The Epidemiology of Porcine Reproductive and Respiratory Syndrome (PRRS) in England: Investigation of risk factors for active PRRS infection and evaluation of surveillance using scenario tree analysis

MSc Veterinary Epidemiology Project Report

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Contents

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) is considered to be one of the most important diseases affecting pigs worldwide. To minimize its devastating impact on pig production, better understanding of the epidemiology of PRRS is required to provide the basis for more effective disease control.

This study is aimed at identifying risk factors for active PRRS infection at farm level and evaluating current PRRS surveillance. Data from 147 farrow-to-finish farms was collected during April 2008 – April 2009 through a cross-sectional survey carried out in England.

Risk factors for active PRRS infection were identified using multivariable logistic regression analysis. A stochastic model based on scenario tree analysis was developed in order to evaluate surveillance.

The results indicated 30.61% (95%CI: 23.07-38.15) prevalence of PRRS infection in the English pig population with a higher proportion of infected farms located in areas with high pig density. In total, 63 (43.45%) farms reported using vaccination against PRRSV. A higher proportion of farms used live vaccine rather than killed vaccine (82.54% vs.17.46%). Farms were more likely to be PRRS positive if they used live virus vaccine (OR=3.65, 95%CI: 1.24-10.73), were located in high pig density areas (OR=4.64, 95%CI: 2.18-9.84) and had dead pigs collected (OR=9.00, 95%CI: 2.40-33.67). However, farms that weaned pigs at the age of 28 days or later had lower odds of being PRRS positive (OR=0.22, 95%CI: 0.07-0.65). An increased frequency of breeding stock brought on the farm identified in univariable analysis after controlling for the effect of herd size and type of farm (indoor/outdoor) was no longer significant (OR=2.87, 95%CI: 0.74-11.25) but was retained in the analysis as it improved the model fit. The probability of an infected farm being detected through passive surveillance was very low (mode=0.0118, 5^{th} and 95^{th} percentiles: 0.0074; 0.0203 respectively). Further, farms which used live virus vaccine had lower probabilities for detection compared to those which did not.

The results indicate that current control measures are not effective and that surveillance needs enhancement. Moreover, biosecurity measures and vaccination need to be evaluated to provide effective control and prevention of the PRRS infection.

Word count: 345

1 Introduction

In Britain and worldwide, porcine reproductive and respiratory syndrome (PRRS) is considered to be one of the most important diseases affecting pigs [1]. This is mainly due to a combination of the impact of various clinical signs on production, predominantly in the acute stage of infection, and the rapid transmissibility of the virus within a susceptible population [2, 3]. The economic impact of PRRS on pig production can be significant, especially if it occurs in herds/regions without previous history of disease. In individual herds, direct costs relate to production losses through high pre and post-weaning mortality and reproductive failure. Indirect costs are mainly associated with disease control. In the USA, the estimated costs of PRRS to the pig industry can reach $560 million per year [4].

The etiological agent of PRRS is a single stranded RNA virus from the family Arteriviridae [5, 6]. There are two genotypes of PRRS virus (PRRSV): European (EU) and North American (NA) [7]. Within the European genotype, four subtypes have been identified [8]. This indicates greater genetic diversity of the virus.

The disease was first reported in the USA in 1987 and by 1990s it spread rapidly throughout North America and to Europe [2]. The first European country to report PRRS was Germany (1990) followed shortly by the Netherlands, Belgium and Spain [9].

In Britain, the first clinical cases were confirmed in 1991 [10]. Since then, the disease has spread and is now considered to be endemic. Its clinical manifestation on individual farms is to a great extent influenced by previous exposure to the virus. In enzootically infected herds, disease can be less severe or even subclinical compared to herds with a naïve pig population. The overall health status of the herd, type of farm and management practices in place on individual farms can influence the dynamics of PRRS.

Various studies have been carried out to investigate the risk factors associated with PRRS in England and elsewhere. Increased herd size, distance to the nearest pig herd [11], pig and herd density, purchase of semen [12], increased purchase of gilts and boars, and total confinement housing [13] were found to be associated with increased risk of PRRS infection. In recent years, the situation on many farms has changed, different breeds are used, changes to management practices were implemented, and new vaccines were introduced. Despite this, PRRS continues to be a major problem for many pig producers. One reason for this might be that vaccines in use are not fully protective against heterologous strains of the virus [14].

Better understanding of PRRS epidemiology in Britain is therefore essential for the development and application of successful control programs. The wide spread of PRRSV and its ability to be reintroduced into herds after eradication [7] left many farmers frustrated and struggling to achieve good production performance.

Monitoring and surveillance play an important role in disease control and eradication. The main objective of the surveillance system is to generate information on detection, monitoring and distribution of disease or infection in the animal population [15]. Two different approaches can be used in data collection: active and passive. Active surveillance is performed periodically, while passive relies on cases being reported to health authorities [16].

For PPRS in the UK, only passive surveillance is currently undertaken and its performance is greatly influenced by sample submissions. Depending on samples received, full post-mortem examination is carried out on pigs and the initial diagnosis includes the use of polymerase chain reaction (PCR) and imunohistochemistry (IHC). In recent years, a slight decrease in submissions was observed, but the proportion of submissions diagnosed with PRRS increased from 2% to 10% [17]. The use of this information can be of limited value if no surveillance enhancement is considered.

This study aims to evaluate the existing surveillance strategy and provide the basis for control and/or eradication of PRRS in England. To achieve this, a better understanding of epidemiology of PRRS is required. Therefore, the objectives of this project are to: **1)** estimate prevalence of PRRS infection in England; **2)** identify risk factors for active PRRSV infection at herd level; and **3)** develop a stochastic model to evaluate the ability of the passive surveillance in place to detect infected farms.

2 Materials and methods

2.1 Study population and data collection

The data used for this study was collected between April 2008 and April 2009 in the context of a cross-sectional study on post-weaning multisystemic wasting syndrome (PMWS) in England.

Farms were recruited through the BPEX PCV2 vaccination scheme and through veterinary practitioners. Inclusion criteria for participation were restricted to farrow-to-finish type of farms.

During a one-day visit to each farm, data on general farm information, management practices, production parameters, health status including vaccination programme, genetics, breeding performance and biosecurity measures, was collected by interviewing the farmer. The questionnaire used was piloted on several farms prior to use. A welfare assessment was also carried out by researchers on each farm.

From each herd, 20 blood samples were collected (six growers, six finishers and two sows). All samples were tested for antibodies to: (1) PRRSV (Biobest-in house ELISA, Biobest Laboratories Ltd., UK); (2) *Actinobaccilus pleuropneumoniae* APP (Swinecheck^{R} ELISA specific for serotypes 3, 6 and 8, Biovet, Saint-Hyacinthe, Quebec, Canada) and (3) swine influenza (H1N1, H1N2) using haemagglutination inhibition test. Porcine circovirus type 2 (PCV2) was detected using a real time PCR protocol described elsewhere [18].

For the purpose of this project, samples collected from growers and finishers on farms which vaccinate for PRRS were re-tested using PRRSV specific real time PCR (AnDiaTec AcuPig, Biobest Laboratories Ltd., UK).

It was calculated that for PRRS diagnosis, a sample of 12 pigs/herd (6 growers and 6 finishers) will be sufficient to detect disease if the within herd prevalence was 22% or higher.

2.2 PRRS case definition

Unvaccinated farms were classified as positive if at least one grower or finisher tested positive for PRRS in the ELISA test, and vaccinated farms were classified as positive if at least one grower or finisher tested positive in the RT-PCR. Sows and weaners were excluded from the case definition in order to accurately estimate active PRRS infection and avoid misclassification due to cross-reaction with vaccine or maternal antibodies.

2.3 Data management and statistical analysis

Data collected during farm visits was entered into several spreadsheets using Microsoft Access 2007 and transferred to Stata 11.2 (StataCorp, Texas) for further analysis.

Data on farm information and biologically plausible factors collected were divided into 6 main groups (see Table 1 for details).

Table 1. Exposure variables included in the risk factor analysis for PRRS

Variable group	Variable description
1. General farm information	Geographic location (by region)
	Number of sites (one/multiple)
	Herd size (measured by number of sows)
	Pig density (total number of pigs within 10 km radius from the farm)
	Farm type: outdoor/ indoor
2. Herd health	*Farmers perception:*
	Herd vaccination program (PPV, PRRS)
	Type of PRRS vaccine used (none/killed virus /live virus)
	Results from serology and PCR:
	Serological results: APP, SI (H1N1, H1N2)
	PCR results: PCV2 (PCR)

3. General management practices	***Herd environment:***
	Ventilation type (natural/artificial/both)
	Lightening (natural/artificial/both)
	Presence of slurry system (yes/no)
	Use of straw yards at any stage of the production (yes/no)
	Presence of other animal species on the farm (yes/no)
	Herd management:
	Number of movements between weaning and finishing
	Mixing of pigs at any stage of the production (yes/no)
	Use of all in all out system at any stage of the production (yes/no)
	Use of sick/hospital pens on farm (yes/no)
	Routine cross-fostering performed (yes/no)
4. Genetics	Breed composition (percentage of breed in the commercial type of pigs):
	Large White (LW)
	Landrace (LD)
	Pietrain (P)
	Duroc (D)
	Hampshire (H)
	Miesham (M)
5. Biosecurity	***Possible route of disease introduction through people:***
	Number of people working on the farm
	Average number of visitors/month
	Number of days pig free
	Use of protective clothes (yes/no)
	Use of boot dips (yes/no)
	Presence of fences around the farm (yes/no)
	Allowed parking on the farm (yes/no)

	Possible route of disease introduction through animals:
	Purchase of boars (yes/no)
	Purchase of gilts (yes/no)
	Purchase of semen (yes/no)
	Disposal of dead pigs (collection/incineration/other)
6. Farm assessment	Weaners stocking density/pen
	Growers stocking density/pen
	Finishers stocking density/pen

The epidemiological unit of the statistical analysis was the farm. The outcome variable was the classification of the farm as PRRS positive or negative.

2.3.1 *Data analysis*

To assess the impact of PRRS on vaccinated and unvaccinated farms, presence of clinical signs and breeding performance was assessed. The difference between individual categories was tested using Kruskal-Wallis test.

Prior to conducting risk factor analysis, the frequency distribution of the outcome for each of the exposures was calculated. Variables with less than 80% of observations recorded were excluded from the multivariable analysis.

Continuous variables were checked for normal distribution using histograms and the skewness-kurtosis test and appropriate log transformation or categorization was performed accordingly. Some new variables were also created i.e. frequency of bringing breeding pigs to the farm.

Univariable analysis of each exposure with binary outcome was performed using Chi-squared test. Only variables with p-value < 0.2 were considered for downstream analysis [19].

Within each exposure group, collinearity checks were carried out using Spearman correlation coefficient for continuous variables and Chi-squared test for categorical variables. Two variables were considered to be collinear if $p<0.01$ or if a correlation coefficient > 7 was obtained. Exposures, based on strengths of the association with the outcome ($p<0.2$) or better

biological plausibility for PRRS and retained after collinearity checks were included in the multivariable logistic regression model.

Confounding was assessed by adding variables into the model starting with exposures with the lowest p-value (strongest association from univariable analysis with the outcome). Each time an exposure was added, confounding was examined and model fit was assessed using likelihood ratio (LR) test. Two priori confounders were considered (herd size and production type) and forced in the model at all times. Ordered categorical exposures were checked for linear trend using LR test.

Finally, a stepwise backward selection process was used starting with all exposures and testing them for statistical significance. Exposures with p-value > 0.05 were removed from the model. All possible two-way interactions were assessed and retained if they improved model fit as determined by LR test ($p < 0.05$) and biological plausibility.

Model fit was assessed using adjusted Hosmer-Lemeshow goodness of fit test [20].

2.4 Evaluation of PRRS surveillance in England

A quantitative methodology for surveillance evaluation based on scenario tree modelling (STM) was described by Martin et al and Hadorn et al [21, 22]. In this method, the chain of events from an animal being infected to being detected is used to calculate the probability of detecting at least one infected animal/herd at a predefined prevalence [21]. This approach has been adopted for the current study in order to calculate the probability that an infected farm is identified through the existing passive surveillance. The scenario tree developed is outlined in Figure 1. Two risk category nodes were used: PRRS live vaccination and pig density (high and low), and two category nodes (use of any PRRS vaccination and type of farm). See Table 2 for details of all input parameters including their sources.

2.4.1 *Model parameterization*

Data from the risk factor analysis was used to model the disease distribution and vaccine use in English pig population. Further, data on PRRS diagnostics and clinical signs observed were obtained retrospectively for a period of April 2008 to April 2009 from: Animal Health and Veterinary Laboratories Agency (AHVLA), literature and one expert opinion from a field veterinarian.

Defining pig population to risk strata was based on 2008 census data of registered pig specialist holdings in England. The pig density map (http://www.thepigsite.com/articles/7/markets-and-economics/2282/british-pig-market) was used to estimate the proportion of farms in high density areas. The proportion of farms with live vaccine and the relative risk (RR) for individual risk strata was calculated based on data from the cross-sectional study. For each stratum, the proportion of the reference population and RR weighted according to the size of stratum population was calculated giving the average adjusted risk (AR) for the population strata equal to one using the formula described by Martin et al[21]:

$$\sum_{l=1}^{L}(AR_l \, x \, PrP_l) = 1$$

where L is the number of risk strata and PrP is the proportion of farms in the *l*th stratum.

The effective probability of infection (EPI) for each risk stratum was calculated by multiplying the design prevalence of PRRS (P*H) obtained from the cross-sectional study with the appropriate AR. Similarly to the risk nodes, the proportion of farms in individual category nodes was estimated using data from cross-sectional study and agricultural statistics.

2.4.2 *Probability of infected farm being detected*

The probability that a randomly chosen farm in England will be identified as true positive was calculated by summing the positive branches of the scenario tree, which equals the system unit sensitivity (SUSe). The probability that an infected farm would be detected through the passive surveillance system was calculated as:

$$\frac{\text{SUSe}}{\sum all\ infected\ animals}$$

Microsoft Excel and Palisade @RISK were used to model probability distributions, and the model was run with 10,000 iterations.

2.4.3 *Evaluation of input parameters*

To account for variation and uncertainty in input parameters used, sensitivity analysis was carried out by varying individual input parameters in the model. The regression coefficients were also obtained using @RISK sensitivity analysis.

Table 2. Description of input parameters for individual tree nodes, including their source and explanation used in the model to assess the passive surveillance system in place for PRRS in England.

Input parameter (including abbreviations)	Value	Source and explanation
Design prevalence P*H	0.300	Cross-sectional study
Risk nodes		
Proportion of farms in high pig density area - HDA	0.284	http://archive.defra.gov.uk/evidence/statistics/foodfarm/landuselivestock/junesurvey/documents/NoofHoldingsbyfarmtypeandcounty.xls
Proportion of farms using live vaccine- LVAC	*RiskBeta*(53,94)	Cross-sectional study
Category nodes		
Proportion of farms vaccinating -VAC	*RiskBeta*(64,83)	Cross-sectional study
Proportion of breeding farms -B	0.60	http://www.ukagriculture.com/statistics/farming_statistics.cfm?strsection=Numbers of Holdings
Detection nodes:		
1. Probability that infected pig shows clinical signs		
PRRS vaccinated breeding farms - VACB	*RiskPert*(0,0.20,0.25)	Cross-sectional study
PRRS vaccinated finishing farms-VACF	*RiskPert*(0,0.20,0.25)	Cross-sectional study
PRRS non-vaccinated breeding farms-NVACB	*RiskPert*(0,0.14,0.40)	Cross-sectional study
PRRS non-vaccinated finishing farms-NVACF	*RiskPert*(0,0.14,0.40)	Cross-sectional study
2. Probability that farmer recognize signs and calls vet		
PRRS vaccinated breeding farms-VACB	*RiskPert*(0.4,0.5,0.6)	Medium probability [22]: less severe clinical signs were expected

		due to vaccination which could go unnoticed compare to non-vaccinated farms
PRRS vaccinated finishing farms-VACF	*RiskPert*(0.1,0.2,0.3)	Low probability [22]: it was believed that clinical signs seen in this type of farms are more difficult to recognize compare to breeding farms plus the effect of vaccination resulted in low probability
PRRS non-vaccinated breeding farms-NVACB	*RiskPert*(0.7,0.8,0.9)	High probability [22]: non vaccinated farms are likely to have more naïve population therefore clinical manifestation would be more apparent
PRRS non-vaccinated finishing farms-NVACF	*RiskPert*(0.4,0.5,0.6)	Medium probability [22]: similar to non-vaccinated breeding farms but clinical signs slightly less severe
3. Probability that vet collects the sample		
PRRS vaccinated breeding farms-VACB	*RiskPert*(0.4,0.5,0.6)	Medium probability [22]: based on the same assumption as above
PRRS vaccinated finishing farms-VACF	*RiskPert*(0.1,0.2,0.3)	Low probability [22]: based on similar assumption as above
PRRS non-vaccinated breeding farms-NVACB	*RiskPert*(0.7,0.8,0.9)	High probability [22]: based on similar assumption as above
PRRS non-vaccinated finishing farms-NVACF	*RiskPert*(0.4,0.5,0.6)	Medium probability [22]: based on similar assumption as above
4. Probability that infected animal test positive		
Sensitivity of PCR test	*RiskUniform*(0.967,0.999)	Evaluation of the AnDiaTec AcuPig PRRSV real time RT-PCR for the detection of NA and EU strains (www.andiatec.com)
Sensitivity of ELISA test	*RiskUniform*(0.961,0.978)	[3]

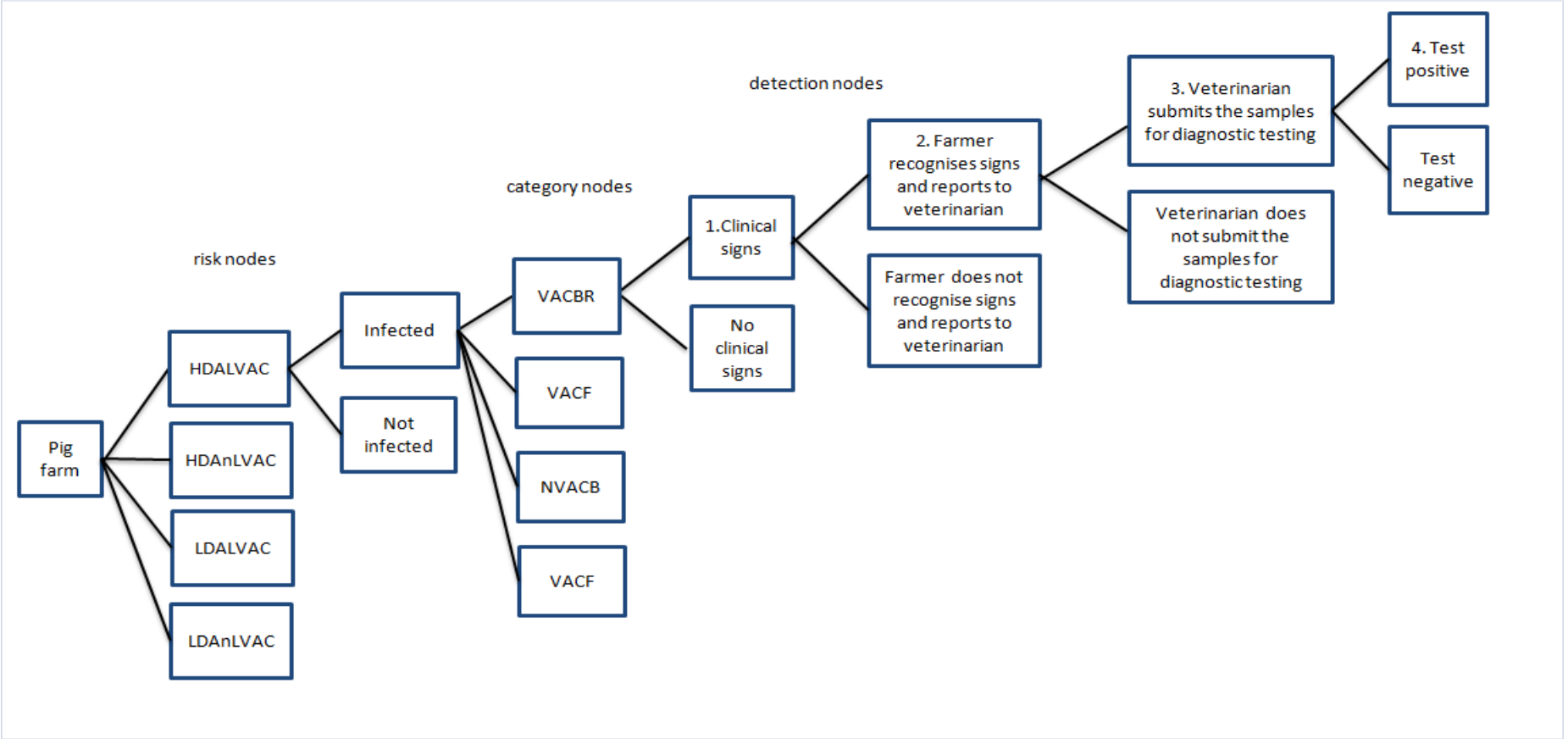


Figure 1. Scenario tree for passive surveillance for PRRS on pig farms in England. See the table above for an explanation of the abbreviations used for individual nodes in this figure. The tree is not completed but would have the same structure as the HDALVAC branch.

3 Results

3.1 Descriptive statistics

In total, 147 farms were recruited in this study between April 2008 and April 2009. Insufficient data was obtained on three farms which were therefore excluded from further analysis.

The median herd size was 300 sows with minimum 16 and maximum 2,000 sows. More than half of farms (56.76%) kept their pigs on one site only. All-indoor type of production was the most common (73.29%) type, with 26.71% of farms keeping either all or most of their pigs outdoor.

Geographically, all regions were represented, but two regions (North Yorkshire and East Anglia) accounted for more than half of recruited farms. Consequently, more than 85% of positive farms were found in this area with very few in the Midlands and none in the South East or South West of England. The geographic distribution of studied farms corresponded to English pig population density.

Regarding biosecurity measures, 43.36% of farms purchased no breeding stock, 18.88% bought breeding stock one to six times per year, and 37.76% farms more than six times per year. Collection of dead pigs was practised on 69.06% of farms compared to 30.94% using on-farm incinerators. Most farms (76.64%) always used protective clothing and 80.45% required visitors to be pig-free with median two days, minimum of one and maximum seven days.

In total, 45 farms (30.61%, 95%CI: 23.07-38.15) tested positive for PRRS and of those, 28 used live vaccine, two used killed vaccine and the remaining 15 used no vaccination (Figure 2). In total, 63 (43.45%) farms vaccinated against PRRSV, with a higher proportion using live vaccine rather than killed vaccine (82.54% vs.17.46%).

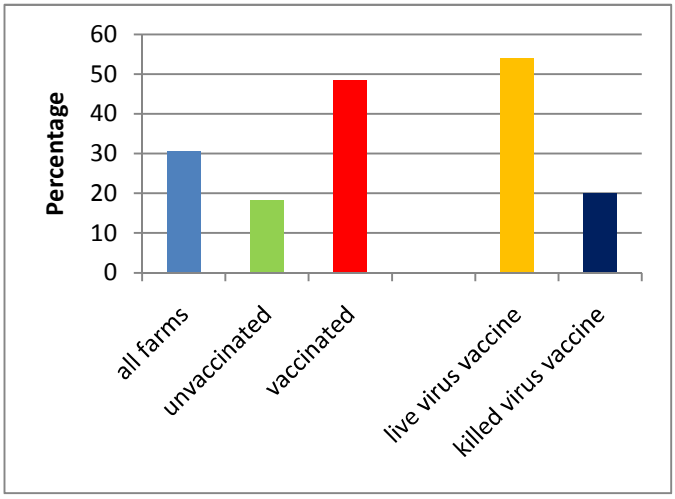
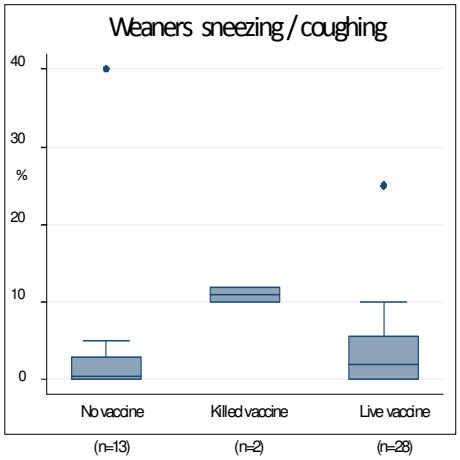
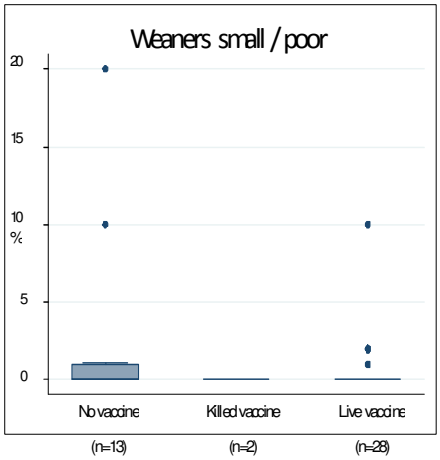


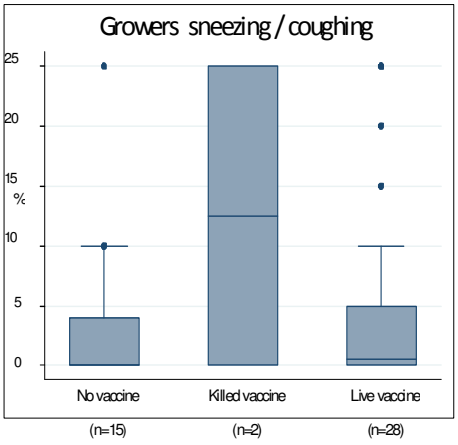
Figure 2. Prevalence of PRRS in England on all farms and according to their vaccination status

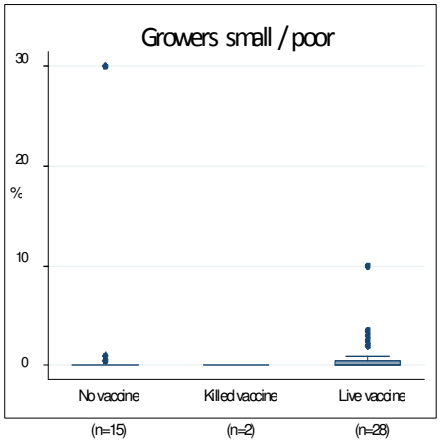
Clinical signs observed in each age group on vaccinating and non-vaccinating farms are presented in Figure 3 for PRRRS positive and negative farms. There was no clear pattern observed, however, on vaccinated farms, the median proportion of pigs showing clinical signs was higher amongst weaners (4-10 weeks of age) and, on non-vaccinated farms, amongst finishers (> 15 weeks). Statistically, there was no evidence of a difference between non-vaccinated farms or farms using live or killed vaccines (Kruskal-Wallis test, p-value > 0.05).

Figure 3a)


Weaners sneezing / coughing
40
30
%
20
10
0
No vaccine
Killed vaccine
Live vaccine
(n=13)
(n=2)
(n=28)


Weaners small / poor
20
15
10
%
5
0
No vaccine
Killed vaccine
Live vaccine
(n=13)
(n=2)
(n=28)


Growers sneezing / coughing
25
20
15
%
10
5
0
No vaccine
Killed vaccine
Live vaccine
(n=15)
(n=2)
(n=28)


Growers small / poor
30
20
%
10
0
No vaccine
Killed vaccine
Live vaccine
(n=15)
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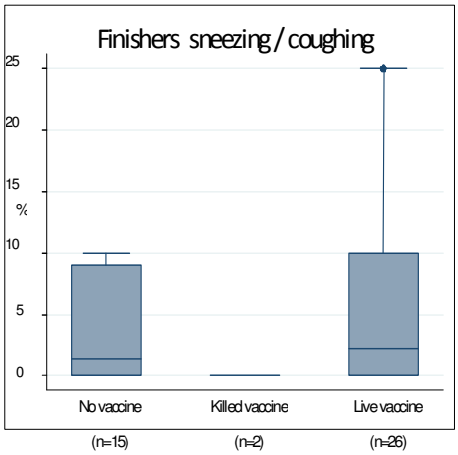
(n=28)


Finishers sneezing / coughing
25
20
15
%
10
5
0
No vaccine
Killed vaccine
Live vaccine
(n=15)
(n=2)
(n=26)

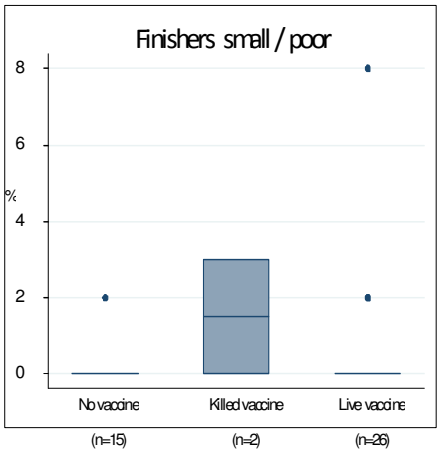

Finishers small / poor
8
6
%
4
2
0
No vaccine
Killed vaccine
Live vaccine
(n=15)
(n=2)
(n=26)

Figure 3b)

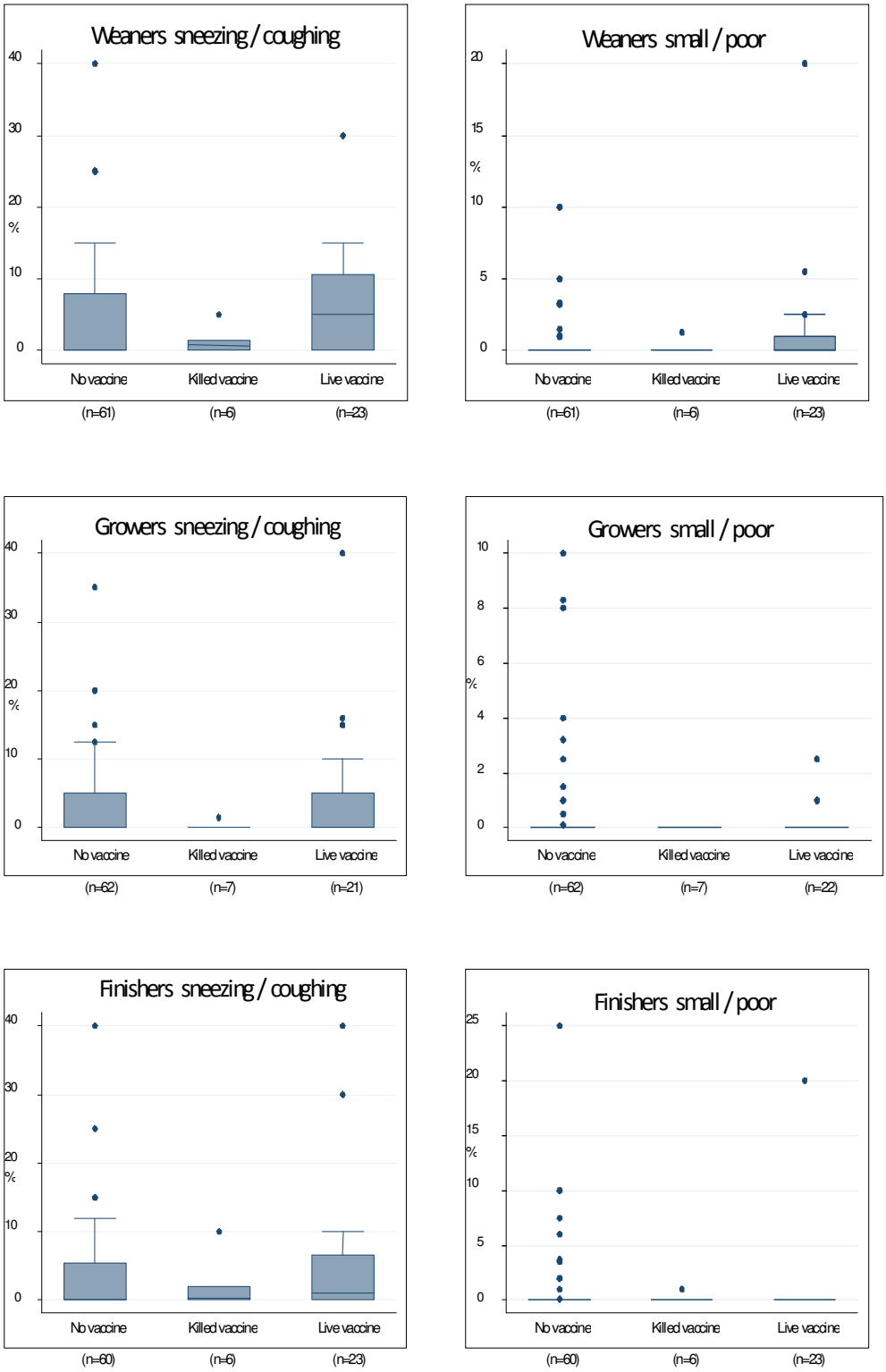


Figure 3a) Percentage of weaners, growers and finishers showing clinical sign on PRRS positive farms by vaccination status. **3b)** Percentage of weaners, growers and finishers showing clinical sign on PRRS negative farms by vaccination status.

3.2 Risk factor analysis

Table 3 summarizes the variables identified as associated with PRRS positive farms in univariable analysis (p ≤0.20) and which were retained after collinearity checks.

Table 3. Descriptive summary of exposures and their crude association with PRRS status

Variable name	value	Number of cases	Number of non cases	OR	95% CI	p-value
Herd size	<250 sows	14(31.82)	41(43.16)	1	-	
	≥250 sows	30(68.18)	54(56.84)	1.62	0.76-3.45	0.20
Farm type:	Outdoor	6(14.63)	25(26.60)	1	-	
	Indoor	35(85.37)	69(73.40)	1.99	0.83-4.78	0.12
PRRS vaccine	None	15(34.09)	67(68.37)	1	-	
	killed	1(2.27)	7(7.14)	1.11	0.21-5.80	
	live	28(63.64)	24(24.49)	5.21	2.38-11.38	<0.001
APP ELISA	negative	7(15.56)	32(31.37)	1	-	
	positive	38(84.44)	70(68.63)	2.48	1.00-6.15	0.05
H1N1	negative	30(68.18)	85(84.16)	1	-	
	positive	14(31.82)	16(15.84)	2.48	1.08-5.68	0.02
Age at weaning in days	<28	30(66.67)	40(40.82)	1	-	
	≥28	15(33.33)	58(59.18)	0.34	0.16-0.72	0.004
Disposal of dead pigs	incineration	6(13.33)	41(42.27)	1	-	
	collection	39(86.67)	56(57.73)	4.29	1.65-11.15	0.001
Frequency of live animals	never	13(29.55)	28(30.77)	1	-	
	1-6/year	2(4.55)	17(18.65)	0.45	0.18-1.14	
	>6/year	20(45.45)	14(15.38)	2.87	1.12-7.34	<0.001
Pig density*	<15 000	19(42.22)	78(77.23)	1	-	
	≥15 000	26(57.78)	23(22.77)	4.64	2.18-9.84	<0.001
Proportion of Pietrain	0	19(43.18)	52(55.32)	1	-	
	0-25	8(18.18)	20(21.28)	1.09	0.41-2.89	
	>25	17(38.64)	22(23.40)	2.11	0.92-4.81	0.177
Other production species present	No	44(80.00)	56(62.22)	1	-	
	Yes	11(20.00)	34(37.78)	0.41	0.18-0.90	0.025

*total number of pigs within 10km radius from the farm

After controlling for the effect of herd size and production type, five risk factors were identified (Table 4).

Table 4. Risk factors for PRRS identified in multivariable logistic regression model, adjusted for herd size and production type, N=127, R^2=0.38. P-value of 0.726 from adjusted Hosmer-Lemeshow goodness of fit test indicated a good model fit.

Variable name	Value	OR	95% CI	p-value*
PRRS vaccine	None	1	-	
	Killed	0.22	0.01-2.98	0.256
	Live	3.65	1.24-10.73	0.019
Frequency of live animals brought on farm	Never	1	-	
	1-6/year	0.28	0.07-1.05	0.060
	>6/year	2.89	0.74-11.25	0.126
Dead pigs disposal	Incineration	1	-	
	Collection	9.00	2.40-33.67	0.001
Pig density (in 10 km radius)	<15 000	1	-	
	>15 000	4.23	1.39-12.88	0.011
Age at weaning in weeks	<28	1	-	
	>=28	0.22	0.07-0.65	0.007

*Wald test p-value

The results suggested that farms with live vaccines were 3.65 times more likely to be PRRS positive compared to non-vaccinated farms.

Further, farms where dead pigs are collected were at greater risk of being PRRS positive compared to those using on-farm incinerators (OR=9.00, 95% CI: 2.40-33.67, p=0.001).

Farms in high pig density areas had higher odds to test positive for PRRS (OR=4.23, 95% CI: 1.39-12.88, p=0.011). Age at weaning ≥ 28 days was, however, identified as a protective factor compared with the baseline (OR=0.22, 95% CI: 0.07-0.65, p=0.007).

Purchase of live animals more than six times per year was no longer statistically significant (p=0.126), however it was retained in the model as it improved overall model fit (LR test p-<0.05).

3.3 Surveillance system analysis

Farms located in high pig density areas using live vaccine had the highest effective probability of infection (EPI) compared to other risk strata. Estimated proportions of pig herds per risk stratum and corresponding EPIs are summarized in Table 5.

Table 5. Effective probability of infection and estimated proportions of pig herds in individual risk strata, N=2962

Risk strata	Percentage (%)	EPI*
HAD + live vaccine	10.24	0.528
HDA + no live vaccine	18.16	0.284
LDA + live vaccine	25.82	0.385
LDA + no live vaccine	45.79	0.207

*EPI=effective probability of infection

The probability of infected farms being detected through passive surveillance is shown in Table 6. When all 2962 pig specialist holding farms were considered, the mode probability was 1.18%. When stratified by risk strata, farms located in high and low density areas which did not use live vaccine had higher mode for the probability of being detected when infected by PRRSV compared to those who used live vaccine. On average, farms located in high density areas had slightly higher mode for detection compared to farms in low density areas.

Table 6. The probability that farms infected with PRRSV will be detected through passive surveillance assuming 30% herd prevalence considering all farms and farms in individual risk strata. The probabilities are shown as proportions.

			Percentiles	
Probability of detection of infected farm	n	Mode	5^{th}	95^{th}
All farms	2962	0.0118	0.0074	0.0203
HAD* + live vaccine	303	0.0044	0.0015	0.0146
HDA + no live vaccine	538	0.0163	0.0065	0.0386
LDA* + live vaccine	765	0.0041	0.0015	0.0147
LDA + no live vaccine	1356	0.0133	0.0066	0.0390

*HDA=high pig density area, LDA=low pig density area

The sensitivity analysis found that probability of an infected pig showing clinical signs as the most important input parameter.

4 Discussion

This study aimed to improve knowledge of the epidemiology of PRRS by identifying possible risk factors for active PRRS infection. When compared to other studies, broader aspects of production and management practices were considered in the risk factor analysis.

The results of this study highlight that PRRSV continues to be a threat for English pig industry. The estimated prevalence of PRRS infection (30.61%; 95% CI: 23.07-38.15) suggest continuous exposure to PRRSV. However, when interpreting this result, the possibility of selection bias needs to be considered. Initial recruitment of farms was conducted through PCV2 vaccination program which could result in a higher proportion of farms with health problems being selected. A proportion (20%) were recruited through practitioners to improve the representativeness of the pig population. A classification of a farm as PRRS positive based on one positive sample was made on less than half of the farms.

To estimate the true prevalence of PRRS infection, sensitivity (Se) and specificity (Sp) of diagnostic tests would need to be considered. Assuming that Se and Sp of Biobest in-house ELISA test is the same as 97.8% and 100% respectively, reported elsewhere [23], would result in no false positives being detected. Less than 100% Se could result in some infected farms being missed; however, results were interpreted at herd-level which improved Se.

Case definition also has to be considered. Due to financial and time constraints, only growers and finishers from vaccinated farms were tested by RT-PCR as relatively common use of PRRSV vaccination has compromised interpretation of ELISA test results of these farms. Ideally, a case definition would be based on the same laboratory test for all farms. Exclusion of sows and weaners from the case definition was supported by the fact that maternal antibodies in piglets can persist up to ten weeks of age [24, 25] and that seropositivity of young stock is more indicative of virus persistence within the population, whereas in adults it could indicate past exposure [11].

This study further identified a high proportion (> 85%) of positive farms in North Yorkshire and East Anglia. However, this result is not surprising as both regions are engaged in intensive production accounting for more than half of the overall pig production in England and thus create a higher proportion of susceptible population for transmission and persistence of infection.

Current knowledge of epidemiology and biology of PRRS was applied when performing statistical analysis. A number of exposures such as: type of ventilation for lactating sows and weaners; type of lighting for lactating sows; presence of slurry; presence of cattle and poultry; and proportion of Duroc in finishing pigs, was found to be associated with PRRS farm status ($p \leq 0.2$). However, due to strong collinearity ($p < 0.001$) with primary exposures, they could not be retained for further analysis.

Using a multivariable logistic regression model, several risk factors were identified. Herd size and production type were considered as priori confounders. Without controlling for their effect, the strength of the association between use of live vaccine and PRRS farm status would have been slightly overestimated (OR=4.12 vs. OR=3.65) and the association between disposal of dead pigs by collection (OR=7.36 vs. OR=9.00), increased frequency of animals brought on the farm (OR=1.98 vs. OR=2.89) and high pig density (OR=2.61 vs. OR=4.23) would have been slightly underestimated.

Interaction between individual pairs of exposures except one (PRRS vaccination type and frequency of live animal brought on farm) was assessed and was not found. In the latter case, interaction term was fitted in the model; however due to insufficient number of observations in individual categories could not be compared using LR test. Normally, this issue could be rectified by regrouping exposures to increase the number of observations in individual categories. In this case, it was important to maintain the original number of categories indicating different exposure levels.

Overall, the risk factors identified were not surprising except the live vaccination. Farms using live virus vaccine were more likely to be PRRS positive compared to those non-vaccinated farms (OR=3.65, 95% CI: 1.24-10.73). Based on results from PCR, it is difficult to say whether the detected virus originated from the vaccine or a different field strain. Live vaccine is licensed for use in breeding or growing pigs. However, in this study, most farmers (93.65%) vaccinated gilts and/or sows. Only four farms vaccinated piglets. Therefore the virus detected in growers or finishers should not have originated from the use of live vaccine, unless the vaccine strain is circulating on the farm. For example, one recent study reported higher detection of vaccine virus in vaccinated pigs compared to non-vaccinated (OR=9.4, p=0.004) [26]. Previously, evidence of presence of vaccine virus in finishing pigs was also demonstrated [27].

In any case, it can be only speculated here that either live vaccine is not protective for the circulating field strain and has no or a limited impact on its circulation within the herd or the

vaccine strain is able to replicate and is transferred to susceptible pigs. The latter would be in disagreement with live virus vaccine requirement of minimal natural transmission [7]. Further investigation targeting open reading frame (ORF) 5 followed by sequencing [28] would be needed in order to confirm the type of virus strain.

In general, vaccination is perceived as an effective control measure and for many pig producers it is the only possible control measure. It is therefore important to mention that no significant difference in presence of clinical signs on vaccinated farms compared to non-vaccinated was observed in this study.

It may also be worth investigating further whether current vaccines are effective in protecting animals from infection or just from disease. For example, vaccination against enzootic pneumonia (EP) is known to prevent clinical disease but does not confer full protection against the infection [29]. The role of secondary pathogens (such as H1N1 and APP found in this study) and the possibility of genetic drift occurring in the virus for which the vaccine strain may not be fully protective (personal communication with M. White, MRCVS) could be considered.

Further results showed that farms located in high pig density areas were at higher risk of being PRRS positive compared to low pig density areas. This finding is in agreement with other studies [11, 12] and can be explained by high transmissibility of the virus within susceptible populations [30]. Aerosol transmission over the short distance has also been demonstrated experimentally [31, 32] and under natural conditions [10]. It is also likely that those farms use the same feed suppliers, transporters or veterinarians, which would increase contact.

This study also highlights the importance of the biosecurity measures in place. Both risk factors: collection of dead pigs and increased frequency of pigs brought on farms, are indicative of an infected pig being the primary source of infection. Direct and indirect transmission through these animals, vehicles and people involved in their handling could explain this finding.

Farms weaning piglets at the age of ≥ 28 days had lower odds of being PRRS positive compared to those weaning at an earlier age. Whilst those farms also tended to use straw yards, protective clothing, hospital pens, slurry systems and had pig clean requirements for visitors (which could have a protective effect), others reports suggest that weaning age may be an important host factor in the epidemiology of respiratory diseases in pigs and that with increased weaning age, the risk of infection decreases [33, 34].

Two risk factors: pig density and use of live virus vaccination, were used for the development of the stochastic model to evaluate passive surveillance. A low probability of an infected farm being detected through this surveillance (mode=0.018, 5^{th} and 95^{th} percentiles: 0.0074; 0.0203 respectively) highlights the importance of underreporting as the main limitation of passive surveillance. Factors impacting reporting and disease control include: the severity of clinical sings and disease awareness amongst farmers; a previous history of PRRS; as well as willingness to report the disease to veterinarians. Lack of financial resources may also contribute to low detection of PRRS.

Lower probability of detecting infection amongst farms using live vaccination could be due to the fact that they are less likely to observe severe clinical signs or believe that clinical signs are due to PRRS infection. This could result in infection going unnoticed with potential spread amongst susceptible pig populations and hinder control measures put in place. It is therefore important to increase awareness amongst farmers and veterinarians that vaccination may not confer solid protection against PRRS which could result in control measures being more effective and consequently improve overall performance of passive surveillance.

Due to the lack of available information, some assumptions were made regarding awareness of disease amongst farmers and veterinarians and the probability of sample submission for diagnostics. Where possible, data from the cross-sectional study, such as proportion of farms using live virus vaccine or pigs showing clinical signs was used to estimate input parameters. In particular, respiratory signs and poor growth observed on PRRS positive farms were considered. When compared with data from AHVLA (unpublished data) for the same period of time, respiratory signs and wasting accounted for the highest frequency of clinical signs seen in PRRS submissions. This, to a certain extent, validates the use of this data. However, as clinical signs were identified to be the most sensitive input parameter, a more robust way of collecting this data should be considered in future.

The model developed here is based not only on the risk factors for PRRS but also highlights the epidemiology and the importance of farmers and veterinarians in detection and control of PRRS.

Given the prevalence of PRRS in the English pig population, passive surveillance has been identified to be performing poorly. Further enhancement in combination with targeted surveillance should be considered.

Overall, when taking distribution of the farms and different management practices into account, this study is considered to be representative of the English pig population. As some of the data was collected retrospectively, the possibility of recall bias should be considered when interpreting the results.

5 Conclusions

The results of this study confirm the high prevalence of PRRS in the English pig population with higher tendency of occurrence in high pig density areas and thus indicate that control measures in place are far from being effective.

The stochastic model developed for the evaluation of passive surveillance suggests a low probability of detection of infected farms. This is of particular concern when considering the possibility of emerging strains of PRRSV.

To improve control measures in place, developing surveillance targeting farms with higher risk of PRRS infection should be considered. Moreover, current biosecurity measures and vaccination should be re-evaluated and tailored to the PRRS situation on individual farms.

Successful control of PRRS will contribute to the improvement of the overall pig health with positive impacts on the competitiveness of British pork.

Word count: 4999

6 References

1. Zuckermann, F.A., et al., Assessment of the efficacy of commercial porcine reproductive and respiratory syndrome virus (PRRSV) vaccines based on measurement of serologic response, frequency of gamma-IFN-producing cells and virological parameters of protection upon challenge. Vet Microbiol, 2007. **123**(1-3): p. 69-85.
2. Christianson, W.T.H.J., Porcine reproductive and respiratory syndrome: A review. Swine Health and Production, 1994. **2**(2): p. 10-28.
3. Anon., The probability of transmission of Porcine Reproductive and Respiratory Syndrome virus (PRRSv) to naive pigs via fresh meat. The EFSA Journal, 2005. **239**: p. 1-85.
4. Neumann, E.J., et al., Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. J Am Vet Med Assoc, 2005. **227**(3): p. 385-92.
5. Wensvoort, G., et al., Mystery swine disease in The Netherlands: the isolation of Lelystad virus. Vet Q, 1991. **13**(3): p. 121-30.
6. Brinton, M.A., et al., Arteriviridae. Virus Taxonomy, 2000. Seventh Report of the International Committee on Taxonomy of Viruses.
7. Mateu, E. and I. Diaz, The challenge of PRRS immunology. Vet J, 2008. **177**(3): p. 345-51.
8. Stadejek, T., et al., Porcine reproductive and respiratory syndrome virus strains of exceptional diversity in eastern Europe support the definition of new genetic subtypes. J Gen Virol, 2006. **87**(Pt 7): p. 1835-41.
9. Paton, D.J., et al., Isolation of a Lelystad virus-like agent from British pigs and scanning electron microscopy of infected macrophages. Vet Microbiol, 1992. **33**(1-4): p. 195-201.
10. Edwards, S., et al., PRRS ("Blue-Eared Pig Disease") in Great Britain. AASP International PRRS Symposium Edition, 1992: p. 32-36.
11. Evans, C.M., G.F. Medley, and L.E. Green, Porcine reproductive and respiratory syndrome virus (PRRSV) in GB pig herds: farm characteristics associated with heterogeneity in seroprevalence. BMC Vet Res, 2008. **4**: p. 48.
12. Mortensen, S., et al., Risk factors for infection of sow herds with porcine reproductive and respiratory syndrome (PRRS) virus. Prev Vet Med, 2002. **53**(1-2): p. 83-101.

13. Weigel, R.M., L.D. Firkins, and G. Scherba, Prevalence and risk factors for infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in swine herds in Illinois (USA). Veterinary Research, 2000. **31**(1): p. 87-88.
14. Kimman, T.G., et al., Challenges for porcine reproductive and respiratory syndrome virus (PRRSV) vaccinology. Vaccine, 2009. **27**(28): p. 3704-3718.
15. OIE, Animal health surveillance. Chapter 1.4., Volume I. In: Terrestrial Animal Health Code, 2011.
16. Hadorn, D.C. and K.D. Stark, Evaluation and optimization of surveillance systems for rare and emerging infectious diseases. Vet Res, 2008. **39**(6): p. 57.
17. Anon., GB surveillance Pig diseases quarterly report, Q4. Veterinary Laboratories Agency, 2008.
18. Yang, Z.Z., et al., Detection of PCV2 DNA by SYBR Green I-based quantitative PCR. J Zhejiang Univ Sci B, 2007. **8**(3): p. 162-9.
19. Dohoo, I., W. Martin, and H. Stryhn, Chapter 15: Model-Building Strategies. In: Veterinary Epidemiologic Research 2009.
20. Hosmer, D.W., et al., A comparison of goodness-of-fit tests for the logistic regression model. Stat Med, 1997. **16**(9): p. 965-80.
21. Martin, P.A., A.R. Cameron, and M. Greiner, Demonstrating freedom from disease using multiple complex data sources 1: a new methodology based on scenario trees. Prev Vet Med, 2007. **79**(2-4): p. 71-97.
22. Hadorn, D.C., et al., Establishing a cost-effective national surveillance system for Bluetongue using scenario tree modelling. Vet Res, 2009. **40**(6): p. 57.
23. Ferrin, N.H., et al., Validation of a blocking enzyme-linked immunosorbent assay for detection of antibodies against porcine reproductive and respiratory syndrome virus. Clin Diagn Lab Immunol, 2004. **11**(3): p. 503-14.
24. Yoon, K.-J., J. Christopher-Hennings, and E.A. Nelson, Diagnosis of PRRS virus. In: PRRS Compendium - Producer Edition, 2003.
25. Murtaugh, M.P., Z. Xiao, and F. Zuckermann, Immunological responses of swine to porcine reproductive and respiratory syndrome virus infection. Viral Immunol, 2002. **15**(4): p. 533-47.
26. grosse Beilage, E., et al., Frequency of PRRS live vaccine virus (European and North American genotype) in vaccinated and non-vaccinated pigs submitted for respiratory tract diagnostics in North-Western Germany. Preventive Veterinary Medicine, 2009. **92**(1-2): p. 31-37.

27. Magar, R. and R. Larochelle, Evaluation of the presence of porcine reproductive and respiratory syndrome virus in pig meat and experimental transmission following oral exposure. Can J Vet Res, 2004. **68**(4): p. 259-66.
28. Christopher-Hennings, J., et al., Porcine reproductive and respiratory syndrome (PRRS) diagnostics: Interpretation and limitations. Journal of Swine Health and Production, 2002. **10**(5): p. 213-218.
29. Haesebrouck, F., et al., Efficacy of vaccines against bacterial diseases in swine: what can we expect? Vet Microbiol, 2004. **100**(3-4): p. 255-68.
30. Nodelijk, G., et al., Introduction, persistence and fade-out of porcine reproductive and respiratory syndrome virus in a Dutch breeding herd: a mathematical analysis. Epidemiology and Infection, 2000. **124**: p. 173-182.
31. Torremorell, M., et al., Airborne transmission of Actinobacillus pleuropneumoniae and porcine reproductive and respiratory syndrome virus in nursery pigs. Am J Vet Res, 1997. **58**(8): p. 828-32.
32. Kristensen, C.S., et al., Experimental airborne transmission of PRRS virus. Vet Microbiol, 2004. **99**(3-4): p. 197-202.
33. Gardner, I.A. and D.W. Hird, Host determinants of pneumonia in slaughter weight swine. Am J Vet Res, 1990. **51**(8): p. 1306-11.
34. Stark, K.D.C., Epidemiological Investigation of the Influence of Environmental Risk Factors on Respiratory Diseases in Swine--A Literature Review. The Veterinary Journal, 2000. **159**(1): p. 37-56.