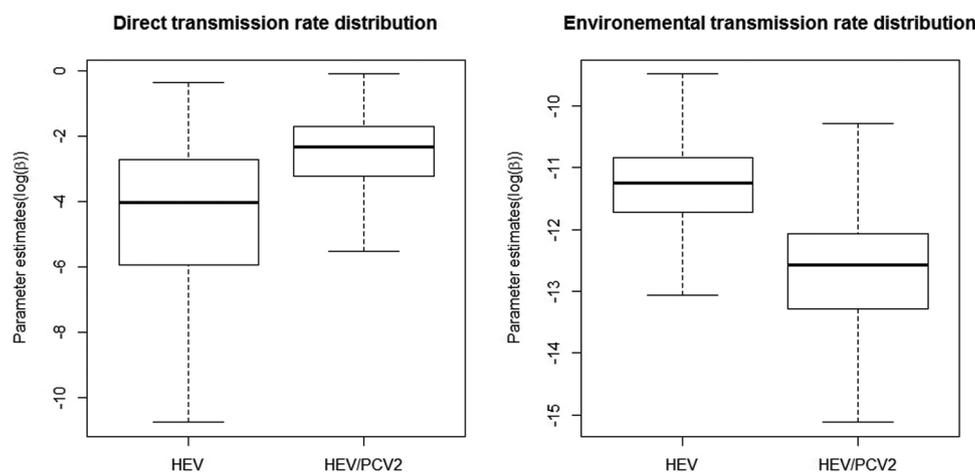


Fig. 6. Distribution of direct and environmental HEV transmission parameters estimated from ten independent Monte-Carlo Markov chains.

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

Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.vetmic.2019.05.010>.

References

- Andraud, M., Grasland, B., Durand, B., Cariolet, R., Jestin, A., Madec, F., Rose, N., 2008. Quantification of porcine circovirus type 2 (PCV-2) within- and between-pen transmission in pigs. *Vet. Res.* 39, 43.
- Andraud, M., Dumarest, M., Cariolet, R., Aylaj, B., Barnaud, E., Eono, F., Pavio, N., Rose, N., 2013. Direct contact and environmental contaminations are responsible for HEV transmission in pigs. *Vet. Res.* 44, 102.
- Andraud, M., Casas, M., Pavio, N., Rose, N., 2014. Early-life hepatitis e infection in pigs: the importance of maternally-derived antibodies. *PLoS One* 9, e105527.
- Barnaud, E., Rogée, S., Garry, P., Rose, N., Pavio, N., 2012. Thermal inactivation of infectious hepatitis e virus in experimentally contaminated food. *Appl. Environ. Microbiol.* 78, 5153–5159.
- Bouwknegt, M., Rutjes, S.A., Reusken, C.B., Stockhofe-Zurwieden, N., Frankena, K., de Jong, M.C., de Roda Husman, A.M., Poel, W.H., 2009. The course of hepatitis E virus infection in pigs after contact-infection and intravenous inoculation. *BMC Vet. Res.* 5, 7.
- Butler, J.E., Lager, K.M., Golde, W., Faaberg, K.S., Sinkora, M., Loving, C., Zhang, Y.L., 2014. Porcine reproductive and respiratory syndrome (PRRS): an immune dysregulatory pandemic. *Immunol. Res.* 59, 81–108.
- Colson, P., Romanet, P., Moal, V., Borentain, P., Purgus, R., Benezech, A., Motte, A., Gerolami, R., 2012. Autochthonous infections with hepatitis E virus genotype 4. *France. Emerg Infect Dis* 18, 1361–1364.
- Darwich, L., Mateu, E., 2012. Immunology of porcine circovirus type 2 (PCV2). *Virus Res.* 164, 61–67.
- de Deus, N., Casas, M., Peralta, B., Nofrarias, M., Pina, S., Martin, M., Segales, J., 2008. Hepatitis E virus infection dynamics and organic distribution in naturally infected pigs in a farrow-to-finish farm. *Vet. Microbiol.* 132, 19–28.
- Doceul, V., Bagdassarian, E., Demange, A., Pavio, N., 2016. Zoonotic hepatitis e virus: classification, animal reservoirs and transmission routes. *Viruses* 8.
- Fablet, C., Rose, N., Bernard, C., Messenger, I., Piel, Y., Grasland, B., 2017. Estimation of the diagnostic performance of two ELISAs to detect PCV2 antibodies in pig sera using a Bayesian method. *J. Virol. Methods* 249, 121–125.
- Gallien, S., Andraud, M., Moro, A., Lediguerher, G., Morin, N., Gauger, P.C., Bigault, L., Paboeuf, F., Berri, M., Rose, N., Grasland, B., 2018. Better horizontal transmission of a US non-InDel strain compared with a French InDel strain of porcine epidemic diarrhoea virus. *Transbound. Emerg. Dis.*
- Grasland, B., Loizel, C., Blanchard, P., Oger, A., Nignol, A.C., Bigarre, L., Morvan, H., Cariolet, R., Jestin, A., 2005. Reproduction of PMWS in immunostimulated SPF piglets transfected with infectious cloned genomic DNA of type 2 porcine circovirus. *Vet. Res.* 36, 685–697.
- Guillois, Y., Abravanel, F., Miura, T., Pavio, N., Vaillant, V., Lhomme, S., Le Guyader, F.S., Rose, N., Le Saux, J.C., King, L.A., Izopet, J., Couturier, E., 2016. High proportion of asymptomatic infections in an outbreak of hepatitis e associated with a spit-roasted piglet, France, 2013. *Clin. Infect. Dis.* 62, 351–357.
- Hosmillo, M., Jeong, Y.J., Kim, H.J., Park, J.G., Nayak, M.K., Alfajaro, M.M., Collantes, T.M., Park, S.J., Ikuta, K., Yunoki, M., Kang, M.I., Park, S.I., Cho, K.O., 2010. Molecular detection of genotype 3 porcine hepatitis E virus in aborted fetuses and their sows. *Arch. Virol.* 155, 1157–1161.
- Jackel, S., Muluneh, A., Pohle, D., Ulber, C., Dahnert, L., Vina-Rodriguez, A., Groschup, M.H., Eiden, M., 2018. Co-infection of pigs with Hepatitis E and porcine circovirus 2, Saxony 2016. *Res. Vet. Sci.* 123, 35–38.
- Lhomme, S., Marion, O., Abravanel, F., Chapuy-Regaud, S., Kamar, N., Izopet, J., 2016. Hepatitis e pathogenesis. *Viruses* 8, 212.
- Lopez-Lopez, P., Risdale, M.L.A., Frias, M., Garcia-Bocanegra, I., Brieva, T., Caballero-Gomez, J., Camacho, A., Fernandez-Molera, V., Machuca, I., Gomez-Villamandos, J.C., Rivero, A., Rivero-Juarez, A., 2018. Risk factors associated with hepatitis E virus in pigs from different production systems. *Vet. Microbiol.* 224, 88–92.
- Martin, M., Segales, J., Huang, F.F., Guenette, D.K., Mateu, E., de Deus, N., Meng, X.J., 2007. Association of hepatitis E virus (HEV) and postweaning multisystemic wasting syndrome (PMWS) with lesions of hepatitis in pigs. *Vet. Microbiol.* 122, 16–24.
- Rosell, C., Segales, J., Domingo, M., 2000. Hepatitis and staging of hepatic damage in pigs naturally infected with porcine circovirus type 2. *Vet. Pathol.* 37, 687–692.
- Salines, M., Barnaud, E., Andraud, M., Eono, F., Renson, P., Bourry, O., Pavio, N., Rose, N., 2015. Hepatitis E virus chronic infection of swine co-infected with Porcine Reproductive and Respiratory Syndrome Virus. *Vet. Res.* 46, 55.
- Salines, M., Andraud, M., Rose, N., 2017. From the epidemiology of hepatitis E virus (HEV) within the swine reservoir to public health risk mitigation strategies: a comprehensive review. *Vet. Res.* 48, 31.
- Salines, M., Demange, A., Stephant, G., Renson, P., Bourry, O., Andraud, M., Rose, N., Pavio, N., 2018. Persistent viremia and presence of hepatitis E virus RNA in pig muscle meat after experimental co-infection with porcine reproductive and respiratory syndrome virus. *Int. J. Food Microbiol.* 292, 144–149.
- Savic, B., Milicevic, V., Bojkovski, J., Kureljusic, B., Ivetic, V., Pavlovic, I., 2010. Detection rates of the swine torque teno viruses (TTVs), porcine circovirus type 2 (PCV2) and hepatitis E virus (HEV) in the livers of pigs with hepatitis. *Vet. Res. Commun.* 34, 641–648.
- Walachowski, S., Dorenlor, V., Lefevre, J., Lunazzi, A., Eono, F., Merbah, T., Eveno, E., Pavio, N., Rose, N., 2014. Risk factors associated with the presence of hepatitis E virus in livers and seroprevalence in slaughter-age pigs: a retrospective study of 90 swine farms in France. *Epidemiol. Infect.* 142, 1934–1944.
- Yang, Y., Shi, R., She, R., Mao, J., Zhao, Y., Du, F., Liu, C., Liu, J., Cheng, M., Zhu, R., Li, W., Wang, X., Soomro, M.H., 2015. Fatal disease associated with Swine Hepatitis E virus and Porcine circovirus 2 co-infection in four weaned pigs in China. *BMC Vet. Res.* 11, 77.