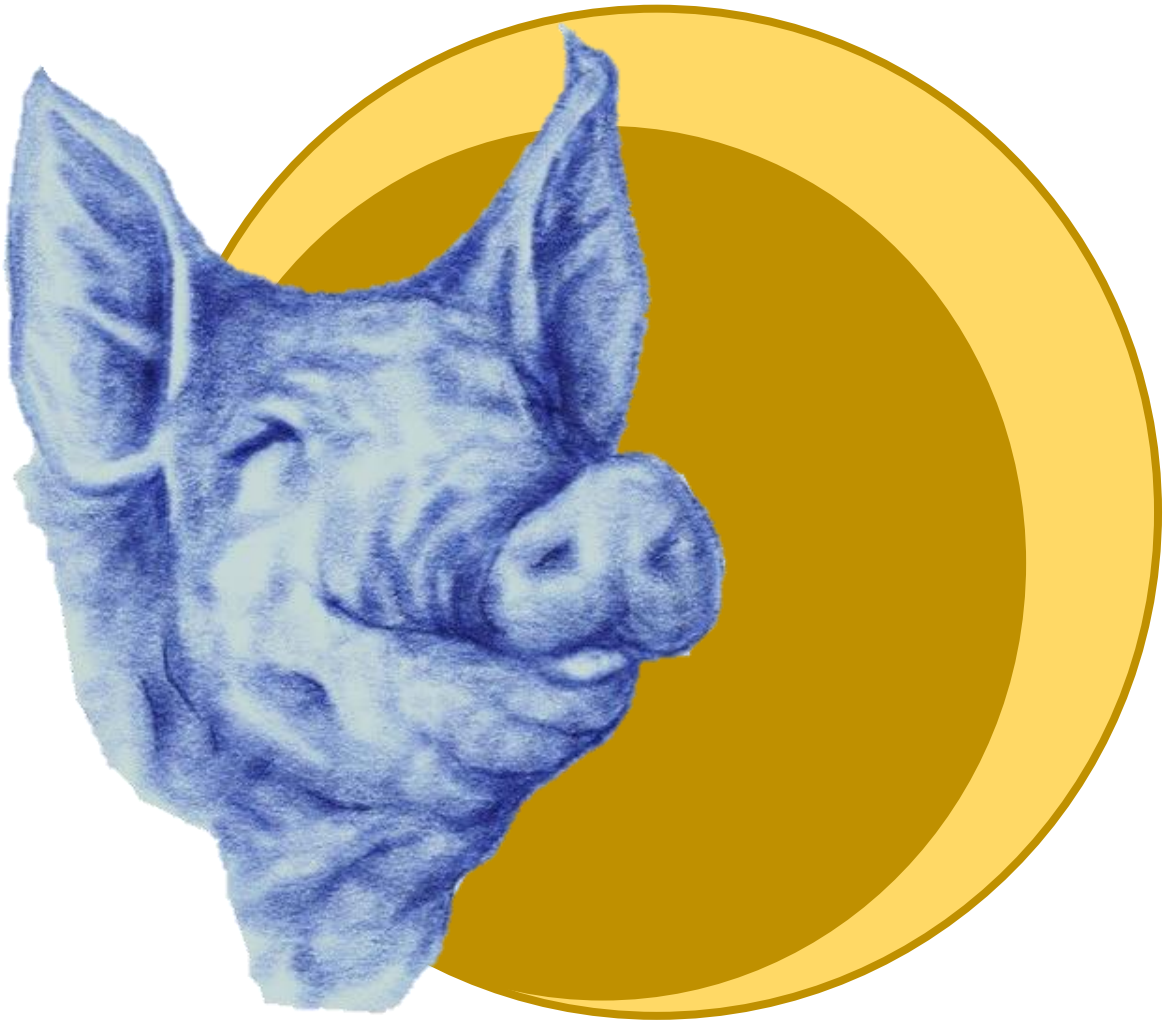
Modelling the flow of Hepatitis E virus infection through the pork production chain and the effects of different management practices on the prevalence at slaughter



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Abstract

A hepatitis E virus (HEV) infection can cause viral hepatitis in humans, pigs are often cited as the main source of zoonotic infections in industrialised countries. HEV-3 and HEV-4 are zoonotic forms of the virus. Human infections can occur through the consumption of undercooked contaminated pork products and contact with contaminated environments. Altering farm management to either push back the peak of HEV-positive pigs or extend the production period so that the majority of pigs have cleared the infection by the time they are slaughtered could help reduce the number of HEV-positive pigs entering the slaughterhouse. Thus, reducing the number of foodborne HEV infections in the Dutch population. A batch of farrow-to-finish pigs was modelled to assist in investigating the effects of different farm management options to reduce HEV-positive pigs at slaughter age. A stochastic MSEIR model with an environmental compartment was utilised in this modelling attempt. Different proposed management approaches in pork production such as altering the time to slaughter, group sizes and the presence of maternal antibodies were compared to a basic scenario. It was concluded, that increasing the number of pigs housed together in a pen did not have a significant effect on the number of HEV-positive pigs at slaughter. Reducing the percentage of maternal antibodies present in a litter to at least 50% significantly reduced the number of HEV-positive pigs at slaughter. A batch with no maternal antibodies present reduced the number of HEV-positive pigs at slaughter age even more drastically. Lastly, extending the production period from 180 to 184 days was the minimum extension needed to significantly reduce the number of HEV-positive pigs at slaughter. In conclusion, the lack of maternal antibodies in a batch appeared to have the biggest effect. In reality, this will be very costly and challenging to implement and maintain. The extension of the fattening period appears to be an easier change at first glance, however, drawbacks become clear upon closer inspection. It is an intervention method very reliant on the infection timing and growth rate of every batch remaining somewhat constant. In reality, these are factors that can often vary amongst farms for several reasons. Future research should look at designing new interventions based on the cleanliness of pig pens both during a batch and between batches as these have been identified as promising parameters to alter to aid in reducing the number of HEV-positive pigs sent to slaughter.

Contents

Introduction

Hepatitis E virus (HEV) is a non-enveloped, single-strand RNA virus belonging to the family *Hepeviridae* (Purdy *et al.,* 2017) which in some cases can cause viral hepatitis in humans (Nicot *et al.,* 2021). Genotypes HEV-3 and HEV-4 have been implicated in zoonotic transmission (Purdy *et al.,* 2017; Mohammad Sultan Khuroo *et al.*, 2016).

HEV-3 is the most frequently encountered genotype associated with human infection in Europe and America (Nicot *et al.,* 2021). Hogema *et al.* (2014) reported an estimated seroprevalence of 27.3% and Arianne *et al.* (2017) reported a seroprevalence of 28.7% for the Dutch population. Most infections are asymptomatic leading to a probable underestimation of HEV cases as many infections may go unnoticed (Lhomme *et al.,* 2020; Mohammad Sultan Khuroo *et al.*, 2016). Additionally, HEV can easily be misdiagnosed as drug-induced liver injury (DILI). The misdiagnoses can be the consequence of clinicians not being aware of the scale of the issue in non-endemic regions. Nonetheless, HEV-3 can cause prolonged viremia, chronic hepatitis, liver fibrosis and cirrhosis in some immunocompromised individuals. Immunocompromised individuals that are especially vulnerable to a severe HEV infection include recipients of solid organ transplants, individuals with human immunodeficiency virus (HIV) and those with haematological neoplasms. Additionally, individuals with chronic alcoholism and/or alcoholic chronic liver disease can acquire HEV superinfections. Interestingly, pregnant individuals who acquire a HEV infection in industrialised countries experience much fewer devastating symptoms compared to individuals in developing countries (Mohammad Sultan Khuroo *et al.*, 2016).

Pigs are commonly cited as the source of zoonotic HEV infections (Purdy *et al.*, 2017; Bouwknegt *et al.*, 2009a; Meng, 2010; Salines *et al.*, 2017), which is corroborated by the findings of Withers *et al.* (2002) who found a higher seroprevalence amongst swine workers in comparison to the general public. HEV infections in pigs are asymptomatic (Mohammad Sultan Khuroo *et al.*, 2016; Salines *et al.*, 2017; Ricci *et al.*, 2017; Kasorndorkbua *et al.*, 2003). Nonetheless, microscopic manifestations can be observed (Mohammad Sultan Khuroo *et al.*, 2016; Kasorndorkbua *et al.*, 2003). In addition, porcine HEV-3 and HEV-4 samples are very similar or indistinguishable from HEV isolated from sporadic human HEV cases (Meng, 2010; Salines *et al.*, 2017). Hogema *et al.* (2021) found that there was no significant difference in the distribution of HEV subtypes between pigs and humans in the Netherlands, thus reinforcing the assumption that pigs are a key source of human infections. Pigs are considered the main reservoir for human infection in industrialised countries (Salines *et al.,* 2017). Other species, such as wild boars, sika deer, mongooses, rabbits (Meng, 2010), cattle, sheep, goats, dogs, cats, horses and rodents have displayed HEV-specific antibodies. However, it has not yet been confirmed that these animals can effectively host and spread the virus (Bouwknegt *et al.,* 2009a).

People can contract HEV through contaminated food and indirect contact through a contaminated environment (Nicot *et al.,* 2021). The consumption of raw/undercooked pork meat, certain pork sausages and pork liver products is often mentioned as a foodborne source of infection (Rodríguez-Lázaro *et al.*, 2018; Salines *et al.,* 2017). The Dutch population consumed 36.4kg of pork per capita in 2020, which resulted in 47% of the meat consumed in the Netherlands being pork (Dagevos *et al.*, 2020). Seroprevalence was found to be lower amongst Dutch vegetarians/vegans/flexitarians (who implemented a vegetarian diet since the age of twelve) who had a relative risk of 0.36 [95%CI:0.23-0.57] compared to the reference group of Dutch blood donors (Alberts *et al.*, 2018).

To reduce foodborne HEV infections in people in The Netherlands, the number of HEV-positive pigs entering the food supply needs to be reduced. The degree of viremia at slaughter can vary depending on the farm management, the age of infection and slaughter age (Ricci *et al.*, 2017). Of the Dutch conventional farms looked at by the 2022 study by Meester *et al.* an average of 73.8% of pigs were found to be seropositive at slaughter on conventional farms compared to 81.4% of organic pigs. Remarkably, within-farm percentages of PCR-positive batches were 44% for conventional farms and only 8.33% for organic farms. As a result, the conventional farms had a risk of 9.61 [CI 95% 5.19-17.8] of delivering at least one PCR-positive pig to slaughter in a batch compared to organic farms. It was theorised that the less compartmentalised nature of organic farms could contribute to these findings. Currently, HEV-free farms appear unattainable even on farms with heightened biosecurity (Meester *et al.,* 2022). Additionally, the widespread dispersion of the virus amongst swine farms points to various infection dynamics possibly linked to farm-specific factors (Salines *et al.,* 2017). Thus, altering on-farm aspects to better time the peak of the infection within a batch could be a viable solution to reducing the number of HEV-positive pigs that end up on the slaughter line. Therefore, shifting back the peak of infectious pigs so that the majority of the pigs have cleared the infection at the time of slaughter or shifting back the age of slaughter.

This project aimed to simulate a conventional farrow-to-finish pig farm and find minimal changes that can possibly be implemented through farm management to produce the least number of HEV-positive pigs at slaughter. Therefore, the project compared different management approaches in pork production, such as altering the time to slaughter, group sizes and the presence of maternal antibodies (MAbs) to a basic scenario.

Materials and Methods

A brief literature review was done on the zoonotic nature of HEV, pig husbandry, HEV infections in pigs and related parameters. The review can be found in the annexe. It was used to better understand the set-up and management practices in pig farms. Certain topics regarding the animal-bound parameters were also further explored. From this collection of literature, the model design and related parameters ranges were decided upon.

The model

A stochastic Maternal immunity (M)-Susceptible(S)-Exposed(E)-Infectious (I)-Recovered (R)-Environment (V) (MSEIR-V) model was made in R (R Core Team, 2018). Additionally, the package SimInf was used (Widgrens *et al.,* 2019). A single MSEIR-V model is depicted in the set of ordinary differential equations (ODEs) below in equations 1-6. A diagram of the basic scenario can be seen later in Figure 5. The diagrams were created in Microsoft Viso (Microsoft Corporation, 2018). Below in Tables 1 and 2, the parameters displayed in Figure 5, 6 and the ODEs are defined. In Tables 1 and 2, the associated values are displayed next to the parameters. Each value is shortly explained below. Each pen within each production section (farrowing, weaning and fattening) has the displayed model running within it whilst pigs are present. Per model run, a batch of pigs will move through all three sections on the farm until slaughter age. The ODEs are based on the model depicted by Meester *et al.* (2021).

$$\frac{dM}{dt} = -\omega M - \mu M \quad (1)$$

$$\frac{dS}{dt} = \omega M - \beta_V \frac{VS}{N} - \mu S \quad (2)$$

$$\frac{dE}{dt} = \beta_V \frac{VS}{N} - \alpha E - \mu E \quad (3)$$

$$\frac{dI}{dt} = \alpha E - \gamma I - \mu I \quad (4)$$

$$\frac{dR}{dt} = \gamma I - \mu R \quad (5)$$

$$\frac{dV}{dt} = \tau \mathrm{I} - \eta dV \quad (6)$$

ODEs of a pen-bound MSEIR-V model. Symbols are defined in Table 1 and 2

Population

The population is comprised of growing pigs on a farrow-to-finish farm. The breeding herd was not considered in any capacity in this model, other than the possible transfer of MAbs. The population of growing pigs is subdivided into three age categories and three different locations, respectively. The piglets aged 0-28 days will be housed in the farrowing section. In the farrowing room, the piglets suckle from their maternal sows. Cross-fostering practices are not included in the model for simplicity. At the end of the 28 days, the piglets are moved to an empty nursery room and weaned. The weaned piglets aged 29-86 days are housed in the nursery section. The pigs are then moved to finishing rooms until they reach slaughter weight assumed to be around 180 days of age (Salines *et al.,* 2020). Below in Figure 1, the flow of pigs through the different sections is depicted.

Figure 1: The flow of a batch of grower pigs from birth to slaughter.

The metapopulation on a farm is subdivided into collectives known as batches. Batches are categorised by the state of the animal (age) and room type (Cador *et al.,* 2016). In the basic scenario, there will be 22 pens consisting of 12 pigs per pen. The division of pigs over pens in the basic scenario can be seen in Figure 2. A group of sows give birth to piglets around the same time, these piglets will then be the future finishing pigs and will move through the system as a batch (Salines *et al.,* 2020). This model depicts a simplified version of the population dynamics of a farrow-to-finish pig farm managed according to a batch-rearing system. This form of management allows for an all-in-all-out system to take place (Bown, 2006). An-all-in-all-out system allows all pigs from the same batch to enter an empty room together and leave the room together. The model will only focus on a single batch at a time due to the segregated nature of the batches on a pig farm.

Environmental layout

There are three environmental stages that the pigs move through according to their age as mentioned above. These three stages are subdivided into separate rooms on a farm. Within rooms there are pens. Similarly aged pigs are subdivided into groups, usually by litter, and housed in the pens. In this model infected pigs can only infect pen mates and not pigs in adjacent pens.

A limited relationship between batches exists through the environment. Farm material and farm workers could facilitate this connection in reality (Salines *et al.,* 2020). However, for simplicity, between batch connections are not considered in this model. All-in-all-out management allows that each batch is managed somewhat independently and limits the connections between batches. These management concepts are further explained in sections 2.4 and 2.5 in the annexe. Thus, between batch spread is not considered in this model.

For this model, a smaller version of the average Dutch farm sizes was chosen. The averages are affected by specialised farms that have much more space dedicated to certain production stages. As a farrow-to-finish farm contains all three production stages, the assumption of smaller areas per stage is made. Additionally, available places are divided into compartments containing different batches. Thus, for one batch within the basic scenario, this model will use twenty-two farrowing pens, twenty-two nursery pens and twenty-two farrowing pens containing 12 pigs per pen. A depiction of the number of pigs per pen for the basic scenario can be seen in Figure 2.

Validation of the model

The basic model will be validated in comparison to the results found by Meester *et al.* (2022). The study looked at five slaughtered batches of 175 conventional farms. Six pigs were randomly selected in each batch and sampled. The samples were then pooled and tested. Thus, if one of the six pigs were positive the batch would be classified as positive. The farm-level percentage of at least one positive batch was 94.4%. The mean positive batches within a farm was 44% (30%-59.6%).

At first, parameters based on a literature study were put into the basic model and checked if similar outcomes as specified by Meester *et al.* (2022) could be obtained. Similar outcomes were not obtained thus, mlrMBO (Bischl *et al.,* 2017) package was used to find the best-fitting parameter estimates to simulate the results obtained by Meester *et al.* (2022). A logarithmic transformation of the squared difference between the obtained outcomes and the outcomes of Meester *et al.* (2022) was used to evaluate the model fit produced by the new set of parameters.

The GENSSI2 (Chis *et al.*, 2011) analysis package was applied in MatLab (The MathWorks Inc.,2022) as several runs of the mrlMBO resulted in a variety of estimated fitted parameters. Only the rate of MAbs loss (ω) was considered a structurally globally identifiable parameter (SGI). Whilst parameters such as the transmission rate (β); the rate at which exposed individuals become infectious (α); the recovery rate (γ); the daily loss of faeces through slates (η); infectious viral load per gram (*v*) where all classified as structurally non-identifiable parameters (SNI). The SNI parameters could not be determined by the use of mrlMBO. The SNI parameters were included in a new mrlMBO run with new narrow upper and lower limits based on literature research. The SGI value was given a broader range.

Therefore, a set of parameter estimates of one of the mrIMBO runs was picked to continue the analysis. These parameter estimates are specified in Tables 1 and 2 in the unshaded cells under the heading "used values". The model reached 85% farm-level prevalence and 40% mean positive batches per farm.

Role of environmental contamination in the spread of HEV amongst pigs

The environment is contaminated by HEV-positive faeces left behind by insufficient cleaning between batches and the defecation of infected pen mates. The infectious pigs contaminate the environment at a specific rate. The environmental contamination rate (τ) is calculated by multiplying the average grams of faeces per pig for each age class per day (*f)* with the infectious viral load (*v*) while also deducting the daily viral decay rate (*d*). The calculation of the viral decay rate (*d*) can be found in section 4 of the annexe.

In a study by Bouwknegt *et al.* (2011) 2 x 10^5 viral copies were found per gram of faeces, however, it was not possible to distinguish which percentage of copies were in the infectious form. A model assumption is that 1/100th of the viral particles are infectious. Thus, the assumed range of infectious viral particles per gram of faeces (*v*) was built around 2000 infectious viral particles per gram of faeces. There was no data available on the proportion of infectious particles at the time of the modelling attempt.

The initial viral load for the environment was calculated with the use of equations 7-9. Symbols used in the equations are partly explained in the captions and the remaining symbols are explained in Table 1. Salines *et al.* (2020) estimated piglets defecate 100g/day on average and fattening pigs 1000g per day. For piglets in the farrowing pen, 80g/day was decided on and for weaned piglets, 120g/day was decided upon based on the above estimate. The surface area per pen is expected to be 1m^2 per fattening pig (Wageningen UR Livestock Research, 2010). As no estimates were found for the weaning and farrowing pens, best guesses were used. The guessed pen size per weaned piglet was 0.4 m^2 and farrowing unit piglet was 0.3 m^2. The daily loss of faeces (η) through the slated flooring was assigned a value based on the best guess estimate. An estimated 97% of faeces produced in each phase were estimated to have passed through the slates into the manure storage pits. With the η estimate, the faeces left per m^2 in a pen is calculated in equation 7. The percentage of faeces cleaned between batches is calculated in Equation 8. The initial faecal load in the environment is calculated in Equation 9. Values for the parameters η and C_i were manually fitted to approximate the same viral load at the start of the simulation as at the end when the same faecal loss percentages were applied. This process was only done to the basic scenario. These fitted values (Table 1), were then applied to all other scenarios.

$$L_i = \frac{f_i \times (1 - \eta) \times n \times t}{P_i \times n} \quad (7)$$

The faeces leftover in a pen per m^2(L). The number of pigs per pen is noted as n and the time spent in the pen is t. The unit of the results is g/m^2. The subscript (i) can be replaced with the value for farrowing (p), weaning (n) or fattening (f) pigs in this and equations 8 to 10.

$$B_i = 1 - \frac{C_i}{L_i} \quad (8)$$

The percentage of faeces cleaned between batches (B).

$$V0 = C_i \times P_i \times n \quad (9)$$

Initial faecal load per pen after cleaning (V0). The number of pigs per pen is noted as n.

$$\tau = [1 - d] \times [(1 - \eta) \times f_i \times v \times I] \quad (10)$$

The rate at which infectious pigs contaminate their environment (τ). The number of infectious individuals is indicated with the capital letter I.

*Table 1: The definitions of symbols used in equations 7-10 pertaining to the environmental load of HEV calculations. Unshaded cells here and in Table 2 were included in the mrlMBO. Parameter estimates marked with *, are best guesses based on literature research. Parameter estimates marked with ** are best guesses with no literature backing due to a lack of literature on the topic. The upper and lower bound limits used in the mrlMBO of variables are indicated if applicable.*

Symbol	Definition	***Lower bound***	***Upper bound***	**Used value**	**Unit**
τ	The rate at which infectious pigs contaminate their environment			*Equation 10*	ge/day^{-1}
η	Faeces through slates **			97	%
C_p	Definition of clean *(farrowing pens)***			20	g/m^2
C_n	Definition of clean *(weaning pens)***			50	g/m^2
C_f	Definition of clean *(fattening pens)***			100	g/m^2
P_p	Pen size per pig *(farrowing pens)***			0.3	m^2
P_n	Pen size per pig *(weaning pen)***			0.4	m^2
P_f	Pen size per pig *(fattening pen)**			1	m^2
f_p	*Faeces per day (pre-weaning piglet)**			80	g/day
f_n	*Faeces per day (Nursery pig)**			120	g/day
f_f	*Faeces per day (Fattening pig)**			1000	g/day
d	*Viral decay rate**	0.0019	0.0021	0.00204475017	day^{-1}
v	*Infectious viral load per gram of faeces**	1800	2200	2199.37010310209	ge/g
ge: genome					

After manual fitting of the initial environmental load to the viral load after between-batch-cleaning, ratios were calculated per section. A ratio of 1.06 was achieved for the farrowing compartment. The weaning and fattening compartments had slightly higher ratios of 1.21 and 1.22 respectively.

Animal bound parameters

This model does not allow for vertical transmission of the virus from the sow to her piglets (Kasorndorkbua *et al.*, 2003).

Maternal Antibodies (M)

MAbs are transferred from the sow to her litter if she possesses antibodies against a specific pathogen (Krog *et al.*, 2019; Andraud *et al.*, 2014). The majority of the MAbs are transferred during colostrum uptake (Kasorndorkbua *et al.*, 2003). A model assumption is all MAbs are transferred through the colostrum within the first 24 hours of life. For simplicity, daily top-ups of MAbs through regular suckling of maternal breast milk will not be included. These antibodies offer protection for a limited period (Kanai *et al.*, 2010). If a litter of piglets is born from a sow with HEV antibodies present the piglets start in the maternal antibody state (equation 1). Alternatively, if a litter of piglets is born from a sow with no HEV antibodies the piglets start in the susceptible state (equation 2). MAbs are reported in piglets until 45 (Andraud *et al.,*2013) up to 60 days of age (Kasorndorkbua *et al.* 2003). If maternal antibodies are below a protective level or absent due to lack of inheritance, the pig is susceptible. In the basic scenario, all piglets are exposed to MAbs and obtain MAbs.

Susceptible (S)

Once susceptible, the pig can enter the exposed state whilst in contact with the contaminated environment. The faecal-oral route is the suspected transmission route of HEV infections amongst pigs (Bouwknegt *et al.*, 2009b). A transmission rate (β) of $2 \cdot 10^{-6}$ ge/g/day ($1 \cdot 10^{-7}$; $7 \cdot 10^{-6}$) has been estimated. This value can be interpreted as the average number of pigs that can be infected by a single genome equivalent present in the environment per day (Andraud *et al.*,2013). Between-pen environmental contamination is rare, but possible in reality. Pens are well segregated and movement between pens is not common practice (Meester *et al.*, 2021). However, our model does not consider it due to the rarity of it.

Exposed (E)

Once infected, the latent period commences. The latent period is 1-2 weeks long according to a review done by Meester *et al.* (2021). The rate of animals moving from the exposed state to the infectious state (α) has a lower bound estimate of 7 $days^{-1}$ and 14 $days^{-1}$.

Infectious (I)

After the latent period, a pig then becomes infectious. The beginning of the infectious period is marked by the start of faecal HEV shedding (Crotta *et al.*, 2018). Whilst infectious a pig contributes to the environmental HEV load. Looking at a review by Meester *et al.* (2021), a large variety of average infectious days have been reported. The shortest being 7 days and the longest being 49 days. After the infectious period, the pig then recovers. The recovery rate (γ) is the inverse of the average infectious period of a pig.

Recovered (R)

A model assumption is that there is no reversion to a susceptible state after immunity is gained through recovery. This assumption is supported by the findings of Sanford *et al.* (2011).

Mortality (μ)

As pigs are largely not affected by the infection (Mohammad Sultan Khuroo *et al.*, 2016; Salines *et al.*, 2017; Ricci *et al.*, 2017; Kasorndorkbua *et al.*, 2003), thus, no additional mortality is included for infected animals. Natural background mortality is included for each production phase. Calculations can be found in section 2.7.1-3 in the annexe.

Table 2: Defined parameters as seen in the ODEs, Figure 5 and Figure 6. The shaded parameters were set with the use of literature. The unshaded parameters were set with the use of model-based optimisation (MBO). A summary of the parameter estimation can be found below. The upper and lower bound limits used in the mrlMBO of variables are indicated if applicable.

Symbol	Definition	Lower bound	Upper bound	Used Value	Unit
μ_{farrow}	The natural death rate of that age group			0.00488	day^{-1}
μ_{wean}	The natural death rate of that age group			0.000331	day^{-1}
μ_{fatten}	The natural death rate of that age group			0.0002589	day^{-1}
ω	The rate of maternal immunity loss	0.0167	0.0222	0.01804674573	day^{-1}
β	The transmission rate from the environment to susceptible pigs	0.0000015	0.0000025	0.00000225893	$ge/g/day^{-1}$
α	The rate at which exposed individuals become infectious	0.095	0.115	0.09937305146	day^{-1}
γ	The recovery rate: reciprocal of time spent in the infectious state	0.028	0.033	0.02884925737	day^{-1}
ge: genome					

Different scenarios

Batch sizes, the presence of MAbs and the production period have been selected to explore the effects of an alternate scenario. The alternate scenario of each factor is compared to the basic scenario. Below the scenarios of the three parameters are described in more detail.

Basic scenario compared to an increased number of pigs per pen

The basic scenario will favour management practices that keep group sizes small. Conventional farrowing crates are extensively used on pig farms. In practice, the pen consists of a restrictive metal crate in which the sow is kept. The flooring around the crate will be utilised by the piglets (Baxter *et al.*, 2011). In this scenario, the nursery pen size will be restricted to a single litter as recommended by van Engen *et al.* (2012) for better biosecurity. Thus, keeping litter mixing to a minimum. These pigs will then remain in the same group when moved to the fattening section. This scenario aims to keep groups as small as possible and mixing to a minimum. This scenario represents the basic scenario. There are 22 farrowing pens, 22 weaning pens and 22 fattening pens all consisting of a maximum of 12 pigs. As time passes some pigs may be removed due to natural mortality. The basic scenario is visualised in Figure 2 below.

Figure 2: The basic scenario: the flow of pigs through a farrow-to-finish farm and pigs per pen.

The alternate scenarios will favour larger groups. Group farrowing systems facilitate the mixing of sows and litters during the farrowing stage. Group farrowing is done to provide more natural conditions for the animals and fulfil social needs (Baxter *et al.,* 2011). Groups of two sows and their litters will be housed in a farrowing pen. Additionally, a larger group size of two litters will be housed together in the nursery stage as specified by van Engen *et al.* (2012) for a more cost-effective way to house weaned piglets. In the first alternate scenario, the group is split into two when moved to the fattening stage. The pigs are not exactly split by litter but by number as at this point the litters will be indistinguishable from each other. In the first alternate scenario, there will be eleven farrowing pens, eleven nursery pens and 22 fattening pens. The farrowing and weaning pens will contain a maximum of 24 pigs per pen. The fattening pens will contain a maximum of 12 pigs per pen. The first alternate scenario is depicted below in Figure 3. The second alternate scenario will favour the extreme of increased batch sizes for all three production stages. The grouping of pigs per production phase can be seen below in Figure 4.

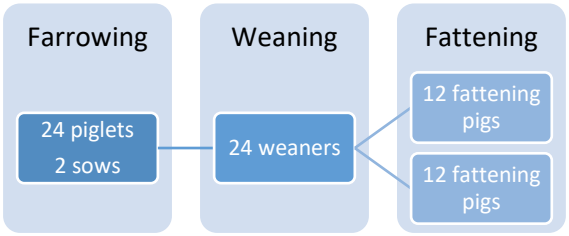

Figure 3: The first alternate scenario: the flow of pigs through a farrow-to-finish farm and pigs per pen- double litter scenario

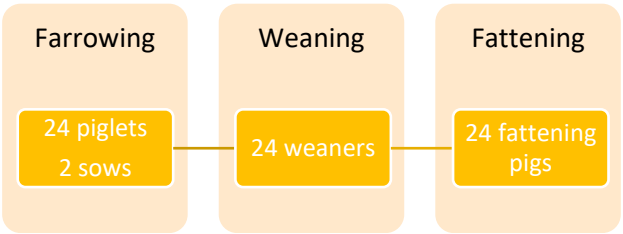

Figure 4: The second alternate scenario: increased pen sizes for all three stages of production. The full double litter scenario is depicted here.

Full Maternal immune batch (basic scenario), reduced and absent MAbs batch

In the review of Meester *et al.,* (2021) looking at several European papers, it was concluded that 50-100% of sows are frequently seropositive on farms. It was also mentioned that the maternal antibodies were demonstrated in 60-100% of piglets born to a sow which was seropositive at the time of the birth. Piglets originating from a fully seropositive breeding herd where 100% of the

piglets have MAbs are depicted in Figure 5 and represent the basic scenario. The first alternate scenario will consist of a batch of piglets where exactly 50% of a litter has MAbs and the other half are fully susceptible at birth. This scenario will contain piglets following the MSEIR model (Figure 5) and the SEIR model (Figure 6) in one batch. The second alternate scenario will be compared to those originating from a fully seronegative breeding herd (Figure 6) and none of the piglets have MAbs.

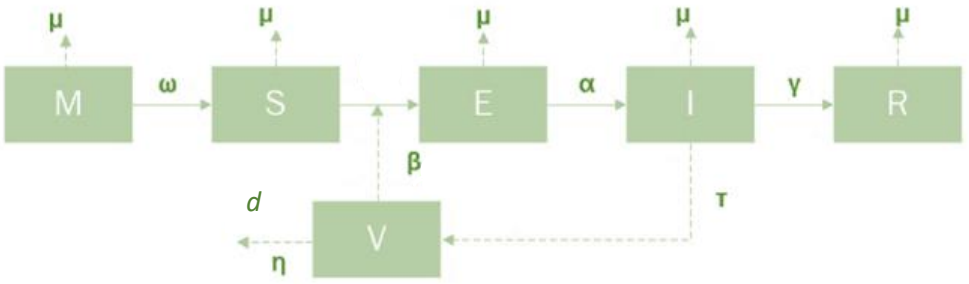

Figure 5: MSEIR model: all piglets born with MAbs start here in the M state. The ODEs used for these piglets are equations 1-6

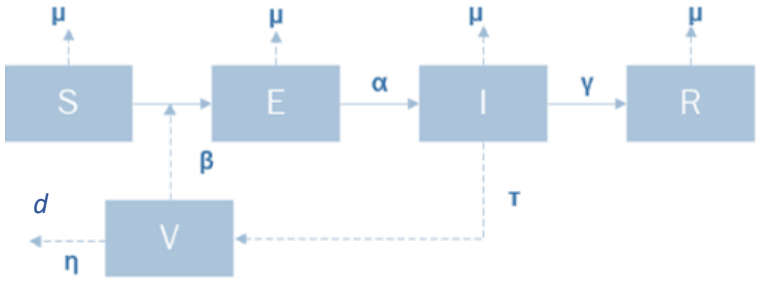

Figure 6: SEIR model: all piglets born without MAbs are susceptible at birth. ODEs used for these piglets are equations 2-6

Basic scenario (94 days) compared to an extended fattening period

An increased production period in the fattening stage could result in the pigs having additional time to clear the infection before slaughter. For the basic scenario, a fattening period of 94 days is used as in the model of Salines *et al.* (2020). The basic scenario also signifies the shortest fattening period. A fattening period of 115 days as described by Meester *et al.* (2023) will be used as the largest extended fattening period. Resulting in a total production time of 180 days and 201 days for the lower and upper bound for the fattening period. Intermediate production periods of 27 and 28 weeks were also tested to try and find significant reductions in the infectious count at slaughter with a minimum extension of the fattening period. The difference between the timelines is visualised below in Figure 7. Once a significant value is reached using the weekly extensions, the day count will be decreased to the last point where significance is reached. This was done to obtain the minimum number of days to extend the production period from the basic scenario to achieve a significant reduction in HEV cases at slaughter without acquiring unnecessary costs due to pigs staying on the farm for unnecessarily long periods.

Farrowing	Weaning	Fattening	Total days	Weeks
28 Days	58 Days	94 Days	180	~26
28 Days	58 Days	103 Days	189	27
28 Days	58 Days	110 Days	196	28
28 Days	58 Days	115 Days	201	29

Figure 7: The timelines of the basic scenario (top) and the alternate scenarios can be visualised. The end of the production period is indicated with a thick line in each timeline.

Evaluation of the different scenarios

Each scenario was executed 500 times. The total number of infectious individuals per batch was recorded. The basic scenario was compared with a permutation test to the alternate scenarios for each farm factor. The tests were also done in R . A seed value of 123 was used for reproducibility. Ten thousand permutations were used to increase the power of the statistical test.

Results

Below are the results of the basic scenario compared to the alternate scenarios of the three different farm factors. The basic scenario is indicated in blue in all graphs. The first alternate scenarios discussed will be the scenarios focusing on increased pen size. Thereafter, the alternate scenarios pertaining to batches where a reduced percentage of MAbs are present. The last alternate scenarios discussed are where the fattening period is extended.

Basic scenario and increased pigs per pen

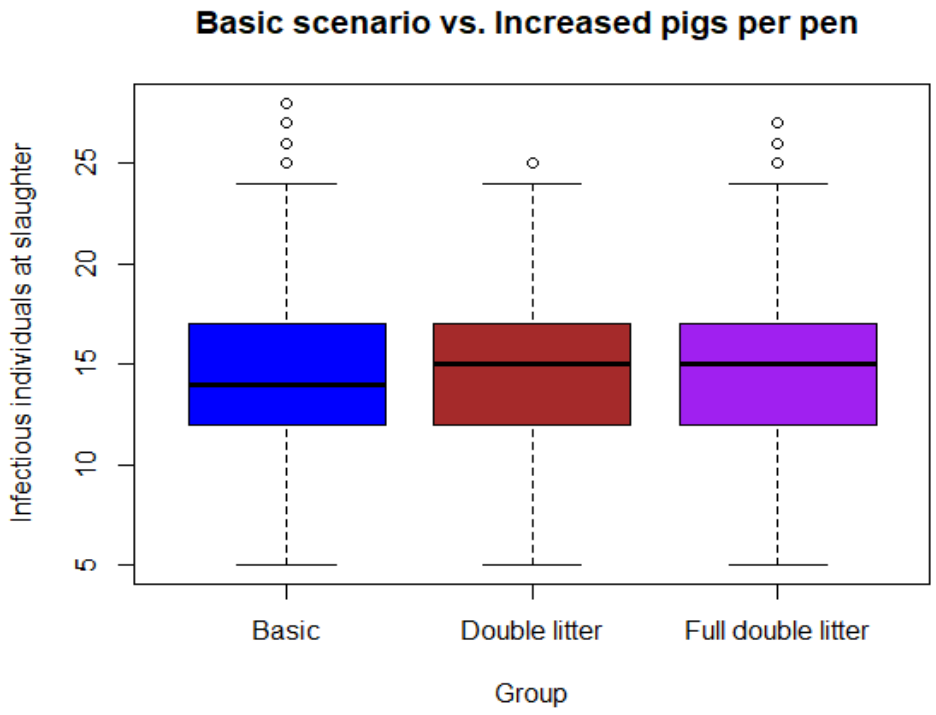

Figure 8: The number of infectious individuals produced by 500 runs of the basic scenario compared to 500 runs of each of the two alternate scenarios that favour more pigs per pen. The "Double litter" bar represents the scenario where 24 pigs are found in the farrowing and weaning pens and then split into 12 pigs per pen for the fattening stage. The "Full double litter" bar indicated the scenario where there are 24 pigs per pen in all three production stages.

The number of pigs in the infectious state at the time of slaughter of the basic scenario and the two alternate scenarios focusing on increasing the number of pigs per pen are depicted in Figure 8. The observed difference between the basic scenario and the situation where only the farrowing and weaning pens have 24 pigs per pen was -0.126. A p-value of 0.5921 was obtained. The middle bar indicates the outcomes of the first scenario. For the second alternate scenario where the pigs per pen was increased in all three production stages a difference of -0.242 was found when compared to the basic scenario. A p-value of 0.3135 was obtained. The results of the second scenario are depicted in the right bar. Both alternate scenarios do not significantly differ in the number of infectious pigs at slaughter age compared to the basic scenario.

Full Maternal immune batch (basic scenario), reduced percentage of MAbs and naïve batch

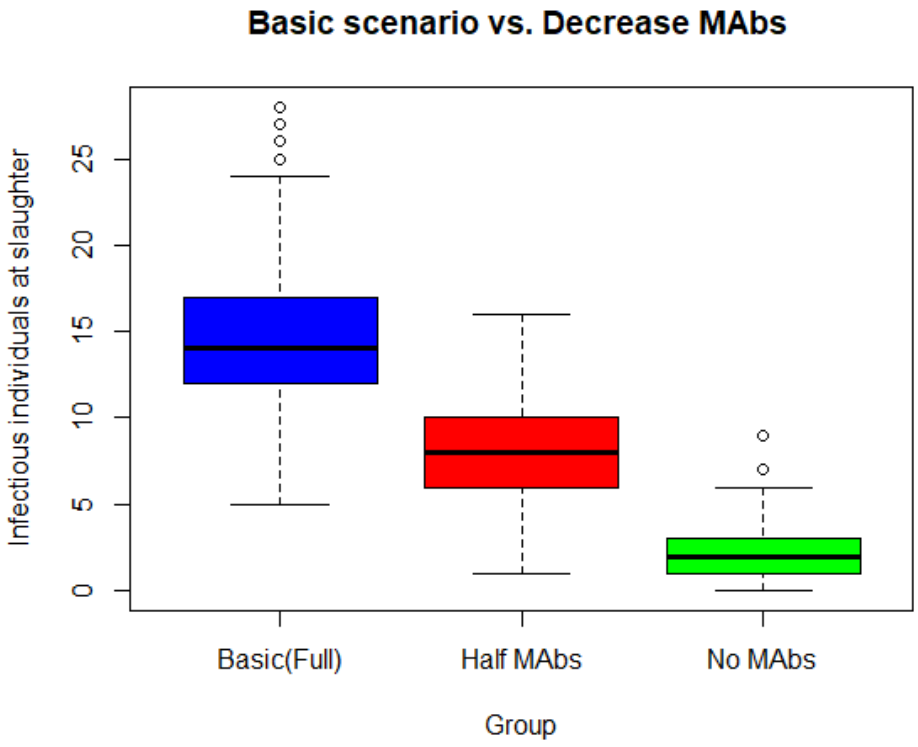

Figure 9: The number of infectious individuals produced by 500 runs of the basic scenario compared to 500 runs of the alternate scenarios where half of all litters have MAbs and none of the litters have MAbs. The results of the scenario where all pigs obtained MAbs is depicted in the "Basic (Full) bar". The results of the scenario where half of a litter obtains MAbs are shown in the "Half MAbs" bar. The results obtained from the situation where no MAbs are present in any of the pigs are displayed in the "No MAbs" bar.

The number of pigs in the infectious state of the basic scenario where each pig has MAbs and the alternate scenarios where decreased or absent MAbs levels are present can be seen in Figure 9. The observed difference between the basic scenario and half MAbs scenario was 6.606. A p-value of <0.0001 was obtained. Comparing the basic scenario and the scenario where no MAbs are present an observed difference of 12.802 was found, which also resulted in a p-value <0.0001. Looking at the trajectory of the infection, the reduction of MAbs allows the peak of infectious individuals to be much earlier in the production period compared to the basic scenario. The larger the reduction in MAbs the earlier the number of infectious pigs peak. In the decreased MAbs scenarios, if a pig is born without MAbs and is not infectious or dead at the time of slaughter, they have recovered by the time they reach slaughter age. The obtained p-values point to a significant difference in the number of infectious individuals produced by the basic scenario compared to the scenarios in which decreased levels of MAbs are present in the batch of pigs.

Basic scenario and extended fattening periods

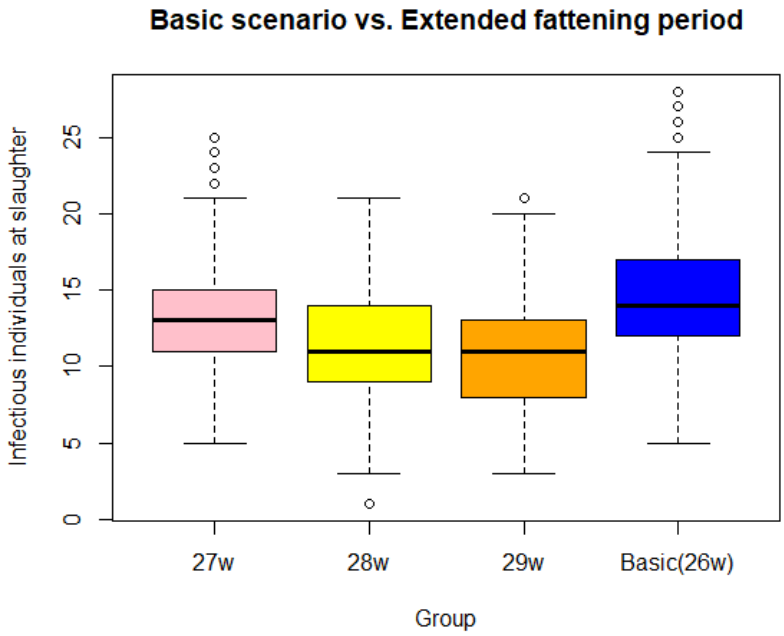

Figure 10: The number of infectious individuals produced by 500 runs of the basic scenario (right) compared to 500 runs with the extended fattening periods. The figure displays the outcome of four scenarios. The basic scenario is indicated as "Basic (26w)" in the right most bar. The total production period of the basic scenario is ~26 weeks with a fattening period of 94 days. The alternate scenarios are indicated to the left of the basic scenario with the associated total production period in weeks. The 27, 28 and 29-week production periods have a the following fattening periods 103, 110 and 115 days respectively.

When comparing the basic scenario to the 27, 28 and 29-week production periods outcomes the observed differences were 1.634, 3.202 and 3.952 respectively. All three resulted in a p-value of <0.0001. Working back from a total production time of 189 days (27 weeks) towards that of the basic scenario (180 days), it was found up to 184 days a p-value of <0.0001 could be achieved. At a period of 184 days, an observed difference of 0.894 and a p-value of 0.0004 was obtained. At 183 days an observed difference of 0.394 and a p-value of 0.0859 was found when compared to the basic scenario. This led to the conclusion that a production period of 184 days and therefore a fattening period of at least 98 days was needed to obtain a significant reduction in HEV cases at slaughter.

Discussion

This modelling attempt aimed to reduce the number of HEV-positive pigs sent to slaughter through implementing alterations at farm level that could aid in the reduction of foodborne HEV infections in the Dutch population. The effectiveness of altered group sizes, varying MAbs levels in a batch and an extended fattening period is discussed in terms of infectious pigs sent to slaughter below.

The scenarios where larger group sizes were favoured failed to produce a significantly different number of infectious pigs at slaughter compared to the basic scenario which favoured smaller group sizes. By adding the mixing of two litters to achieve larger group sizes, it was hypothesised that the peak of infection would shift to an earlier point in the production cycle. As the total number of pigs in the batch remained the same, the animals were divided into bigger groups. The model also only allows for within-pen transmission. Thus, once one pig is infectious they would have the opportunity to contaminate the environment of 23 other pigs and possibly infect these pigs depending on their state. In the basic scenario, the infectious pig will only have the opportunity to contaminate the environment of a maximum of 11 other pigs and possibly infect these pigs depending on their state. Thus, it was hypothesized that more pigs per pen would facilitate the efficient spread of HEV. This in turn could lead to more pigs having acquired HEV and having cleared the infection at slaughter age. The hypothesis was based on a theory expressed by Meester *et al*. (2022) that the less compartmentalised nature of organic farms could aid organic farms in delivering fewer PCR-positive pigs to slaughter compared to conventional farms despite having a higher seroprevalence at slaughter. The intervention intended to shift the peak of infectious pigs to an earlier time in production. However, no significant difference between the basic scenario and the alternate scenarios was found. It appears, that increasing the number of pigs per pen has little effect on the number of infectious pigs delivered to slaughter.

When looking at the basic scenario (the entire batch is exposed to MAbs) compared to the batches where MAbs levels are decreased or absent, it can be seen that the scenario where no MAbs were present led to the biggest significant decrease in infectious state pigs at slaughter. These findings are in line with those of Crotta *et al.* (2018), who reported a decrease in the number of piglets exposed to MAbs led to a decrease in the prevalence of viraemic pigs at slaughter. The inverse was also reported, if the MAbs coverage increased amongst the piglets the number of infected pigs at slaughter increased. It is important to note that the Crotta *et al.* (2018) results are based on a modelling attempt and not field research. There is uncertainty pertaining to the reversion to a susceptible state after recovery after an extended period, thus, one cannot say if in reality these results will be obtained. If reversion to a susceptible state occurs, the pigs could get infected towards the end of the production cycle, which could result in an increase in the number of infectious pigs at slaughter.

In addition to the above-mentioned uncertainty, controlling the number of sows with HEV antibodies may be challenging in practice. Testing the breeding herd sows for HEV antibodies and replacing HEV-antibody-positive sows with HEV-antibody-negative sows will be a very costly measure for farmers. Additionally, maintaining a HEV-antibody-free status in the breeding herd may be very challenging, especially on an integrated farrow-to-finish farm where HEV is possibly still present in the pork production herd. As demonstrated by Meester *et al*. (2022), even farms with

heightened biosecurity did not attain a HEV-free status. Thus, in reality, creating a breeding herd with continuously no HEV antibodies appears unrealistic at present. However, if one could control for this and reversion to a susceptible state was not possible, this measure would appear to allow for the biggest reduction in infectious individuals at slaughter. Nonetheless, management efforts looking at reducing the spread of HEV in the breeding herd should be explored as even a reduction in MAbs levels within a batch could lead to a valuable decrease in infectious pigs at slaughter.

When comparing the basic scenario where pigs were slaughtered at 180 days of age to the alternate scenario where pigs were slaughtered at a later age, one can see the alternate scenarios performed significantly better. HEV seropositivity is expected to rise with age and the opposite is true for prevalence (Meester *et al.,* 2023). The peak of infection is thought to occur between 84 and 105 days of age on pig farms (Berto *et al.,* 2012). This period is at the start of the fattening phase. A logical explanation is that the longer fattening period allows pigs a longer time to clear their infections before slaughter. The results of Meester *et al.* (2022), showed that despite having a high seroprevalence at slaughter organic pigs had less infectious pigs at slaughter compared to conventional farms. This implies that HEV infections are not avoided to a greater extent in organic farms, but more of the infectious individuals have cleared the infection by the time slaughter weight is reached. The length of time to reach slaughter weight differs between organically and conventionally reared pigs. As organic pig production usually facilitates slower body mass accumulation compared to conventional farming practices (Quander-Stoll *et al.* 2021). It could be proposed that this difference could account for some of the variation between the two production systems described by Meester *et al.* (2022).

Additionally, it is important to note that even amongst conventional pig farms the length of the production cycle may differ amongst farms. In pig production, the length of time pigs stay on a farm is dependent on their growth rate. Pigs stay on the farm until slaughter weight is reached. If the pigs grow rapidly due to, for example, an efficient feed conversion ratio, the pigs will reach slaughter weight earlier than slower-growing pigs where an inefficient feed conversion ratio is present. Thus, growth rate can have a direct impact on the length of the production cycle. This means a production cycle of 180 days does not apply to every farm and possibly not every batch of that farm. Nonetheless, farmers could reduce the growth rate of the pigs to achieve a longer stay before slaughter. In practice, this option could drive up production costs due to the resources needed to support the animals during this extended stay due to less efficient growth. This option will impact the profit that can be made by a farmer. If the timing of the peak of the infection remains the same as in the basic scenario, extending the fattening period could help reduce the number of infectious pigs at slaughter. Achieving a production cycle of at least 184 days would significantly reduce the number of infectious pigs at slaughter if the peak of the infection occurs at a similar time as before in the case of the model. However, this method is very sensitive to a change in the timing of the peak of infectious pigs which can vary amongst farms due to different management practices. If this peak shifts to a later stage the intervention will be ineffective. If the measure is no longer effective the farmer will be acquiring additional costs for no added benefit. Therefore, keeping conditions between batches similar is essential to the effectiveness of this measure once a suitable extension period based on the timing of the peak of infectious pigs is uncovered.

Validating the model was a considerable challenge. Data originating from 175 farms of which each had five batches sampled was used to validate the model. There can be a large heterogeneity of

various circumstances between farms which could affect the HEV prevalence at slaughter. Circumstances such as hygiene, biosecurity, infrastructure, percentage with MAbs, co-infections and cleaning efficacy between batches often vary between farms in reality. In addition, several variables were based on best guess estimates which could differ from reality. In the case of the model, there is homogeneity in the input parameters, for example, cleanliness and a lack of co-infections. The homogeneity does not completely reflect the complexity of the situation. Future modelling attempts should try and incorporate more heterogeneity into the model to achieve a better representation of reality.

Additionally, in reality, the infection dynamic on a pig farm is far more intricate than the simplified model design created. In the model, the only sources of infection pressure are infectious HEV copies either left behind from the previous batch due to insufficient cleaning and the excretion of infectious pen mates. A 2023 presentation at the 35th VEEC conference by M. Meester, focused on unpublished findings, mentioned several introductions of HEV are likely to occur during the stay of a batch on a farm. This statement is supported by a phylogenetic analysis of HEV strains on pig farms. The model does not take into account several routes of HEV introduction onto a farm. These alternate routes are yet to be studied in more detail. Once better understood, these alternate routes of introduction can be incorporated in future modelling attempts to better reflect reality.

All but one parameter used in the MBO was found to be SNI. The transmission rate; the rate at which exposed individuals become infectious; the recovery rate; the daily loss of faeces through slates; and the infectious viral load per gram of faeces where all classified as SNI parameters. More experimental research should be done to better estimate the SNI parameters that were identified by the GENSS2 analysis. Having a better understanding of various input parameters would lead to more accurate modelling.

There is a large variation in the number of days a pig remains infectious reported in literature. Several reasons ranging from how the infection is established, how an individual responds to an infection and the presence of co-infections could contribute to this range. Generally, a dose-response relationship affects the probability of an individual becoming infected by a pathogen. The route of exposure also affects the course of the infection. Most studies done to determine the latent period and infectious period use intravenous and oral inoculations with a specific viral dose to establish infection. As demonstrated by Meester *et al.* (2021), these factors have a large impact on the results. HEV transmission experiments often have different results compared to what is observed in pig farms. Latent and infectious period estimates are generally longer in field observations compared to experimental results (Salines *et al.* 2015). The above factors made it complicated to decide on an appropriate range for the infectious period. More research needs to be done on the infectious period under field conditions to model the course of an HEV infection more accurately in pig farms.

Persistently infected pigs caused by co-infections were not considered in this modelling attempt. Persistently infected pigs may occur more frequently than initially thought (Sanford *et al.,* 2011). Immune-modulating infections could alter the course of a HEV infection. Immunosuppressive conditions have been found to lead to chronic HEV infections in humans. Porcine reproductive and respiratory syndrome (PRRSV) can impair the immune response of a pig that is infected (Salines *et al.*

2015). PRRSV has been considered endemic in The Netherlands since 1991 (Nodelijk *et al.,* 2003). Salines *et al.* (2015) reported modified HEV dynamics in the presence of PRRSV. An increase in the HEV latent period and the infectious period was observed in the PRRSV/HEV-positive group compared to the HEV-positive group. A co-infection also significantly increased the viral shedding in the inoculated group, however, this was not observed in the contact-infected group of the study. Nonetheless, the sample size was small, therefore the possibility remains that increased viral shedding can also be present in contact-infected pigs. Seroconversion is also delayed by the presence of PRRSV. Including persistently infected pigs in a batch could make the model more representative of reality, especially as PRRSV is endemic in The Netherlands.

A model assumption is that immunity is gained after infection once recovered based on the findings of Sanford *et al.* (2011). The study challenged previously inoculated and recovered pigs with various HEV strains and found a protective effect due to gained immunity. However, the 12 weeks between HEV exposure in the study is much shorter than the production period of a pig (~26 weeks). Therefore, one does not know if immunity wanes after a period longer than 12 weeks. Additionally, the study started with 8-week-old pigs. In reality, pigs may be exposed at a younger age and have a less developed immune system. This could alter the immune response observed. To better understand and model the course of a HEV infection in the context of pigs on a farm for an extended period of time, more research exploring the immunity gained by pigs post-recovery needs to be done.

Another limitation of this study is that the initial viral load was manually fitted to be approximately the same at the start of the batch and after cleaning, exclusively for the basic scenario. The different alternate scenarios could have a different equilibrium of HEV particles remaining between batches. A model improvement would be to individually fit this parameter to each alternate scenario, possibly in an automated and more efficient way. This will allow for more accurate modelling of the alternate scenarios and as a result more accurate conclusions drawn from them.

During manually fitting the initial environmental load it was discovered that the variables pertaining to the daily percentage of faeces falling through the slates and the definition of what is clean once the area has been cleaned between batches have been found to have drastic effects on the outcome of the simulation. Meester *et al.* (2022) hypothesised that the closed flooring with bedding materials which can hold more faeces as seen in organic farms compared to the slated floors encountered in conventional farms could be another reason HEV is less prevalent amongst organic slaughter-age pigs. This could be modelled as a lesser percentage of daily faeces loss resulting in a greater build-up of faeces in pens. The build-up of contamination in the environment could facilitate more infection pressure and a faster spread of HEV amongst the pigs. Future modelling attempts could also aim to use this finding to explore options to better time the onset of infection to reduce the number of infectious pigs entering the food chain.

In conclusion, two of the alternate scenarios tested resulted in significantly lower infectious pigs delivered to slaughter. The lack of MAbs in a batch appeared to have the biggest effect. However, in practice, testing for sows with HEV antibodies and replacing them with HEV antibody-free sows will be costly and impractical for farmers, as well as very difficult to maintain. However, it does show that shifting the peak of infectious individuals to an earlier point in time is a promising objective to

explore. The extension of the fattening period appears to be a more easily implemented change at first glance, however, upon closer inspection drawbacks come to light. It is an intervention method very reliant on the infection timing and growth rate of every batch remaining somewhat constant. As the timing of the peak of the infection could alternate between farms for various reasons, the extension period is not a one-size-fits-all solution. Rather an option to possibly further investigate in a more individualised way. Future research should look at designing new interventions based on the cleanliness of pig pens both during a batch and between batches as these have been identified as promising parameters to alter in this model.

Acknowledgement

I would like to thank Dr Boris V. Schmid for contributing to the completion of this project as my supervisor. In addition to his supervisory input, he contributed ideas and coded elements during the design of the model in R. Parts solely coded by him were the environmental load section written in C code and the GENSSI2 analysis MatLab.

Annexe

Section 1: Zoonotic transmission routes of HEV

1.1 Foodborne transmission

Foodborne transmission can occur though the consumption of food product having been made from raw materials obtained from an infected animal or food products that have become contaminated through HEV-positive materials. Thus, the consumption of pork products can increase the risk of developing a HEV infection. The consumption of food products containing raw or undercooked porcine liver has been highlighted as a risk factor. The consumption of game meat, such as deer and wild boar meat, has also been highlighted as a risk factor. The consumption of shellfish can also be considered a risk factor in some cases (Ricci *et al.*, 2017).

1.2 Environmental transmission

Although this project will only focus on preventing foodborne transmission, environmental transmission can also cause HEV infections in humans. Especially for people participating in occupations where people have direct contact or indirect contact with infectious animals is considered a risk factor. The direct contact farmers and veterinarians have with infectious animals puts them at a higher risk of contracting HEV. Indirect contact for example forest workers have with wild boar and deer through their daily tasks puts them in a higher risk category (Ricci *et al.*, 2017).

Section 2: Pig Husbandry-farm set -up

2.1 The farrowing environment

The sows are housed in farrowing crates. The flooring of the crates has a solid portion and the rest is covered with a fine grate to allow for the throughflow of manure. The crate usually contains one sow and her piglets until weaning (Wageningen UR Livestock Research, 2010). After every litter leaves the farrowing pen it must be washed and disinfected (van Engen & Scheepens, 2007).

According to the KWIN 2022-2023, the average Dutch pig farm with farrowing crates has five compartments with 44 farrowing crates resulting in 220 places in total. These estimates consider breeding farms and not only farrow-to-finish farms and can therefore be expected to be lower in less-specialised farms. An arbitrary value of 22 farrowing crates for a single sow compartment was decided upon for the basic scenario.

2.2 The Nursery Environment

In some practices, piglets remain in the farrowing pen during the weaning phase to reduce stress whilst the sow is removed while in other practices a specific section of a farm is allocated as a nursery for piglets (van Engen *et al.*, 2012).

Often 12-26 piglets are kept together in a nursery pen, thus, one to two litters are kept together (Wageningen UR Livestock Research, 2010; van Engen *et al.*, 2012). One aims to keep mixings of litters to a minimum when changing from one compartment to the next (Wageningen UR Livestock Research, 2010). In an optimal situation one can move a litter at a time or two litters at a time if the one litter is marked to keep them separate (van Engen *et al.*, 2012). By not mixing litters one can

reduce the risk of disease spreading between litters (van Engen *et al.*, 2012). The cost of housing larger groups is often lower. However, the performance of smaller groups is often better (Hulsen & Scheepens, 2013).

On the average Dutch pig farm according to the KWIN 2022-2023, there are eight compartments with 22 pens with space to accommodate 26 weaners. This results in a total of 4576 places for weaners on the farm. These estimates consider breeding farms and not only farrow-to-finish farms and can therefore be expected to be lower in less-specialised farms. Considering the desire to test a scenario which favours small groups and one that favours large groups, an arbitrary value of 22 weaning pens for a single compartment was decided upon for the basic scenario. It was also decided to keep 12 pigs per pen in the weaning compartment for the basic scenario to favour smaller group sizes.

2.3 The fattening environment

By avoiding mixing when moving weaners to finishing rooms one can help reduce the spread of disease and reduce the risk of fighting amongst pigs (van Engen *et al.*, 2012).

The spread of pathogens between various compartmentalisation levels needs to be controlled. By having solid pen partitions, one reduces the risk of spreading pathogens to neighbouring pens. Additionally, cleaning and disinfecting the pens is essential to prevent pathogens from spreading from one batch to the next (Roozen *et al.*, 2007).

According to the KWIN 2022-2023, the average Dutch pig farm has 5040 places for fattening pigs. These farms are set up with seventeen compartments with 24 pens to house twelve pigs per pen. Another estimate stated around 12-15 pigs can kept in a pen in a fattening compartment with around $1m^2$ per pig (Wageningen UR Livestock Research, 2010). These estimates consider breeding farms and not only farrow-to-finish farms and can therefore be expected to be lower in less-specialised farms. For the basic scenario, 22 fattening pens were decided upon to accommodate a maximum of 12 pigs. In the basic scenario, litter will not be mixed and pen-mates will remain pen-mates during every shift if still alive at that point in time.

2.4 Batch farrowing

In batch systems, sows are divided into groups which allows mating and subsequently farrowing to occur in intervals allowing for batches of similarly aged piglets to move through the system. This system supports the use of an all-in-all-out system.

The alternative is continuous farrowing. Where breeding and farrowing are happening daily. With this management system, it is hard to break disease cycles within a farm (Hines, 2023).

2.5 All-in-all-out production

An all-in-all-out production system requires a room to be fully emptied, cleaned and disinfected before the next batch of pigs are brought in. Implementing this practice the infection pressure is lowered within the room. By maintaining this practice one could avoid pathogens jumping from one batch to another (Hulsen & Scheepens, 2013).

This system also allows for more optimal management of the room to best suit the needs of animals in a specific age range (Wageningen UR Livestock Research, 2010).

2.6 Walking routes

Walking routes are often maintained on pig farms to prevent the spread of disease between batches (Hulsen & Scheepens, 2013). The route usually starts from cleaner regions and moves toward dirtier regions within the farm. For example, the farmer can start their route at the showers and change into clean overalls then proceed to move through the farm from young toward older batch compartments, then lastly go to the dirtiest places like the manure storage if need be (Wageningen UR Livestock Research, 2010; Hulsen & Scheepens, 2013). To maintain the assumption that within-pen transmission is the only source of infection for pigs, it is assumed that walking routes and other biosecurity measures are maintained on the farm. This allows for HEV contamination not to be spilled back from older batches to younger batches.

2.7 Mortality in each production stage

2.7.1 Farrowing stage

The total mortality for Dutch pig farms at this stage is 12.8%. This includes stillborn piglets and piglets that die up until weaning takes place (Bigvitaliteit, 2009). Stillborn piglets are seemingly healthy but have possibly died during the birthing process (van Engen & Scheepens, 2007). The piglets stay in this phase for 28 days. The average sow delivers 30 weaned piglets per year in The Netherlands (Blanken *et al.*, 2022). Most breeds have 1.8 to 2.2 farrowing moments per year. The breed of the pig used on the farm can significantly impact the litter size and mortality observed. Breeds with higher litter sizes often have higher mortality rates within the litter. A study looking at six breeds concluded a mean litter of twelve piglets was born, of which eleven were born alive and nine were eventually successfully weaned (Nowak *et al.*, 2020). The values reported by Nowak *et al.* (2020) result in a 25% mortality rate. This is much higher than that reported in the Dutch situation. Assuming the average litter size of eleven piglets being born alive, one could assume that 1-2 piglets died during farrowing. As the above-mentioned averages are from Polish and US farms, one could assume practices could differ accounting for fewer piglets lost during the farrowing stage. In this model, twelve piglets per sow are assumed. The 12.8% mortality rate of the Dutch situation will be used. A daily mortality rate of 0.00488 is used. The daily mortality calculation is described below. The daily mortality for the farrowing section can be seen in calculation 1.

<u>Adjusted daily mortality rate calculation method (for all three stages):</u>

1-daily mortality rate = survival rate

Survival rate $^{number\ of\ days}$ = percentage of pigs surviving

1- the percentage of pigs surviving= total mortality over the time

<u>Calculation 1: Adjusted daily mortality rate calculation:</u>

$(1-((1-0.00488)^{28})) = 0.1280075 \approx 12.8\%$

2.7.2 Nursery stage

The mortality rate drops after weaning is complete. The mortality for pigs after weaning is 1.9% (Bigvitaliteit, 2009). Assuming a mortality rate of 1.9% over 58 days, a daily mortality rate of 0.000331 is expected. The calculation of the daily rate can be found below in calculation 2.

Calculation 2: Adjusted daily mortality rate calculation:

$(1-((1-0.000331)^{58})) = 0.01901801 \approx 1.9\%$

2.7.3 Fattening stage

Looking at the KWIN 2022-2023 fattening pig mortality was around 2.4% in conventional Dutch pig farms (Blanken *et al.*, 2022). Assuming a 2.4% mortality rate over 94 days one could expect a 0.0002589 daily mortality amongst the finishing pigs. The calculation of the daily mortality rate of the fattening section can be visualised below in calculation 3.

Calculation 3: Adjusted daily mortality rate calculation:

$(1-((1- 0.0002589)^{94})) = 0.02404593 \approx 2.4\%$

2.8 Farm types in The Netherlands

Approximately 30% of pig farms in the Netherlands are farrow-to-finish farms. Most Dutch pig farms are specialised in the sense that they either produce weaned piglets or finisher pigs. The number of farrow-to-finish in the Netherlands was estimated to be 511 farms with around 3348 animals per farm (Wageningen UR Livestock Research, 2023). The average size of a Dutch farrow-to-finish farm in 2009 was 309 sows and 1800 grower-finishers (Kemp *et al.*, 2011).

Despite farrow-to-finish farms being in the minority of farm types in The Netherlands, a farrow-to-finish farm will be modelled to not factor in the mixing that could occur during transport and to keep cleaning assumptions constant between stages. The cleanliness standard and as a result the level of HEV-infected piglets may differ if several breeding farms are connected to form a batch of fattening pigs.

Section 3: Disease transmission

3.1 Vertical transmission

Morozov *et al.* (2015) reported that the data of their study favoured the possibility of vertical transmission. They reported that several six-day-old piglets were found to be HEV positive in a specific pathogen-free Göttingen minipig farm in Denmark. In human HEV-1 the placenta is a site of viral replication. It was hypothesised that this may also be the case in pigs.

A 2003 study by Kasorndorkbua *et al.* where twelve gilts were inoculated at 80 days of gestation found no clinical signs in either the sows or piglets once born. Additionally, no significant differences in performance between the piglets of inoculated sows and control group sows. No evidence of vertical transmission was found.

Another study looked at 159 aborted/stillborn foetuses and the sera samples of 45 corresponding maternal sows to investigate transplacental transmission. HEV RNA was found in the livers of several of the foetuses and their corresponding sows. It is important to note that in all HEV-positive foetuses, a co-infection of porcine circovirus type 2 (PCV2) was found. A PCV2 infection can lead to reproductive failure in pregnant sows. It is possible that a PCV2 co-infection can encourage transplacental HEV infection of foetuses (Hosmillo *et al.*, 2010).

As the Morozov *et* al. (2015) study does not mention testing for the presence of PCV2, the possibility of transplacental infection will not be considered.

3.2 Maternal Antibodies (MAbs)

MAbs which can be transmitted from a sow to her offspring through colostrum have been shown to have protective effects against several infectious agents (Kanai *et al.*, 2010). It is important to note with some diseases MAbs only provide partial protection by reducing clinical expression, through the suppression of within-piglet viral replication (Andraud *et al.*, 2014).

It has been shown that MAbs can be successfully transferred from HEV-Ab-positive sows to their piglets. A clear correlation between sow antibody levels and piglet antibody levels has been found (Krog *et al.*, 2019; Andraud *et al.*, 2014). Kasorndorkbua *et al.* (2003) and Kanai *et al.* (2010) found maternally derived anti-HEV immunoglobulin (IgG) antibodies in piglets from HEV inoculated gilts. The MAbs were passively passed onto the piglets through the consumption of colostrum (Kasorndorkbua *et al.*, 2003). Seropositivity amongst sows in a French herd ranged from 70% to 90% (Andraud *et al.*, 2014).

Krog *et al*. (2019) report that the level of MAbs had no significant impact on the HEV infection dynamics later observed within the litter. The protective role of MAbs in the case of HEV is unclear in this study.

Kasorndorkbua *et al.* (2003) found that MAbs lasted until around 2 months of age. Kanai *et al.* (2010) reported a rapid decline in MAbs until 50 days of age. This led to seroconversion taking place around 60 days of age in piglets with MAbs and 50 days of age in piglets without MAbs after exposure to HEV. The difference in seroconversion between the two was found to be significant. Viremia was detected in the MAbs litter at 60 days of age and in the non-MAbs litter at 40 days of age. The peak of HEV-RNA in the serum of these two litters was seen for the MAbs litter at 90 days of age and the non-MAbs at 60 days of age. Both groups ceased shedding at 120 days of age.

Kanai *et al.* (2010) concluded that there was a delay in onset of viremia and seroconversion in litters who had MAbs. Nonetheless, both piglets with and without maternal antibodies shed virus in a similar time frame and for a similar time. The similarity in faecal shedding whilst having the difference in rate of viremia and seroconversion might point to a delayed immune response to a HEV exposure when a piglet has MAbs. A mean duration of passive immunity of 46 (95% CI 42-50) days was concluded by Andraud *et al.* (2013).

3.3 Transmission

Natural infection is expected to occur through the faecal-oral route (Bouwknegt *et al.*, 2009b). The environmental viral load is related to the accumulation of virus particles in the environment through the faecal shedding of infectious animals. The density of virus in the environment is related to the number of shedding pigs and the viral load in their faeces which is dependent on where they stand in the course of their infection. A viral shedding peak occurs between days 8 to 17 post-infection. Additionally, a large inter-variability in the amount of virus each pig sheds has been reported (Andraud *et al.,* 2013). In a study by Bouwknegt *et al.* (2011) 2 x 10^5 viral copies were found per gram

of faeces, however, it was not possible to distinguish which percentage of copies were in the infectious form. For simplicity, an arbitrary 100th of the total HEV RNA count will be used as the infectious viral count per gram of faeces. Piglets defecate an average of 100g/day and around 1000g/day whilst fattening pigs (Salines *et al.*, 2020). For the purpose of this study, pre-weaning piglets will presumably produce 80g/day, post-weaning piglets 120g/day and fattening pigs 1000g/day.

Cleaning reduces the viral load in the environment, this is especially crucial to ensure that rooms are as pathogen-free as possible for new batches. However, several practices can result in sub-optimal cleanliness of the rooms leading to the new batches being exposed to the pathogens of the previous batches. Disinfectants will not work properly if there is organic matter still left over after the washing step whilst cleaning pig pens (van Engen & Scheepens, 2007). Applying disinfectant to a completely dry pen can make the disinfectant less effective. Whilst the pen dries a protective biofilm can form around microorganisms in the pen (Roozen *et al.*, 2007). HEV viral particles are fairly stable in acidic and mild alkaline environments. HEV is vulnerable to disinfection methods which make use of chlorine (Mohammad Sultan Khuroo *et al.*, 2016). Additionally, housing and farm material cleanliness and cleanability can play a role in the persistence of HEV on a farm. Concrete flooring in pig pens is associated with between-batch transmission of HEV. Concrete is a porous material and is harder to clean compared to other flooring materials like steel or rubber.

Additionally, the use of unclean driving boards to move pigs from one location to the next can aid in the mechanical spread of HEV between litters and batches. The cleanliness and the cleanability of the boots worn on the farm can aid in between litter and batch spread of HEV on a farm. The presence of flies in the environment can also aid in the mechanical spread of HEV between litters and batches (Meester *et al.*, 2023). There is little to no information available on the quantification of HEV left in the environment after ineffective cleaning and disinfection. Nonetheless, it appears to play a key role in the persistence of HEV on a farm.

The transmission rate is a crucial parameter in epidemiological model building. It plays an essential role in mimicking reality through the use of modelling (Buch *et al.*, 2023). The transmission rate within a pen is $2 \cdot 10^{-6}$ g/ge/day ($1 \cdot 10^{-7}$; $7 \cdot 10^{-6}$). This value can be interpreted as the average number of pigs that can be infected by a single genome equivalent present in the environment (Andraud *et al.,*2013). Between-pen environmental contamination is rare, but possible. This is due to pens being well segregated and movement between pens is not common practice (Meester *et al.,* 2021).

3.4 Latent period

The latent period is 1-2 weeks long according to a review done by Meester *et al.* (2021). Bouwknegt *et al.* (2009b), reported a difference in the course of infection in contact-infected pigs and intravenously (iv) inoculated pigs. Iv-inoculated pigs have a significantly shorter period to the onset of faecal shedding and a significantly longer period of faecal shedding. Contact infected pigs had a latent period of 7 days (95% CI 5-10).

3.5 Infectious pigs

Viremia compared to shedding

Viremia and faecal shedding both occur once a pig is infected with HEV, these two states often overlap but not always. Viremia is defined as being able to detect the virus in the bloodstream of the animal. In this stage, one can assume there is a virus accumulation in the liver and other organs. It is the viraemic state that can be infectious to humans through foodborne transmission. Usually, during this stage, the pigs also excrete the virus through faecal shedding. The beginning of the infectious period (in relation to pig-to-pig transmission) is considered when faecal shedding starts and was found to last 9.7 (8.2-11.3) days on average (Andraud *et al.,* 2013). Nonetheless, being considered non-viraemic does not exclude the possibility of faecal shedding (Crotta *et al.*, 2018). Williams *et al.* (2001) reported extrahepatic HEV in the colon and small intestine tissue. Making it a possibility that the virus replicates in the intestines before it reaches the liver (Crotta *et al.*, 2018). Looking at the review by Meester *et al.* (2021), the typical viraemic period lasts around 1-2 weeks while faecal shedding can last 1-7 weeks. As previously discussed the course of the infection differs between the route of infection and the Meester *et al.* (2021) paper created these averages on several infection routes.

Bouwknegt *et al.* (2009b) reported a faecal shedding period of 23 days (19-28) for contact-infected pigs. It was also reported that viremia started 13 days (8-17) after faecal shedding started and lasted 11 days (8-13). Below in Figure 11, the depiction of the infectious period as described by Bouwknegt *et al.* (2009b) can be visualised.

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Shed																								
Viremia																								

Figure 11: The infectious period of a contact-infected pig as described by Bouwknegt et al. (2009b)

However, there are few papers employing contact exposure in their methods and a wide variation in results is observed by Meester *et al.* (2021). The shedding period will be used for the infectious period as this is more relevant to the spread of HEV through a farm.

One can assume an infectious period between 17-24 days in trial settings according to Meester *et al.* (2021). Immune-modulating co-infections such as PRRSV are known to delay the onset of shedding and the onset of an immune response to a HEV infection. The period of viral shedding was extended with the presence of a co-infection with PRRSV (Salines *et al.*, 2015).

3.6 Recovered pigs

Recovery can be classified as the elimination of infectious viral particles from the host (Griffin, 2022). Despite viremia having ceased in a recovered animal, HEV RNA can still be detected in the faeces, bile, liver and intestines of that animal (Crotta *et al.*, 2018). Recovery does not equate to the simultaneous elimination of viral RNA (Griffin, 2022). Nonetheless, for how long the virus can be detected in its infectious form in these internal organs is unknown (Crotta *et al.*, 2018).

Despite having ceased shedding viral particles in their faeces, 23% of pigs had detectable levels of HEV RNA in various organs including the liver and lymph nodes (Kanai *et al.*, 2010). Viremia ceased around 27 days post-infection, but positive-strand HEV RNA was still at detectable levels in some organs (Williams *et al.*, 2001). Viral RNA is detectable in certain organs for an extended period after the animal has shifted from the infectious state to the recovered state. However, no literature was found on the infectivity of the HEV RNA.

It appears previous infection confers protective immunity based on the results of Sanford *et al.* (2011). Additionally, the results suggested infection by one genotype of HEV can elicit a protective immune response against other genotypes of HEV.

4. Daily Viral Decay rate

In a study looking at the decay rate of HEV, an initial viral load of $5x10^5$ focus forming units per millilitre (ffu/ml) was placed onto stainless steel disks. After 24 hours of desiccation, $8.7x10^3$ ffu/ml was remaining on the disk *(Wißmann et al., 2023).* Below the formula used to calculate the daily decay is depicted and the calculation is shown in calculation 4.

$$k = -\frac{1}{24h \times per\ day} \times \ln\left(\frac{N_t}{N_0}\right)$$

$N_{(t)}$: Viral concentration at time (t)

N_0: Initial viral concentration

k: rate of viral decay

ln: Natural logarithm

Calculation 4: The daily viral decay of HEV

$$0.00195 = -\frac{1}{24h \times per\ day} \times \ln\left(\frac{8.7 \times 10^3}{5 \times 10^5}\right)$$

5. R output

Table 3: R output displaying the HEV viral load after between batch cleaning and the initial HEV viral load at which the simulation started on. The ratio between the two is also displayed.

pen	HEV_after_cleaning	HEV_initial	ratio
<chr>	<dbl>	<dbl>	<dbl>
1 farrowing	5025937.	4750639.	1.06
2 fattening	25658878.	21113953.	1.22
3 weaning	5095474.	4222791.	1.21

Table 4: The parameter estimates obtained with the use of the MBO as presented by R.

```
gamma              alpha
0.02884925737          0.09937305146

beta
0.00000225893

omega           hev_decay
0.01804674573       0.00204475017

infectious_viral_load_per_gram
2199.37010310209
```

Below, the top value displays the percentage of farms that produced at least one positive batch with the use of the above-stipulated parameters. The lower value displays the average percentage of positive batches a single farm produced if the above parameters were applied. Both were obtained from the R run and sampling methods as described by Meester *et al.* (2022).

```
> print(farmprev)
[1] 85.14286

> print(intrafarmprev)
[1] 40.0
```

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