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This is the peer-reviewed, manuscript version of an article published in *Microbial Risk Analysis*. The version of record is available from the journal site:

<http://dx.doi.org/10.1016/j.mran.2018.02.001>.

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The full details of the published version of the article are as follows:

TITLE: 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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JOURNAL: Microbial Risk Analysis

PUBLISHER: Elsevier

PUBLICATION DATE: 9 February 2018 (online)

DOI: 10.1016/j.mran.2018.02.001

**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

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

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

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

## 53 **2. MATERIAL AND METHODS**

### 54 **2.1. Baseline model**

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

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

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

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

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

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

$$75 \frac{dM}{dT} = -\delta M$$

$$76 \frac{dNv}{dT} = \delta M - \beta NvV$$

$$77 \frac{dV}{dT} = \beta NvV - \gamma V$$

$$78 \frac{dR}{dT} = \gamma V$$

79 Where:  $\delta$  is the decay rate of the population with maternal immunity ( $M$ ),  $\beta$  is the transition  
80 rate from Not-viraemic ( $Nv$ ) to viraemic ( $V$ ) and  $\gamma$  represents the recovery rate from the  
81 viraemic status.

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

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

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

## 98 **2.2. Infectious status of the pigs in the different compartments.**

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

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

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

$$112 Nv_F^+ = Uniform(7; 60) \text{ days}$$

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

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

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

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

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

### 135 **2.3. Expert opinion**

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

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

143 Results were included into a discrete distribution:



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

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  $i^{\text{th}}$  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  $HEV_V^+ = \text{predicted proportion of viraemic at } dpDay$

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**

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

175 In order to quantify the impact of the uncertainty in  $Nv_F^+$ ,  $R_L^+$ ,  $R_G^+$  and  $R_F^+$ , the results of two  
176 scenarios were compared; ‘Scenario A’, with the distribution describing  $Nv_F^+$ ,  $R_L^+$ ,  $R_G^+$  and  $R_F^+$ ,  
177 fixed to the value corresponding to their 5<sup>th</sup> percentile and ‘Scenario B’ where the  
178 distributions were fixed to the value corresponding to the 95<sup>th</sup> percentile. In addition, as a  
179 sensitivity analysis for the experts’ estimates, all the relevant outputs were calculated  
180 removing the opinions related to the lower level of confidence (i.e. “not confident”).

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

190 To this end, two discrete distributions including all the percentage deviations to be explored  
191 were used to calculate the new  $\delta$  and  $\beta$  at each iteration as follow:

$$192 \quad \delta_{new} = \delta + (\delta * \Delta(\delta))$$

$$193 \quad \beta_{new} = \beta + (\beta * \Delta(\beta))$$

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

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

201 of immune animals ( $M$ ) is decreased by (5%, 10%, 50%, 90%, 100%) and increased by (5%,  
202 10%, 45%) were simulated.

### 203 **3. RESULTS**

#### 204 **3.1. Baseline model**

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

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

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

213 When  $\beta$  was kept to its baseline value, a reduction in  $\delta$  equal to a value between 0.05% and  
214 80.0% of its baseline value led to an increased proportion of viraemic pigs entering the  
215 slaughterhouse. Similarly, reducing  $\beta$  by an amount between 0.05% and 40.0% of its baseline  
216 value would lead to an increase in  $HEV_V^+$  at  $dpDay$ . In both cases, an increase in the baseline  
217 value of  $\delta$  and  $\beta$  would generate a lower proportion of viraemic pigs at the end of the  
218 production cycle.

219 A similar effect was observed, when  $\delta$  and  $\beta$  were kept constant and the effect of changes in  
220 the number of piglets covered by maternal immunity at  $t_0$  was assessed (Figure 3).

221 Results indicated that for example, a 10% reduction in the number of piglets acquiring  
222 antibodies from colostrum would lead to a decrease in the prevalence of infected pigs at  
223 slaughter equal to ~8% of the baseline (12.5%). On the other hand, if all the pigs were covered  
224 by maternal immunity at  $t_0$  (+45% of the baseline which is equal to the whole population of  
225 560 pigs) the simulated proportion of viraemic pigs at slaughter would be expected to increase  
226 to 19.8%.

#### 227 **3.2. Expert opinion results**

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

230 violin plots in figure 4. The violin plot describing the uncertainty in the number of days  
231 infectious HEV remains detectable in livers of animals recovered from viremia  $R_L^+$  ranged  
232 from 0 to 120 days with a median value of 11 and 7, 23 and 51 at 25<sup>th</sup>, 75<sup>th</sup> and 95<sup>th</sup> percentile  
233 respectively. The violin plot describing the uncertainty in the number of days infectious HEV  
234 remains detectable in gallbladder/bile of animals recovered from viremia  $R_G^+$  ranged from 0  
235 to 180 days with a median value of 23 and 7, 40 and 83 at 25<sup>th</sup>, 75<sup>th</sup> and 95<sup>th</sup> percentile  
236 respectively. When the experts' estimates corresponding to the lower level of confidence  
237 were removed, the new distribution for  $R_L^+$ , ( $R_{Lc}^+$ ) ranged from 0 to 45 with median value of  
238 8 and 6, 8 and 22 at 25<sup>th</sup>, 75<sup>th</sup> and 95<sup>th</sup> percentile. Similarly, the new distribution for  $R_G^+$ , ( $R_{Gc}^+$ )  
239 ranged from 0 to 60 with median value of 14 and 7, 14 and 37 at the 25<sup>th</sup>, 75<sup>th</sup> and 95<sup>th</sup>  
240 percentile respectively.

### 241 **3.3. Predicted proportion of $HEV_L^+$ , $HEV_G^+$ and $HEV_F^+$ .**

242 The probability of a random pig excreting infectious HEV with faeces at depopulation ranged  
243 from 25.3% to 30.8% with a median value of 27.5% and 26.8% and 29.1% at 25<sup>th</sup> and 75<sup>th</sup>  
244 percentile. The probability of a random pig entering the slaughterhouse with infectious HEV  
245 in liver ranged from 13.8% to 94.4% with a median value of 20.0% and 17.5% and 32.9% at  
246 25<sup>th</sup> and 75<sup>th</sup> percentile. Finally, the probability of infectious HEV in gallbladder/bile ranged  
247 from 13.8% to 94.7% with a median value of 29.2% and 17.5% and 47.2% at 25<sup>th</sup> and 75<sup>th</sup>  
248 percentile respectively. Particularly for  $HEV_L^+$  and  $HEV_G^+$ , as these probabilities were totally  
249 dependent upon  $R_L^+$  and  $R_G^+$ , the shapes of their distributions were compatible with the  
250 results obtained from the expert opinion (Figure 4).

### 251 **3.4. Sensitivity analysis**

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

259 When all the uncertainty distributions describing  $Nv_F^+$ ,  $R_L^+$ ,  $R_G^+$  and  $R_F^+$  were fixed to the 5<sup>th</sup>  
260 percentile (i.e. Scenario A),  $HEV_L^+$  and  $HEV_G^+$  resulted 13.8%, the same value as  $HEV_V^+$ ,  
261 this is because the 5<sup>th</sup> percentile of both  $R_L^+$ ,  $R_G^+$  is 0, while  $HEV_F^+$  resulted 25.3%. When the  
262 values at 95<sup>th</sup> percentile were used (i.e. Scenario B)  $HEV_L^+$ ,  $HEV_G^+$  and  $HEV_F^+$  resulted  
263 63.8%, 90.05% and 30.8% respectively. Those results indicate a relevant effect of the  
264 uncertainty in those parameters.

#### 265 **4. Discussion**

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

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

279 Only extreme scenarios where the rate of decay of maternal immunity was 80% lower than  
280 the baseline and the infection rate 40% lower led to a reduction in the predicted proportion  
281 of viraemic pigs at slaughterhouse (Figure 2). Smaller reductions would have the opposite  
282 effect on the infectious fraction at slaughter age. This is due to the fact is that animals would  
283 be infected at a later age and the prevalence peak would consequently shift towards the  
284 slaughter age as already observed by Backer at al. [24]. For the same reason, paradoxically,  
285 the behaviour of the model in response to changes in both parameters indicated that if a  
286 given 'threshold' reduction is not achieved, the positive effect can be observed by decreasing  
287 the infection age so that prevalence peaks earlier. The same logic applies when changing the  
288 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

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

### 322 **Main assumptions**

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

### 327 **Conclusions**

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

### 336 **Acknowledgments**

337 The study was part of project funded by the Agriculture and Horticulture Development  
338 Board (AHDB-Pork). [ref. 5140004020]

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423

424

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
$R_F^+$	$Discrete(14, \dots, 21; 1, \dots, 1)$	Number of days HEV RNA remains detectable in faeces of animals recovered from viraemia	Days	[31, 32]

$HEV_{Nv_F^+}$	$(HEV_V^+ \text{ at } dpDay + Nv_F^+)$	Predicted proportion of Not-viraemic animals excreting HEV with faeces at $dpDay + Nv_F^+$	%	//
$HEV_{R_L^+}$	<i>recovered at: <math>dpDay - R_L^+</math></i>	Proportion of recovered animals with infected liver	%	//
$HEV_{R_G^+}$	<i>recovered at: <math>dpDay - R_G^+</math></i>	Proportion of recovered animals with infected gallbladder/bile	%	//
$HEV_{R_F^+}$	<i>recovered at: <math>dpDay - R_F^+</math></i>	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 $dpDay$	%	//

430

431 *Table 3. Estimates of transmission parameters ( $\delta$  = rate of decay of maternal immunity,*  
432  *$\beta$  = infection rate,  $\gamma$  = recovery rate) for a MSIR model of hepatitis E transmission, obtained*  
433 *using the Generalized Reduced Gradient (GRG) algorithm to fit the data from De Deus et al.*  
434 *[29].*

Parameter	Value
$\delta$	3.1E-02
$\beta$	2.5E-04
$\gamma$	3.5E-02
$M(t_0)$	384
$S(t_0)$	175
$I(t_0)$	1

435

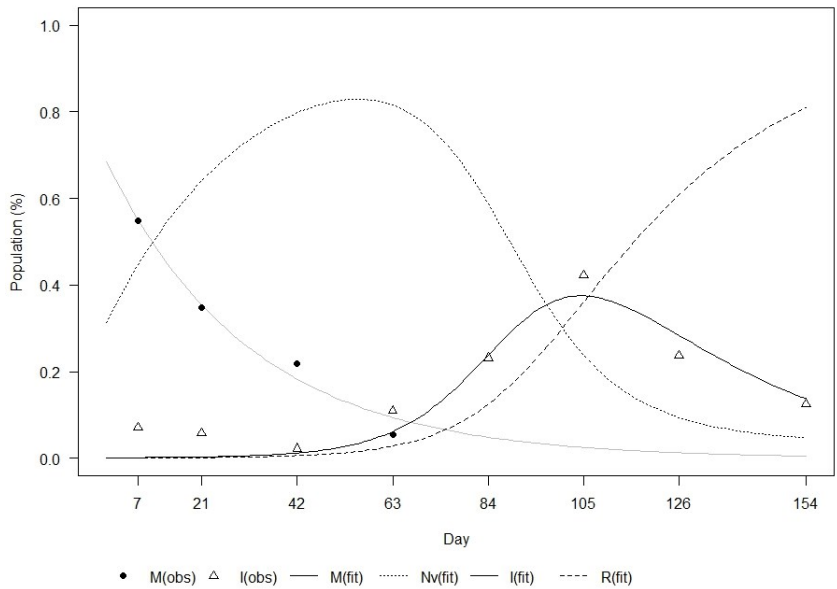
436 Table 4. Predicted proportions of viraemic animals ( $HEV_V^+$ ), animals with infected  
 437 liver ( $HEV_L^+$ ), gallbladder/bile ( $HEV_G^+$ ) and animals actively excreting HEV with faeces  
 438 ( $HEV_F^+$ ) at  $dpDay$  in the baseline model (baseline) and when opinions from experts with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

	Median	Min	25 <sup>th</sup> percentile	75 <sup>th</sup> 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%
$HEV_L^+$ (s)	18.1%	13.8%	16.9%	28.3%	53.5%
$HEV_G^+$ (s)	26,6%	13.8%	17.5%	43.6%	72.4%

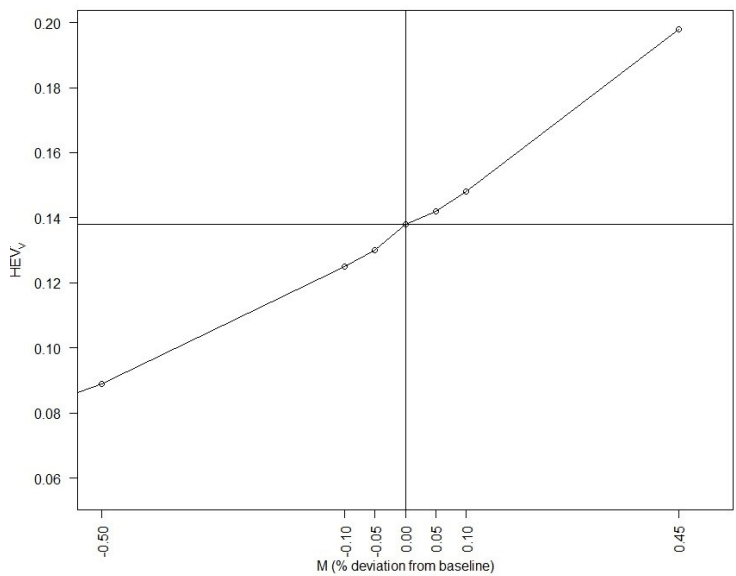
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443

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

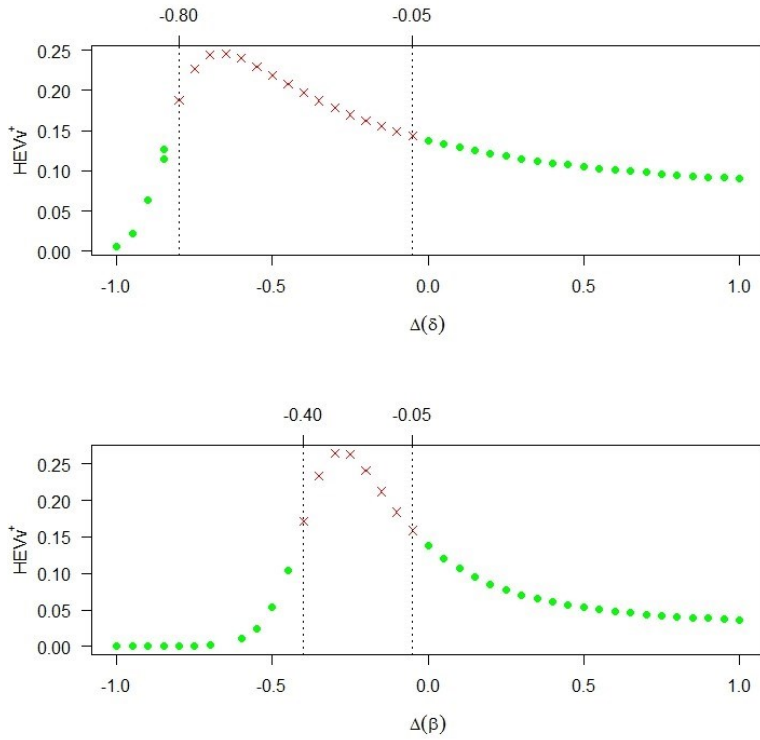


448  
 449 Figure 2 results of simulated scenarios assessing the behaviour of  $HEV_v^+$  as a function of the rate of decay of animals covered by maternal  
 450 antibodies ( $\delta$ ) and the infection rate ( $\beta$ ). In the upper graph,  $\beta$  was kept constant and equal to the value used in the baseline model and only  
 451 variation in  $\delta$  was assessed. In the lower graph,  $\delta$  was kept constant and the effects of variation in  $\beta$  were explored. In both the graphs, the  
 452 crossed points indicate the percentage variations in  $\delta$  and  $\beta$  leading to an increased proportion of viraemic pigs at slaughter compared to the  
 453 baseline.

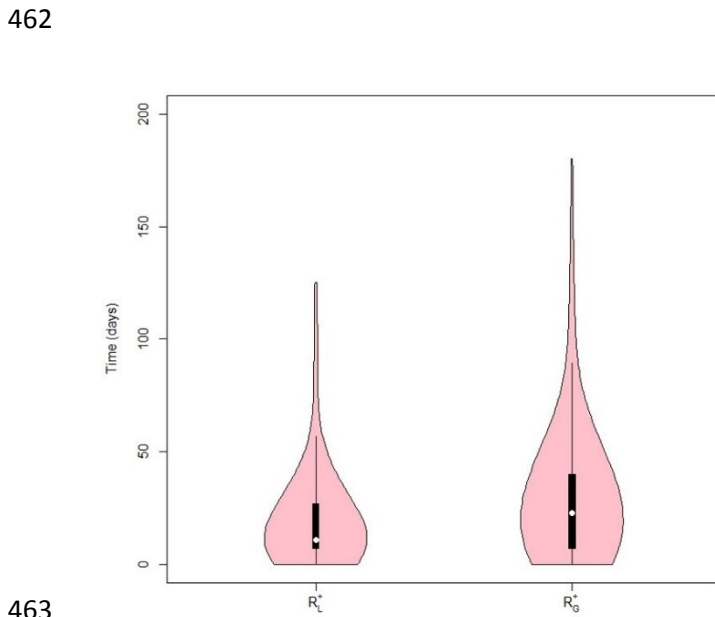


454  
 455

456 Figure 3 graphical representation of changes in baseline proportion of viraemic pigs entering the slaughterhouse when the baseline number  
 457 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.



458  
 459 Figure 4 Violin plots representing the uncertainty in the length of time infectious HEV can be considered detectable in liver ( $R_L^+$ ) and  
 460 gallbladder/bile ( $R_G^+$ ) after recovery from the viraemic phase. For each 'violin', the white dot and thick internal lines represent the median  
 461 and 25<sup>th</sup>/75<sup>th</sup> percentiles, while the total height of the violin represents the range of the data



463  
 22