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Quantitative risk assessment of hepatitis E virus: modelling the occurrence of viraemic pigs and the presence of the virus in organs of food safety interest.

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KEYWORDS

Risk assessment, HEV, foodborne pathogen, zoonotic disease, pork, pigs, transmission model

Running title

Modelling the occurrence of hepatitis E virus in organs of slaughter-age pigs

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ABSTRACT

Hepatitis E virus (HEV) is a zoonotic pathogen with consumption of pork and derived products identified in different countries as a risk factor for human exposure to HEV. Great efforts have been made to understand the dynamics of virus transmission within domestic swine populations through modelling. However, from a food safety prospective, it is critical to integrate the parameters involved in the transmission dynamics with those governing the actual presence of HEV in the bloodstream, the liver, gallbladder or faeces. To date, several aspects related to the pathogenesis of the disease are still unknown or characterized by significant levels of uncertainty, making this conjunction challenging. We used published serological data obtained from pigs in a farrow-to-finish farm to implement an Immune-Susceptible-Infected-Recovered (MSIR) model reproducing the on-farm dynamics that lead to the occurrence of viraemic pigs at slaughter. Expert opinion on the length of time infectious HEV can be detected in liver, gallbladder/bile and faeces after recovery from viraemic status were used to inform a stochastic model aimed at estimating the expected proportion of viraemic pigs ($HEV_V{}^+$), pigs with infectious HEV in liver ($HEV_L{}^+$), gallbladder/bile ($HEV_G{}^+$) and faeces ($HEV_F{}^+$) entering the slaughterhouse. To simulate the potential effect of on-farm mitigation strategies, we estimated the changes in outcomes of interest as a function of variations in the baseline transmission parameters. The model predicted a proportion of viraemic pigs entering the slaughterhouse of 13.8% while the proportions of $HEV_L{}^+$, $HEV_G{}^+$ and $HEV_F{}^+$ ranged from 13.8% to 94.4%, 13.8% to 94.7% and from 25.3% to 30.8% respectively, due to the uncertainty surrounding the experts' opinions. Variations in MSIR model's parameters alert of the need to carefully consider in the application of mitigation strategies aimed at delaying the decay of maternal immunity or the peak of the within herd transmission. When the rate of decay of maternal immunity and the transmission rate were decreased between 80% and 5% and 40% and 5% from the baseline values respectively, adverse effects on $HEV_V{}^+$ were observed. The model highlights the relevance of specific aspects in the pathogenesis of the disease from a food safety prospective and it was developed to be easily reproducible and updatable as soon as accurate data becomes available. As presented, the model can be directly connected to existing or future pig-related models to estimate the significance of the identified parameters on the risk of human exposure to HEV through consumption of pork products.

1. INTRODUCTION

The European Food Safety Authority (EFSA) recognises hepatitis E as an emerging public health concern in Europe with a complex epidemiology that includes foodborne transmission [1].Hepatitis E virus (HEV) is a non-enveloped positive-stranded RNA virus; four different genotypes, each including several subtypes, have been identified so far and linked to specific geographical distributions and host ranges [2]. Genotypes G1 and G2 have been isolated only in humans and are associated with epidemics in Asia, Africa and Central America [3] whereas G3 and G4 are zoonotic and circulate in humans and several animals, particularly pigs and other mammalian species [3-6]. Hepatitis E is usually a mild, self-limiting infection but some cases may develop into a fulminant form with reported mortality rates ranging from 1 to 4% and up to 25% in pregnant woman [7].

A high seroprevalence of zoonotic HEV is reported in pig populations of industrialized countries [8-12] and HEV RNA has been isolated from processed pork products, especially those containing liver [13-15]. A recent case-control study associated the consumption of processed pork products with indigenous HEV infection [16] in England and Wales and several studies indicated meat products as a source of infection in humans [17-19]. This evidence and the ubiquitous nature of the virus in animals -particularly in domestic pigs- raises public health concern for zoonotic infection through direct contact with infected animals or through the consumption of animal meats.

With particular reference to the risk of infection through consumption of meat products, the likely impact of HEV on food safety can be quantified adopting a probabilistic approach and estimating the probability of exposure to the virus through consumption of pork products. Recently, two quantitative risk assessment (QRA) have been published, both aimed at estimating the probability of human exposure to HEV through consumption of pork liver and liver sausages in Switzerland [20, 21]. These models considered the food products rather than individual pigs as the starting point, therefore, the farm level dynamics describing the infectious status of the animals entering the slaughterhouse and the events occurring at processing stage were not explored.

Understanding the role of the dynamics leading to viraemic pigs at slaughter is critical because the presence of HEV in bloodstream is considered as the plausible vehicle for the zoonotic

transmission of the virus in humans [22]. Moreover, in prospective of future implementation of comprehensive 'farm-to-fork' QRA, it is important to identify the key biological parameters governing the presence of HEV not only in pigs' meat but also in the key offal of major interest as food products (i.e. liver) or as potential source of cross-contamination at slaughter (i.e. faeces, intestine or bile).

In recent years, several studies explored and implemented mathematical models to estimate the transmission parameters of HEV within different domestic swine populations in different countries [8, 23-25]. These studies were based on field data and represent a valuable contribution for the understanding of HEV in-field transmission dynamics and the role of factors influencing the probability of infection (e.g. environmental contamination, maternal immunity). However, these models were parameterized using longitudinal data obtained from faecal or serological samples but the actual presence of the virus in the bloodstream and in key organs of food safety interest were not considered. Furthermore, pathogenesis of hepatitis E is still poorly understood [26-28], and predicting the presence of the virus in the internal organs over time is challenging given the scarcity of data from dedicated experimental studies.

Following these considerations, the objectives of this study were to: (i) implement a baseline model reproducing the dynamics of HEV infection in a closed population of naturally infected pigs in a farrow-to-finish farm; (ii) estimate the expected proportion of pigs entering the slaughterhouse with infected livers, gallbladder/bile, and excreting virus in faeces and, (iii) quantify the effect of the uncertainty and data gaps in the parameters underlying those estimations.

2. MATERIAL AND METHODS

2.1. Baseline model

Data reported from the longitudinal study conducted by De Deus et al., [29] were used to estimate the parameters of a compartmental model describing the viraemic status of a closed population of pigs over time.

This study was identified as a part of a literature screening conducted in February 2017 on studies reporting longitudinal data on HEV infection preferably in naturally infected swine herds. The PubMed search engine of the MEDLINE database was used with the query:

"(Hepatitis E[Title] AND Longitudinal[Title] AND Pigs[title] OR Hepatitis E[Title] AND Naturally infected[Title] AND Pigs[title])" and six items were found. Amongst the candidate studies, De Deus et al. [29], was considered as the most easily reproducible to implement the baseline model to be used for the purpose of this work.

In that study, 45 piglets from 19 sows from the same weekly farrowing batch were randomly selected and serially bled at 1, 3, 6, 9, 12, 15, 18 and 22 weeks of age. Serum samples were tested for specific anti-HEV antibodies by ELISA and the presence of HEV RNA was assessed by means of a semi-nested RT-PCR.

As the authors reported the proportion of piglets showing evidence of maternal immunity, an MSIR model (an extension of the Susceptible-Infectious-Recovered (SIR) model that includes the M class for maternally-derived immunity) was used to describe the transition of the population among the compartments in time. The observed number of immune and viraemic pigs in the original study are reported in table 1.

The model is described by the set of ordinary differential equations:

$$\frac{dM}{dT} = -\delta M$$

$$\frac{dNv}{dT} = \delta M - \beta NvV$$

$$\frac{dV}{dT} = \beta NvV - \gamma V$$

$$\frac{dR}{dT} = \gamma V$$

Where: δ is the decay rate of the population with maternal immunity (M), β is the transition rate from Not-viraemic (Nv) to viraemic (V) and γ represents the recovery rate from the viraemic status.

The 45 monitored piglets were sampled from a number of sows representing 8% of the total sow population (total number of sows in the farm = 240). The hypergeometric process was used to estimate at each i^{th} sampling time the most likely number of seropositive or infected animals if the same proportion of piglets were sampled from the overall sow population.

The estimated proportions of seropositive and infected pigs at each sampling point were used to estimate the rates of decay of animals with maternal immunity (δ), of infection (β) and of recovery (γ). The system of differential equations was first informed by tentative values for the unknown parameters and the reduced gradient algorithm (GRG) for nonlinear problems was then used to estimate the set of parameters that minimizes the residuals from observed and predicted values. A convergence tolerance of 0.0001 was selected as the acceptable relative change in the absolute value of the target (difference in residuals) indicating the objective function value is changing very slowly as algorithm progresses from point to point.

The parameterized system of differential equations allows to estimate the number of immune, not-viraemic, infected and recovered animals at any point in time, therefore, it was used to obtain the proportions of interest at the day of depopulation (*dpDay*) when animals are sent to the slaughterhouse (consistent to De Deus et al., *dpDay* was set to 154).

2.2. Infectious status of the pigs in the different compartments.

The status of individual pigs at *dpDay* was used to infer the expected proportions of viraemic animals ($HEV_V{}^+$), animals with infected livers ($HEV_L{}^+$), gallbladder/bile ($HEV_G{}^+$) and animals excreting virus in their faeces ($HEV_F{}^+$). To this end, the following evidence and assumptions about not-viraemic, viraemic and recovered animals were combined:

(i) Not-viraemic. Animals belonging to this category are not in the viraemic phase and specific anti HEV antibodies are not present. In not-viraemic animals, the presence of the virus in faeces cannot be excluded. In fact, extra-hepatic sites of virus replication have been identified [30] and it is possible that the virus replicates in the intestinal tract before reaching the liver. The presence of genomic HEV RNA in faeces has been reported from a number of days before the onset of viremia ranging from: 10-60 [31], 7-28 [32], and 8.3-17 days [22].

In the model it is assumed that the not yet infected animals are excreting the virus with faeces from a minimum of 7 to a maximum of 60 days before the onset of the viraemic phase. The uncertainty in this length of time (Nv_F^+) is described by the rounded Uniform distribution:

$$Nv_F^+ = Uniform(7; 60)\ days$$

In the model, the overall proportion of not-viraemic animals excreting HEV RNA with faeces ($HEV_{Nv_{F^+}}$) at *dpDay* is equal to the proportion of not-viraemic animals which is predicted to

become infected from *dpDay* to *dpDay* + Nv_F^+ (i.e. the animals are assumed to have the virus detectable in faeces at least Nv_F^+ days before onset of viremia).

(ii) Viraemic. In these animals, the virus is detectable in the bloodstream. It is assumed that in viraemic animals the liver and the organs, where the virus is known to accumulate and replicate, are infected. Viraemic pigs are also assumed to actively excrete virus with faeces during this stage.

(iii) Recovered. Animals belonging to this category recovered from viraemia and anti-HEV IgG are detectable in the bloodstream. Genomic HEV RNA might still be detectable in the faeces and key internal organs such as liver, bile and intestine [33].

The length of time during which the virus can be detected in the liver and target organs in animals recovered from viraemia, and whether the virus is present in its infective form in these animals is unknown. Some indication of virus persistence is shown from results of an experimental study conducted in Italy where HEV RNA was detected in the liver of one pig that had recovered from viremia 7 days before [34]. However, as the pig was sacrificed, it was not possible to estimate for how long the virus could have remained present in liver after recovery from viremia. In the study by De Deus et al. [29], HEV RNA was observed in the livers and faeces of two non-viraemic pigs but unfortunately from reported results, it is not possible to ascertain whether the same animals had been viraemic previously. Furthermore, it cannot be ruled out that the presence of the virus in the liver of these animals simply indicated the pre-viraemic phase.

2.3. Expert opinion

In the model, the expected length of time the virus is still detectable in liver (R_L^+) and gallbladder/bile (R_G^+) after recovery from viraemia were obtained by expert opinion.

Ten international experts agreed to provide their opinion about the minimum (MIN) and maximum (MAX) value of the delta time period elapsing from the resolution of viraemic phase to the absence of infectious HEV from the liver and gallbladder/bile. For each estimation, interviewees were also asked to give a score on a scale from 1 (not confident) to 4 (confident) to describe how confident they were with their own estimations.

Results were included into a discrete distribution:

144 $R_L^+, R_G^+ = Discrete(\{x_i\}; \{p_i\})\ days$

145 where $\{x_i\}$ is the vector of the ranges modelled as rounded Uniform distributions (with the
146 Minimum and Maximum values identified by each i[th] expert being the distribution's
147 parameters) and $\{p_i\}$ is the vector of the weights given to each opinion. This way, each
148 expert's distribution has a chance to be sampled proportional to its level of confidence.

149 The expected proportion of recovered animals excreting the virus with faeces ($HEV_{R_{F^+}}$) was
150 estimated assuming that infectious HEV remains detectible in faeces at least for a length of
151 time *(R_F^+)* ranging from 14 to 21 days after recovery from viraemia [32, 35]. Again, a discrete
152 uniform distribution was used to assume that every number of days within the range 14-21 is
153 equally probable.

154 All the proportions of recovered animals with virus present in the liver *($HEV_{R_{L^+}}$)*,
155 gallbladder/bile ($HEV_{R_{G^+}}$) and faeces ($HEV_{R_{F^+}}$) were obtained from the calculated
156 proportions of recovered animals on day: $(dpDay - R_L^+)$, *$(dpDay - R_G^+)$* and $(dpDay - R_F^+)$
157 respectively.

158 Finally, the overall proportions of viraemic animals ($HEV_V{}^+$), animals with infected
159 livers ($HEV_L{}^+$), animals with infected gallbladder/bile ($HEV_G{}^+$) and animals actively
160 excreting HEV with faeces ($HEV_F{}^+$) were estimated as:

161 $HEV_V{}^+ = predicted\ proportion\ of\ viraemic\ at\ dpDay$

162 $HEV_L{}^+ = HEV_V{}^+ + HEV_{R_{L^+}}$

163 $HEV_G{}^+ = HEV_V{}^+ + HEV_{R_{G^+}}$

164 $HEV_F{}^+ = HEV_V{}^+ + HEV_{Nv_{F^+}} + HEV_{R_{F^+}}$

165 All the outcomes of the model were obtained by means of Monte Carlo simulation (500,000
166 iterations). The risk analysis software @Risk (version 7.0.1 for Excel, Palisade Corporation,
167 Newfield, NY) was used for the simulations and the sensitivity analysis. Statistical software R
168 3.3.0 was used for the graphical display of results. The inputs and expected outcomes of the
169 baseline model are presented in table 2.

170 **2.4. Assessment of the uncertainty and variability in model inputs**

All the estimations for the parameters of interest *($HEV_V{}^+$, $HEV_L{}^+$, $HEV_G{}^+$* and *$HEV_F{}^+$)* obtained in the baseline model, are strictly dependent upon the uncertainty distributions describing Nv_F^+, R_L^+, R_G^+, R_F^+, the value of *dpDay* and the parameters of the differential equations describing transitions across population compartments over time.

In order to quantify the impact of the uncertainty in Nv_F^+, R_L^+, R_G^+ and R_F^+, the results of two scenarios were compared; 'Scenario A', with the distribution describing Nv_F^+, R_L^+, R_G^+ and R_F^+, fixed to the value corresponding to their 5th percentile and 'Scenario B' where the distributions were fixed to the value corresponding to the 95th percentile. In addition, as a sensitivity analysis for the experts' estimates, all the relevant outputs were calculated removing the opinions related to the lower level of confidence (i.e. "not confident").

With respect to δ and β, those parameters are assumed to intrinsically incorporate all the biological and managerial factors affecting the decay rate in the proportion of animals covered by maternal immunity and those facilitating or preventing the transmission of HEV within animals. As indicated by several studies, these parameters are likely to be influenced by environmental and husbandry practices [23, 29, 36, 37]; however, accurate estimations of the effects of different management and environmental practices on the model's parameters are currently not available. Therefore, a number of arbitrary combinations were explored and the behaviour of the main model's outcome (i.e. $HEV_V{}^+$) as a function of deviations of ± 100% (by 5%) in both δ and β was assessed by calculating the outcome for each ith combination.

To this end, two discrete distributions including all the percentage deviations to be explored were used to calculate the new δ and β at each iteration as follow:

$$\delta_{new} = \delta + (\delta * \Delta(\delta))$$

$$\beta_{new} = \beta + (\beta * \Delta(\beta))$$

$\Delta(\delta)$ and $\Delta(\beta)$ are the two equal discrete distributions: $Discrete(-1, -0.95, \dots, +0.95, +1)$ that were used to modulate the changes in the original parameters during simulations.

Additionally, as the maternal antibodies are transmitted to piglets through colostrum of seropositive sows, the number of piglets protected by maternal immunity can be reasonably assumed to be directly dependent on the number of seropositive sows and the cross-fostering rate at farrowing. In order to test the impact of mitigation strategies aimed at reducing the number of piglets covered by maternal immunity, 5 scenarios in which the baseline number

of immune animals (*M*) is decreased by (5%, 10%, 50%, 90%, 100%) and increased by (5%, 10%, 45%) were simulated.

3. RESULTS

3.1. Baseline model

The parameters of the differential equations maximizing the chances of obtaining the observed values are reported in table 2. Figure 1 provides a graphical representation comparing the predicted dynamics with the data observed by De Deus et al. [29].

Our model predicted a proportion of 13.8% viraemic pigs at depopulation, which is consistent with the proportion observed by De Deus et al. [29] (i.e. 12.5%).

The results of different scenarios implemented to evaluate the effects on $HEV_V{}^+$ of hypothetical interventions aimed at increasing/reducing the infection rate or the decay of maternal immunity are summarized in figure 2.

When β was kept to its baseline value, a reduction in δ equal to a value between 0.05% and 80.0% of its baseline value led to an increased proportion of viraemic pigs entering the slaughterhouse. Similarly, reducing β by an amount between 0.05% and 40.0% of its baseline value would lead to an increase in $HEV_V{}^+$at *dpDay*. In both cases, an increase in the baseline value of δ and β would generate a lower proportion of viraemic pigs at the end of the production cycle.

A similar effect was observed, when δ and β were kept constant and the effect of changes in the number of piglets covered by maternal immunity at t_0 was assessed (Figure 3).

Results indicated that for example, a 10% reduction in the number of piglets acquiring antibodies from colostrum would lead to a decrease in the prevalence of infected pigs at slaughter equal to ~8% of the baseline (12.5%). On the other hand, if all the pigs were covered by maternal immunity at t_0 (+45% of the baseline which is equal to the whole population of 560 pigs) the simulated proportion of viraemic pigs at slaughter would be expected to increase to 19.8%.

3.2. Expert opinion results

Results of questionnaires submitted to experts investigating the persistency of infectious HEV in liver and gallbladder/bile from animals recovered from the viraemic phase are reported as

violin plots in figure 4. The violin plot describing the uncertainty in the number of days infectious HEV remains detectable in livers of animals recovered from viremia R_L^+ ranged from 0 to 120 days with a median value of 11 and 7, 23 and 51 at 25th, 75th and 95th percentile respectively. The violin plot describing the uncertainty in the number of days infectious HEV remains detectable in gallbladder/bile of animals recovered from viremia R_G^+ ranged from 0 to 180 days with a median value of 23 and 7, 40 and 83 at 25th, 75th and 95th percentile respectively. When the experts' estimates corresponding to the lower level of confidence were removed, the new distribution for R_L^+, (R_{Lc}^+) ranged from 0 to 45 with median value of 8 and 6, 8 and 22 at 25th, 75th and 95th percentile. Similarly, the new distribution for R_G^+, (R_{Gc}^+) ranged from 0 to 60 with median value of 14 and 7, 14 and 37 at the 25th, 75th and 95th percentile respectively.

3.3. Predicted proportion of $HEV_L{}^+$, $HEV_G{}^+$ and $HEV_F{}^+$.

The probability of a random pig excreting infectious HEV with faeces at depopulation ranged from 25.3% to 30.8% with a median value of 27.5% and 26.8% and 29.1% at 25th and 75th percentile. The probability of a random pig entering the slaughterhouse with infectious HEV in liver ranged from 13.8% to 94.4% with a median value of 20.0% and 17.5% and 32.9% at 25th and 75th percentile. Finally, the probability of infectious HEV in gallbladder/bile ranged from 13.8% to 94.7% with a median value of 29.2% and 17.5% and 47.2% at 25th and 75th percentile respectively. Particularly for $HEV_L{}^+$ and $HEV_G{}^+$, as these probabilities were totally dependent upon R_L^+ and R_G^+, the shapes of their distributions were compatible with the results obtained from the expert opinion (Figure 4).

3.4. Sensitivity analysis

The predicted proportions animals with infected liver ($HEV_L{}^+$) and gallbladder/bile ($HEV_G{}^+$) at $dpDay$ when answers with a low level of confidence were removed (i.e. R_{Lc}^+ and R_{Gc}^+ are used during simulation) are reported in table 4. The less confident experts were also those providing the higher upper limits in the individual discrete distributions describing both R_L^+ and R_G^+; Removing their estimations generated remarkable differences in the distributions' (right) tails while the values at 25th, 50th and 75th percentiles remained compatible with the baseline.

When all the uncertainty distributions describing Nv_F^+, R_L^+, R_G^+ and R_F^+ were fixed to the 5th percentile (i.e. Scenario A), $HEV_L{}^+$ and $HEV_G{}^+$ resulted 13.8%, the same value as $HEV_V{}^+$, this is because the 5th percentile of both R_L^+, R_G^+ is 0, while $HEV_F{}^+$ resulted 25.3%. When the values at 95th percentile were used (i.e. Scenario B) $HEV_L{}^+$, $HEV_G{}^+$and $HEV_F{}^+$resulted 63.8%, 90.05% and 30.8% respectively. Those results indicate a relevant effect of the uncertainty in those parameters.

4. Discussion

We used observed longitudinal data on hepatitis E infection in a closed pig population to adapt a MSIR model in order to reproduce and explore the dynamics leading to animals carrying HEV entering the slaughterhouse.

In recent years, several studies aimed at estimating the transmission parameters of HEV in pigs have been published [8, 23, 24]. It should be considered that the main objective of this study was to assess the practical consequences of variations in the model parameters rather than to provide an improved method for model parameterization. For this reason, the simple method we used to obtain the best parameter estimates was considered adequate for the scope of this study. When the parameters of the model were modified to simulate the effects of hypothetical strategies that could modify the rates at which maternal immunity declines or pig-to-pig transmission occurs; undesirable effects (i.e. delay in the prevalence peak leading to more viraemic pigs at depopulation) were observed suggesting great caution when considering such measures.

Only extreme scenarios where the rate of decay of maternal immunity was 80% lower than the baseline and the infection rate 40% lower led to a reduction in the predicted proportion of viraemic pigs at slaughterhouse (Figure 2). Smaller reductions would have the opposite effect on the infectious fraction at slaughter age. This is due to the fact is that animals would be infected at a later age and the prevalence peak would consequently shift towards the slaughter age as already observed by Backer at al. [24]. For the same reason, paradoxically, the behaviour of the model in response to changes in both parameters indicated that if a given 'threshold' reduction is not achieved, the positive effect can be observed by decreasing the infection age so that prevalence peaks earlier. The same logic applies when changing the proportion of piglets covered by maternal immunity at t_0 (figure 3). It should be considered

289 that both the parameters related to number of piglets covered by maternal immunity at t_0
290 and the rate of decay of maternal immunity are strictly related to the infectious status of the
291 sows (and previous exposure to HEV) and the management of the piglets. In fact, the presence
292 of anti-HEV antibodies in the colostrum is conditional to the seropositive status of the sow
293 and the serological titres of piglets at one week of age was found to be highly correlated with
294 those of the dams [37]. This evidence suggests that the decay of the maternal immunity at
295 individual level (and thus in the population), is also strictly related to the amount of antibodies
296 each piglet acquired through colostrum ingestion. Furthermore, cross-fostering rate at
297 farrowing has been found to be a significant risk factor for the presence of viraemic pigs at
298 slaughter [38]. All the available evidence indicate that mitigation strategies aimed at reducing
299 the number of seropositive sows at farrowing could lead to an overall decrease in the number
300 of viraemic pigs entering the slaughterhouse.

301 To our knowledge, this is the first attempt at exploring HEV dynamics considering the
302 persistence of infectious HEV in animals recovered from the viraemic phase. This feature is of
303 critical importance in terms of the public health implications of HEV infection in slaughter pigs
304 and essential for any future probabilistic assessment of human HEV exposure through the
305 consumption of pork products. In fact, on one hand, some of those organs are food product
306 themselves and the consumption of products containing pork liver has been identified as risk
307 factor for human HEV infection [13, 15, 39, 40]. Furthermore, the presence of active virus in
308 this organ, in bile and in faeces, might lead to cross-contamination during the evisceration
309 procedures at the slaughterhouse where the rupture of the guts or gallbladder may occur.

310 In this study, we made use of the 'expert opinion' to overcome the lack of data/evidence on
311 key aspects of the hepatitis E pathogenesis.

312 Although the estimations we obtained for the parameters R_L^+ and R_G^+ are characterized by
313 considerable uncertainty (reflecting the actual lack of knowledge in this key aspect of HEV
314 pathogenesis), this approach gave us the opportunity to assess the extent of this key data gap
315 and the importance of the uncertainty surrounding those parameters from a food safety
316 prospective. Lack of data against with this results can be compared (i.e. longitudinal data
317 including the proportions of recovered pigs with infected livers and gallbladders at
318 depopulation) prevented a proper validation of the model outcomes. Generating knowledge
319 to fill the identified gaps might be challenging, but essential in order to conduct a sound

quantitative assessment of exposure to HEV through consumption of pork meat or products made with pork meat.

Main assumptions

Consistently with available evidence [31, 32] and the above referenced studies estimating the rate of HEV transmission by means of SIR models, the main assumptions made in the structure of the model are: (i) homogeneous mixing of the pigs within the herd and (ii) no reversion back to the viraemic stage once immunity is developed.

Conclusions

We developed a stochastic model suitable to estimate the expected proportions of pigs carrying hepatitis E virus in their blood, liver, gallbladder/bile and faeces when entering the slaughterhouse. Thus, the model extends previous simulation frameworks that were restricted to viraemic animals to include all groups of animals of relevance from a food safety perspective. Although considerable uncertainty exists regarding key parameters of the model, it allows a critical evaluation of the potential consequences of on-farm mitigation strategies and a quantification of the impact of the most important gaps in knowledge in the pathogenesis of HEV.

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425 **TABLES**

426 *Table 1. Observed number of pigs with evidence of maternal derived immunity (M) and*
427 *viraemia (V) in time as reported by De Deus et al., [29].*

sampling week	Sample size	$M\ (HEV\ IgG^{+})$	$I\ (HEV_V{}^{+})$
1	42	23	3
3	43	15	3
6	41	9	1
9	36	5	4
12	30	Na	7
15	26	Na	11
18	21	Na	5
22	16	Na	2

428

429 *Table 2. Overview of the model inputs and expected outcomes of the baseline model.*

Input	Distribution/Function	Description	Unit	Source
dpDay	Constant	Day of depopulation	Day	[29]
$HEV_V{}^{+}$	Infected at *dpDay*	Predicted proportion of viraemic animals at *dpDay*	%	//
Nv_F^{+}	Uniform (7;60)	Number of days Not-viraemic animals are excreting HEV with faeces before onset of viraemia	Days	[31, 32]
R_L^{+}	$Discrete(\{x_i\};\{p_i\}$	Number of days infectious HEV remains detectable in livers of animals recovered from viraemia	Days	Expert opinion
R_G^{+}	$Discrete(\{x_i\};\{p_i\}$	Number of days infectious HEV RNA remains detectable in gallbladder of animals recovered from viraemia	Days	Expert opinion
R_F^{+}	$Discrete(14,..,21;1,..,1)$	Number of days HEV RNA remains detectable in faeces of animals recovered from viraemia	Days	[31, 32]

$HEV_{Nv_{F^+}}$	$(HEV_V{}^+ at\ dpDay + Nv_F^+)$	Predicted proportion of Not-viraemic animals excreting HEV with faeces at $dpDay + Nv_F^+$	%	//
$HEV_{R_{L^+}}$	$recovered\ at: dpDay - R_L^+$	Proportion of recovered animals with infected liver	%	//
$HEV_{R_{G^+}}$	$recovered\ at: dpDay - R_G^+$	Proportion of recovered animals with infected gallbladder/bile	%	//
$HEV_{R_{F^+}}$	$recovered\ at: dpDay - R_F^+$	Proportion of recovered animals excreting HEV with faeces	%	//
$HEV_L{}^+$	$HEV_V{}^+ + HEV_{R_{L^+}}$	Overall proportion of animals with infected liver at *dpDay*	%	//
$HEV_G{}^+$	$HEV_V{}^+ + HEV_{R_{G^+}}$	Overall proportion of animals with infected gallbladder/bile at *dpDay*	%	//
$HEV_F{}^+$	$HEV_V{}^+ + HEV_{Nv_{F^+}} + HEV_{R_{F^+}}$	Overall proportion of animals excreting HEV with faeces at *dpDay*	%	//

Table 3. Estimates of transmission parameters (δ = rate of decay of maternal immunity, β = infection rate, γ = recovery rate) for a MSIR model of hepatitis E transmission, obtained using the Generalized Reduced Gradient (GRG) algorithm to fit the data from De Deus et al. [29].

Parameter	Value
δ	3.1E-02
β	2.5E-04
γ	3.5E-02
M (t0)	384
S (t0)	175
I (t0)	1

436 *Table 4. Predicted proportions of viraemic animals* ($HEV_V{}^+$), *animals with infected*
437 *liver* ($HEV_L{}^+$), *gallbladder/bile* ($HEV_G{}^+$) *and animals actively excreting HEV with faeces*
438 ($HEV_F{}^+$) *at* $dpDay$ *in the baseline model (baseline) and when opinions from experts with a*
439 *level of confidence equal to 1 were removed (**s**). The values representing the median, 25th and*
440 *75th percentiles of the outputs' distributions are reported together with the Minimum and*
441 *Maximum.*

	Median	Min	25th percentile	75th percentile	Max
$HEV_L{}^+$(Baseline)	20.0%	13.8%	17.5%	32.9%	94.4%
$HEV_G{}^+$(Baseline)	29.2%	13.8%	17.5%	47.2%	94.7%
$HEV_F{}^+$(Baseline)	27.5%	25.3%	26.8%	29.1%	30.8%
$HEV_L{}^+(\mathbf{s})$	18.1%	13.8%	16.9%	28.3%	53.5%
$HEV_G{}^+$ $(\mathbf{s})$	26,6%	13.8%	17.5%	43.6%	72.4%

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Figure 1 comparison between the population dynamics after parameterization of the set of differential equation describing a MSIR model and observed data reported by De Deus et al. (2008). Solid grey = covered by passive immunity (M), short dash = Not-viraemic (Nv), solid black = infected (I), long dash = Recovered (R). Solid circle and triangles are the proportions of animals with maternal immunity and infected observed by De Deus et al. (2008).

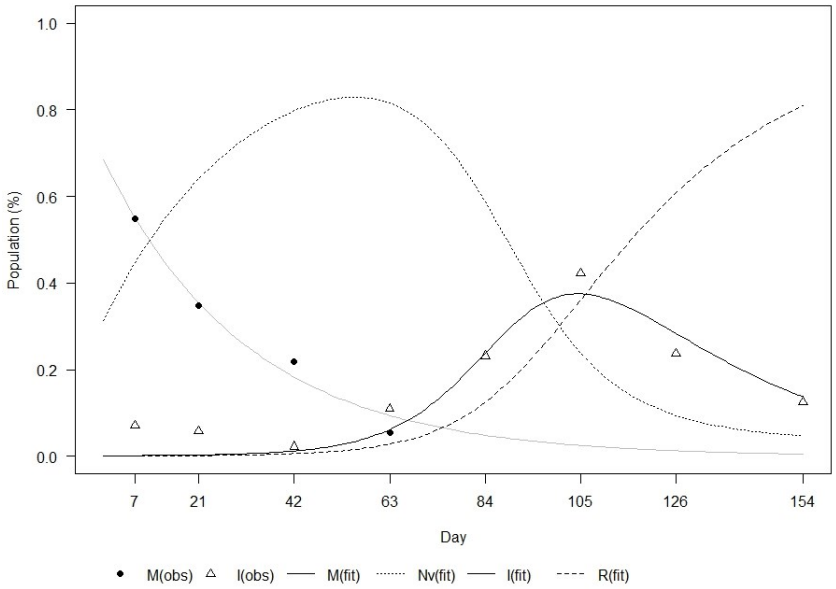

Figure 2 results of simulated scenarios assessing the behaviour of $HEV_V{}^+$ as a function of the rate of decay of animals covered by maternal antibodies (δ) and the infection rate (β). In the upper graph, β was kept constant and equal to the value used in the baseline model and only variation in δ was assessed. In the lower graph, δ was kept constant and the effects of variation in β were explored. In both the graphs, the crossed points indicate the percentage variations in δ and β leading to an increased proportion of viraemic pigs at slaughter compared to the baseline.

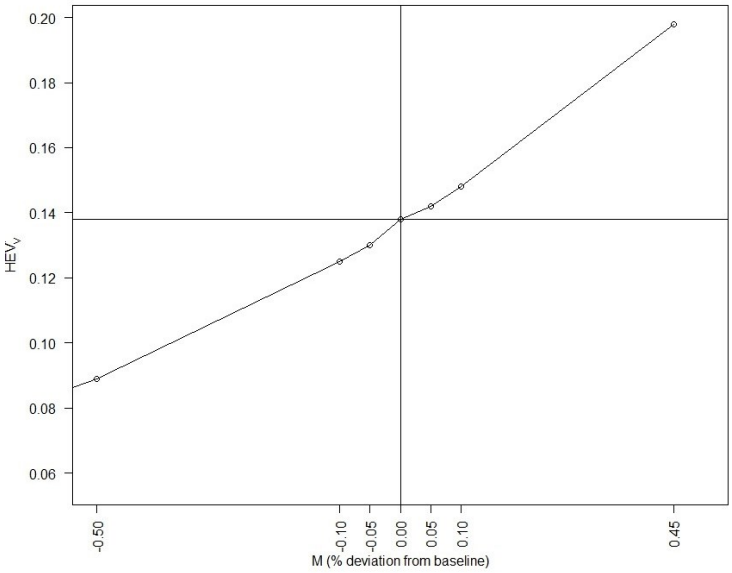

Figure 3 graphical representation of changes in baseline proportion of viraemic pigs entering the slaughterhouse when the baseline number of piglets covered by maternal immunity at t_0 decreased by 5%, 10% and 50% or increased by 5%, 10% and 45% from the baseline value.

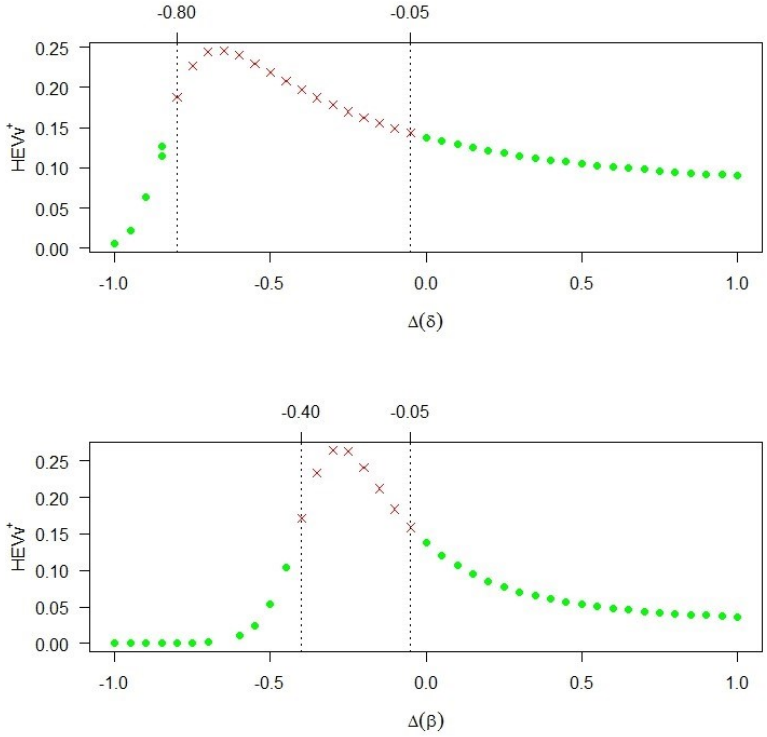

Figure 4 Violin plots representing the uncertainty in the length of time infectious HEV can be considered detectable in liver (R_L^+) and gallbladder/bile (R_G^+) after recovery from the viraemic phase. For each 'violin', the white dot and thick internal lines represent the median and 25th/75th percentiles, while the total height of the violin represents the range of the data