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Short communication

Persistent viremia and presence of hepatitis E virus RNA in pig muscle meat after experimental co-infection with porcine reproductive and respiratory syndrome virus

Morgane Salines[a,b], Antonin Demange[c,d,e], Gaël Stéphant[c,d,e], Patricia Renson[a,b,f], Olivier Bourry[a,b], Mathieu Andraud[a,b], Nicolas Rose[a,b,1], Nicole Pavio[c,d,e,*,1]

[a] ANSES, French Agency for Food, Environmental and Occupational Health & Safety, Ploufragan/Plouzané Laboratory, BP53, 22440 Ploufragan, France
[b] Bretagne-Loire University, Rennes, France
[c] ANSES, Laboratoire de Santé Animale, UMR 1161 Virology, 94706 Maisons-Alfort, France
[d] INRA, UMR 1161 Virology, 94706 Maisons-Alfort, France
[e] Ecole Nationale Vétérinaire d'Alfort, UMR 1161 Virology, 94706 Maisons-Alfort, France
[f] Union des Groupements de Producteurs de Viande de Bretagne (UGPVB), 104 rue Eugène Pottier, 35065 Rennes, cedex, France

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ABSTRACT

Although hepatitis E virus (HEV) transmission has been demonstrated after consumption of products containing infected pig liver, human cases can be also associated with other pig meat products, such as sausages. Data on HEV viremia and dissemination in muscle meat of infected animals are still sparse, especially during long-term infection. Previously, we have shown that experimental co-infection of pigs with HEV and porcine reproductive and respiratory syndrome virus (PRRSV) lengthens HEV infection up to 49 days and increases the likelihood of the presence of HEV RNA in the liver of the pig at a later stage of infection. In the present study, we show that during experimental HEV-PRRSV co-infection, prolonged HEV viremia, up to 49 days post-inoculation (dpi), is detected. The long-term viremia observed was statistically associated with the absence of HEV seroconversion. HEV RNA was also frequently detected, at a late stage of infection (49 dpi), in the three different types of muscle tested: femoral biceps, psoas major or diaphragm pillar. The HEV RNA load could reach up to $1 \cdot 10^6$ genome copies per gram of muscle. Detection of HEV in muscle meat was statistically associated with high HEV loads in corresponding liver and fecal samples. The presence of HEV in pig blood, femoral biceps and major psoas, corresponding to ham and tenderloin muscles respectively, is of concern for the food industry. Hence, these results indicate new potential risks for consumers and public health regarding pork products.

1. Introduction

Hepatitis E virus (HEV) is responsible for acute and occasionally chronic hepatitis in humans after enteric transmission. In developed countries, it is mainly of zoonotic origin, with pigs being the major reservoir (Pavio et al., 2017). Confirmed cases of zoonotic transmission have been associated with the consumption of raw or undercooked food products containing infected pig liver (e.g. pig liver sausages) (Colson et al., 2010; Guillois et al., 2016; Renou et al., 2014). More generally, case-control studies have identified the consumption of pig meat products as a major factor associated with HEV infections. Said and colleagues (2017) demonstrated, using epidemiological data collected from confirmed cases, that consuming ham and/or sausages from a given British supermarket brand was statistically associated with a higher risk of having an HEV infection (Said et al., 2017). Faber et al. (2018) collected exposure data from notified hepatitis E cases in Germany, with individually matched population controls, using a semi-standardized telephone interview. They identified ready-to-eat pork products (e.g. raw ham, frankfurter, spreadable sausages made of raw meat, liver sausage or liver pâté) as major sources for autochthonous hepatitis E (Faber et al., 2018). Data on the prevalence of HEV in pork products, other than in pig livers, are still very sparse and few publications report on the detection of HEV RNA in different categories of pig meat (e.g. sausages) (Berto et al., 2012; Di Bartolo et al., 2012;

* Corresponding author at: UMR 1161 Virology, ANSES, Laboratoire de Santé Animale, 94706 Maisons-Alfort, France.
E-mail addresses: morgane.salines@anses.fr (M. Salines), patricia.renson@anses.fr (P. Renson), olivier.bourry@anses.fr (O. Bourry), mathieu.andraud@anses.fr (M. Andraud), nicolas.rose@anses.fr (N. Rose), nicole.pavio@anses.fr (N. Pavio).
[1] These senior authors contributed equally to this article.

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Intharasongkroh et al., 2017; Szabo et al., 2015). One study found up to 20% of raw sausages (salami, without liver added) to be positive for HEV RNA (Szabo et al., 2015).

HEV RNA has been frequently detected in liver, bile or fecal samples from slaughtered pigs, (Salines et al., 2017), but until now, few studies have examined the presence of HEV RNA in other organs or tissues of naturally infected pigs at slaughterhouse time. In one report, HEV RNA was amplified in several organs and tissues, such as the bladder (10/43) or tonsils (3/43) of slaughtered pigs (n = 43) (Leblanc et al., 2010). In this study, none of the loin samples tested were HEV-positive (Leblanc et al., 2010). In a second report, HEV RNA was present along the different stages of the pork production chain, from the carcass dissection to liver removal steps and in pig lingual muscle, with an estimated prevalence of 2.7% (n = 112) (Di Bartolo et al., 2012).

HEV replicates in the liver but HEV RNA can be amplified in other pig organs and tissues after experimental infections (Bouwknegt et al., 2009; Williams et al., 2001). The detection of HEV-negative strands (replication intermediate) suggests that HEV can replicate in extra-hepatic sites, such as the small intestine, lymph nodes, and colon (Williams et al., 2001). In another study, positive HEV RNA hybridization signals were also detected in the liver, small and large intestine, tonsil, spleen, and kidney (Choi and Chae, 2003), supporting the presence of HEV in organs other than liver. In the study described by Bouwknegt et al. (2009), where the course of HEV infection was determined in pigs after intravenous inoculation and contact-infection, HEV RNA was detected in the longissimus, biceps femoris and iliopsoas, of both animal categories (Bouwknegt et al., 2009). The authors could not determine whether this was due to intrinsic and/or extrinsic contamination (i.e. cross-contamination with blood during necropsy).

HEV infection in pigs is usually acute, asymptomatic and self-resolving within 3 weeks (Salines et al., 2017). However, like in humans, where chronic cases are observed in solid organ transplant recipients under immunosuppressive treatment (Kamar et al., 2017), experimental HEV infection of pigs under active immune suppression led to chronic HEV shedding, lasting up to 13 weeks (Cao et al., 2017). In natural rearing conditions, pig immune responses can be modulated by frequent intercurrent infection with immune-modulating porcine viruses (e.g. porcine reproductive and respiratory syndrome virus) (Rahe and Murtaugh, 2017). Long-term HEV infection may influence the within-host course and HEV dissemination in organs. No study has addressed the question of the presence of HEV in pig organs during chronic infection, which is important regarding the risk of HEV presence in pig blood or meat at slaughter time. We have previously shown that co-infection with HEV and porcine reproductive and respiratory syndrome virus (PRRSV) affects the HEV time course. PRRSV co-infection with HEV extended HEV fecal shedding by a factor of 5, and increased the frequency of HEV RNA detection in pig livers at late stages of infection (49 days) (Andraud et al., 2013; Salines et al., 2015). These results suggest that HEV pathogenesis and dissemination could be affected by PRRSV co-infection. Thus, the aim of the present study was to assess the presence of HEV in serum and muscle meat of pigs in the context of PRRSV co-infection, after a natural route of inoculation.

2. Materials and methods

2.1. HEV/PRRSV co-infection experiment

Experimental HEV/PRRSV co-infection of Specific-Pathogen-Free (SPF) pigs was previously described (Salines et al., 2015). Briefly, 18 Large-White piglets were randomly allocated to 3 independent pens (3 inoculated and 3 contact piglets per pen). In each pen, the inoculated piglets received: *(i)* orally 10^8 HEV RNA copies in a volume of 10 mL of a genotype 3 HEV suspension (strain FR-SHEV3e, Genbank accession number JQ953665) prepared according to the protocol described in Andraud et al. (2013); and *(ii)* 2.5 mL per nostril of a PRRSV suspension (strain PRRS-FR-2005-29-24-1 “Finistere”, genotype 1, subtype 1, Genbank accession number KY366411) titrating 10^5 $TCID_{50}$/mL. Two negative control pigs were included in a separate room. The protocol was approved by the Anses/ENVA/UPEC Ethics Committee (Approval No. 16 with the French National Committee for Ethics in animal experimentation). Since HEV is a zoonotic agent, biosecurity measures were applied to prevent any transmission from pigs to animal care handlers.

2.2. Sample collection

Blood samples were collected once a week until the end of the study (49 dpi). For euthanasia, anesthesia was carried out with intravenous injection of 1 g/50 kg live weight of Nesdonal® (thiopental-sodium, Merial, Lyon, France). This anesthesia is highly reproducible and has no impact on the quality of bleeding thereafter. Exsanguination was then performed by cutting deeply with a sharp blade into the carotid artery on both sides, with the anesthetized pigs hung by the legs. The carcasses were processed 20 min after exsanguination to ensure the absence of remaining blood flow. Necropsy was performed and liver and muscles samples (femoral biceps, psoas major and diaphragm pillar) were collected and kept frozen until used. To avoid cross-contamination, each muscle sample was handled using single use sterile materials (gloves, clamps, blades, and tips).

2.3. RNA extraction

RNA extractions from serum, fecal or muscle juice samples were performed manually using the QIAamp Viral RNA extraction Mini kit (QIAGEN, Illkirch, France), according to the manufacturer's instructions, except that sample size was 200 µL. Fecal samples were solubilized in a 10% phosphate buffered saline suspension. Muscle juices were recovered after one cycle of freeze and thaw at −20 °C, from 20 g of each muscle (Feurer et al., 2018). Comparison of HEV recovery rate from muscle juice or from muscle homogenate (Fast-prep 24, MP Biomedicals, Illkirch, France), was performed after spiking with a viral suspension of HEV3 (Genbank accession number EF494700), and showed similar results (data not shown).

2.4. HEV RNA quantification

HEV RNA quantification in serum, liver and muscles samples was performed, after RNA extraction, using real-time quantitative RT-PCR targeting HEV ORF3 (Jothikumar et al., 2006). Standard quantification curves were produced by plotting the quantification cycle (Cq) values against the logarithm of the input copy numbers of a standard RNA. Standard RNA was obtained after in vitro transcription of a plasmid pCDNA 3.1 ORF 2–3 HEV, as previously described (Barnaud et al., 2012). Results were expressed in HEV RNA copy number per gram of feces or muscle or per milliliter of serum (RNA copies/g or RNA copies/mL).

2.5. HEV serology

HEV serology was previously determined, and is presented in Fig. 3 of the publication on HEV/PRRSV co-infection of pigs (Salines et al., 2015). Briefly, anti-HEV antibodies were detected using the HEV ELISA 4.0v kit (MP Diagnostics, Illkirch, France), according to the manufacturer's instructions, except the serum quantity used (10 µL instead of 20 µL). Samples were positive when the optical density at 450 nm wavelength was higher than the threshold defined as the mean for negative controls + 0.3.

2.6. Statistical analysis

Time to viremia onset, viremia duration and period between shedding and viremia were estimated using a parametric survival model.

Table 1
HEV RNA quantification in serum, muscle, feces and liver samples from HEV/PRRSV co-infected animals and contact pigs (n = 20).

		Viremia kinetic (RNA copies/mL serum)							HEV RNA in muscle at 49 dpi (RNA copies/g)			HEV RNA in feces at 49 dpi (RNA copies/g)	HEV RNA in liver at 49 dpi (RNA copies/g)
	dpi	7	14	21	28	35	42	49	FB	PM	DP		
	Control	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Control	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Group 1	Inoculated	nd	nd	nd	$3.56 \cdot 10^3$	$1.43 \cdot 10^4$	$1.91 \cdot 10^4$	$1.70 \cdot 10^3$	nd	nd	nd	$1.49 \cdot 10^7$	$1.46 \cdot 10^6$
	Inoculated	nd	nd	$4.22 \cdot 10^4$	$3.85 \cdot 10^4$	$7.70 \cdot 10^4$	$2.14 \cdot 10^4$	$4.02 \cdot 10^3$	nd	nd	nd	$5.85 \cdot 10^7$	$1.87 \cdot 10^6$
	Inoculated	nd	nd	$1.33 \cdot 10^4$	$2.92 \cdot 10^4$	$7.49 \cdot 10^4$	$5.21 \cdot 10^4$	$2.15 \cdot 10^4$	nd	nd	$1.43 \cdot 10^4$	$6.40 \cdot 10^7$	$1.02 \cdot 10^6$
	Contact	nd	nd	nd	nd	$2.12 \cdot 10^4$	$2.80 \cdot 10^4$	$9.44 \cdot 10^3$	nd	nd	nd	$4.87 \cdot 10^7$	$8.72 \cdot 10^5$
	Contact	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	$3.49 \cdot 10^5$	nd
	Contact	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	$1.20 \cdot 10^6$	nd
Group 2	Inoculated	nd	nd	$4.51 \cdot 10^3$	$2.07 \cdot 10^4$	$2.24 \cdot 10^4$	nd	nd	$4.33 \cdot 10^3$	nd	$6.23 \cdot 10^3$	$1.45 \cdot 10^5$	$1.56 \cdot 10^3$
	Inoculated	nd	nd	$2.16 \cdot 10^4$	$2.43 \cdot 10^4$	$2.05 \cdot 10^4$	$1.24 \cdot 10^4$	nd	nd	$2.18 \cdot 10^4$	$2.70 \cdot 10^3$	$6.80 \cdot 10^6$	$2.42 \cdot 10^6$
	Inoculated	nd	nd	$6.31 \cdot 10^4$	$1.69 \cdot 10^4$	$5.55 \cdot 10^4$	$2.80 \cdot 10^4$	nd	$8.14 \cdot 10^3$	$3.55 \cdot 10^3$	$5.40 \cdot 10^3$	$4.86 \cdot 10^6$	$3.59 \cdot 10^5$
	Contact	nd	nd	nd	nd	nd	$2.64 \cdot 10^4$	$7.33 \cdot 10^3$	$5.91 \cdot 10^4$	$6.75 \cdot 10^5$	$6.42 \cdot 10^3$	$5.55 \cdot 10^7$	$1.12 \cdot 10^6$
	Contact	nd	nd	nd	nd	nd	$2.65 \cdot 10^4$	$6.82 \cdot 10^3$	$2.12 \cdot 10^4$	$2.28 \cdot 10^3$	$3.72 \cdot 10^3$	$5.24 \cdot 10^6$	$1.69 \cdot 10^6$
	Contact	nd	nd	nd	nd	$1.76 \cdot 10^4$	$6.64 \cdot 10^4$	$2.94 \cdot 10^3$	$6.92 \cdot 10^3$	nd	nd	$3.07 \cdot 10^8$	$3.44 \cdot 10^6$
Group 3	Inoculated	nd	nd	nd	$4.40 \cdot 10^3$	$2.08 \cdot 10^4$	$2.11 \cdot 10^4$	$1.22 \cdot 10^4$	nd	nd	nd	$2.08 \cdot 10^8$	$3.24 \cdot 10^6$
	Inoculated	nd	nd	nd	nd	$8.68 \cdot 10^3$	$1.72 \cdot 10^4$	$1.92 \cdot 10^4$	nd	$1.59 \cdot 10^4$	$6.17 \cdot 10^3$	$5.75 \cdot 10^7$	$2.04 \cdot 10^4$
	Inoculated	nd	nd	$4.10 \cdot 10^4$	$1.95 \cdot 10^4$	$1.46 \cdot 10^4$	$1.00 \cdot 10^4$	$1.15 \cdot 10^3$	nd	$1.09 \cdot 10^6$	$1.62 \cdot 10^4$	$5.61 \cdot 10^7$	$9.63 \cdot 10^5$
	Contact	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	$7.39 \cdot 10^5$	nd
	Contact	nd	nd	nd	nd	nd	nd	nd	$5.32 \cdot 10^3$	nd	nd	$1.03 \cdot 10^8$	$5.87 \cdot 10^5$
	Contact	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	$8.29 \cdot 10^5$	nd

Quantitative RT-PCR results on individual serum samples (HEV RNA copies/mL of serum) at each sampling time and from fecal, liver and muscle samples at necropsy (HEV RNA copies/g). Shaded zones correspond to periods in which infected individuals were viremic and to HEV-positive fecal, liver and muscle samples. dpi: days post infection; nd: not detected; FB: femoral biceps; PM: psoas major; DP: diaphragm pillar.

Two parametric models were tested (lognormal and Weibull distributions of survival times) and compared using Akaike Information Criterion (AIC). Statistical associations between viral RNA quantities in the different matrices were evaluated using Pearson correlation tests. The links between HEV quantities in serum and muscles and the seroconversion as regards HEV (as binary variable) were assessed using Kruskal–Wallis tests. Statistics were analyzed using R software (Ihaka and Gentleman, 1996).

3. Results

The results for HEV RNA quantification in serum, muscle, liver and fecal samples are presented in Table 1. HEV RNA was detected in the serum samples of all inoculated animals at 35 dpi and in 45% of contact pigs at 42 dpi (Table 1, Fig. 1). HEV RNA yields in the sera ranged from $1.1 \cdot 10^3$ to $7.7 \cdot 10^4$ RNA copies/mL. Time-to viremia onset, viremia duration and period between HEV shedding and viremia were fitted to lognormal distributions. On average, HEV viremia, in both inoculated and contact pigs, started at 23.4 dpi [95% confidence interval 21.2–25.7] and lasted 28.8 days [95% CI 18.6–44.8]. The delay between HEV fecal excretion and viremia was on average 7.9 days [95% CI 5.8–11.0], in both inoculated and contact infected pigs. At 49 dpi, HEV RNA quantities in the serum and feces of inoculated pigs were statistically correlated (correlation coefficient CC = 0.83, p-value < 0.01). In contact pigs, significant associations were found between HEV RNA levels in serum and liver (CC = 0.82, p-value < 0.01).

Results on HEV seroconversion of infected pigs have been already published (Salines et al., 2015). Briefly, 4 out of 9 inoculated animals produced anti-HEV antibodies between 35 and 49 dpi, and 7 out of 9 contact individuals seroconverted between 42 and 49 dpi. Statistical analysis indicated that at 49 dpi, in both inoculated and contact pigs, high viral load in serum was significantly associated with the absence of seroconversion during the study period (p-value < 0.01 and p-value < 0.05, respectively).

HEV RNA was detected in the three types of muscles tested: femoral biceps, psoas major and diaphragm pillar (Fig. 2), with quantities ranging from $2.3 \cdot 10^3$ to $1.1 \cdot 10^6$ RNA copies/g. No significant differences in the proportions of positive samples, nor in the mean viral RNA levels, were found between the three types of muscles (p-value > 0.1).

No statistical associations were found between the different parameters tested: HEV RNA yields in serum and in muscle samples at 49 dpi; viremia duration and HEV presence in muscle; time to viremia onset and HEV presence in muscles; HEV presence in muscle and seroconversion; HEV quantities in muscle and seroconversion (p-value > 0.1). In contrast, in contact infected pigs, statistical associations were found between HEV RNA levels in muscle and liver (CC = 0.79, p-value < 0.01), and HEV RNA levels in muscle and feces (CC = 0.68, p-value < 0.05).

PRRSV viremia was detected in all inoculated animals and in all contact infected animals, except one that did not show any detectable PRRSV viremia (results shown in Salines et al., 2015).

4. Discussion and conclusions

Motivated by studies suggesting the presence of HEV in pig blood, muscle or meat products without pig liver (Berto et al., 2012; Di Bartolo et al., 2012; Grierson et al., 2015; Szabo et al., 2015) and by a previous study showing unusually long lasting HEV excretion after PRRSV co-infection (Salines et al., 2015), the presence of HEV RNA was investigated in serum and muscle meat of experimentally HEV/PRRSV co-infected pigs. We found that HEV RNA was frequently detected in both serum and muscles of co-infected pigs. Viremia started 7.9 days after initial fecal shedding and lasted 28.8 days. In a previous study by Bouwknegt et al. (2009), (where HEV transmission and dissemination were studied using a different setting, with different pigs, HEV strains, methods of detection and after intravenous inoculation), HEV contact infected pigs exhibited viremia starting after 13 days of fecal excretion and lasting 11 days (Bouwknegt et al., 2009). In the present study, earlier and longer viremia was observed in the HEV/PRRSV co-infected pigs, which may suggest that PRRSV co-infection modulates HEV physiopathology and length of viremia. The presence of HEV RNA in pig serum has been described in several studies performed at the slaughterhouse (for review Salines et al., 2017); hence the present data

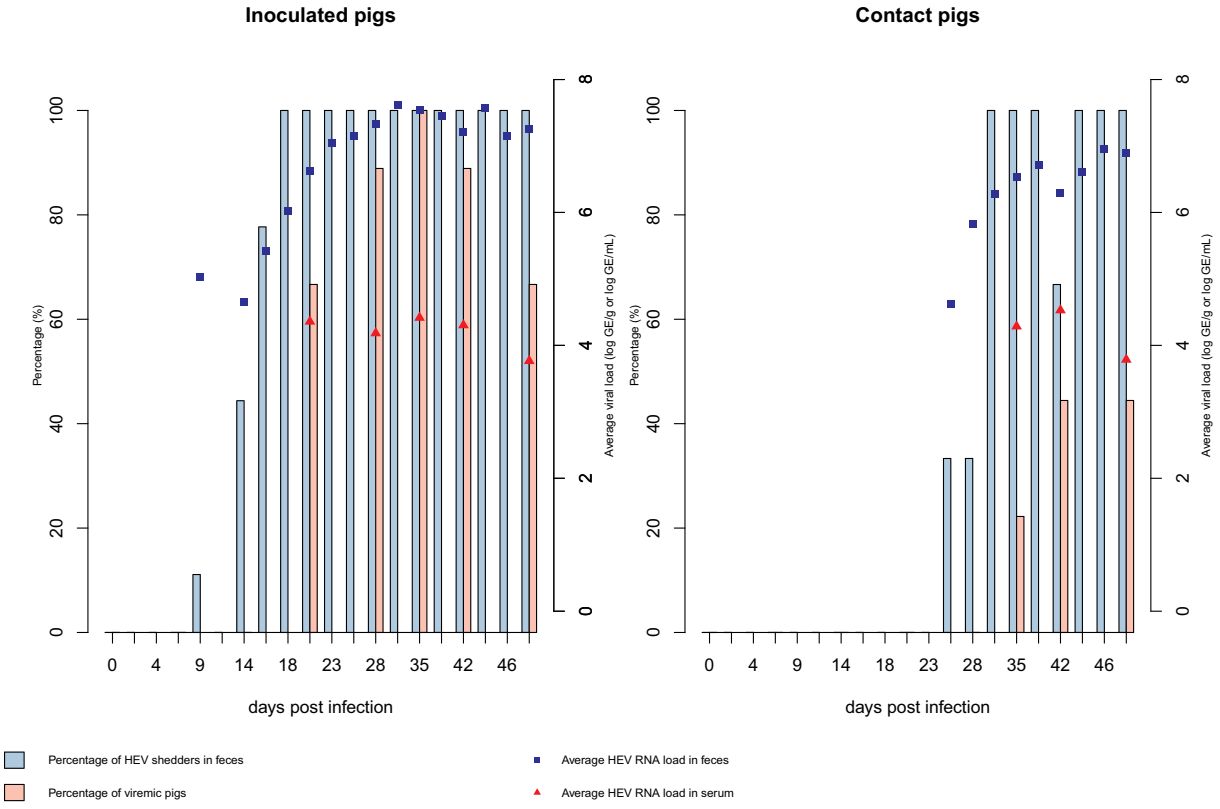

Fig. 1. Percentage of HEV shedding and viremic pigs and average HEV RNA copy numbers in feces and serum (log RNA copies/g or log RNA copies/mL) of HEV/PRRSV co-infected pigs (n = 18).

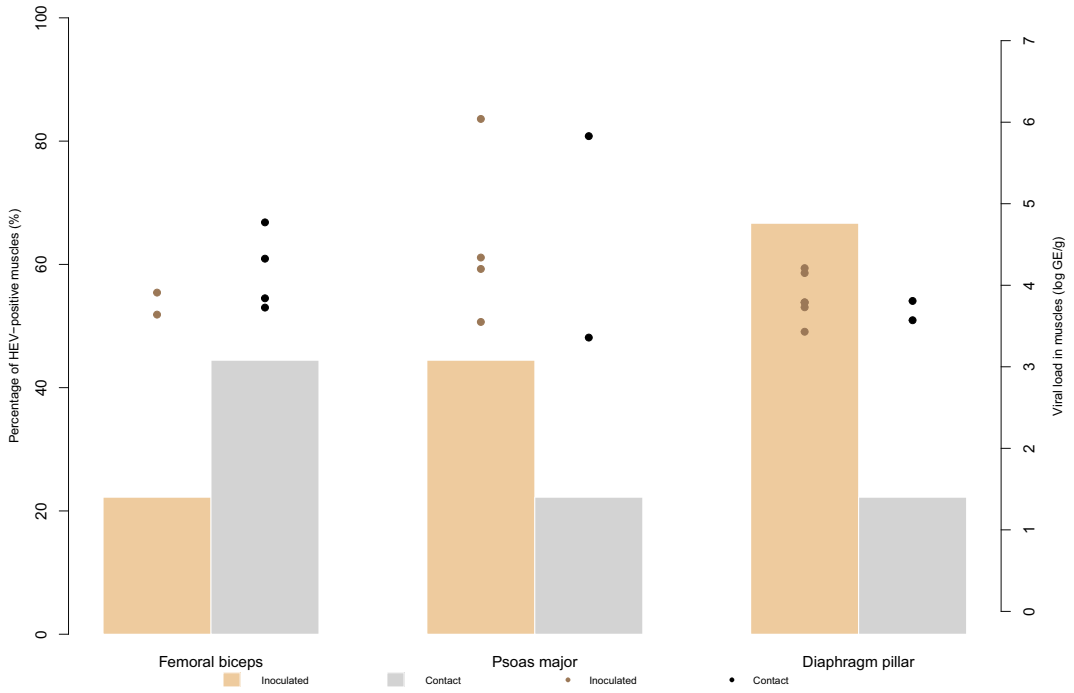

Fig. 2. Percentage of HEV-positive muscle (bars) and HEV RNA copies (log RNA copies/g) in positive muscle (dots) of HEV/PRRSV co-infected pigs (n = 18).

support the possible risk of HEV exposure through any pig blood-derived products insufficiently heated, used in the food industry.

In the present study as well, muscles of both infected and contact pigs were HEV-positive at 49 dpi. Bouwknegt et al. (2009) reported that only a few animals of the HEV-infected group were found HEV-positive in muscle up to 32 days after fecal shedding (Bouwknegt et al., 2009). These findings therefore suggest that muscle from HEV/PRRSV co-infected pigs would be more likely to contain HEV at a later stage than during HEV-only infection.

In our previous results, we have shown that HEV-PRRSV co-infection was associated with delays in HEV seroconversion (Salines et al., 2015). PRRSV infection has an impact on innate immunity that also

affects the development of an effective adaptive immune response, such as production of neutralizing antibodies (Rahe and Murtaugh, 2017). Here, we observed prolonged and high-level viremia in the absence of seroconversion (Salines et al., 2015), in agreement with a lack of virus neutralization. Furthermore, for the 3 inoculated pigs of group 2, HEV RNA was amplified at high levels in muscle, but not detected in serum at 49 days post inoculation (Table 1), which is a rather unique finding. It can be hypothesized that, in some circumstances, induced by the co-infection with PRRSV, HEV may replicate in muscle cells, in spite of the absence of apparent HEV replication in muscle, as shown in one experimental infection (no negative-strand of HEV RNA detected pig muscle) (Williams et al., 2001). Another possible explanation would be that long-term viremia favors the interaction of HEV particles with heparan sulfate expressed at the surface of muscle cells. Indeed, it has been shown that heparan sulfate proteoglycans (HSPGs) are required for cellular binding of the hepatitis E virus ORF2 capsid protein and for viral infection (Kalia et al., 2009). In skeletal muscle, HSPGs are the major proteoglycans (PGs) in the basal lamina and on the cellular surface of myocytes (Sanes et al., 1986). This class of polysaccharides is highly expressed and plays a major role in the functional integrity of skeletal muscle (Jenniskens et al., 2006). In humans, two cases of HEV-associated severe myositis have been described (Del Bello et al., 2012; Mengel et al., 2016). One of them reports on a liver transplant recipient with acute hepatitis E, associated with Guillain-Barre syndrome (Del Bello et al., 2012). The patient developed severe muscle weakness and his condition worsened. HEV viremia was found by RT-PCR, but HEV RNA was undetectable in cerebrospinal fluid. A biopsy of the left biceps showed myopathic changes, with a significant percentage of necrotic muscle fibers (10%), and signs of inflammation. The presence of HEV RNA in the muscle was not investigated in the biopsy. It would be of interest to test for the accumulation of HEV particles in muscle in humans, in cases of severe myositis. The presence of HEV in pig muscle, as observed during the present study, may also have an impact on the understanding of HEV physiopathology.

Although the oral infectious dose of HEV in humans is unknown, in pigs it is estimated to be 10^5 HEV RNA copies (Andraud et al., 2013). Here, up to $6 \cdot 10^5$ HEV RNA copies/g of muscle (psoas major) were quantified. It is therefore possible that these HEV quantities are sufficient to induce an infection in case of consumption of infected raw or undercooked meat. To prevent such exposure of consumers, in the absence of surveillance of HEV in pig meat, consumers should be advised to cook pork products very well.

The present findings highlight that pig meat products such as ham and tenderloin may contain HEV, under specific circumstances. Studies in natural conditions of pig breeding, with multi-pathogen exposure, would provide new insights into HEV dissemination in pigs.

A study conducted on 1034 pig muscles collected in French slaughterhouses did not show any HEV-positive sample, not even in pigs with HEV-positive liver (Feurer et al., 2018). Comparison with the present study is limited since the parts of muscle collected were different (gluteus medius or semi-membranosus), and no indication was provided regarding the pigs' PRRSV status. Hence, based on our study, investigation on the presence of HEV-positive muscles at the slaughterhouse should be conducted with a larger sample, stratified on the farm PRRSV status, and collecting femoral biceps and psoas major muscles.

Ham and tenderloin muscle can be consumed dried or undercooked (rare), respectively. HEV infectivity has not been directly assessed in drying conditions, but HEV remains infectious after 28 days at room temperature (Johne et al., 2016). Sufficient cooking, 20 min at 71 °C, inactivates HEV in food products contaminated artificially (Barnaud et al., 2012).

From our observations, co-infections with swine pathogens impairing the immune response against HEV may increase the risk of contaminated pig meat and products entering the food chain. Further studies are required to investigate whether other intercurrent infections (porcine circovirus-2), exposure to immunomodulatory molecules (toxins), or stress conditions would have an impact on the HEV infection course.

In conclusion, HEV contamination of pig meat and, not only of pig livers, has to be considered when assessing the HEV risk related to the consumption of pork products from a public health perspective, and surveillance plans should be implemented in the pork chain. We found that the presence of HEV in muscle might be predictable from the fecal viral genome load, which would be of great interest for easier detection of infected animals at the slaughterhouse. Testing fecal samples could therefore make it possible to identify pigs at risk of introducing infected meat into the food chain.

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Authors' contributions

MS analyzed the data and drafted the manuscript. AD and GS analyzed the HEV samples and interpreted the results. PR and OB analyzed and interpreted the results from PRRSV samples. MA participated in data analysis. NR designed and coordinated the study, and participated in animal experiments and data analysis. NP supervised the HEV laboratory work and contributed to the coordination of the study and manuscript writing. All co-authors revised the manuscript and approved the final submitted version.

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