Universiteit Utrecht

Faculty of Veterinary Medicine

Honours Programme Master thesis

By

Carmijn B. Meulenbroek

Investigating the Epidemiology of Hepatitis E Virus on Dutch Pig Farms

A retrospective case control study associating risk factors with non-infected batches of slaughter pigs and a cross-sectional determination of the pen-level Hepatitis E Virus prevalence on Dutch pig farms

Supervisors

Dr. T.J. Tobias (PhD, DVM, MSc)
M. Meester (DVM, MSc)

Date: 24th August 2021
Student number: 6148905
Email: c.b.meulenbroek@students.uu.nl
Master of Farm Animal Health and Veterinary Public Health

Preface

This research project was carried out as part of the Honours Programme of the Master of Veterinary Medicine at Utrecht University. This programme gives students who are interested in performing veterinary research the opportunity to conduct studies within one of the research facilities of the Faculty of Veterinary Medicine. Additionally, this report is my master thesis for the master of Farm Animal Health and Veterinary Public Health.

I was given the opportunity to contribute to the research that is executed within the bigger framework of the HEVentie project from September 2020 till September 2021. The HEVentie project is devoted to the investigation of the epidemiology of Hepatitis E virus on Dutch pig farms, aiming to identify effective intervention strategies. The project is funded by the Topsector Agri & Food and the Dutch Ministry of Economic Affairs via the public private partnership '1Health4Food' in the Netherlands. Within the Topsector, private industry, knowledge institutes and the government are working together on innovations for safe and healthy food for 9 billion people in a resilient world.

This report entails two separate papers, composed according to the publishing guidelines established by the journal Preventive Veterinary Medicine. First, a general introduction is presented, followed by the two papers in separate chapters. The report ends with a general discussion integrating both papers. For both studies, a Layman summary was added to increase the readability for farmers and other interested people from the pig sector.

Carmijn Meulenbroek
24th of August 2021

Table of Contents

Chapter 1

General Introduction

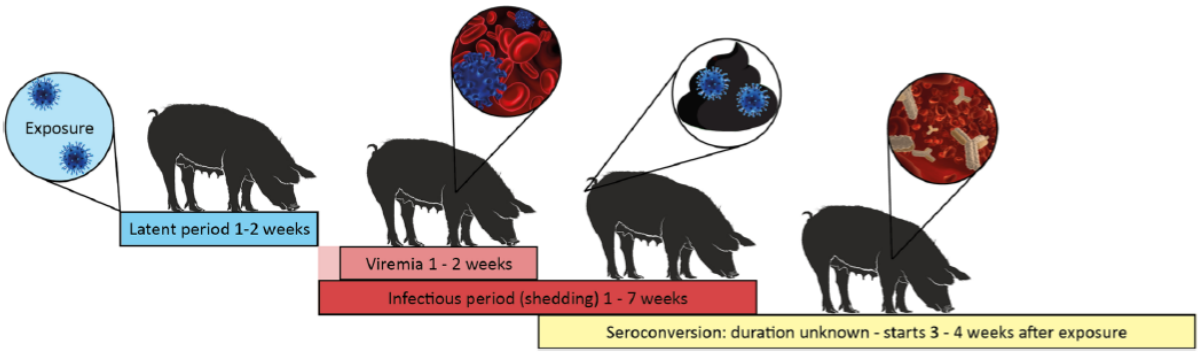
Hepatitis E virus (HEV) is the major cause of viral hepatitis globally (Pallerla et al., 2020). In most cases, an HEV infection results in either a self-limiting acute hepatitis or it runs an asymptomatic course (Kamar et al., 2014). However, patients may develop symptoms such as jaundice and, moreover, death may occur (Dalton et al., 2008; Turner et al., 2010).

HEV is a quasi-enveloped single-stranded RNA virus and member of the family *Hepeviridae* (Ji et al., 2021; Smith et al., 2014). Currently, eight HEV genotypes (Gt) are known, from which five are able to cause human infection (Fenaux et al., 2019). HEV infections acquired in high income countries are mostly caused by Gt3 and Gt4 (Harrison & DiCaprio, 2018), which are considered zoonotic. It has been shown that HEV is able to infect several animal species (e.g. domesticated and feral swine, deer, chickens, mongooses, rabbits and rats) (Hsu & Tsai, 2014; Johne et al., 2010; Linares et al., 2018; Meng et al., 1997; Nakamura et al., 2006; Reuter et al., 2009; Zhao et al., 2009). Currently, pigs and wild boar are considered to be the main source for human HEV infections (Harrison & DiCaprio, 2018). Indeed, presence of HEV has been reported in pigs and pork in many countries (Barnaud et al., 2012; Berto et al., 2012; Di Bartolo et al., 2012; Feagins et al., 2007; Jori et al., 2016; Rutjes et al., 2014a; Walachowski et al., 2014). In addition, researchers have been able to infect pigs using virus isolated from pig livers obtained from grocery stores, which demonstrates the infectivity of HEV in pork (Feagins et al., 2007). Moreover, it has been shown that human cases can be traced back to HEV present in pigs and pork by phylogenetic analysis of HEV strains (Riveiro-Barciela et al., 2015; Widdowson et al., 2003). As HEV infected pork is a foodborne health risk, the presence of HEV on pig farms can be considered a risk for public health. Therefore, mitigation of HEV presence on pig farms is necessary.

To enable identification of effective intervention strategies, it is vital to study the transmission of HEV on pig farms. This report entails two studies investigating the epidemiology of HEV. It should be noted that the epidemiology of infectious diseases on pig farms is complex, due to the clustering of animals on various levels. Firstly, pigs are clustered at pen-level. Secondly, a group of pens forms a farm compartment. In this report, a group of pigs housed in a single farm compartment will be referred to as a 'batch'. Thirdly, an age cohort of pigs usually consists of several farm compartments housing pigs of similar age. Regarding age cohorts, new-born piglets start in the farrowing phase, followed by the nursery phase and the consecutive fattening phase. Table 1 contains the terminology used in this report. For epidemiological analysis, random samplings have to account for individual as well as a priori cluster level variation. Besides the cluster levels, transmission by indirect contact between clusters may occur, as well as the exchange of potentially infected pigs in case of improper 'all-in/all-out' practices.

Pigs infect each other via faecal-oral transmission (Bouwknegt et al., 2011). The course of HEV infection has been studied in experimentally infected pigs and has been summarized by Meester et al., (2021) in Figure 1. Infected pigs first enter a latent stage (duration: one to two weeks), followed by a stage in which they are viraemic (duration: one to two weeks) and deemed infectious for others due to faecal shedding (duration: one to seven weeks). Eventually, seroconversion occurs three to four weeks after the pig is exposed to HEV (duration: unknown) (Meester et al., 2021). Infected pigs do not show any clinical signs (Meng et al., 1997).

FIGURE 1: HEV INFECTION IN EXPERIMENTALLY INFECTED PIGS (MEESTER ET AL., 2021)



In this report, the terms 'HEV-free batch' and 'HEV-infected batch' are frequently used. A batch of slaughter pigs is defined to be 'HEV free' if both serological and virological analysis of blood serum samples show negative results. Absence of both anti-HEV antibodies and HEV-RNA indicates that the sampled animals have not been infected with HEV until the moment of sampling. On the other hand, presence of anti-HEV antibodies implies that pigs have been infected with HEV during their lives and detection of HEV-RNA suggests an infection at the moment of slaughter. Therefore, batches of slaughter pigs from which serological and/or virological analysis of blood serum samples show positive results are defined as HEV-infected batches.

The overarching goal of this report is to contribute to the epidemiological investigation of Hepatitis E virus on pig farms, in order to enable mitigation of HEV on pig farms.

Table 1: Overview of used terminology

Age cohort	Group of pigs of similar age, usually housed in several farm compartments. Age cohorts in consecutive order: farrowing phase – nursery phase – fattening phase.
Farrowing phase	Piglets housed in the farrowing room. Phase starts at birth and ends the age of weaning (~4-6 weeks).
Nursery phase	Phase starts after weaning and ends when the piglets are relocated to new pens with more space per pig (~10 weeks/25 kg).
Fattening phase	Phase starts after relocation of weaned piglets and ends at the moment of slaughter. Pigs in this phase are called fattening pigs.
Batch	Group of similar aged pigs housed in one farm compartment.
Farm compartment	Farm section consisting of a group of pens housing pigs separated from other farm sections by a door and central corridor.
HEV-free	Serological and virological analysis of blood serum samples show negative results (no detection of HEV-RNA or anti-HEV antibodies).
HEV-infected	Serological and/or virological analysis of blood serum samples show positive results (no detection of HEV-RNA and/or anti-HEV antibodies).

Chapter 2

To be submitted to: Preventive Veterinary Medicine

Risk Factors Associated with Slaughter Batches Free from Hepatitis E virus on Infected Pig Farms

Highlights

- This study investigates factors affecting the HEV transmission between farm compartments by associating risk factors to the infection status of batches of slaughter pigs.
- An association was found between the ratio of HEV-free and HEV-infected batches per farm and the following potential risk factors: 'building year of the oldest barn', 'the washing frequency of clothes', 'cleaning procedure includes sweeping of the floor of the central corridor'.
- Future research should investigate the presence of causal relations between the washing frequency of clothes and within-farm HEV transmission on pig farms.
- Future research should investigate the presence of causal relations between including sweeping of the floor of the central corridor in the cleaning procedure and within-farm HEV transmission on pig farms.
- The effect of using different construction materials on the within-farm HEV transmission should be explored in future research.

Layman summary

Hepatitis E virus (HEV) is a virus that can infect both pigs and humans and is present on most pig farms and in pork. To reduce the foodborne zoonotic risk, it is necessary to lower the number of pig farms infected with HEV. Recent research suggests that investigating the transmission of HEV between farm compartments is promising to identify potentially effective mitigation strategies.

This study aimed to identify risk factors associated with occurrence of HEV-free batches of slaughter pigs on infected farms. Considering the faecal-oral transmission route of HEV, it was decided to focus on risk factors regarding between-batch transmission within farm compartments. A case-control study based on results from a previous prevalence study was performed on 73 Dutch pig farms. The presence of potential risk factors on farms was determined using a questionnaire and farm hygiene inspection. A total of 136 potential risk factors were investigated using statistical analysis. It was observed the potential risk factors 'building year of the oldest barn', 'the washing frequency of clothes', 'cleaning procedure includes sweeping of the floor of the central corridor' could explain the differences in within-farm HEV transmission best. Since this study identifies associations, the causal mechanisms underlying these findings need to be studied before effective mitigation strategies can be identified.

Abstract

Hepatitis E virus (HEV) is virus persistently present on most pig farms, which can infect both pigs and humans. Therefore, investigation of within-farm transmission of HEV is vital. Recent research suggests that investigating the variation of HEV infection on batch-level is promising to identify potentially effective mitigation measures. This study aimed to identify risk factors associated with occurrence of HEV-free batches of slaughter pigs on infected farms. Considering the faecal-oral transmission route of HEV, it was decided to focus on risk factors regarding between-batch transmission within farm compartments.

A retrospective case-control study was performed on 73 Dutch pig farms. The presence of potential risk factors on farms was determined using a questionnaire and farm hygiene inspection. A total of 136 potential risk factors were investigated using an aggregated logistic regression modelling approach with the ratio of HEV-free and HEV-infected batches of slaughter pigs per farm as the outcome variable. The best model fit was achieved by the model containing the potential risk factors 'building year of the oldest barn', 'the washing frequency of clothes', 'cleaning procedure includes sweeping of the floor of the central corridor'. Since this study identifies associations, the causal mechanisms underlying these findings need to be studied before effective mitigation strategies can be identified.

Keywords

Hepatitis E Virus, Zoonosis, Veterinary public health, Risk factors, On-farm persistence, Risk mitigation, Pigs

Introduction

Considering the reported farm-level HEV seroprevalence which ranges from 30 to 98% (Salines et al., 2017), and the results of a recently conducted Dutch study showing that anti-HEV antibodies were detected on all investigated farms (Meester et al. *in preparation*), HEV presence on pig farms is clear. It has been hypothesized that pig farms remain infected over time, i.e. are persistently infected with HEV (Meester et al., 2021). This hypothesis has been based on phylogenetic analyses showing that pig farms often have a unique HEV strain which remains closely related over time (Wang et al., 2019). Consequently, it has been indicated that risk mitigation regarding HEV on pig farms should be directed towards lowering the transmission of HEV within farms (Meester et al., 2021).

Regarding the within-farm transmission dynamics of HEV, it has been shown that the seroprevalence of HEV in pigs increases with age (Leblanc et al., 2007) and it has been determined that the peak prevalence of faecal HEV shedding pigs occurs at the age of 90 days (Salines et al., 2017). While most farms are thought to be persistently infected with HEV (Meester et al., 2021), a recent study by M. Meester et al. demonstrated the ability of persistently infected pig farms to deliver HEV-free batches of pigs to the slaughter house (*manuscript in preparation*). These findings indicate that not all pigs have been infected with HEV at the time of slaughter. Moreover, it can be suggested that not all slaughter-aged pigs raised on persistently infected pig farms become infected with HEV. Since pigs of similar age are clustered in a farm compartment (defined as 'batch'), it can be suggested that that HEV transmission between farm compartments can be prevented. Considering the on-farm persistence of HEV (Meester et al., 2021), preventing transmission of HEV between farm compartments is the most promising method to enable HEV mitigation on pig farms.

Previous studies have identified possible mitigation strategies on pig farms, based on risk factor analyses that associate risk factors with the farm-level seroprevalence or prevalence of HEV in pig livers, blood and faeces (Lopez-Lopez et al., 2018; Walachowski et al., 2014). Reported risk factors associated with a high seroprevalence include mingling practices in the nursery stage, a small gap between the level of manure in the pit and the slatted floor in pens housing fattening pigs (Walachowski et al., 2014). Risk factors associated with the presence of HEV in pig livers include a high cross-fostering rate and the use of specific boots for swine activities (Walachowski et al., 2014). Implementation of a quarantine period and usage of a sanitary ford are risk factors reported to be associated with the prevalence of HEV in sows and fattening pigs (Lopez-Lopez et al., 2018). The possible presence of variability in infection between batches of pigs was not investigated in these studies.

Scientific evidence is lacking regarding the identification of factors contributing to the prevention of HEV transmission between farm compartments. This hinders design and implementation of effective management measures and with that the mitigation of HEV presence on pig farms. Therefore, the aim of this study is to identify risk factors that are associated with the ability of infected pig farms to keep compartments, or actually batches of pigs, free from HEV. Within a larger framework study, an extensive risk factor study was conducted with this exact purpose. Considering the great number of factors regarding farm management and the need for sufficiently detailed farm management examination, it was decided to focus this risk factor analysis on specific farm management factors. Since the transmission route of HEV in pigs is faecal-oral (Bouwknegt et al., 2008), it was decided to focus on the factors that potentially affect the transmission of HEV between batches of pigs consecutively housed in one farm compartment (defined as between-batch transmission). A retrospective case control study was performed concentrated on risk factors affecting the between-batch transmission of HEV within farm compartments with the ratio of the number of HEV-free and the number HEV-infected batches of slaughter pigs per farm as outcome variable.

Materials and methods

A retrospective case control study was performed focussing on the transmission of HEV between batches of pigs consecutively housed in one farm compartment with the ratio of the number of HEV-free and the number HEV-infected batches of slaughter pigs per farm as outcome variable. Farm selection and the identification of HEV-free and HEV-infected batches were based on the serological and virological results from a previously performed HEV seroprevalence study (Meester et al, *in preparation*). A detailed farm management examination was carried out conducting an interview with the farmer that entailed a questionnaire and hygiene inspection, resulting in the identification of present possible risk factors. Both the interview guide and study were approved by the ethical research board of Utrecht University, before the start of the research. Prior to the administration of the questionnaire and execution of the hygiene inspection, all farmers signed an informed consent.

Sample collection and analysis

The prevalence study from which the serological and virological results were derived, was conducted between January and August 2019, involving 215 Dutch pig farms delivering slaughter pigs to slaughterhouses of a major slaughter company in the Netherlands. A detailed description of the serum sample collection is described by M. Meester et al. (*in preparation*). Briefly, blood samples were collected from five to twelve slaughter pigs per slaughter batch during exsanguination. Sampled pigs were randomly selected from each batch. Per farm, between two and 24 batches were sampled.

The serological and virological analyses are described by M. Meester et al. (*in preparation*). Briefly, presence of IgM and IgG anti-HEV antibodies was detected in individual sera using an in-house pig specific sandwich enzyme-linked immunosorbent assay (ELISA), as recommended by van der Poel et al., (2014). For the detection of HEV RNA, sera of pigs were pooled per slaughter batch (total of 200 µl). Extraction of HEV RNA was carried out using the Direct-zol 96 kit (Zymo Research). HEV RNA detection was conducted by real time RT-PCR using the Taqman Fast virus-1 step master mix (Applied biosystems) on the LightCycler 480 (Roche), according to Jothikumar et al. (Jothikumar et al., 2006).

Farm selection and data collection

A selection of the farms sampled by M. Meester et al. *(in preparation*) were included in the current risk factor analysis, based on the reported serological and virological results. Two groups of farms were selected for the current risk factor analysis. For the first group of farms, the inclusion criteria were: (1) at least one batch was identified as HEV-free, and (2) a total seroprevalence of <=80%. The second group of farms consisted of farms without HEV-free batches. Farms with both the highest seroprevalence and highest virological prevalence were selected. A total of 143 farms were approached, from which 73 agreed to participate.

All 73 participating farms were visited between July and November 2020, during which a questionnaire was administered, and an inspection of farm hygiene was performed. Farms were visited by two research assistants, from a team of eight students with a background from an applied agricultural or veterinary university. Research assistants received training precedent to visiting farms. Farm visits occurred in pairs of researchers, blinded to the HEV status of the farm. The questionnaire entailed a structured interview with the farmer or farm manager. The interview consisted of approximately 200 questions. The performed hygiene inspection comprised approximately 80 points of interest. The interview was conducted in Dutch. The used questionnaire and hygiene inspection can be found in original language in Appendix A and Appendix B, respectively.

In this study, a specific part of the farm management factors are considered. After consultation of porcine and public health experts, a total of 138 questions and points of interest were selected that were assumed to represent the main potential risk factors regarding the between-batch transmission of HEV. These potential risk factors entail six categories: (a) hygiene, cleaning, and disinfection measures, (b) management of the manure pit, (c) compliance to the 'all-in/all-out' principle (i.e. whether all pigs are relocated simultaneously), (d) duration of sanitary vacancy of farm compartments after pigs have been moved, (e) composition of flooring and percentage of slatted flooring, (f) general impression of farm hygiene and tidiness. The remaining risk factors will be investigated in another study within the HEVentie project.

Statistical analysis

Outcome variable

Given the unequal number of slaughter batches per farm for which laboratory results were available, the a priori probability to detect HEV-free batches differs per farm. In the data analysis, it was decided to account for this effect by using aggregated logistic regression modelling on the ratio of the number of HEV-free to the number of HEV-infected batches of slaughter pigs per farm.

Based on the virological and serological results from M. Meester et al. (in preparation), the batches of slaughter pigs delivered by the 73 selected farms were dichotomized: (1) batches that had both a seroprevalence and PCR result of 0% (to be defined as HEV-free batches and as such as 'cases') (2) batches with a seroprevalence and/or virological prevalence of more than 0% (to be defined as non-HEV-free batches and controls).

Explanatory variables and statistical testing

Explanatory variables consisted of a single question from the questionnaire or point of interest from the hygiene inspection. Levels of explanatory variables consisted of the predefined answer options. In case of open questions, answers had to be categorized to enable statistical analysis. Therefore, all answers to open questions were summarized and categorized, based on biological relevance, with a minimum of four observations per category. All continuous variables were transformed into categorical variables. Based on biological relevance, and in conjunction with members of the project team, cut-off-values were determined based on assumed biological relevance, with a minimum of four observations per category. Each reasonable cut-off-value resulted in a variable that was further analysed. Five steps were taken to analyse the association between the explanatory variables and the outcome variable.

Firstly, it was ensured that each level of the investigated explanatory variables contained at least four observations, to enable any statistical analysis. Variables with all observations in one level were excluded from further analysis. Additionally, variables with two levels of which at least one of the levels contained less than four observations were removed from the dataset. Variables with at least three levels were further analysed in step two, independent of the number of observations per level.

Secondly, pilot runs of the logistic regression model were executed, which showed that the minimum of four observations per level had to be increased to eight to allow model calculations. Therefore, it was decided to merge levels containing less than eight observations per category, based on biological relevance. If the biological relevance did not allow levels to be merged, a minimum of four observations per level was allowed. Considering the limited number of observations in the dataset, a maximum of four levels per explanatory variable was tolerated. In case an explanatory variable contained more than four levels, levels were merged, based on assumed biological relevance. Additionally, pilot runs indicated the necessity to add a category containing all missing values (NAs) to explanatory variables containing more than 9 NAs, to allow model calculations.

Thirdly, univariate analysis was performed to assess the statistical link between each explanatory variable and the defined outcome variable. A total of 105 variables were included in the univariate analysis. Aggregated logistic regression with random farm effects was used to investigate associations between the outcome variable and each explanatory variable. For variables addressing practices in the farrowing or nursery phase, data subsets for these specific farm groups were composed. Evaluation of model fit to the data was performed using Akaike Information Citerion (AIC) (Burnham et al., 2002). A lower AIC indicates a better model fit, explaining the variability in the data better. The AIC contains a penalty for the number of explanatory variables. Models with similar AIC but less explanatory variables are preferred over models with more included variables and only marginally lower AIC. To assess the attribution to explaining the data by an explanatory variable, for each variable a separate model was created, and the AIC was compared to a model without explanatory variables ('empty model'). For each model with one variable, the odds ratios and its 95% confidence interval (95% CI) and the difference in

AIC between the empty model and the model containing the variable were calculated.

Fourthly, selection of variables to be included in the multivariate analysis was carried out based on a drop in AIC of at least one point compared to the empty model. Variables that were thought to be biologically irrelevant were excluded from further analysis after consultation with the project team, since such variables may deteriorate the multivariate model. Similarly, variables with a minimum of eight NA's were eliminated, since these variables drastically lower the power of the multivariate analysis.

Fifthly, multivariate analysis was performed on all remaining variables using a combination of forward and backward model selection based on AIC. The final model was determined based on the model with the lowest AIC. Additionally, the controlled odds ratios (ORs) and the 95% confidence intervals (95%CI) for the variables in this model were calculated.

Reading, management and analysis of variables were facilitated using several R packages: "plyr", "readxl, "dplyr", "tidyverse", "lme4" (RStudio Team, 2020).

Results

Description of included farms

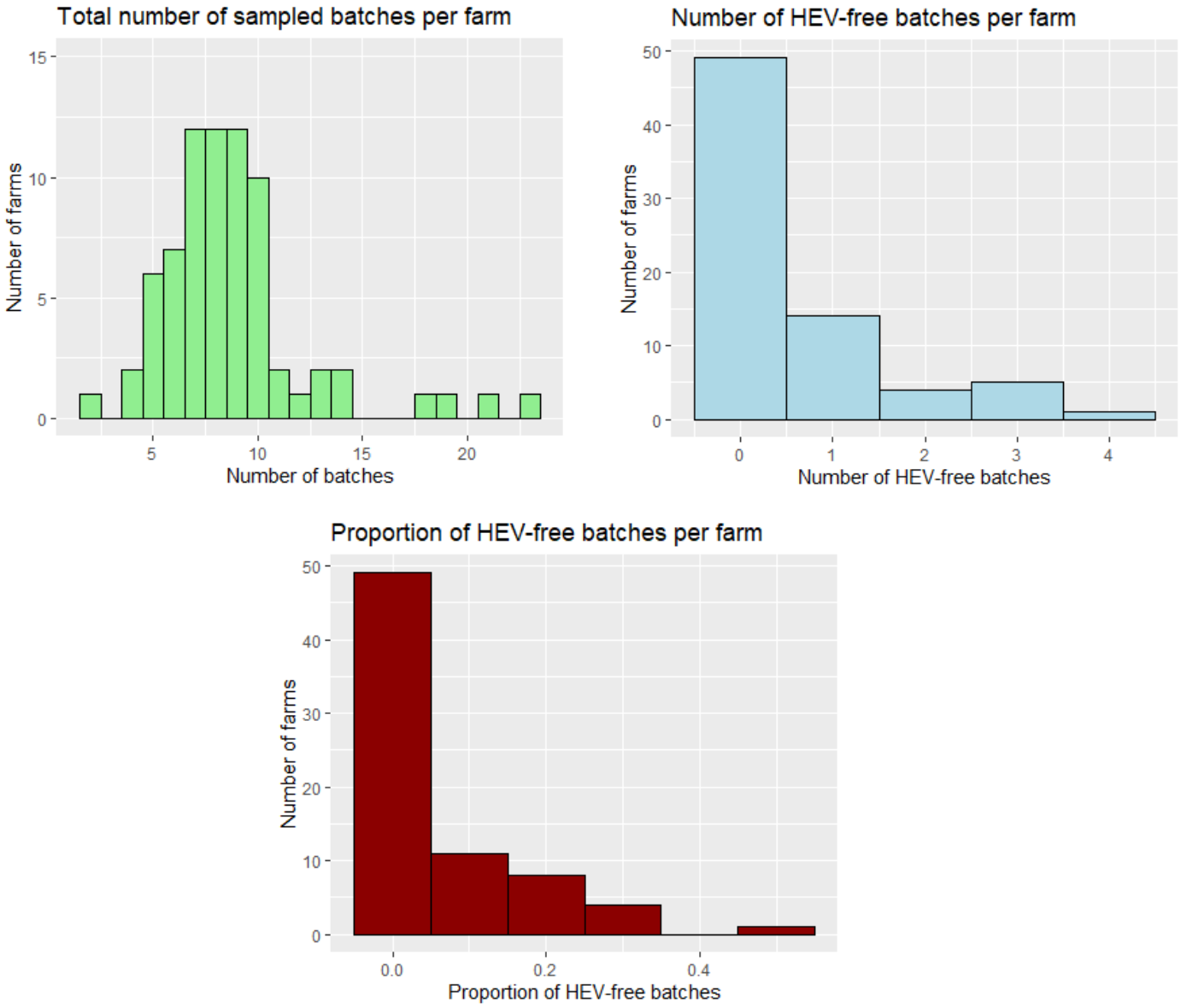
The questionnaire and hygiene check were executed on 73 selected farms. Table 2 enables comparison of farm characteristics of farms with and without cases. It shows that more farms delivering HEV-infected batches than farms delivering HEV-free batches were included in the study in each farm system, each farm type and in total. Regarding farm characteristics, the mean and standard deviation of the number of farm compartments from which pigs compose a slaughter batch, are equal for farms with and without cases. For both the farms delivering HEV-free batches of slaughter pigs and farms without cases, the standard deviation of the number of fattening pigs slaughtered in 2019 per farm is noticeably high, compared to the mean of this farm characteristic.

Table 2: Comparison of the farms with and without cases

		Farms with 0 cases (HEV-free batches)	Farms with 1 or more cases (HEV-free batches)
		Number of farms	
Total		49	24
Farm system			
	Farrow-to-finish farm	17	6
	Weaning-to-finish farm	3	0
	Fattening pigs farm	29	18
Farm type			
	Conventional	41	20
	Organic	8	4
		Mean +/- sd	
Farm characteristics			
	Number of fattening pigs slaughtered in 2019 per farm	8812 +/- 7407	8131 +/- 7039
	Number of farm compartments from which pigs compose a slaughter batch	4 +/- 2	4 +/- 2

Of the 639 batches from which data was considered in this study, 41 were identified as HEV-free. The number of collected batches per farm ranged between 2 and 23, with a median of 8 batches per farm. Since the outcome variable, the ratio of the number of HEV-free and HEV-infected batches per farm, is too complex to present in a single figure, Figure 2 displays the input for the outcome variable: the total number of sampled batches per farm, the number of HEV-free batches per farm and the proportion of HEV-free batches per farm. It can be seen that the majority of farms did not deliver any HEV-free batches. Consequently, the proportion of delivered HEV-free batches per farm is predominantly zero.

FIGURE 2: PRESENTATION OF THE OUTCOME VARIABLE 'RATIO OF HEV-FREE AND HEV-INFECTED BATCHES' BY ITS INPUT



Univariate and multivariate analysis

A total of 136 explanatory variables were considered in this study. Due to a lack of observations per level, several explanatory variables had to be excluded from further analysis, resulting in 105 variables that were investigated in the univariate analysis. Six out of the 105 variables investigated in the univariate analysis reduced the AIC by more than 1 point, thus improved the model fit (Table 3). These variables are: washing frequency of clothes (AIC: -3.17), rubber flooring in fattening pigs pen (AIC: -3.42), cleaning procedure includes sweeping of the floor of the central corridor (AIC: -2.14), building year oldest barn (AIC: -1.66), effective chemical in most used disinfection chemical (AIC: -1.27) and distance from manure pit to slatted floor (AIC: -1.09). Significant odds ratios were obtained for two variables. Firstly, both a daily and monthly washing frequency of clothes were associated with a higher ratio of HEV-free batches compared to weekly washing frequency (OR = 5.45 (95%CI 1.45-20.45) and OR = 7.64 (95%CI 1.42-41.35), respectively). Secondly, implementing rubber flooring in fattening pig pens was significantly associated with higher ratio of HEV-free batches (OR = 3.37 (95%CI: 1.26-9.07). Odds ratios for the remaining risk factors investigated in the univariate analysis are given in Appendix C. The potential risk factors for which odds ratios could not be obtained, due to a lack of observations per category are listed in Appendix D.

Out of the six variables that reduced the AIC by more than 1 point, four variables were included in the multivariate analysis. The variable regarding rubber floors was not included in the multivariate analysis, since the assumed biological relevance of a rather small surface covered by rubber was thought to be neglectable. Confounding with factors such as feeding systems was thought to be more likely. Similarly, the variable concerning the distance between the slatted floor and manure pit was excluded from the multivariate analysis, since the high number of missing values drastically reduced the power of the study. Consequently, the variables 'building year of the oldest barn', 'cleaning procedure includes sweeping of the floor of the central corridor', 'the washing frequency of clothes' and 'the effective chemical in the most used disinfection chemical' were included in the multivariate analysis. The best model fit was achieved by the model that included 'building year of the oldest barn', 'sweeping the floor', 'the washing frequency of clothes' (Table 3). Significant odds ratios were observed for the washing frequency of clothes and the building year of the oldest barn. Similar to the results from the univariate analysis, a daily and monthly washing frequency of clothes were associated with a higher ratio of HEV-free batches compared to weekly washing frequency (OR = 3.57 (95%CI 1.10-11.54) and OR = 4.36 (95%CI 1.06-17.94), respectively). Regarding the building year of the oldest barn, it was observed that a building year after 1970 significantly increased the odds on delivering HEV-free batches of slaughter pigs. The variable 'cleaning procedure includes sweeping of the floor of the central corridor' did not result in significant odds ratios, but the acquired odds ratio indicates that including sweeping of the floor of the central corridor in the cleaning procedure is associated with a lower occurrence of HEV-free batches (OR = 0.28 (95%CI 0.06-1.36)).

Table 3: Results from the univariate and multivariate analysis

	Univariate analysis[a]		Multivariate analysis
Potential risk factors	Difference in AIC to the empty model	Odds ratio (95% CI)[bc]	Odds ratio (95% CI)[b]
Washing frequency of clothes			
Weekly (n=27)	-3.71	1	1
Daily (n=23)		5.45* (1.45-20.45)	3.57* (1.10-11.54)
Monthly or after each batch (n=7)		7.65* (1.42-41.35)	4.36* (1.06-17.94)
Every time the clothes have been in contact with animals or are dirty (n=14)		1.98 (0.41-9.66)	1.45 (0.35-6.15)
Rubber flooring in fattening pigs pen			
False (n=59)	-3.42	1	
True (n=13)		3.37* (1.26-9.07)	Variable not evaluated[c]
Cleaning procedure includes sweeping of the floor of the central corridor			
False (n=59)	-2.14	1	1
True (n=14)		0.22 (0.04 - 1.11)	0.28 (0.06-1.36)
Building year oldest barn			
Before or in 1970 (n=15)	-1.66	1	1
After 1970 (n=59)		3.49 (0.88-13.86)	8.28* (1.00-67.89)
Effective chemical in most used disinfection chemical			
No disinfection chemical reported (n=29)	-1.27	1	Not retained[d]
Ammonium chloride + glutaraldehyde (n=26)		0.45 (0.17-2.23)	
Hydrogen peroxide (n=5)		2.91 (0.89-9.51)	
Other effective chemical (n=6)		1.08 (0.24-4.81)	
Distance from manure pit to slatted floor			Variable not evaluated[e]
Less than 60 cm	-1.09	1	
More than 60 cm		0.42 (0.16-1.12)	

[a] Solely results regarding variables lowering the AIC by at least 1 point are displayed. Remaining explanatory variables are listed in Appendix C.
[b] 95%CI: 95% confidence interval.
[c] Due to assumed absence of biological relevance, this variable was excluded from multivariate analysis.
[d] This model was tested in the multivariate analysis, but was not retained in model selection procedure based on AIC.
[e] Due to high number of missing values, this variable was excluded from multivariate analysis.
*Significant odds ratio

Discussion and conclusion

This study aimed to identify risk factors associated with pig farms that manage to keep compartments of the farm free from HEV infection. Therefore, a risk factor analysis was carried out with the outcome variable defined as the ratio of the number of HEV-free batches to the number of HEV-infected batches. In our study set, 73 farms were visited of which 24 (0.33 proportion) farms had at least delivered one HEV-free batch of pigs to the slaughterhouse.

The risk factor analysis has identified several associations with the outcome variable. The univariate analysis showed that model improvement was attained by six variables. After the multivariate analysis, the best model was determined, which consisted of the variables 'building year of the oldest barn', 'the washing frequency of clothes', 'cleaning procedure includes sweeping of the floor of the central corridor'. Significant odds ratios were observed for the first two variables. The association between the building year of a barn and HEV transmission has not been reported yet. Regarding the hygiene management related variables, similar results have been obtained by Lopez-Lopez et al., (2018), who have related biosecurity measures to the presence of HEV in blood of pigs. Additionally, Walachowski et al., (2013) have reported an association between a lack of hygiene measures and the proportion of slaughter pigs delivered with HEV-positive livers. Both papers do not specify which hygiene measures were taken into account, apart from a single example per paper that do not correspond to the potential risk factors investigated in this study. Therefore, no comparisons can be made regarding specific hygiene measures affecting HEV transmission.

Walachowski et al., (2013) reported that a small gap between the pit manure and slatted floor in pens housing fattening pigs increased HEV seroprevalence, we found that a distance from manure pit to slatted floor of more than 60 centimetres was associated with a lower occurrence of HEV-free batches. While the comparison of the investigated cut-off values of the hight between the slatted floor and manure pit is hindered by the unspecified term 'small gap' used by Walachowski et al., (2013), it is unlikely that biological mechanisms underly this discrepancy, considering the faecal-oral transmission route of HEV (Bouwknegt et al., 2011). Presumably, the gap measured in our study does not reflect the situation in time of the housing of sampled slaughter batches, since the time period between sample collection and performance of the hygiene inspection was significant. Additionally, solely the less reliable univariate results are available. This may have resulted in the contra intuitive results for this variable. Furthermore, Walachowski et al., (2013) reported that the within-herd HEV seroprevelance was associated with a down period in the nursery phase of less than four days. Similarly, this study investigated the down period in the nursery phase with a cut-off value was one day. However, implementation of the variable resulted in no model improvement (AIC=1.97). This may have been caused by the use of a different cut-ff value for the duration of the down period. Researching the effect

of a different cut-off value can be useful, but was not feasible in this study, due to the low number of farms with a down period of more than three days.

Before our results can be used to identify possible intervention strategies to prevent HEV transmission between farm compartments, several nuances should be emphasized. Firstly, a common phenomenon in statistics entails that analysing datasets with many variables and a limited number of observations may result in a number of significant associations, even if there is no causal relation (J.C. Vernooij, *personal communication*). Consequently, model improvement provided by including variables could be based on coincidence rather than biological mechanisms. Furthermore, investigating many variables regarding one subject increases the chance that several explanatory variables are correlated. For instance, if a farm has a strict hygiene protocol, it is likely that the farmer showers before each farm visit and wears gloves. Since the factors are associated, both factors will be associated to the outcome variable if only one of the factors hinders the transmission of HEV. Therefore, associated explanatory variables may interfere with the model. The used multivariate analysis corrects for associated explanatory variables, but the relatively large differences between the odds ratios in the univariate and multivariate analysis of the variable regarding the building year of the farm indicates that associated variables may have interfered with the model (J.C. Vernooij, *personal communication*). Consequently, the multivariate model might contain associations that do not affect HEV transmission, indicating the need for studies investigating the causal relation between the identified risk factors and HEV transmission.

Secondly, the effect of confounding factors should not be overlooked. Confounding factors affect both the explanatory variable and the outcome variable, suggesting a non-existent relationship. For example, it is implausible that the building year of the oldest barn affects biological processes, while it is likely that the cleanability of the farm may be affected by the wear and tear of construction materials or the use of modern materials like plastics instead of wood. Another example is the implementation of rubber flooring in fattening pig pens. Rubber flooring could be associated with the use of certain feeding systems. If this feeding system is associated to the introduction of HEV in a farm compartment, the use of rubber flooring can be associated to the outcome variable in this study, without an underlying biological mechanism. It must be stated, however, that feeding system was not investigated in this study or and previous risk factor analyses do not report such associations.

Thirdly, a low number of observations per category may cause a less precise estimate for the odds ratio, resulting in interference with interpretation of the results. This can be expected for the contradictive result concerning the monthly and daily washing frequency of clothes. The effect of both a higher and lower washing frequency are similar effect on the outcome variable, while this seems biologically illogical. This may be explained by the low number of observations in the monthly washing category (n=7). Similarly, the low number of farms using hydrogen peroxide and peracetic acid (n=5) may have caused the opposite effect of different effective chemicals in disinfection chemicals.

Fourthly, the questionnaire and hygiene inspection demanded for quantitative data over qualitative data. Farmers were asked to indicate which pre-defined category reflected their farm practices best, resulting in exposure misclassification. Additionally, farmers were asked about information from a year ago, which may have led to recall bias. Since eight research assistants administered the questionnaire and executed the hygiene inspection, interviewer bias cannot be ruled out.

Fifthly, the use of a univariate analysis does not allow variables to be analysed in a combination of possibly relevant factors. Therefore, it is possible that not all relevant explanatory variables have been selected for the multivariate analysis. For example, using a certain disinfection chemical may not have a major effect on HEV transmission if it is not used frequently. On the other hand, disinfecting frequently with an impotent disinfection chemical will not alter HEV transmission. Thus, it is necessary to analyse the use of disinfection chemicals and disinfection frequency simultaneously. To overcome this problem, future studies may consider using LASSO regression (Ranstam & Cook, 2018).

Seventhly, a considerable time period of approximately 18 months is present between the collection of the data from which the outcome variable has been derived and the administration of the questionnaire and execution of the hygiene check on which the explanatory variables are based. Therefore, particularly the explanatory variables regarding the hygiene check may not reflect the situation before sampling very well. For instance, our finding that a smaller distance to the manure pit is associated with a higher odds on a HEV-free batch is not in line with the finding of Walachowski et al., who reported that HEV seroprevalence increased if there was a small gap between the slatted floor and the pit manure (Walachowski et al., 2014). Moreover, considering the faecal-oral transmission route of HEV (Bouwknegt et al., 2011), it is likely that an increased exposure to manure will result in increased HEV transmission. This discrepancy might be explained by the time period between the sample collection and performance of the hygiene inspection.

Eightly, care should be taken when interpreting the serological and virological results regarding outcome variable. The samples have been taken from six pigs per slaughter batch. The small sample size per batch implies some uncertainty about the batch infection status based on PCR or ELISA. Additionally, as farmers combine pigs originating from four farm compartments in average into one slaughter batch, the slaughter batch result might reflect the HEV status of more than one farm compartment.

Lastly, the current risk factor analysis focusses on the transmission of HEV between concurrent batches housed in the same farm compartment. Other transmission routes than between-batch transmission within a farm compartment should not be overlooked. This is indicated by the variable regarding the cleaning procedure of the central corridor, since the variable addresses the hygiene protocol between farm compartments, rather than transmission between concurrent batches within a farm compartment. The improvement of the model fit provided by this variable implies that the

investigation of the contribution of transmission between farm compartments to the on-farm persistence of HEV warrant further investigation.

In conclusion, we identified the 'building year of the oldest barn', 'the washing frequency of clothes', 'cleaning procedure includes sweeping of the floor of the central corridor' as risk factors potentially enabling HEV mitigation on pig farms. Since this study was carried out on 73 farms that were selected from a group of 215 randomly selected Dutch farms based on several inclusion criteria and farmers participated voluntarily, selection and participation bias may have affected the generalisability of the group of farms that participated. However, it is not likely that this has affected the identification of potential risk factors affecting HEV transmission, since the biological mechanisms underlying HEV transmission are similar for all pig farms.

Future research should investigate the causal relation between the identified potential risk factors and the within-farm HEV transmission, since the statistics used in the current risk factor analysis only allow to identify associations, and no causal relations. To assess the causal relationship, an experimental case-control study investigating HEV transmission should be carried out. Based on this risk factor analysis, an interesting approach for the potential risk factor 'the washing frequency of clothes', would be to implement a weekly washing frequency as control, and a daily and monthly washing frequency as case. The case-control design for the implementation of sweeping in the cleaning procedure of the central corridor is straight-forward. Regarding the building year of the oldest barn, a case-control study in experimental setting would be complex and, moreover, no intervention strategies can be derived from such studies. Thus, the investigation of mechanisms underlying the possible effect on transmission, such as the use of different construction materials, is recommended. Additionally, researchers should investigate other transmission routes of HEV withing farms than transmission between farm compartments, for example on pen-level.

If the experimental studies would reveal a causal relation between the washing frequency of clothes, the implementation of sweeping in the cleaning procedure of the central corridor, or the use of certain construction materials and HEV transmission within farms, effective intervention strategies can be identified and tested, hopefully contributing to the mitigation of the public health risk associated with HEV presence on pig farms.

Chapter 3

To be submitted to: Preventive Veterinary Medicine as Short Communication.

Short Communication: Exploration of The Pen-Level Prevalence of Hepatitis E Virus on Pig Farms

Highlights

- We were able to determine the pen-level prevalence (divided into the between-pen and within-pen prevalence) of HEV-shedding pigs on pig farms, and the presence of variability in this prevalence is clear.
- Pen-level investigation of HEV transmission on pig farms is a promising and feasible method.
- Considering the possibility of between-farm differences in HEV transmission dynamics, researchers may need to conduct farm-specific pilot studies before designing pen-level epidemiological research.

Layman summary

Hepatitis E virus (HEV) is a virus that can infect both humans and pigs, which is present on most pig farms. To reduce the foodborne zoonotic risk, it is necessary to gain a better understanding of how HEV is transmitted on pig farms. Since all pig farms are infected with HEV, it has been indicated that efforts to reduce the number of pigs infected with HEV on pig farms should be directed towards lowering the transmission of HEV within pig farms, rather than between farms. Transmission of HEV within pens is inevitable and we suggest that investigating the transmission of HEV on pen-level may be promising. Since studies on pen-level cannot be conducted without knowledge about the prevalence of HEV on pen-level, we explored the pen-level prevalence of HEV-shedding pigs on four Dutch pig farms. On these farms, faecal swab samples were taken from pigs in four age categories. Both the proportion of pens housing HEV shedding animals (between-pen prevalence) and the proportion of HEV shedding animals in positive tested pens (within-pen prevalence) were determined. Additionally, the prevalence of HEV-shedding pigs on farm-level and age cohort-level was calculated. HEV-shedding animals were identified on three of the four investigated farms, all in the age category 'last week of fattening phase'. Both the between-pen prevalence and within-pen prevalence varied greatly between farms. The between-pen prevalence and within-pen ranged from 0 to 0.75 and 0.14 to 1, respectively. These results indicate that pen-level epidemiological research is not only a promising, but also a feasible method. Future research should take into account that HEV transmission dynamics may vary between farms.

Abstract

Hepatitis E virus (HEV) is a zoonotic virus that is persistently present on most pig farms. The epidemiology of HEV on pig farms remains to be elucidated. Considering the persistent presence of HEV on pig farms, it has been indicated that mitigation of HEV on pig farms should be focussing on within-farm transmission. The R0 of HEV is high and transmission between pigs occurs through the faecal-oral transmission route, resulting in inevitable transmission of HEV within pens. Consequently, we suggest that epidemiological research with a pen housing pigs may be promising. This study aims to enable pen-level epidemiological studies. Therefore, a cross-sectional exploration of the pen-level prevalence of HEV-shedding pigs was carried out. Faecal swab samples were taken from pigs in four age categories on four Dutch pig farms. Both the proportion of pens housing HEV shedding animals (between-pen prevalence) and the proportion of HEV shedding animals in positive tested pens (within-pen prevalence) were determined. Additionally, the prevalence of HEV-shedding pigs on farm-level and age cohort-level was calculated. HEV-shedding animals were identified on three of the four investigated farms, all in the age category 'last week of fattening phase'. Both the between-pen prevalence and within-pen prevalence varied greatly between farms. The between-pen prevalence and within-pen ranged from 0 to 0.75 and 0.14 to 1, respectively. These results indicate that pen-level epidemiological research is not only a promising, but also a feasible method. Future research should take into account that HEV transmission dynamics may vary between farms.

Keywords

Hepatitis E Virus, Zoonosis, Veterinary public health, Pen-level research, Prevalence, Pigs

Introduction

Numerous studies have been devoted to the investigation of the epidemiology of HEV on pig farms, aiming to contribute to the mitigation of HEV on pig farms. These studies have either been devoted to the investigation of HEV epidemiology on farm-level (Lopez-Lopez et al., 2018; Rutjes et al., 2007, 2014b; Walachowski et al., 2014), batch-level (Fernández-Barredo et al., 2016; McCreary et al., 2008) or animal-level (Bouwknegt et al., 2008; Rose et al., 2011). While these studies have provided several insights, the epidemiology of HEV on pig farms remains to be elucidated (Salines et al., 2017).

Pig farms are suggested to be persistently infected with HEV (Meester et al., 2021), since phylogenetic analyses have shown that pig farms often have a unique HEV strain which remains closely related over time (Wang et al., 2019). Reducing the number of HEV infections in pigs should therefore be concentrated at preventing transmission within farms. Within farms, transmission of HEV may occur within pens, between pens and between farm compartments. Therefore, research investigating the transmission of HEV on these levels is favourable. However, HEV transmission within pens is unavoidable, given faecal-oral transmission and the high R0 that is reported (Bouwknegt et al., 2008). Consequently, investigation of animal-level epidemiology of HEV may be inefficient. Thus, epidemiological research with either farm compartment or pen as observational unit can be considered as the most promising method to identify intervention strategies resulting in mitigation of HEV on pig farms.

This study focusses on enabling pen-level epidemiological research. To allow pen-level epidemiological studies to be conducted, it is vital to explore the pen-level HEV prevalence and the variability in the pen-level prevalence between pens within and between pig farms. Consequently, this study aims to explore the proportion of pens housing HEV-shedding pigs and the proportion of HEV-shedding pigs per pen on pig farms. Since this is, to the knowledge of the author, the first study to explore the pen-level concept in an observational setting, the farm-level and age cohort-level prevalence of faecal HEV shedding in pigs was determined to permit comparisons to available scientific studies.

Materials and methods

This cross-sectional study aimed to explore the pen-level prevalence of faecal HEV shedding in pigs. Additionally, the farm-level and the age cohort-level faecal HEV shedding prevalence was determined. The Animal Welfare Body from Utrecht University was consulted and concluded that the study was exempt from an animal ethical evaluation, as the project did not include procedures according to the European Directive on the protection of animals used for scientific purposes (EC/2010/63).

Study design, sample collection and farm selection

Four Dutch pig farms participated in this study. Farms were selected based on the virological and serological research from a previous prevalence study conducted in 2019. The prevalence study from which the serological and virological results were derived, was conducted between January and August 2019, involving 215 Dutch pig farms delivering slaughter pigs to slaughterhouses of a major slaughter company in the Netherlands. A detailed description of the serum sample collection is described by M. Meester et al. (in preparation). Briefly, blood samples were collected from five to twelve slaughter pigs per slaughter batch during exsanguination. Sampled pigs were randomly selected from each batch. Per farm between two and 24 batches were sampled.

The serological and virological analyses have been described by M. Meester et al. (in preparation). Briefly, presence of IgM and IgG anti-HEV antibodies was detected in individual sera using an in-house pig specific sandwich enzyme-linked immunosorbent assay (ELISA), as recommended by Van der Poel et al., (2014). For the detection of HEV RNA, sera of pigs were pooled per slaughter batch (total of 200 µl). Extraction of HEV RNA was carried out using the Direct-zol 96 kit (Zymo Research). HEV RNA detection was conducted by real time RT-PCR using the Taqman Fast virus-1 step master mix (Applied biosystems) on the LightCycler 480 (Roche), according to Jothikumar et al. (Jothikumar et al., 2006).

For the current cross-sectional study, the inclusion criteria based on the previous prevalence study (M. Meester et al., in preparation) were: (1) a seroprevalence of more than 70% and (2) detection of HEV-RNA in at least one of the pooled serum samples.

On all investigated farms, a cross-sectional study was conducted in May 2021. Faecal swab samples were acquired from pigs of four age groups: (1) first week of nursery phase (~5 wks of age), (2) last week of nursery phase (~9 wks of age), (3) second week of fattening phase (~12 wks of age) and (4) last week of fattening phase (~24 wks of age)). Sampling moments 1 and 2 reveal the presence of infectious pigs on moments of the mingling of piglets. The major occasions for transmission from infected pigs to either pigs or humans are reflected by sampling moments 3 and 4.

Feacal swab samples were acquired directly from the rectum, using a cotton wool swab for 10 seconds. As HEV may also be present in the environment (Ianiro et al., 2021), researchers ensured that cotton wool swabs only came into contact with the pigs rectum. During farm visits, gloves were changed between each pen, hairnets were changed between each farm compartment and boots were changed

between the weaning and fattening phase. Samples were taken from 7 pens per age category. The sampled pens were randomly selected from, but evenly distributed over, the available farm compartments housing the animals of interest. In every pen, each individual pig was sampled. If a farm housed more than 20 pigs per pen, 20 pigs per pen were sampled. Only animals that were showing signs of severe disease were excluded from sampling. Table 4 displays the number of taken faecal samples. Swab samples were stored at room temperature upon arrival at the laboratory on the same day.

Molecular detection of HEV

For the detection of HEV RNA, 2ml tryptosephosphate 2.95% with gentamycin was added to the cotton wool swab sample. After vortexing, samples were incubated for 30 minutes, enabling the faeces to soak off. Samples were vortexed again and centrifuged at 2,500 *g*. The supernatant was separated and stored. Subsequently, a was pool pen sample was made by taking 40 µL supernatant per sample from each individual pig sample. Awaiting further analysis, samples were stored at -80 °C.

Samples were analysed at Wageningen Bioveterinary Research. First, all pooled samples were tested. On 100 µl of every pooled sample, RNA isolation was carried out using the Quick-DNA/RNA Viral MagBead kit (Zymo Research), following the manufacturer's recommendations. Molecular detection of HEV RNA was conducted using real-time RT-PCR following the protocol recommended by Jothikumar, including the inclusion of negative controls in each run (Jothikumar et al., 2006). Upon a positive pool sample result, individual samples were tested following the methods as described above.

Prevalence calculations

This study explores the farm-level, age cohort-level and pen-level prevalence of faecal HEV shedding in pigs on four pig farms. A pig was considered to shed HEV if HEV-RNA was detected in the individual faecal sample of the pig. To determine the pen-level HEV prevalence, the proportion of pens housing HEV-shedding pigs per farm was determined (to be defined as between-pen prevalence). Subsequently, in pens housing HEV-shedding pigs, the proportion of HEV-shedding pigs per pen was determined (to be defined as within-pen prevalence). The age cohort-level HEV prevalence was calculated for both age categories per age cohort, to enable identification of variation within age cohorts. The HEV prevalence on age cohort level was derived from the proportion of HEV-shedding animals per batch. Based on the proportion of farms housing HEV-shedding pigs, the farm-level HEV prevalence was calculated.

Results

Faecal presence of HEV-RNA was detected in on three farms: Farms A, C and D. Therefore, the farm-scale HEV prevalence of HEV shedding pigs is 0.75 (prevalence not shown in Table 4).

Table 4 presents the age cohort-level virological results and accompanying prevalence of HEV shedding pigs per farm. It can be seen that HEV-RNA was solely detected in the faeces of pigs in the age category 'last week of fattening phase'. On Farm A, the prevalence of HEV-shedding animals in this age category was 0.09. On Farms C and D, the prevalence was 0.60 and 0.02, respectively. In the other three age categories 'first week of nursery phase', 'last week of nursery phase' and 'second week of fattening phase', no HEV-shedding pigs were identified.

The pen-level prevalence of HEV shedding pigs was determined on two levels: (1) the between-pen prevalence of HEV-shedding pigs, and (2) the within-pen prevalence the prevalence of HEV shedding pigs. Table 4 lists the number of sampled animals and pens, virological results and accompanying prevalence per farm and age category. Analysis of the samples pooled per pen resulted in a between-pen prevalence of 0.14, 0.75 and 0.14 on Farms A, C and D, respectively. On farm A, HEV shedding animals were detected in solely one pen. In this pen, seven out of the eleven were shown to shed HEV, resulting in a within-pen-level prevalence of 0.64. While the number of pens housing HEV-shedding pigs, namely one, was identical for Farms A and D, Farm D was shown to have a lower within-pen prevalence: only one of the seven sampled pigs was shown to shed HEV. The highest between-pen and within-pen prevalence of HEV shedding pigs was identified at Farm C: six out of the eleven sampled pens ware shown to house HEV-shedding pigs. Within these pens, the within-pen HEV prevalence ranged from 0.45 to 1.00.

Figure 3 combines the age cohort-level and pen-level results. It presents the between-pen proportion of HEV shedding pigs, while pointing out that variation in the proportions were solely obtained for pens in the age category 'last week of fattening phase', since no HEV-RNA was detected in the faeces of pigs in the remaining age categories.

FIGURE 3: THE BETWEEN-PEN PREVALENCE OF HEV-SHEDDING PIGS DIFFERS PER AGE CATEGORY AND PER FARM

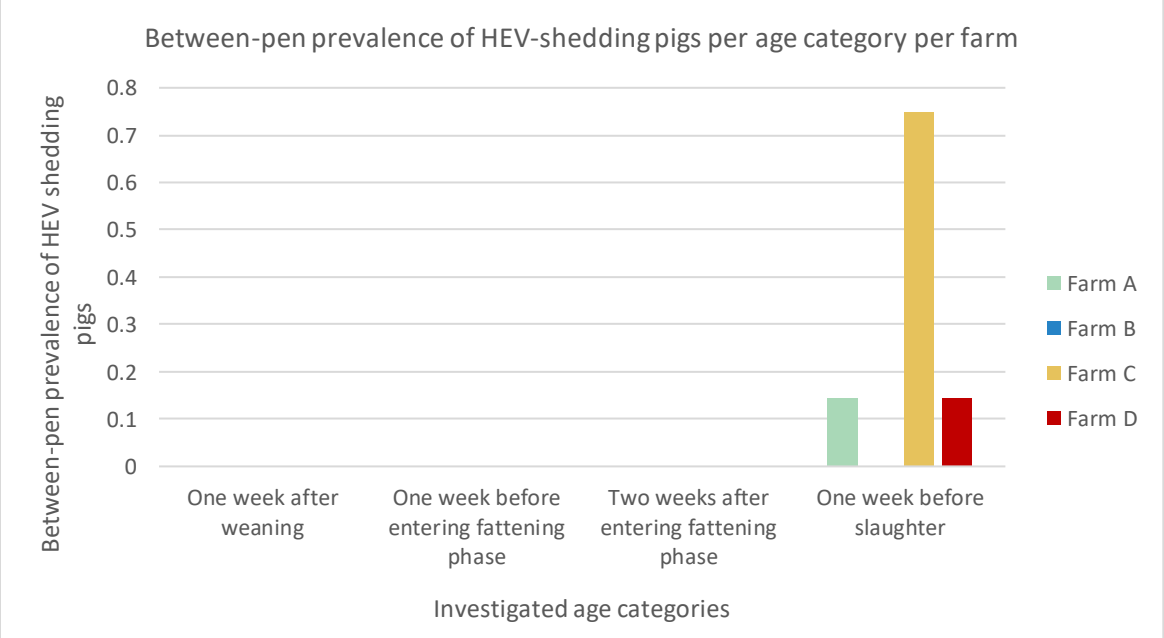


Table 4: The pen-level prevalence (between-pen and within-pen) and the age cohort-level prevalence

Farm	Age category	Number of HEV positive pooled pen samples/number of sampled pens (between-pen prevalence[a])	Number of HEV shedding animals per positive tested pen/number of sampled animals in the pen (within-pen prevalence[b])*	Number of HEV shedding animals in total/total number of sampled pigs (age cohort-level prevalence[c])
A	First week of nursing phase	0/7 (0.00)		0/138 (0.00)
	Last week of nursing phase	0/7 (0.00)		0/139 (0.00)
	Second week of growing phase	0/7 (0.00)		0/105 (0.00)
	Last week of growing phase	1/7 (0.14)	7/11 (0.64)	7/75 (0.09)
B	First week of nursing phase	0/7 (0.00)		0/140 (0.00)
	Last week of nursing phase	0/7 (0.00)		0/142 (0.00)
	Second week of growing phase	0/7 (0.00)		0/141 (0.00)
	Last week of growing phase	0/7 (0.00)		0/139 (0.00)
C	First week of nursing phase	0/6 (0.00)		0/84 (0.00)
	Last week of nursing phase	0/6 (0.00)		0/90 (0.00)
	Second week of growing phase	0/8 (0.00)		0/85 (0.00)
	Last week of growing phase	6/8 (0.75)	10/10 (1.00) 8/9 (0.89) 4/8 (0.5) 8/9 (0.89) 9/10 (0.90) 5/11 (0.45)	44/73 (0.60)
D	First week of nursing phase	0/7 (0.00)		0/139 (0.00)
	Last week of nursing phase	0/7 (0.00)		0/93 (0.00)
	Second week of growing phase	0/7 (0.00)		0/70 (0.00)
	Last week of growing phase	1/7 (0.14)	1/7 (0.14)	1/51 (0.02)

[a] The between-pen prevalence of HEV shedding pigs was calculated as the proportion of sampled pens in which HEV-RNA was detected.

[b] The within-pen prevalence of HEV shedding pigs was calculated as the proportion of individual faecal samples in which HEV-RNA was detected per positive tested pen.

[c] The batch-level prevalence of HEV shedding pas was calculated as the proportion of total number of individual faecal samples in which HEV-RNA was detected per age category.

Discussion and conclusion

This cross-sectional study explores the prevalence of HEV in pigs on pen-level. Since this is, to the knowledge of the author, the first study to explore the pen-level epidemiology of HEV in an observational setting, the farm-level and age cohort-level prevalence of faecal HEV shedding on the investigated pig farms was determined to permit comparisons to available scientific studies.

We found a farm-level prevalence of HEV shedding animals of 75%, since HEV-RNA was detected in faeces of pigs on three out of the four investigated farms. According to a review performed by Salines et al., (2017), the reported farm-scale virological HEV prevalence in literature ranges from 10% to 100%. Thus, our result is in line with previous literature. However, it is remarkable that no HEV-shedding animals were detected in farm B, considering our inclusion criterium that farms should have delivered batches of slaughter pigs with a seroprevalence of 70% of higher. It should be noted that the period in which pigs shed HEV is relatively short (1-7 weeks (Meester et al., 2021)). Moreover, the study design is cross-sectional and does not allow pigs to be followed up longitudinally. Therefore, it is more likely that HEV-shedding pigs were present on this farm and that no samples were taken from these animals at the moment they were shedding HEV.

On age category-level, our prevalence of HEV shedding animals was zero in the age categories 'first week of nursery phase', 'last week of nursery phase' and 'second week of fattening phase'. Similar to our results, a recent cross-sectional study performed by Ianiro et al., (2021) reported absence of HEV shedding in weaner pigs. Others did report HEV shedding in pigs in the nursery phase (Fernández-Barredo et al., 2016; Forgách et al., 2010; Steyer et al., 2011) and, moreover it has been suggested that regrouping piglets before entering the nursery phase increases HEV prevalence (Walachowski et al., 2014). Thus, the presence of HEV shedding pigs in the nursery phase differs per farm. Our finding of absence of HEV-shedding pigs in the second week of the fattening phase is noticeable. We were unable to find any papers reporting similar results. Furthermore, a meta-regression analysis based on 31 studies performed by Salines et al., (2017), resulted in a peak prevalence of fecal HEV shedding pigs aged ~90 days, corresponding with the age of the investigated ~85-days-old pigs in the age category 'second week of fattening phase'. Since all samples were handled similarly and sufficient care was taken regarding the obtaining, storing and analysis of the samples, it is unlikely that loss of RNA in the samples has caused the absence of HEV detection. Thus, presumably the HEV shedding pattern on the farms investigated in this study is different from that of farms investigated in previous studies. This could be due to our selection criteria that farms should deliver a relatively high proportion of viraemic slaughter pigs, while others often include a random selection of pig farms. Solely in the age category 'last week of nursery phase', HEV shedding pigs were detected, with an age cohort-prevalence ranging from 0.02 to 0.6. The prevalence of HEV in 6-month-pigs has been reported to range from 0% (Sasaki et al., 2018) to 15% (Honing et al., 2011). The meta-regression analysis by Salines et al. (2017) showed a prevalence of 6.1% in 185-days-old pigs. Thus, our prevalence in pigs in the last week of the fattening phase exceeds the

reported prevalence in pigs of similar age. This is in line with our finding that the proportion of HEV-shedding pigs in the first week of fattening phase is lower than has been reported in literature. Infected pigs shed HEV for one to seven weeks and seroconvert during that period (Meester et al., 2021). To leave sufficient susceptible animals in the last stage of the fattening phase, which is necessary to enable the relatively high number of infected pigs in the last stage of the fattening phase, the number of infected pigs in the first stage of the fattening pigs needs to be relatively low. This corresponds with the relatively low prevalence in the pigs in the age category 'second week of fattening phase', and the relatively high prevalence in the age category 'last week of fattening phase' found in this study.

In conclusion, our findings are partly in line with previous research, but also indicate a HEV shedding pattern with a later onset of HEV infection than is usually reported. This may be due to our selection of farms delivering a relatively high proportion of viraemic slaughter pigs, while other studies have investigated a random selection of pig farms.

This study is, to the knowledge of the author, the first to explore the observational pen-level prevalence of HEV shedding pigs on pig farms. While the used selection criteria for the investigated farms reduce the generalisability of the found pen-level prevalence, we were able to determine the between-pen and within-pen prevalence and the presence of variability in the prevalence of HEV on pen-level is clear. Considering the need for investigation of HEV transmission within farms, which is underlined by the finding that most pig farms are persistently infected with HEV (Meester et al., 2021), and the conclusion that at transmission of HEV within pens is inevitable (Meester et al., 2021), given faecal-oral transmission and the high R0 that is reported (Bouwknegt et al., 2008), future studies may consider using pens housing pigs as unit of observation in studies investigating HEV epidemiology on pig farms. Such studies should focus on explaining the source of infection for that unit of observation and potential mitigation strategies.

However, this study indicates that study design of pen-level epidemiological research might be complex. Firstly, it is noticeable that the pen-level and age cohort-level prevalence of HEV shedding animals shows major differences per farm, on both batch-level and pen-level, indicating that HEV shedding patterns may vary greatly between farms. Variability in HEV shedding pattern between farms has been reported by others (McCreary et al., 2008; Nakai et al., 2006). Secondly, we have shown that shedding patters on a farm may be different than can be expected based on literature. While literature indicates a peak prevalence in shedding in pigs aged 12 weeks, we were unable to detect HEV shedding in 12-weeks-old pigs on all farms. Additionally, the prevalence of HEV-shedding animals in the last stage of the fattening phase exceeds prevalences reported in literature. Therefore, it might be beneficial for researchers planning to study HEV epidemiology on pen-level to consider calculating sample sizes and determining appropriate sampling moments for each individual participating farm.

Pens housing pigs may not be the sole useful unit of observation regarding research studying HEV transmission within pig farms. Using farm compartments as unit of observation is another interesting approach. To permit such studies, a study similar to this cross-sectional research should be conducted. Based on our results, sampling moments would preferably mainly take place during the fattening phase, since we detected no HEV shedding pigs in the nursery phase, but since HEV shedding patterns may vary between farms, the nursery phase should not be overlooked.

This study has several limitations. Firstly, no blood samples have been collected. Serological analysis would have enabled identification of HEV infection prior to and between sampling moments, since seroconversion occurs three to four weeks after exposure and the period of faecal HEV shedding is relatively short (one to seven weeks) (Meester et al., 2021). However, regarding animal welfare, the use of faecal samples is preferred, since taking blood samples causes more discomfort, pain, stress and injury to the animal (Risalde et al., 2020). Secondly, the cross-sectional study design does not allow longitudinal following of the infection status of pigs. Since pigs from consecutive batches are housed in totally different farm compartments, they may have been exposed to HEV at a different moment in their lives, resulting in different HEV transmission dynamics between batches. Therefore, sampling animals from different batches might not reveal the actual HEV shedding pattern. Thirdly, all blood samples were pooled before virological analysis. Consequently, pigs shedding a low amount of virus may have been overlooked.

In conclusion, pen-level investigation of HEV epidemiology on pig farms is a promising a feasible method, that will hopefully contribute to the mitigation of HEV on pig farms. Considering the major differences in the prevalence of HEV shedding animals on both batch-level and pen-level, researchers should consider performing farm-specific pilot studies before investigating HEV transmission within pig farms.

Chapter 4

General discussion

This report entails two studies that are part of the HEVentie project, a project aiming to identify effective intervention strategies enabling mitigation of HEV on pig farms. The overarching objective of this report is to contribute to the investigation of the within-farm transmission of HEV on pig farms. This is vital, since HEV in pigs poses a risk for public health (Ricci et al., 2017) and most pig farms are persistently infected with HEV (Meester et al., 2021). In this chapter, I discuss the results of both studies from the overarching perspective and present recommendations for future studies.

Considering the construction of most pig farms, within-farm HEV transmission can be investigated on two levels: the level of farm compartments and the level of pens housing pigs. The first paper presented in this report (chapter 2) studies the transmission of HEV between farm compartments. This study shows that several hygiene measures are associated with occurrence of batches escaping from HEV infection on pig farms, but causal associations and clarification of biologically relevant mechanisms remain to be determined. The second paper (chapter 3) explores the pen-level epidemiology of HEV. The between-pen and within-pen HEV prevalence were calculated and varied greatly between and within farms. No HEV-shedding pigs were identified in all age categories, except in the last week of the fattening phase. Consequently, it was suggested that HEV shedding patterns differ per farm. Additionally, on one of the four investigated farms total absence of HEV shedding was shown, even though the inclusion criterion was a relatively high seroprevalence. Combining the identification of HEV-free batches of slaughter pigs on infected pig farms (chapter 2) and the identification of variation in pen-level HEV prevalence (chapter 3), suggest that several factors affect within-farm HEV transmission. Therefore, future work is needed to identify mitigation strategies that prevent HEV transmission within farms.

In this report, the term HEV-free was defined as a batch of pigs from which blood serum samples tested negative during serological and virological analysis. However, HEV has been demonstrated in many other organs in infected pigs, such as liver, lymph nodes and kidneys (Krog et al., 2019). Since it has been indicated that presence of HEV-RNA in faeces is indicative for presence of HEV in organs (Krog et al., 2019), future research should consider combining virological results from faecal samples with virological and serological results from serum samples to improve our definition of 'HEV-free'.

As has been discussed in chapter 2, a batch of slaughter pigs is often composed of pigs derived from several farm compartments. Consequently, batches of slaughter pigs do not perfectly reflect the group of pigs that was housed in a specific farm compartment. Additionally, the concept of farm compartments is not applicable to many organic farms. Furthermore, it is likely that transmission of HEV within farm compartments is dependent on transmission of HEV on pen-level. Pen-level is likely to be the smallest efficient unit of observations, since transmission of HEV within pens in inevitable (Meester et al.,

2021). Therefore, pen-level epidemiological studies could be preferred over research on the level farm compartments.

Recent literature has stated that it is likely that the farm house environment acts as a continuous source of HEV (Ianiro et al., 2021). Considering the faecal-oral transmission route of HEV (Bouwknegt et al., 2011), an interesting approach regarding exploring within-farm HEV transmission would be to investigate the contribution of environmental HEV contamination on pig farms as a source of infection. Since the amount of environmental HEV contamination may be affected by the number of HEV shedding pigs in the pen in the previous batch, the shedding pattern of HEV within a pen may depend on the shedding pattern of HEV in the same pen in the previous batch. Thus, a longitudinal study following up consecutive batches of pigs would be of interest.

Integrating all recommendations results in a suggestion for future research within the HEVentie project: a longitudinal Randomized Controlled Trial investigating the effect of implementation of hygiene measures on pen-level. The outcome variable of this study would be a characteristic of the shedding pattern in the pen, such as the period until at least one pig starts shedding HEV. The intervention should be based on the hygiene measures identified in the risk factor analysis presented in this study. One cluster of pens forms the cohort without intervention (defined as untreated control cohort), and the other cluster of pens is treated with several predefined hygiene measures (defined as treated cohort). Within the untreated control cohort, the load of environmental contamination may differ per pen, dependant on the shedding pattern in the pen in the previous batch. It is not likely that exact same relation is present in the treated cohort, since hygiene measures will lower the environmental load of HEV. Therefore, the outcome variable will not only be dependent on the classification of treated and untreated control cohort, but also on the shedding pattern in the pen in the previous batch and a possible interaction between both cohorts and shedding pattern. Therefore, it is vital to appoint a characteristic reflecting the HEV shedding pattern in the previous batch. Logically, this characteristic would resemble the characteristic on which the outcome variable is based, so that several concurrent batches can be investigated with a minimum number of samples. In case this outcome variable is the time until at least one pig start shedding HEV, the statistical analysis could entail a survival analysis. To assess the effect of the intervention, the hazard ratio can be calculated. It would be logical to assess the effect of the effect of the period until shedding in the previous batch for the treated cohort and non-treated control cohort separately, since it is likely that hygiene measures will interact with the environmental contamination. Therefore, a survival analysis with separate strata for the treated cohort and non-treated control cohort should be carried out and the hazard ratios should for the different periods until shedding in the previous batch should be calculated. This study design will not only investigate a causal relation between hygiene measures and HEV shedding patterns, but also the effect of environmental contamination on HEV shedding patterns. Execution of this research will hopefully contribute to the intervention strategies that are vital to mitigate HEV on pig farms.

The aim of implementing such intervention strategies is to mitigate the public health risk associated with HEV presence on pig farms. However, it should be noted that if HEV transmission is reduced insufficiently, it can be expected that an increased proportion of actively infected pigs will be slaughtered (Meester et al., 2021), due to a later onset of HEV infection. In other words, if HEV mitigation strategies on pig farms lower HEV transmission insufficiently, less seroconverted and more viraemic slaughter pigs will be delivered to the slaughterhouse. While the presence of anti-HEV antibodies is likely to be harmless, presence of infectious HEV in pork poses a risk for public health (Ricci et al., 2017). Thus, the implementation of mitigation strategies may increase the risk for public health if premature conclusions about the effectivity of such strategies are drawn. Therefore, the effect of strategies contributing to HEV mitigation on pig farms should be proven to be sufficiently effective, before implementing any mitigation strategies.

HEV presence has been demonstrated not only in pigs, but also in wild boars (Adlhoch et al., 2009) and many other wild animals (Ricci et al., 2017). Additionally, HEV has been detected in water (Rutjes et al., 2009), and the origin of drinking water on pig farms has been shown to be associated with the risk of delivering slaughter pigs with HEV-positive livers (Walachowski et al., 2014). Consequently, I hypothesize that mitigation of HEV on pig farms is not solely dependent on HEV transmission within pig farm, but also on the introduction of HEV from various sources, such as drinking water and, particularly on organic pig farms, contact with (faeces of) wild animals. Thus, the desired approach to deal with the public health risk associated with presence of HEV in pork is a One Health approach. Only if researchers will engage in interdisciplinary collaborations, mitigation of HEV on pig farms will be successful.

Acknowledgements

I would like to thank my supervisors Tijs Tobias and Marina Meester for all their enthusiasm and guidance. Even though my project had to take a slightly different turn and I had to perform most of my activities from home, they were devoted to come up with a challenging and interesting research project for me. Moreover, they were there for me, answered the many questions I asked them and provided feedback whenever I needed it. Consequently, participating in this project has taught me a lot and has kindled my interest in conducting veterinary and epidemiological research.

During this year, many have contributed to this Honour Programme project. I would like to thank all the members of the HEVentie consortium, particularly dr. Martijn Bouwkegt, prof. Arjan Stegeman and prof. Wim van der Poel, for their insightful suggestions and discussions. Renate Hakze-van der Koning and Mirlin Spaninks played a crucial role in designing and executing the lab work, and educating me about it. Additionally, all presenters and participants from the Pig Topic and Epidemiological meetings have shown me how valuable participating in a research community can be. Lastly, I would like to thank the students with whom I worked on several parts of this project for all the (pig-related) knowledge they passed on to me.

References (53)

Adlhoch, C., Wolf, A., Meisel, H., Kaiser, M., Ellerbrok, H., & Pauli, G. (2009). High HEV presence in four different wild boar populations in East and West Germany. *Veterinary Microbiology*, *139*(3–4), 270–278. https://doi.org/10.1016/J.VETMIC.2009.06.032

Barnaud, E., Rogée, S., Garry, P., Rose, N., & Pavio, N. (2012). Thermal inactivation of infectious hepatitis E virus in experimentally contaminated food. *Applied and Environmental Microbiology*, *78*(15), 5153–5159. https://doi.org/10.1128/AEM.00436-12

Berto, A., Martelli, F., Grierson, S., & Banks, M. (2012). Hepatitis e virus in pork food chain, United Kingdom, 2009-2010. *Emerging Infectious Diseases*, *18*(8), 1358–1360. https://doi.org/10.3201/eid1808.111647

Bouwknegt, M., Frankena, K., Rutjes, S. A., Wellenberg, G. J., Husman, A. M. de R., Poel, W. H. M. van der, & Jong, M. C. M. de. (2008). Estimation of hepatitis E virus transmission among pigs due to contact-exposure. *Veterinary Research*, *39*(5), 1. https://doi.org/10.1051/VETRES:2008017

Bouwknegt, M., Teunis, P. F. M., Frankena, K., de Jong, M. C. M., & de Roda Husman, A. M. (2011). Estimation of the Likelihood of Fecal-Oral HEV Transmission Among Pigs. *Risk Analysis*, *31*(6), 940–950. https://doi.org/10.1111/j.1539-6924.2010.01546.x

Burnham, K. P., Anderson, D. R., & Burnham, K. P. (2002). *Model selection and multimodel inference : a practical information-theoretic approach*.

Dalton, H. R., Stableforth, W., Thurairajah, P., Hazeldine, S., Remnarace, R., Usama, W., Farrington, L., Hamad, N., Sieberhagen, C., Ellis, V., Mitchell, J., Hussaini, S. H., Banks, M., Ijaz, S., & Bendall, R. P. (2008). Autochthonous hepatitis e in Southwest England: Natural history, complications and seasonal variation, and hepatitis e virus IgG seroprevalence in blood donors, the elderly and patients with chronic liver disease. *European Journal of Gastroenterology and Hepatology*, *20*(8), 784–790. https://doi.org/10.1097/MEG.0B013E3282F5195A

Di Bartolo, I., Diez-Valcarce, M., Vasickova, P., Kralik, P., Hernandez, M., Angeloni, G., Ostanello, F., Bouwknegt, M., Rodríguez-Lázaro, D., Pavlik, I., & Ruggeri, F. M. (2012). Hepatitis E virus in pork production chain in Czech Republic, Italy, and Spain, 2010. *Emerging Infectious Diseases*, *18*(8), 1282–1289. https://doi.org/10.3201/eid1808.111783

Feagins, A. R., Opriessnig, T., Guenette, D. K., Halbur, P. G., & Meng, X. J. (2007). Detection and characterization of infectious Hepatitis E virus from commmercial pig livers sold in local grocery in the USA. *Journal of General Virology*, *88*(3), 912–917. https://doi.org/10.1099/vir.0.82613-0

Fenaux, H., Chassaing, M., Berger, S., Gantzer, C., Bertrand, I., & Schvoerer, E. (2019). Transmission of hepatitis E virus by water: An issue still pending in industrialized countries. In *Water Research* (Vol. 151, pp. 144–157). Elsevier Ltd. https://doi.org/10.1016/j.watres.2018.12.014

Fernández-Barredo, S., Galiana, C., García, A., Vega, S., Gómez, M. T., & Pérez-Gracia, M. T. (2016). Detection of Hepatitis E Virus Shedding in Feces of Pigs at Different Stages of Production Using Reverse Transcription-Polymerase Chain Reaction: *Http://Dx.Doi.Org.Proxy.Library.Uu.Nl/10.1177/104063870601800506*, *18*(5), 462–465. https://doi.org/10.1177/104063870601800506

Forgách, P., Nowotny, N., Erdélyi, K., Boncz, A., Zentai, J., Szucs, G., Reuter, G., & Bakonyi, T. (2010). Detection of Hepatitis E virus in samples of animal origin collected in Hungary. *Veterinary Microbiology*, *143*(2–4), 106–116. https://doi.org/10.1016/J.VETMIC.2009.11.004

Harrison, L., & DiCaprio, E. (2018). Hepatitis E Virus: An Emerging Foodborne Pathogen. In *Frontiers in Sustainable Food Systems* (Vol. 2, p. 14). Frontiers Media S.A. https://doi.org/10.3389/fsufs.2018.00014

Honing, R. W. H. der, Coillie, E. van, Antonis, A. F. G., & Poel, W. H. M. van der. (2011). First Isolation of Hepatitis E Virus Genotype 4 in Europe through Swine Surveillance in the Netherlands and Belgium. *PLOS ONE*, *6*(8), e22673. https://doi.org/10.1371/JOURNAL.PONE.0022673

Hsu, I. W.-Y., & Tsai, H.-J. (2014). Avian Hepatitis E Virus in Chickens, Taiwan, 2013. *Emerging Infectious Diseases*, *20*(1), 149. https://doi.org/10.3201/EID2001.131224

Ianiro, G., Chelli, E., Sabato, L. De, Monini, M., Ostanello, F., & Bartolo, I. Di. (2021). Long-term surveillance for hepatitis E virus in an Italian two-site farrow-to-finish swine farm. *Zoonoses and Public Health*, *68*(5), 474–482. https://doi.org/10.1111/ZPH.12837

Ji, H., Chen, S., He, Q., Wang, W., Gong, S., Qian, Z., Zhang, Y., Wei, D., Yu, W., & Huang, F. (2021). The different replication between nonenveloped and quasi-enveloped hepatitis E virus. *Journal of Medical Virology*, 1–11. https://doi.org/10.1002/JMV.27121

Johne, R., Plenge-Bönig, A., Hess, M., Ulrich, R. G., Reetz, J., & Schielke, A. (2010). Detection of a novel hepatitis E-like virus in faeces of wild rats using a nested broad-spectrum RT-PCR. *Journal of General Virology*, *91*(3), 750–758. https://doi.org/10.1099/vir.0.016584-0

Jori, F., Laval, M., Maestrini, O., Casabianca, F., Charrier, F., & Pavio, N. (2016). Assessment of Domestic Pigs, Wild Boars and Feral Hybrid Pigs as Reservoirs of Hepatitis E Virus in Corsica, France. *Viruses*, *8*(8), 236. https://doi.org/10.3390/v8080236

Jothikumar, N., Cromeans, T. L., Robertson, B. H., Meng, X. J., & Hill, V. R. (2006). A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *Journal of Virological Methods*, *131*(1), 65–71. https://doi.org/10.1016/j.jviromet.2005.07.004

Kamar, N., Dalton, H. R., Abravanel, F., & Izopet, J. (2014). Hepatitis E virus infection. In *Clinical Microbiology Reviews* (Vol. 27, Issue 1, pp. 116–138). American Society for Microbiology (ASM).

https://doi.org/10.1128/CMR.00057-13

Krog, J., Larsen, L. E., & Breum, S. Ø. (2019). Tracing Hepatitis E Virus in Pigs From Birth to Slaughter. *Frontiers in Veterinary Science*, *6*(FEB), 50. https://doi.org/10.3389/fvets.2019.00050

Leblanc, D., Ward, P., Gagné, M. J., Poitras, E., Müller, P., Trottier, Y. L., Simard, C., & Houde, A. (2007). Presence of hepatitis E virus in a naturally infected swine herd from nursery to slaughter. *International Journal of Food Microbiology*, *117*(2), 160–166. https://doi.org/10.1016/j.ijfoodmicro.2007.03.008

Linares, M., Hicks, C., Bowman, A. S., Hoet, A., & Stull, J. W. (2018). Infectious agents in feral swine in Ohio, USA (2009-2015): A low but evolving risk to agriculture and public health. *Veterinary and Animal Science*, *6*, 81–85. https://doi.org/10.1016/J.VAS.2018.06.002

Lopez-Lopez, P., Risalde, M. de los A., Frias, M., García-Bocanegra, I., Brieva, T., Caballero-Gomez, J., Camacho, A., Fernández-Molera, V., Machuca, I., Gomez-Villamandos, J. C., Rivero, A., & Rivero-Juarez, A. (2018). Risk factors associated with hepatitis E virus in pigs from different production systems. *Veterinary Microbiology*, *224*, 88–92. https://doi.org/10.1016/j.vetmic.2018.08.020

McCreary, C., Martelli, F., Grierson, S., Ostanello, F., Nevel, A., & Banks, M. (2008). Excretion of hepatitis E virus by pigs of different ages and its presence in slurry stores in the United Kingdom. *Veterinary Record*, *163*(9), 261–265. https://doi.org/10.1136/VR.163.9.261

Meester, M., Tobias, T. J., Bouwknegt, M., Kusters, N. E., Stegeman, J. A., & van der Poel, W. H. M. (2021). Infection dynamics and persistence of hepatitis E virus on pig farms – a review. In *Porcine Health Management* (Vol. 7, Issue 1, pp. 1–16). BioMed Central Ltd. https://doi.org/10.1186/s40813-021-00189-z

Meng, X. J., Purcell, R. H., Halbur, P. G., Lehman, J. R., Webb, D. M., Tsareva, T. S., Haynes, J. S., Thacker, B. J., & Emerson, S. U. (1997). A novel virus in swine is closely related to the human hepatitis E virus. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(18), 9860–9865. https://doi.org/10.1073/pnas.94.18.9860

Nakai, I., Kato, K., Miyazaki, A., Yoshii, M., Li, T.-C., Takeda, N., Tsunemitsu, H., & Ikeda, H. (2006). DIFFERENT FECAL SHEDDING PATTERNS OF TWO COMMON STRAINS OF HEPATITIS E VIRUS AT THREE JAPANESE SWINE FARMS. *The American Journal of Tropical Medicine and Hygiene*, *75*(6), 1171–1177. https://doi.org/10.4269/AJTMH.2006.75.1171

Nakamura, M., Takahashi, K., Taira, K., Taira, M., Ohno, A., Sakugawa, H., Arai, M., & Mishiro, S. (2006). Hepatitis E virus infection in wild mongooses of Okinawa, Japan: Demonstration of anti-HEV antibodies and a full-genome nucleotide sequence. *Hepatology Research*, *34*(3), 137–140. https://doi.org/10.1016/j.hepres.2005.10.010

Pallerla, S. R., Harms, D., Johne, R., Todt, D., Steinmann, E., Schemmerer, M., Wenzel, J. J., Hofmann, J., Shih, J. W. K., Wedemeyer, H., Bock, C.-T., & Velavan, T. P. (2020). Hepatitis E Virus Infection: Circulation, Molecular Epidemiology, and Impact on Global Health. *Pathogens*, *9*(10), 1–21. https://doi.org/10.3390/PATHOGENS9100856

Ranstam, J., & Cook, J. A. (2018). LASSO regression. *British Journal of Surgery*, *105*(10), 1348–1348. https://doi.org/10.1002/BJS.10895

Reuter, G., Fodor, D., Forgách, P., Kátai, A., & Szucs, G. (2009). Characterization and zoonotic potential of endemic hepatitis E virus (HEV) strains in humans and animals in Hungary. *Journal of Clinical Virology*, *44*(4), 277–281. https://doi.org/10.1016/j.jcv.2009.01.008

Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernandez Escamez, P. S., Herman, L., Koutsoumanis, K., Lindqvist, R., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., Threlfall, J., … Girones, R. (2017). Public health risks associated with hepatitis E virus (HEV) as a food-borne pathogen. *EFSA Journal*, *15*(7). https://doi.org/10.2903/j.efsa.2017.4886

Risalde, M. A., Rivero-Juarez, A., Frias, M., Olivas, I., Lopez-Lopez, P., García-Bocanegra, I., Brieva, T., Caballero-Gómez, J., Camacho, A., Fernández-Molera, V., Gómez-Villamandos, J. C., & Rivero, A. (2020). Evaluation of a non-invasive screening approach to determine hepatitis e virus status of pig farms. *Veterinary Record*, *187*(7), 272. https://doi.org/10.1136/vr.105840

Riveiro-Barciela, M., Mínguez, B., Gironés, R., Rodriguez-Frías, F., Quer, J., & Buti, M. (2015). Phylogenetic Demonstration of Hepatitis E Infection Transmitted by Pork Meat Ingestion. *Journal of Clinical Gastroenterology*, *49*(2), 165–168. https://doi.org/10.1097/MCG.0000000000000113

Rose, N., Lunazzi, A., Dorenlor, V., Merbah, T., Eono, F., Eloit, M., Madec, F., & Pavio, N. (2011). High prevalence of Hepatitis E virus in French domestic pigs. *Comparative Immunology, Microbiology and Infectious Diseases*, *34*(5), 419–427. https://doi.org/10.1016/j.cimid.2011.07.003

RStudio Team. (2020). *RStudio: Integrated Development Environment for R. RStudio*. PBC. http://www.rstudio.com/

Rutjes, S. A., Bouwknegt, M., Van Der Giessen, J. W., De Roda Husman, A. M., & Reusken, C. B. E. M. (2014a). Seroprevalence of hepatitis e virus in pigs from different farming systems in the Netherlands. *Journal of Food Protection*, *77*(4), 640–642. https://doi.org/10.4315/0362-028X.JFP-13-302

Rutjes, S. A., Bouwknegt, M., Van Der Giessen, J. W., De Roda Husman, A., & Reusken, C. B. E. M. (2014b). Seroprevalence of hepatitis e virus in pigs from different farming systems in the Netherlands. *Journal of Food Protection*, *77*(4), 640–642. https://doi.org/10.4315/0362-028X.JFP-13-302

Rutjes, S. A., Lodder, W. J., Bouwknegt, M., & de Roda Husman, A. M. (2007). Increased hepatitis E virus prevalence on Dutch pig farms from 33 to 55% by using appropriate internal quality controls for RT-PCR. *Journal of Virological Methods*, *143*(1), 112–116. https://doi.org/10.1016/j.jviromet.2007.01.030

Rutjes, S. A., Lodder, W. J., Lodder-Verschoor, F., Van Den Berg, H. J. L., Vennema, H., Duizer, E., Koopmans, M., & De Roda Husman, A. M. (2009). Sources of hepatitis E virus genotype 3 in the Netherlands. *Emerging Infectious Diseases*, *15*(3), 381–387. https://doi.org/10.3201/eid1503.071472

Salines, M., Andraud, M., & Rose, N. (2017). From the epidemiology of hepatitis e virus (HEV) within the swine reservoir to public health risk mitigation strategies: A comprehensive review. *Veterinary Research*, *48*(1), 31. https://doi.org/10.1186/s13567-017-0436-3

Sasaki, Y., Haruna, M., Uema, M., Noda, M., & Yamada, Y. (2018). Prevalence and Phylogenetic Analysis of Hepatitis E Virus among Pigs in Japan. *Japanese Journal of Infectious Diseases*, *71*(1), 75–78. https://doi.org/10.7883/YOKEN.JJID.2017.235

Smith, D. B., Simmonds, P., Group, members of the I. C. on the T. of V. H. S., Jameel, S., Emerson, S. U., Harrison, T. J., Meng, X.-J., Okamoto, H., Poel, W. H. M. Van der, & Purdy, M. A. (2014). Consensus proposals for classification of the family Hepeviridae. *The Journal of General Virology*, *95*(Pt 10), 2223. https://doi.org/10.1099/VIR.0.068429-0

Steyer, A., Naglič, T., Močilnik, T., Poljšak-Prijatelj, M., & Poljak, M. (2011). Hepatitis E virus in domestic pigs and surface waters in Slovenia: Prevalence and molecular characterization of a novel genotype 3 lineage. *Infection, Genetics and Evolution*, *11*(7), 1732–1737. https://doi.org/10.1016/J.MEEGID.2011.07.007

Turner, J., Godkin, A., Neville, P., Kingham, J., & Ch'ng, C. L. (2010). Clinical characteristics of hepatitis E in a "Non-Endemic" population. *Journal of Medical Virology*, *82*(11), 1899–1902. https://doi.org/10.1002/jmv.21905

van der Poel, W. H. M., Pavio, N., van der Goot, J., van Es, M., Martin, M., & Engel, B. (2014). Development and validation of a genotype 3 recombinant protein-based immunoassay for hepatitis E virus serology in swine. *Brazilian Journal of Medical and Biological Research*, *47*(4), 334–339. https://doi.org/10.1590/1414-431X20133249

Walachowski, S., Dorenlor, V., Lefevre, J., Lunazzi, A., Eono, F., Merbah, T., Eveno, E., Pavio, N., & Rose, N. (2014). Risk factors associated with the presence of hepatitis E virus in livers and seroprevalence in slaughter-age pigs: A retrospective study of 90 swine farms in France. *Epidemiology and Infection*, *142*(9), 1934–1944. https://doi.org/10.1017/S0950268813003063

Wang, H., Karlsson, M., Lindberg, M., Nyström, K., & Norder, H. (2019). Hepatitis E virus strains infecting Swedish domestic pigs are unique for each pig farm and remain in the farm for at least 2 years. *Transboundary and Emerging Diseases*, *66*(3), 1314–1323. https://doi.org/10.1111/TBED.13153

Widdowson, M. A., Jaspers, W. J. M., Van Der Poel, W. H. M., Verschoor, F., De Roda Husman, A. M., Winter, H. L. J., Zaaijer, H. L., & Koopmans, M. (2003). Cluster of cases of acute hepatitis associated with hepatitis E virus infection acquired in The Netherlands. *Clinical Infectious Diseases*, *36*(1), 29–33. https://doi.org/10.1086/345439

Zhao, C., Ma, Z., Harrison, T. J., Feng, R., Zhang, C., Qiao, Z., Fan, J., Ma, H., Li, M., Song, A., & Wang, Y. (2009). A novel genotype of hepatitis E virus prevalent among farmed rabbits in China. *Journal of Medical Virology*, *81*(8), 1371–1379. https://doi.org/10.1002/jmv.21536

Appendices

Appendix A

The questionnaire used to identify present potential risk factors in the original language

1B. Bedrijfstype

13 Welke diercategorieën worden op dit varkensbedrijf gehouden (onder 1 UBN)?

14 Welk van onderstaande bedrijfstypes past het beste bij dit bedrijf?

15 Zijn er op hetzelfde erf nog andere bedrijfstakken dan het houden van varkens?

16 Zo ja, welke bedrijfstakken zijn er naast varkens houden? :

- 16a Melkveehouderij
- 16b vleeskoeienhouderij
- 16c schapenhouderij
- 16d geitenhouderij
- 16e leghennenhouderij
- 16f vleeskuikenhouderij
- 16g paardenhouderij
- 16h akkerbouw
- 16i mestopslag
- 16j mesttransport
- 16k loonwerk
- 16l anders, namelijk:

17 Welke functie heeft u op dit varkensbedrijf?

18 Hoeveel varkensbedrijven heeft u, of de eigenaar, in totaal?

19 Heeft u in 2019 of 2020 uw bedrijf uitgebreid?

20 En zo ja hoe heeft u dan uitgebreid?

1C. Medewerkers

21 Met hoeveel FTE wordt gemiddeld op dit bedrijf gewerkt, buiten de eigena(a)r(en) om?

22 Hoeveel mensen werken er op dit bedrijf (inclusief eigenaren, buiten piekdagen om zoals tijdens spenen)?

23 Werkt/werken de eigenaren mee in de stal?

24 Hoeveel van deze mensen werken ook op andere varkensbedrijven?

25 Hoe wordt omgegaan met piekarbeid, zoals tijdens spenen of reinigen?

- a. Extra personeel (bijv. op nul uren basis)
- b. Een extern bedrijf of ZZP'ers worden ingehuurd
- c. Familie werkt mee
- d. Er wordt langer of harder doorgewerkt
- e. Met piekarbeid gaat men anders om, namelijk:

1D. Hepatitis E?

26 Heeft u vóór ons project wel eens van HEV gehoord?

27 Vindt u HEV een belangrijk onderwerp in de varkenshouderij?

Als het bedrijf alleen vleesvarkens heeft, of biggen en vleesvarkens, vraag dan hoeveel km de leverancier ervandaan ligt, en vraag naar het nummer en mailadres van de leverancier van zijn dieren, dan kunnen we de extra vragen hopelijk aan die boer stellen...

28 Hoeveel kilometer zit dit bedrijf van het vermeerderingsbedrijf af? km

29 Zouden we de volgende gegevens mogen van het vermeerderingsbedrijf?:

29a Het telefoonnummer van het bedrijf waar u het vaakst biggen van

29b Het emailadres

29c Het huisnummer

29d en als laatste de postcode

Aan de hand van de vraag 13 'Wat voor diercategorieën heeft u op dit bedrijf onder 1 UBN', kies je nu de juiste 'vragenlijst deel 2-4 Specifiek'.

Check voordat je verder gaat goed of alle vragen zijn beantwoord.

Let op, de IDs die je hierboven hebt ingevuld, moet je ook invullen bij de IDs in vragenlijst deel 2-4 en deel 5!

Vragenlijst specifiek - 2,3,4 - vermeerdering en vleesvarkensbedrijven

2a. Dier en dierplaatsen - algemeen

30 Hoeveel varkensgebouwen heeft het bedrijf?

31 Zijn de dieren per categorie apart gehuisvest?

32 In welk jaar is de oudste stal op het bedrijf gebouwd?

33 In welk jaar is de nieuwste stal op het bedrijf gebouwd?

34 Heeft u hokken met een uitloop naar buiten? ☐

35 Indien ja, bij welke diercategorieën? ☐ Kraamstal ☐ Gespeende biggen ☐ Dragende zeugen ☐ Vleesvarkens

36 Een hoeveel weken systeem heeft dit bedrijf (of had het in 2019)?

37 Indien anders, wat voor systeem dan?

38 Van welke KI-organisatie betrekt u sperma?

39 Welke eindbeer heeft u in 2019 het meest gebruikt?

40 Heeft dit bedrijf haar eigen opfok? ☐

41 Indien ja, wordt er aan rotatiekruising gedaan? ☐

42 Welke genetica hebben de eerste en tweede worps zeugen nu?

43 Koopt u gelten aan? ☐

44 Zo ja, wat is de herkomst van de gelten die u aankoopt?

2a. Dier en dierplaatsen - per diercategorie

45 Hoeveel zeugenplaatsen had u in 2019 in de kraamstal?

47 Hoeveel biggen heeft u in 2019 gespeend?

48 Hoeveel rondes speenbiggen draaide u in 2019 per afdeling?

49 Hoeveel biggenplaatsen had u in 2019 gemiddeld per hok?

51 Hoeveel vleesvarkens heeft u in 2019 gemiddeld afgeleverd?

52 Hoeveel rondes vleesvarkens draaide u in 2019 per afdeling?

53 Hoeveel vleesvarkensplaatsen had u in 2019 gemiddeld per hok?

2b. Dierziektes en behandelingen - algemeen

54 In welke Salmonella categorie viel dit varkensbedrijf bij de vleesvarkens in 2019?

55 Heeft u in 2019 tenminste een trimester gehad met Salmonella score 2 of 3? ☐

56 Hoevaak had dit bedrijf in 2019 een klinische uitbraak met PRRS?

57 Is de diagnose PRRS minimaal 1x bevestigd met diagnostiek? ☐

58 Hoe vaak had dit bedrijf in 2019 een klinische uitbraak griep?

59 Is die griep vorig jaar minimaal 1x bevestigd met diagnostiek? ☐

2b. Dierziektes en behandelingen - per diercategorie

60 Wat was uw DDD (Antibiotica) in 2019 (per diercategorie)?

Voor zeugen en zuigende biggen?

Voor gespeende biggen?

Voor vleesvarkens?

61/62/63 Waartegen vaccineert/ent u op dit bedrijf (per diercategorie)?

Zeugen tijdens dracht en biggen in kraamstal:	Speenbiggen:	Vleesvarkens:
☐ PCV2	☐ PCV2	☐ PCV2
☐ Mycoplasma hyopneumoniae	☐ Mycoplasma hyopneumoniae	☐ Mycoplasma hyopneumoniae
☐ PRRS	☐ PRRS	☐ PRRS
☐ E coli	☐ E coli	☐ Salmonella
☐ PIA	☐ PIA	☐ PIA
☐ Rota	☐ Glässer	☐ Glässer
☐ Vlekziekte	☐ APP	☐ APP
☐ Parvo		☐ Influenza/griep
		☐ Vlekziekte
Anders, namelijk:	Anders, namelijk:	Anders, namelijk:

64 Welke vaccins geeft u naaldloos (per cat.)

naaldloos kraam	naaldloos speen	naaldloos vlv

65a Na hoeveel zuigende biggen spuiten, vervangt u de naald?

65b Na hoeveel gespeende biggen spuiten, vervangt u de naald?

65c Na hoeveel vleesvarkens spuiten, vervangt u de naald?

2c. Technische prestaties - kraamstal/gespeende biggen

66 Wat was de gemiddeld uitval tot spenen in 2019? %

67 Wat was de gemiddelde speenleeftijd van uw biggen in 2019? dagen

68 Hoe vaak heeft u in 2019 uw biggen gewogen tijdens spenen?

69 Anders, namelijk

70 Wat was in 2019 het gemiddelde speengewicht van uw biggen?

71 Wat was in 2019 gemiddelde uitval na spenen van uw biggen? %

2c. Technische prestaties - vleesvarkens

72 Op hoeveel weken legt u normaal gesproken vleesvarkens op? weken

73 Wat was in 2019 de gemiddelde GpDpD van uw vleesvarkens? (1 lokatie) gram per dag

74 Welke voederconversie EW hadden de vleesvarkens van deze locatie in 2019?

75 Wat was in 2019 de gemiddelde uitval bij de vleesvarkens? %

76 Hoeveel dagen na opleg gingen uw vlv in 2019 gemiddeld naar de slacht? dagen

77 Hoeveel dagen zit er gemiddeld tussen het jongste en oudste vleesvarken bij slacht? dagen

78 Uit hoeveel verschillende afdelingen levert u aan het slachthuis in 1x?

216a Op welke datum gaan de nu oudste vleesvarkens naar de slacht?

216b Op welke datum gaat de kop van de eenna oudste vleesvarkens naar de slacht?

3a. Voer

79/80/81 Per diercategorie, welk voer wordt aangeboden?	Zuigende biggen	Gespeende biggen	Vleesvarkens
	☐ melk ☐ brok ☐ brij (zelf gemengd) ☐ brij (brok met water)	☐ brok ☐ brij (zelf gemengd) ☐ brij (brok met water)	☐ brok ☐ brij (zelf gemengd) ☐ brij (brok met water)
82 Per diercategorie, via welk systeem krijgen ze voer?			
83 Per categorie, voert u met de hand of automatisch?			

84 Hoe vaak per jaar voert u voersilo's helemaal leeg?

85 Wordt er voer aangezuurd? ☐

86 Heeft u een restvoerkar / restvoerkarren? ☑

87 Wat doet u met restvoer van een bepaalde afdeling?

3a. Water

88 Waar komt het water vandaan dat u de varkens geeft?

89a Indien van een bron, sinds welk jaar is de bron in gebruik?

89b Indien van een bron, wat is de afstand tussen de bron en de mestput? meter

89c Indien bron, waarmee maakt u het geschikt voor drinkwater?

89d Het wordt anders geschikt gemaakt voor drinkwater, namelijk:

90/91 Voor welke diercategorieën zuurt u wel eens water aan? ☐ geen een ☐ gespeende biggen ☐ vleesvarkens

92Welk product gebruikt u om water aan te zuren?

93 Op welke wijze werd in 2019 (en nu) het drinkwatersysteem gereinigd?

94 Reiniging gebeurt anders, namelijk

95 Welke hoofdstof zit in het middel waarmee water wordt gereinigd?

96 Indien de stof niet bekend is, welk product (naam) wordt gebruikt?

3a. Mest

	Kraamstal	Gespeende biggen	Vleesvarkens
97 Om de hoeveel rondes laat u de mestput leeglopen, per diercategorie			
Anders, namelijk:			
99 Hoe vaak komt hokbevuiling voor op dit bedrijf, per diercategorie			

100 Hoe vaak ziet u schuim/mest boven de roosters uitkomen?

101 Bij welke diercategorie zit er dan mest/schuim boven rooster?

4a. Hygiënesluis

105 Is er een douche in de hygiënesluis? ☐

106 Door wie wordt de douche gebruikt bij het ingaan van de stal?
- ☐ niemand
- ☐ voervoorlichter
- ☐ manager/eigenaar
- ☐ bezoekers
- ☐ personeel
- ☐ andere erfbetreders zoals een loodgieter
- ☐ dierenarts
- ☐ Hangt af van contact met andere varkens

107 Wat voor kleding dragen mensen die de stal ingaan?

108 Hoe vaak dragen ze bedrijfskleding?

109 Wat voor schoeisel dragen mensen die de stal ingaan?

110 Hoe vaak dragen ze dat schoeisel?

4b. Laad- en losplaats

111 Zijn de laad- en losplaats op dit bedrijf op dezelfde plek? ☐

112 Gebruikt u eigen transportwagen voor verplaatsen van varkens? ☐

113 Wordt de transportwagen gereinigd op de laad/losplaats? ☐

115 Kunnen varkens die de stal hebben verlaten, terug naar binnen? ☐

116 Lopen de transporteurs de stal in en uit tijdens laden/lossen? ☐

117 Moeten dieren tijdens laden/lossen langs een andere diercategorie dan hun eigen (bijv. vleesvarkens langs kraamstal)? ☐

118 Hoe vaak reinigt u de laad/losplaats?

119 In een andere frequentie, namelijk

4c. Quarantaine stal

120 Heeft dit bedrijf een quarantainestal? ☐

122 Heeft de quarantainestal een aparte mestput? ☐

123 Heeft de quarantainestal een aparte luchtinlaat? ☐

124 Hoe lang staat de quarantainestal per keer ongeveer leeg? dagen

4d. Contact met dieren/ongedierte

125 Hebben de varkens op dit bedrijf wel eens contact met huisdieren ☐

127 Ander huisdier, namelijk

126 Indien ze contact hebben met huisdieren: welke huisdieren?

128 Hebben de varkens op dit bedrijf wel eens contact met:
- ☐ varkens van extern bedrijf
- ☐ ongedierte
- ☐ ander vee van dit, of extern bedrijf

129 Wat beschouwt u als ongedierte?

130 Per type ongedierte, in welke mate komt het hier voor:
- Ratten
- Vliegen
- Muizen
- Muggen

130 ander ongedierte, namelijk

Mate van voorkomen van ander ongedierte

131 Wie bestrijdt ongedierte op dit bedrijf?

132 Heeft u een vastgelegd protocol om ongedierte te bestrijden? ☐

133 Op welke manier worden vliegen bestreden?

134 Is de bestrijding van ongedierte succesvol of gaat het moeizaam?

Vragenlijst Bioveiligheid - 5 - Vermeerdering en vleesvarkens bedrijven

5a. Reiniging

135 Er volgen een aantal locaties en voorwerpen in stallen. Kunt u per onderdeel aangeven óf u die schoonmaakt, hoe u dat doet en hoe vaak u die schoonmaakt?

136 Maakt u onderstaande onderdelen schoon en zo ja hoe vaak?

137 Of als het antwoord er niet tussen zit, anders namelijk:

136a Hok kraamstal		
136b Hok speenstal		
136c Hok vleesvarkens		
136d Gang tussen afdelingen		
136e Verrijking		
136f Plafond afdeling		
136g Onder roosters		
136h Laarzen		
136i Kleding/overall		
136j Schotjes		
136k Quarantaine		
136l Uitloop		

Voor de volgende onderdelen, in welke stappen maakt u het schoon?

138 Hok Kraamstal

- ☐ Bezemschoon?
- ☐ Installaties verwijderen?
- ☐ Inweken?
- ☐ Mét inweekmiddel / vuilbreker?
- ☐ Schoonspuiten?

Als ja bij schoonspuiten, waarmee?

139 Hok Biggenstal

- ☐ Bezemschoon?
- ☐ Installaties verwijderen?
- ☐ Inweken?
- ☐ Mét inweekmiddel / vuilbreker?
- ☐ Schoonspuiten?

Als ja bij schoonspuiten, waarmee?

140 Hok Vleesvarkensstal

- ☐ Bezemschoon?
- ☐ Installaties verwijderen?
- ☐ Inweken?
- ☐ Mét inweekmiddel / vuilbreker?
- ☐ Schoonspuiten?

Als ja bij schoonspuiten, waarmee?

141 Gang tussen afdelingen

- ☐ Bezemschoon?
- ☐ Installaties verwijderen?
- ☐ Inweken?
- ☐ Mét inweekmiddel / vuilbreker?
- ☐ Schoonspuiten?

Als ja bij schoonspuiten, waarmee?

142 Laarzen

- ☐ Laarzenborstel?
- ☐ Inweken?
- ☐ Schoonspuiten?

Als ja bij schoonspuiten, waarmee?

143 Quarantaine stal (indien aanw.)

- ☐ Bezemschoon?
- ☐ Inweken?
- ☐ Mét inweekmiddel / vuilbreker?
- ☐ Schoonspuiten?

Als ja bij schoonspuiten, waarmee?

144 Uitloop (indien aanwezig)

- ☐ Bezemschoon?
- ☐ Inweken?
- ☐ Mét inweekmiddel / vuilbreker?
- ☐ Schoonspuiten?

5b. Desinfectie

145 Wat is desinfectie volgens u?

146 Hoeveel tijd vindt u dat er tussen reiniging en desinfectie van een hok zou moeten zitten?

147 Hoe lang zit er op dit bedrijf minimaal tussen reinigen en desinfectie van hokken?

149 Desinfecteert u deze onderdelen en zo ja, hoe vaak?		150 Of als het antwoord er niet tussen zit, anders namelijk:
149a Hok kraamstal		
149b Hok speenstal		
149c Hok vleesvarkens		
149d Gang tussen afdelingen		
149e Verrijking		
149f Laarzen		
149g Schotjes		
149h Quarantaine stal		
149i Uitloop		

151 Welke desinfectantia heeft u in 2019 allemaal gebruikt?

152 Met welk middel desinfecteerde u in 2019 het meest/vaakst?

153 Spoelt u na desinfectie na met water? ☐

154 Zo ja, met wat voor water spoelt u na?

155 In hoeveel % van de gevallen is afdeling nog nat als er nieuwe dieren inkomen? %

5c. Direct Contact - Per diercategorie

Direct contact - Kraamstal

156 Wat zijn redenen voor u om biggen over te leggen?

- ☐ uniformiteit
- ☐ toomgrootte
- ☐ zwakke biggen
- ☐ conditie zeug
- ☐ big heeft geen speen
- ☐ aantal biggen gespeend vorige pariteit

Anders, namelijk:

157 Op welk moment na de geboorte legt u over?

158 Houdt u per zeug bij hoeveel biggen worden af- of bijgelegd? ☐

159 Hoeveel % van uw biggen worden gemiddeld overgelegd?

160 In hoeveel % van keren draaide u kraamafdelingen in 2019 in 1 keer leeg?

161 Waarom draait u een kraamafdeling soms niet in 1x vol of leeg?
☐ ziekte ☐ kraamstallen
☐ pleegzeugen ☐ conditie zeug
Anders, namelijk:

162 Heeft u een ziekenboeg in de kraamstal? ☐

163 Indien ja, hoe vaak staat de kraam ziekenboeg per jaar ongeveer leeg?

Direct contact - gespeende biggen

164 Op basis van welke factoren zet u gespeende biggen bij elkaar?
☐ gewicht ☐ Net hoe het uitkomt
☐ geslacht ☐ tomen (koppels) bij elkaar
☐ zelfde afdeling als kraamstal Anders:

165 Wie verplaatst(en) biggen van de kraam- naar de speenafdeling?

166 Lopen de biggen van kraam naar speen of gaan ze in een kar?

167 In hoeveel % van keren draaide u speenafdelingen in 2019 in 1 keer leeg?

168 Als de afdeling deels leeggaat, waar gaan overgebleven biggen dan heen?

169 Waarom draait u een speenafdeling soms niet in 1 keer leeg?
☐ ziekte ☐ te weinig hokken ☐ uniformiteit
☐ verkoop van deel biggen Anders:

170 Heeft u een ziekenboeg in de gespeende biggenstal? ☐

171 Indien ja - Hoe vaak staat de speen ziekenboeg per jaar ongeveer leeg?

Direct contact - vleesvarkens

172 Op basis van welke factoren zet u varkens tijdens opleggen in hetzelfde hok?
☐ geslacht ☐ zelfde groep als in speenafdeling ☐ Net wat uitkomt
☐ gewicht ☐ Zelfde varkens als in hun speenhok Anders:

173 Doet u aan dubbel opleggen? (of een kwart = ook ja) ☐

174 Wie verplaatst de varkens van de speenafdeling naar de vleesvarkens?

175 In hoeveel % van de keren haalde u vlv'ens afdelingen in 2019 in 1x leeg?

176 Als afdelingen deels leeg gaan,waar gaan overgebleven vlv'ens dan heen?

177 Waarom draait u een vleesvarkensafdeling soms niet in 1 keer leeg?
☐ ziekte ☐ uniformiteit
☐ te weinig hokken anders:

178 Heeft u een ziekenboeg in de vleesvarkensstal? ☐

179 Indien ja - Hoe vaak staat de vlv'ens ziekenboeg per jaar ongeveer leeg?

180 Mogen dieren vanaf een ziekenboeg weer terug naar gezonde dieren? ☐

181 Indien dit mag, waar gaan die dieren dan heen?

Restafdeling

182 Heeft dit bedrijf een restafdeling? ☐

183 Indien het een restafdeling heeft, hoe vaak staat de restafdeling leeg?

185 Welke dieren staan er in de restafdeling als er geen restdieren in zitten?

186a Zijn er hokken waar u alleen in kunt komen via een ander hok? ☐

186b Indien ja, bij welke diercategorie(ën) zijn dit soort hokken aanwezig?

5d. Indirect Contact - Per diercategorie

Indirect contact tussen afdelingen

187 Wanneer doet u/personeel een behandelrondje?

188 Bent u (of personeel) op 1 dag bij meerdere diercategorieën (in meerdere stallen) te vinden? ☐

189 Wanneer draagt u handschoenen in de stal? Anders:

191 Indien u ze draagt, hoe vaak per dag wisselt u uw handschoenen?

	in de kraamstal	in de biggenstal	in de vleesvarkensstal
192 Op welke volgorde loopt u langs afdelingen (per stal)?			
193 Heeft u in deze stallen een aparte hygiënesluis?	☐	☐	☐
194 Wanneer gebruikt men die sluis daadwerkelijk?			
195 Anders, namelijk			

Indirect contact tussen leeftijden

199 Hoe lang staat de kraamafdeling leeg voordat nieuwe zeugen erin komen? dagen

200 Waar worden de zeugen gedoucht?

201 Wat doet u om aangekochte gelten te laten wennen aan de omgeving?

202 Welke materialen geeft u gelten om door te smetten? (bij aankoop alleen!)

203 Hoe lang staat een speenafdeling leeg voor er nieuwe biggen in komen? dagen

204 Hoe lang staat een vlv'ens afdeling leeg voor er nieuwe vlv'ens in komen? ____ dagen

Indirect contact via materialen

205 Biedt u varkens wel eens verrijking aan die ze op kunnen maken? ☐

206 Indien u dat aanbiedt, welke materialen zijn dit dan?

☐ jute ☐ stro
☐ touw ☐ zaagsel
☐ luzerne ☐ houtkrullen
Anders, namelijk: ____

207 Is er verrijking die u verplaatst van de ene naar de andere afdeling? ☐

208 Indien ja, welke verrijking dan? ____

209 Indien ja, wat doet u met de verrijking voordat u het verplaatst? ____

210 Zijn er spullen die bij meerdere diercategorieën worden gebruikt? ☐ Voorbeelden zijn schotjes, stroppen, gehoorbeschermers, weegschalen, voerkarren, rammelaar et cetera.

211 Indien ja, welke materialen gebruikt u in meerdere stallen? ____

212 Waar legt u in eerste instantie een varken neer dat net gestorven is? ____

213 Komen dode varkens wel eens in andere afdelingen dan waar ze dood zijn gegaan? ☐

214 Komen varkens wel eens in contact met restanten van dode varkens waar ze geen hok mee hebben gedeeld? ☐

215 Zou u ervoor openstaan om nog eens deel te nemen aan onderzoek binnen dit project? ____

De enquête is af! Vraag naar de locatie (afdelingsnummers) van de volgende onderdelen voor de visuele check:

- Kraamafdeling die leegstaat
- Kraamafdeling met zeugen die net gebigd hebben of aan het biggen zijn
- Kraamafdeling met biggen van ongeveer 1 week oud
- Quarantainestal
- Speenafdeling met jongste biggen
- Speenafdeling met oudste biggen
- Indien van toepassing ziekenboeg en restafdeling gespeende biggen
- Vleesvarkensafdeling met jongste varkens
- Vleesvarkensafdeling met oudste biggen
- Indien van toepassing ziekenboeg en restafdeling vleesvarkens
- Laad/losplaats (of alleen laadplaats als geen aankoop)

Appendix B
Hygiene inspection used to identify presence of potential risk factors

HEVentie - Risicofactor studie - Visuele Check

ID

1. Hygiënesluis

1a Moet je door duidelijke erfafscheiding om het erf op te komen? ☐
1b Moet je een hygiënesluis passeren om de stal binnen te komen? ☐
2 Heeft de hygiënesluis een afscheiding tussen buiten en binnen? ☐
3 Kun je je handen wassen in de sluis met zeep en water? ☐
4 Ligt er een doos handschoenen die open is en gebruikt lijkt? ☐
5 Is er een douche aanwezig? ☐
6 Is de douche vandaag gebruikt? (plasje/handdoek/shampoo) ☐
7 Is jou gevraagd de douche te gebruiken voor entree? ☐
8 Moet je van kleding (shirt en broek) verwisselen? ☐
9 Moet je een overall aantrekken? ☐

10 Beoordeel hoe schoon de overall is, kies het best passende antwoord:
1 Schoon: Komt opgevouwen uit de kast of droog van de lijn, geen zichtbare mest
2 Oogt schoon: Hangt aan kapstok, geen mest, of materialen in zakken, droog
3 Oogt vies: Zichtbaar vuil of mest eraan, materialen in de zakken zoals spuitjes
4 Oogt zeer vies, duidelijk al lange tijd niet gewassen
5 Anders namelijk

10a Anders, namelijk (overall):

11 Moet je bedrijfslaarzen aantrekken? ☐

12 Waar kun je de laarzen vinden?
1 Op de kop aan een rek
2 Op de grond, op vaste plek
3 Op de grond, niet op vaste plek
4 Anders, namelijk

12a Anders, namelijk (laars)

13 Hebben de laarzen profiel?
1 Ja, allemaal
2 Nee ze zijn zonder profiel
3 Deel is met en deel zonder profiel

14 Beoordeel de hygiëne van de laarzen: Schoon of vies?
1 Schoon
2 Vies

15 Indien de laarzen vies zijn, in welk(e) opzicht(en)?
☐ 15a Stoffig
☐ 15b Mest aan onderkant laars
☐ 15c Mest aan zij- of bovenkant van de laars
☐ 15d Bloed op de laars

2. Laad- en losplaats

16 Wat kun je aanvinken over de Laad-/losplaats?

☐ 16a Goed te reinigen
☐ 16b Op een plek waar voer geleverd wordt
☐ 16c Op een plek waar varkens overheen lopen bij verplaatsing
☐ 16d Op een plek waar veehouder/personeel langs moet lopen
☐ 16e Heeft dezelfde oprit als waar alle andere auto's oprijden
☐ 16f Sluit direct aan op een stal met dieren
☐ 16g Is in de buurt van een luchtinlaat van een varkensstal
☐ 16h Op een plek waar mest wordt opgehaald?

17 Oogt de laad-/losplaats schoon? ☐

3. Kraamstal

18 Vink aan wat je aantreft bij de ingang van de kraamstal:
☐ 18a Aparte kleding ☐ 18b Aparte overall ☐ 18c Aparte laarzen
☐ 18d Plek om laarzen goed schoon te maken vóór entree kraamstal
☐ 18e Plek om handen te wassen vóór entree kraamstal
☐ 18f Mogelijkheid om nieuwe handschoenen aan te doen

19 Is je verteld op welke volgorde je moet lopen in kraamstal? ☐

20 Zo ja, moet je van jong naar oud lopen in de kraamstal? ☐

21 Hoeveel materialen liggen er in gang die niet in gang horen?
1 Geen
2 Een paar materialen
3 Veel materialen
4 De gang ligt vol

22 Hoeveel dode vliegen liggen in de gang?
1 Geen

24 Is er een douche voor zeugen in gang tussen dragend en kraam? ☐

In de kraamstal ga je naar drie afdelingen: een afdeling die helemaal leeg is, een afdeling met zeugen die op dat moment aan het biggen zijn of bijna gaan biggen en een afdeling met biggen van ongeveer een week oud.
Op een bedrijf met een meerwekensysteem kan het voorkomen dat er geen afdeling is met pas geboren biggen of biggen van een week oud. Ga dan naar de afdeling met de jongste biggen die er wel zijn, en schrijf hieronder op hoe oud die biggen zijn.

Bij meerwekensysteem: hoe oud zijn de jongste biggen in de afdeling? ☐

3a. Afdeling die volledig leeg is - geen zeugen en geen biggen

28 Hoe schoon is de lege afdeling? Kies het best passende antwoord:

1 Zeer schoon, geen zichtbare mest, gang leeg, voertrog en verrijking ook schoon
2 Schoon, geen zichtbare mest
3 Niet schoon, zichtbare mest aanwezig
4 Niet schoon, zichtbare mest, stof

3b. Afdeling met zeugen die bijna gaan biggen/aan het biggen zijn

25 Zit er in de hokken zonder biggen mest die niet van de zeug kan zijn? ☐

3c. Afdeling met tomen van +/- 1 week oud

26 Wordt er bijgehouden hoeveel biggen bij een zeug zijn overgelegd? Of hoeveel totaal in die afdeling? ☐

27 Kies het 1e en 3e hok links in de afdeling, en het 2e en achterste hok rechts. Tel het aantal levende biggen in die hokken.

27a Aantal biggen in 1e links ☐
27b Aantal biggen 3e links ☐
27c Aantal biggen in 2e rechts ☐
27d Aantal biggen in achterste rechs ☐

4. Gespeende biggenstal

29 Hoe ben je van kraamstal naar gespeend gelopen?

1 Binnendoor
2 Buitenom

30 Vink aan wat je moet doen om vanuit de kraamstal de gespeende biggenstal in te komen

☐ 30a Van stal naar stal met apart schoeisel (bijv rubberen klompen)
☐ 30b Kleding verwisselen ☐ 30c Overall verwisselen
☐ 30d laarzen verwisselen ☐ 30e Opnieuw douchen

30 En vink aan of het volgende mogelijk is bij entree van de gespeende biggenstal

☐ 30f Laarzen goed schoonmaken voor entree biggenstal
☐ 30g Handen wassen voor entree biggenstal
☐ 30h Mogelijkheid om nieuwe handschoenen aan te doen

31 Is je verteld op welke volgorde je moet lopen in biggenstal? ☐

32 Zo ja, moet je van jong naar oud lopen in de biggenstal? ☐

33 Hoeveel materialen liggen er in gang die niet in gang horen?

1 Geen
2 Een paar materialen
3 Veel materialen
4 De gang ligt vol

34 Hoeveel dode vliegen liggen in de gang?

1 Geen
2 Weinig
3 Veel
4 Zeer veel

35 Hoeveel feces van ratten/muizen ligt er in de gang?

1 Geen
2 Weinig
3 Veel
4 Zeer veel

In de gespeende biggenstal ga je naar twee afdelingen: een afdeling met biggen die die week zijn gespeend en een afdeling met biggen die bijna worden opgelegd.
Op een bedrijf met een meerwekensysteem kan het voorkomen dat er geen afdeling is met pas gespeende biggen of biggen die bijna worden opgelegd. Ga dan naar de afdeling met de jongste biggen die er wel zijn, en schrijf hieronder op hoe oud die biggen zijn.

Hoe oud zijn de gespeende biggen in de jongste afdeling?:

Hoe oud zijnde gespeende biggen in de oudste afdeling?:

Kies in beide afdelingen het 2e hok rechts en het achterste hok links en vul in		**4a. Afdeling met de pas gespeende biggen**		**4b. Afdeling met biggen vlak voor opleg**	
		36 2e hok rechts	37 Achterste hok links	38 2e hok rechts	39 Achterste hok links
Breedte van het hok (cm)					
Lengte van het hok (cm)					
Het percentage van de vloer dat dicht is		%	%	%	%
Is de vloer nat of droog?					
Van welk materiaal is de vloer gemaakt?	Beton	☐	☐	☐	☐
	Plastic	☐	☐	☐	☐
	Rubber	☐	☐	☐	☐
	Staal	☐	☐	☐	☐
	Anders namelijk				
Afstand van de vloer tot de mest in put (cm)					
Aantal biggen in het hok					
Geslacht van biggen					

4. Vervolg gespeende biggen stal

40 Is bij de oudste biggen de datum van spenen of geboorte genoteerd? ☐

41 Welke datum kun je dan vinden?:
1 Datum van geboorte
2 Datum van spenen
3 Week van geboorte

42 Oudste biggen: Noteer de datum van de oudste van die afdeling

43 Was er een speenhok dat je alleen viá een ander hok kon betreden? ☐

5. Vleesvarkensstal

44 Hoe ben je van speenstal naar de vleesvarkensstal gelopen?
1 Binnendoor
2 Buitenom

45 Vink aan wat je moet doen om van de gespeende biggen stal de vleesvarkensstal binnen te komen.
- ☐ 45a Van stal naar stal met apart schoeisel (bijv rubberen klompen)
- ☐ 45b Kleding verwisselen
- ☐ 45c Overal verwisselen
- ☐ 45d Laarzen verwisselen
- ☐ 45e Opnieuw douchen

45 En vink aan of het volgende mogelijk is bij entree van de vleesvarkensstal
- ☐ 45f Laarzen goed schoonmaken voor entree van de stal
- ☐ 45g Plek om handen te wassen met zeep voor entree vlvstal
- ☐ 45h Mogelijkheid om nieuwe handschoenen aan te doen

46 Is je verteld op welke volgorde je moet lopen in de vlvstal? ☐

47 Moet je van jong naar oud lopen in de vlvstal? ☐

48 Hoeveel materialen liggen er in gang die niet in de gang horen?
1 Geen
2 Een paar materialen
3 Veel materialen
4 De gang ligt vol

49 Hoeveel dode vliegen liggen in de gang?
1 Geen
2 Weinig
3 Veel
4 Zeer veel

50 Hoeveel feces van ratten/muizen ligt er in de gang?
1 Geen
2 Weinig
3 Veel
4 Zeer veel

In de vleesvarkensstal ga je naar twee afdelingen: De afdeling met net opgelegde varkens en de afdeling met varkens die het éérst naar de slacht gaan.

In beide afdelingen kies je twee hokken, en je vult de volgende gegevens in voor die twee hokken:

Kies in beide afdelingen het 2e hok rechts en het achterste hok links en vul in		5a. Afdeling met pas opgelegde vlv (51)		5b. Afdeling met vlv nét voor de slacht (52)	
Breedte van het hok (cm)					
Lengte van het hok (cm)					
IHet percentage van de vloer dat dicht is		%	%	%	%
Is de vloer in het hok nat of droog?					
Van welk materiaal is de vloer gemaakt?	Beton	☐	☐	☐	☐
	Plastic	☐	☐	☐	☐
	Rubber	☐	☐	☐	☐
	Staal	☐	☐	☐	☐
	Anders namelijk				
Afstand van de vloer tot aan de mest in put					
Aantal vleesvarkens in het hok					
Het geslacht van de vleesvarkens					

53 Jongste vleesvarkens: Is/zijn er datum/data bekend over dit hok/afdeling? ☐

54a Zo ja, noteer hoogste geboorte/speendatum die je kunt vinden:

54b Zo ja, noteer de hoogste oplegdatum die je kunt vinden:

55 Oudste vleesvarkens: Is er een datum genoteerd bij dit hok/afdeling ☐

55a Zo ja, noteer hoogste geboortedatum/speendatum die je kunt vinden:

55b Zo ja, noteer de hoogste oplegdatum die je kunt vinden:

56 Heb je een vlv hok gezien dat je alleen via een ander hok kon betreden? ☐

6. Restafdeling en ziekenboeg

57a Is er een restafdeling in de gespeende biggenstal? ☐

57b Hoeveel biggen zitten er in de speen restafdeling? 0

57c Hebben de biggen in de reststal allemaal ongeveer dezelfde grootte? ☐

57d Vergelijk de grootte van de kleinste big in de restafdeling, met de jongste speenbig die je in de gespeende biggenafdeling zag. Is de big in de restafdeling kleiner, even groot of groter dan de jongste speenbiggen?
1 Kleiner
2 Even groot
3 Groter

57e Vergelijk de grootte van de grootste big in de restafdeling, met de oudste speenbig die je in de gespeende biggenafdeling zag. Is de big in de restafdeling kleiner, even groot of groter dan de oudste speenbiggen?
1 Kleiner
2 Even groot
3 Groter

58a Is er een restafdeling in de vleesvarkensstal? ☐

58b Hoeveel varkens zitten er in de vlees restafdeling? 0

58c Hebben de vlv in de restafdeling allemaal ongeveer dezelfde grootte? ☐

58d Is het kleinste varken in de restafdeling, kleiner, even groot, of groter dan de jongste vleesvarkens?
1 Kleiner
2 Even groot
3 Groter

58e Is het grootste varken in de restafdeling, kleiner, even groot, of groter dan de oudste vleesvarkens?
1 Kleiner
2 Even groot
3 Groter

59a Is er een ziekenboeg in de gespeende biggenstal ☐

59b Hoeveel dieren zitten er in de speen ziekenboeg? 0

59c Zijn de biggen in de ziekenboeg ongeveer even groot? ☐

59d Lopen biggen die de speenstal inkomen, langs de ziekenboeg? ☐

59e Kan veehouder zijn controlerondje makkelijk eindigen bij zieken? ☐

59f Zijn er aparte laarzen of een desinfectiebak voor de ziekenboeg? ☐

60a Is er een ziekenboeg in de vleesvarkensstal ☐

60b Hoeveel dieren zitten er in de vlees ziekenboeg? 0

60c Zijn de vleesvarkens in de ziekenboeg ongeveer even groot? ☐

60d Lopen vlv die voor het eerst in stal komen, langs de ziekenboeg? ☐

60e Kan de veehouder zijn controlerondje makkelijk eindigen bij zieken? ☐

60f Zijn er aparte laarzen of een desinfectiebak voor de ziekenboeg? ☐

7. Quarantaine

61 Is de quarantainestal een apart gebouw? ☐ 62 Zo nee, in welke stal zit de quarantaine afdeling?

63 Moet je omkleden bij het betreden van de quarantaine afdeling? ☐

64 Moet je je laarzen wisselen bij betreden van de quarantaine afdeling? ☐

65 Kun je je laarzen goed schoonmaken voor betreden van quarantaine? ☐

66 Zie je dingen die passen bij het doorsmetten van gelten? ☐

67 Zo ja, wat voor materialen die hierbij passen, zie je?

8. Uitloop van kraamstal/speenstal of vleesvarkensstal

68 Is de uitloop verhard of niet?

1 Ja verhard
2 Nee onverhard
3 Een deel is onverhard

69 Is de uitloop vloer dicht of van roosters?

1 Volledig dicht
2 Volledig van roosters
3 Deels van roosters

70 Denk je dat de uitloop goed schoon te maken is? ☐

9. Terug naar het geheel...

71 Heb je een huisdier in de stal gezien? ☐

72 Zo ja, welk dier of welke dieren?

73 Heb je huisdieren direct om de stal heen gezien? (kantine) ☐

74 Droeg veehouder of personeel handschoenen terwijl je er was?

1 Ja
2 Nee
3 Een deel van de mensen
4 Ik heb niemand in de stal gezien

75 Heb je in de kraamstal dingen gezien die passen bij doorsmetten? ☐ 76 Indien ja, wat heb je dan gezien?

77 Heb je veel voerbakken in de gang gezien zónder afdekking? ☐

78 Heb je veel ongediertebestrijding gezien (bijv val/jampotjes) ☐

79 Geef algemene score voor hoe netjes dit bedrijf is van buiten

1 Zeer rommelig
2 Rommelig
3 Niet netjes, niet rommelig
4 Netjes
5 Zeer netjes

80 Geef algemene score voor hoe schoon dit bedrijf is van binnen

1 Zeer schoon
2 Schoon
3 Niet zo vies, ook niet zo schoon
4 Vies
5 Zeer vies

Appendix C

Odds ratios for explanatory variables lowering the AIC with less than one point

Potential risk factor	AIC	Levels	OR[a] (95%CI)[bc]
Frequency emptying manure storage farrowing phase	1.81	Less often than between every batch of pigs (n=15)	1
		Between every batch of pigs or more often (n=8)	0.54 (0.04-8.22)
Frequency pen fouling fattening phase	2.54	(Almost) never (n=21)	1
		Sometimes (n=27)	1.07 (0.36-3.21)
		Often (n=25)	0.55 (0.16-1.87)
Building year newest barn	1.80	Build in or before 2000 (n=9)	1
		Build after 2000 (n=64)	1.42 (0.3-6.85)
Frequency emptying manure storage nursery phase	0.79	Less often than between every batch of pigs (n=17)	1
		Between every batch of pigs or more often (n=9)	0.21 (0.01-4.58)
Frequency emptying manure storage fattening phase	2.00	Less often than between every batch of pigs (n=47)	1
		Between every batch of pigs or more often (n=26)	1.02 (0.38-2.72)
Frequency pen fouling fattening phase	2.00	Never (n=9)	1
		Sometimes/often (n=14)	0.99 (0.07-13.96)
Frequency foam presence above slatted floor (in relation to manure)	2.37	Never (n=35)	1
		Sometimes (n=14)	1.96 (0.61-6.37)
		Several times per year (n=24)	0.9 (0.31-2.65)
Cleaning frequency central corridor	0.91	Yearly or less often (n=10)	1
		After contact with animals has ended (n=23)	1.77 (0.4-7.87)
		Between every batch of pigs (n=24)	1.3 (0.29-5.9)
		Every week (n=13)	0.21 (0.02-2.46)
Cleaning frequency enrichment	1.94	Yearly or less often (n=15)	1
		Between every batch of pigs (n=55)	1.2 (0.27-5.29)
Cleaning frequency ceiling	-0.30	Yearly or less often (n=34)	1
		Between every batch of pigs (n=38)	2.07 (0.8-5.4)
Cleaning frequency underside slatted floor	-0.49	Never (n=67)	1
		Yearly or more often (n=7)	3.45 (0.76-15.6)
Cleaning frequency boots	4.25	Yearly or less often (n=12)	1
		Daily or after contact with animals (n=30)	0.66 (0.17-2.62)
		Monthly (n=13)	0.64 (0.12-3.47)

		Weekly (n=16)	1.43 (0.34-6.03)
Cleaning frequency sorting boards	0.07	Yearly or less often (n=15)	1
		After contact with animals (n=28)	3.32 (0.86-12.83)
		Between every batch of pigs (n=20)	0.97 (0.2-4.78)
		Weekly (n=27)	2.88 (0.44-18.68)
Cleaning frequency outdoor space	2.99	Yearly or less often (n=10)	1
		Between every batch of pigs (n=4)	0.82 (0.05-13.38)
		Outdoor space is not present on the farm (n=59)	1.83 (0.4-8.39)
Cleaning frequency pen fattening phase	0.55	Yearly or less often (n=16)	1
		Between every batch of pigs (n=56)	2.18 (0.59-8.03)
Cleaning procedure pen farrowing phase includes sweeping the floor	1.75	FALSE (n=18)	1
		TRUE (n=5)	2.11 (0.11-41.93)
Cleaning procedure pen farrowing phase includes soaking before hosing down	1.74	FALSE (n=6)	1
		TRUE (n=17)	0.48 (0.03-7.74)
Cleaning procedure pen farrowing phase includes use of cleaning agent during soaking	1.31	FALSE (n=7)	1
		TRUE (n=16)	0.34 (0.03-3.7)
Cleaning procedure pen nursery phase includes sweeping the floor	1.84	FALSE (n=20)	1
		TRUE (n=6)	1.91 (0.08-47.5)
Cleaning procedure pen nurery phase includes soaking before hosing down	1.84	TRUE (n=7)	1
		FALSE (n=19)	0.53 (0.03-11.04)
Cleaning procedure pen nursery phase includes use of cleaning agent during soaking	1.85	TRUE (n=10)	1
		FALSE (n=16)	0.56 (0.04-8.93)
Cleaning procedure pen fattening phase includes sweeping the floor	0.41	FALSE (n=51)	1
		TRUE (n=22)	1.87 (0.7-4.98)
Cleaning procedure pen fattening phase includes soaking before hosing down	1.86	FALSE (n=17)	1
		TRUE (n=56)	1.25 (0.39-4)
Cleaning procedure pen fattening phase includes use	1.43	FALSE (n=38)	1

of cleaning agent during soaking			
		TRUE (n=35)	1.44 (0.55-3.78)
Cleaning procedure central corridor includes sweeping the floor	-2.14	FALSE (n=59)	1
		TRUE (n=14)	0.22 (0.04-1.11)
Cleaning procedure central corridor includes soaking before hosing down	1.69	FALSE (n=48)	1
		TRUE (n=25)	0.75 (0.27-2.08)
Cleaning procedure central corridor includes use of cleaning agent during soaking	0.98	FALSE (n=62)	1
		TRUE (n=11)	1.88 (0.55-6.4)
Cleaning procedure central corridor includes hosing down surfaces	1.71	FALSE (n=7)	1
		TRUE (n=66)	1.63 (0.26-10.39)
Cleaning procedure boots includes use of a brush	1.09	FALSE (n=45)	1
		TRUE (n=28)	0.61 (0.22-1.69)
Cleaning procedure boots includes soaking before hosing down	0.59	FALSE (n=68)	1
		TRUE (n=5)	2.83 (0.53-15.23)
Cleaning procedure boots includes spray-cleaning	1.86	FALSE (n=23)	1
		TRUE (n=50)	0.82 (0.3-2.26)
Water pressure and temperature used for spray-cleaning boots	1.27	Cold water with low pressure (n=49)	1
		Cold water with high pressure (n=4)	2.41 (0.35-16.59)
Cleaning procedure outdoor space includes sweeping the floor	0.90	FALSE (n=64)	1
		TRUE (n=9)	0.41 (0.07-2.35)
Cleaning procedure outdoor space includes soaking before hosing down	0.90	FALSE (n=67)	1
		TRUE (n=6)	0.41 (0.07-2.35)
Cleaning procedure outdoor space includes hosing down surfaces	1.91	FALSE (n=63)	1
		TRUE (n=10)	0.81 (0.2-3.35)
Farmers interpretation of the word 'desinfection'	1.78	Farmer is not convinced that disinfection is necessary and/or disinfection is not carried out (n=13)	1
		Farmer describes the aim of disinfection measures (n=31)	1.24 (0.36-4.26)

		Farmer describes execution of disinfection measures (n=21)	0.52 (0.12-2.18)
Farmers opinion on optimal time between cleaning and disinfection	3.51	No opinion (n=21)	1
		12 hours or less (n=18)	1.27 (0.34-4.74)
		24 hours or more (n=10)	0.64 (0.11-3.61)
		After pens are no longer wet (n=24)	1.98 (0.59-6.64)
Time period between cleaning and disinfection during the cleaning procedure	3.53	0-24 hours (n=31)	1
		24 hours or more (n=13)	1.42 (0.41-4.87)
		Unknown/no disinfection measures executed (n=29)	0.91 (0.32-2.65)
Disinfection frequency boots	-0.04	Yearly or less often (n=)	1
		Between every batch of pigs or weekly (n=)	2.28 (0.79-6.63)
		Daily or after each contact with animals (n=)	0.59 (0.18-1.97)
Disinfection frequency central corridor	-0.04	Never (n=37)	1
		Yearly (n=11)	0.91 (0.2-4.14)
		Between every batch of pigs (n=22)	2.41 (0.83-6.95)
Disinfection frequency sorting boards	0.96	Yearly or less often (n=47)	1
		More often than yearly (n=23)	1.67 (0.62-4.53)
Disinfection frequency pen farrowing phase	1.03	Yearly or less often (n=7)	1
		Between every batch of pigs (n=16)	4.39 (0.21-90.01)
Disinfection frequency pen nursery phase	0.30	Yearly or less often (n=10)	1
		Between every batch of pigs (n=16)	6.88 (0.36-131.63)
	2.74	Never (n=30)	1
Disinfection frequency pen fattening phase		Yearly (n=9)	2.14 (0.48-9.62)
		Between every batch of pigs or weekly (n=31)	1.6 (0.56-4.63)
	-1.27		
Surfaces are hosed down after disinfection	2.68	FALSE (n=30)	1
		No disinfection measures executed (n=27)	0.64 (0.22-1.84)
		TRUE (n=16)	0.52 (0.15-1.83)

Water used for hosing down surfaces after disinfection originates from	1.00	Spring water (n=12)	1
		Tap water (n=6)	0.62 (0.04-9.37)
		No disinfection measures executed or hosing down (n=56)	2.55 (0.57-11.31)
Compliance to the 'all-in/all-out' principle farrowing phase	1.16	Less than 100% (n=9)	1
		1 (n=14)	0.32 (0.03-3.57)
Reason for incompliance to 'all-in/all-out' principle in farrowing phase is uniformity of piglets	1.80	FALSE (n=16)	1
		TRUE (n=7)	1.87 (0.13-27.4)
Compliance to the 'all-in/all-out' principle farrowing phase	0.99	Less than 100% (n=15)	1
		1 (n=11)	3.99 (0.25-62.92)
Destination of weaned piglets not ready to enter the growing phase	1.99	Stays in farm compartment (n=8)	1
		Located to different farm compartment (n=8)	1.46 (0-1584.58)
Reason for incompliance to 'all-in/all-out' principle in nursery phase is sale of piglets	2.00	FALSE (n=22)	1
		TRUE (n=4)	0.93 (0.02-49.25)
Reason for incompliance to 'all-in/all-out' principle in nursery phase is shortage of available pens in growing phase	1.87	FALSE (n=21)	1
		TRUE (n=5)	1.94 (0.06-60.23)
Reason for incompliance to 'all-in/all-out' principle in nursery phase is shortage of uniformity of piglets	-1.97	FALSE (n=19)	1
		TRUE (n=7)	0 (0-inf)
Compliance to the 'all-in/all-out' principle fattening phase	0.42	25% or more (n=15)	1
		Less than 25% (n=58)	0.51 (0.18-1.46)
Destination of fattening pigs not ready to go to slaughter	2.58	Stays in farm compartment (n=25)	1
		Located to farm compartment for residual pigs (n=28)	1.11 (0.38-3.24)
		Located to different farm compartment (n=14)	2.03 (0.6-6.83)

Reason for incompliance to 'all-in/all-out' principle in fattening phase is illness of the pigs	1.45	FALSE (n=66)	1
		TRUE (n=7)	0.52 (0.08-3.16)
Reason for incompliance to 'all-in/all-out' principle in fattening phase is number of available pens	-1.00	FALSE (n=69)	1
		TRUE (n=4)	0 (0-inf)
Reason for incompliance to 'all-in/all-out' principle in fattening phase is uniformity of pigs	1.94	FALSE (n=8)	1
		TRUE (n=65)	1.21 (0.26-5.63)
The time period in which sows and piglets are absent in a compartment farrowing phase	1.71	1 day or less (n=12)	1
		More than 1 day (n=7)	0.32 (0.01-16.27)
The time period in which sows and piglets are absent in a compartment nursery phase	1.97	1 day or less (n=10)	1
		More than 1 day (n=14)	0.69 (0.01-32.91)
The time period in which sows and piglets are absent in a compartment fattening phase	-0.98	4 days or less (n=52)	1
		More than 4 days (n=14)	0.31 (0.07-1.28)
Concrete flooring of pens nursery phase	0.24	Present (n=7)	1
		Not present (n=17)	0.09 (0-2.57)
Plastic flooring of pens nursery phase	1.53	Present (n=10)	1
		Not present (n=14)	0.27 (0.01-9.95)
Rubber flooring of pens nursery phase	1.99	Present (n=19)	1
		Not present (n=5)	0.8 (0.01-79.22)
Flooring of pens nursery phase different than mentioned above	2.00	Present (n=20)	1
		Not present (n=4)	0.96 (0.01-146.75)
Percentage of slatted floor in pen nursery phase	1.63	20% or less (n=10)	1
		More than 20% (n=12)	0.34 (0.01-9.56)
Distance between the manure pit and slatted floor pen nursery phase	-0.06	60 cm or less (n=12)	1

		More than 60 cm (n=8)	17.64 (0.71-438.42)
Rubber flooring of pens growing and phattening phase	-3.42	Present (n=59)	1
		Not present (n=13)	3.37 (1.26-9.07)
Steel flooring of pens growing and phattening phase	1.96	Present (n=63)	1
		Not present (n=9)	1.17 (0.27-4.95)
Flooring of pens fattening phase different than mentioned above	1.81	Present (n=61)	1
		Not present (n=11)	0.74 (0.18-2.96)
Percentage of slatted floor in pen nursery phase	0.87	40% or less (n=21)	1
		More than 40% (n=49)	1.82 (0.58-5.64)
Distance between the manure pit and slatted floor pen fattening phase	-1.09	60 cm or less (n=26)	1
		More than 60cm (n=39)	0.42 (0.16-1.12)
Classification of the appearance of outside of the farm	1.00	Messy (n=10)	1
		Between tidy and messy (n=14)	0.22 (0.03-1.54)
		Tidy (n=48)	0.77 (0.22-2.66)
Classification of the appearance of inside of the farm	2.74	Clean (n=33)	1
		Between clean and dirty (n=25)	1.12 (0.41-3.05)
		Dirty (n=14)	0.51 (0.13-2.06)
	-2.22		
Farm type	1.60	Conventional (n=61)	1
		Organic (n=12)	0.65 (0.16-2.55)
Farm system	1.42	Farrow-to-finish farm (n= 23)	1
		Weaning-to-finish farm (n= 3)	0 (0-inf)
		Fattening pigs farm (n= 47)	1.12 (0.40-3.19)

[a] OR: odds ratio
[b] 95%CI: 95% confidence interval
[c] For the reference levels, the 95%CI is not available.

Appendix D

List of variables with less than 4 observations per level, consequently excluded from univariate analysis

Cleaning procedure central corridor includes uninstalling systems
Cleaning procedure farrowing phase includes hosing down pens
Cleaning procedure farrowing phase pens includes uninstalling systems (e.g. enrichment)
Cleaning procedure fattening phase includes hosing down pens
Cleaning procedure fattening phase pens includes uninstalling systems (e.g. enrichment)
Cleaning procedure nursery phase includes hosing down pens
Cleaning procedure nursery phase pens includes uninstalling systems (e.g. enrichment)
Cleaning procedure outdoor space includes use of cleaning agent during soaking
Cleaning procedure quarantine includes hosing down quarantine
Cleaning procedure quarantine includes soaking before hosing down
Cleaning procedure quarantine includes sweeping the floor
Cleaning procedure quarantine includes use of cleaning agent during soaking process
Concrete flooring of pens grower and finishing phase
Disinfection frequency pen farrowing phase
Disinfection frequency pen nursery phase
Disinfection frequency quarantantine
Disinfection frequency quarantantine
Manure of piglets was observed in at least one pen housing a gestating sow in farrowing room
Plastic flooring of pens grower and finishing phase
Reason for incompliance to 'all-in/all-out' principle in farrowing room is condition of the sow
Reason for incompliance to 'all-in/all-out' principle in farrowing room is limited availability of foster sows
Reason for incompliance to 'all-in/all-out' principle in farrowing room is limited number of farrowing pens
Reason for incompliance to 'all-in/all-out' principle in farrowing room is sickness of piglets
Reason for incompliance to 'all-in/all-out' principle in nursery room is condition of the sow
Water pressure and temperature used for hosing down central corridor
Water pressure and temperature used for hosing down pen farrowing phase
Water pressure and temperature used for hosing down pen fattening phase
Water pressure and temperature used for hosing down pen nursery phase
Water pressure and temperature used for hosing down quarantine