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Epidemiological investigations in regard to porcine reproductive and respiratory syndrome (PRRS) in Quebec, Canada. Part 2: Prevalence and risk factors in breeding sites

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major threat for swine industry and understanding factors involved in its epidemiology is undoubtedly essential for disease control. As a part of a larger project, a cross-sectional study was performed on breeding sites in a moderate density area of swine production in Quebec to estimate the prevalence of PRRSV infected sites and to evaluate if characteristics of sites and biosecurity practices, either as specific measures or as a global score, were associated with PRRSV status. A questionnaire and diagnostic procedures were performed on 54 breeding sites between September 2006 and August 2008. A biosecurity score that had been previously computed using two-step clustering procedure was used, classifying breeding sites into two biosecurity patterns (high vs. low) according to 21 specific biosecurity measures. The apparent prevalence of PRRSV infected sites was 74.0% (95% CI, 60.3–85.0). Univariable and multivariable logistic regression models with robust standard errors adjusting for potential clustering of sites due to same ownership were computed. In a first multivariable model evaluating characteristics of sites and specific biosecurity variables, four main effects were significantly associated ($P < 0.05$) with PRRSV positive status: large pig inventory (OR: 10.7), proximity to closest pig site (OR: 7.3), absence of shower (OR: 8.7) and free access to the main entrance of the site by the rendering truck (OR: 7.0). In a second multivariable model including a global biosecurity score as a surrogate for a specific pattern of biosecurity measures, this score was not retained in the final model. The adjusted population attributable fractions were 16% for the proximity to closest pig site variable, 27% for the absence of shower variable, and 10% for the free access to main entrance of the site by the rendering truck. These two latter biosecurity measures, manageable directly on the site, should be prioritized and be part of any intervention strategy designed for PRRSV control.

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1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is caused by a spherical, enveloped, single-stranded and positive-sense RNA virus belonging to the family of *Arteriviridae* (Cavanagh, 1997). The disease is responsible for major economic losses in swine industry, affecting all stages of production (Neumann et al., 2005). In breeding herds, the virus induces late-term abortions, reduces farrowing rate, leads to the production of heterogeneous litters (stillborn, mummified or weak piglets) and to a decrease in the number of weaned piglets (Christianson and Joo, 1994). In weaner-finisher phases, the infection is

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associated with respiratory signs, increased mortality rate and a decrease in growth performances (Christianson and Joo, 1994).

The important heterogeneity observed among PRRS virus (PRRSV) strains of both known genotypes, the European and the North American, combined with the absence of complete protection following heterologous challenge complicate disease management (Meng et al., 1995a,b; Lager et al., 1999; Meng, 2000; Forstberg, 2002; Prieto et al., 2008). In fact, protection against a heterologous PRRSV strain is not warranted through commercial vaccination since both modified-live vaccines currently available are based on a single PRRSV strain each. Avoiding introduction of the virus into a herd is therefore essential, but difficult, considering the several means of transmission reported in the literature. Introduction of infected semen or boars are important risk factors for occurrence of the disease in a herd (Le Potier et al., 1997; Mortensen et al., 2002). Purchase of gilts from PRRSV positive herd or movement of other types of infected animals between different stages of production represents also a known mechanism of transmission between production sites (Mousing et al., 1997; Larochelle et al., 2003). Furthermore, vehicles could be an important actor of regional spread of PRRSV since they are capable of conveying the virus over significant distances on their wheels (Dee et al., 2002). Other fomites carried by people, including boots, coveralls and exterior surfaces of shipping containers are also suspected as potential ways of virus introduction (Dee et al., 2002; Otake et al., 2002a; Pitkin et al., 2009). In addition, live mechanical vectors should be considered in the epidemiology of the disease: flying insects and potentially, rodents, waterfowl, as well as other wild and domestic animals (Hooper et al., 1994; Zimmerman et al., 1997; Otake et al., 2002b; Trincado et al., 2004). Finally, since viable PRRSV is isolated from aerosols sampled several kilometres from an infected population source, this latter mechanism can also favour area spread of the virus between different production sites (Otake et al., 2010).

Considering the several pathways of PRRSV transmission, internal and external biosecurity could be implemented to slow down the transmission of an endemic strain within a herd as well as between herds (Amass and Clark, 1999; Dargatz et al., 2002; Barrington et al., 2006). The effectiveness of all generally recommended biosecurity measures against PRRSV introduction has not yet been assessed in field studies, but some experiments have been conducted on strategies to limit mechanical transmission of the virus through aerosols, vehicles visiting the site or personnel/fomites entering the barn (Dee et al., 2004, 2007, 2010). PRRSV-negative herd survival was found to be positively associated with sanitation procedure for employees and visitors entering the site, restrictions on employee access to site and thermo-assisted drying of vehicles (Holtkamp et al., 2010a). Relationship between biosecurity measures implemented in the field and PRRSV status of production sites is important to assess in order to plan preventive measures to avoid transmission of the virus between sites in a perspective of regional management of the disease. Therefore, the objectives of this study were to estimate the prevalence of PRRSV infected sites and to determine whether herd and neighbourhood characteristics of sites and biosecurity practices, evaluated as specific measures or as a global score, were associated with PRRSV status of breeding sites in a moderate density area of swine production in Quebec.

2. Materials and methods

2.1. Study design and source population

A cross-sectional study was conducted in the Estrie region of the province of Quebec, Canada, between September 2006 and August 2008. This region represents a moderate density area of swine production with 44 pigs/km^2 (Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, 2010). The source population was all production sites housing sows. Site was defined as one or more barns located within 300 m from another, belonging to the same owner (individual or corporate), and having the same animal source(s). In order to select sites, all producers listed into the Quebec Federation of Pork Producers (FPPQ) database and registered as owner of sow operation(s) were contacted. A written description of the project and a participation form to be signed and returned were initially sent to producers, and participation was on a voluntary basis. For refusal or in the absence of a response, producers were contacted by phone to seek for their participation or to inquire for reasons of refusal.

2.2. Questionnaire

A questionnaire was developed to assess the potential risk factors reported in the literature for introduction of PRRSV within a site. Four veterinarians specialized in swine production and three producers were consulted to assess relevance, clarity and completeness of questions. This questionnaire (in French) comprised mostly semi-closed questions, and is available upon request from the first author. The questionnaire was filled out by the first author during a 45 min live interview with the owner of independent farm or the employee for farms under contract. When it was impossible to perform live interview (mainly because the producer was not available on the site), the questionnaire was completed during a phone interview. All questions pertained to practices at the time of questionnaire completion with the exception of questions regarding gilt purchases (previous six months). Data were obtained on different herd and neighbourhood characteristics of participating sites (Table 1) and on specific external biosecurity practices (Table 2).

2.3. Sampling strategy and laboratory analysis

Assessment of PRRSV status of production sites varied according to PRRS history and clinical signs at the time of sampling as reported by the producer. Sites housing at least one pig positive for presence of PRRSV or antibodies were classified as infected. All diagnostic procedures were done at the Faculty of Veterinary Medicine of the University of Montreal in St. Hyacinthe. The procedures were approved

Table 1
Descriptive statistics for herd and neighbourhood characteristics for breeding sites in Estrie, Quebec, Canada (52 sites).

Variables	Breeding sites $n = 52$
Categorical variables	*n* (%)
Production type	
Farrow-to-wean	11 (21)
Farrow-to-grow	8 (15)
Farrow-to-finish	33 (63)
Ownership	
Independent producer	48 (92)
Contract producer	4 (8)
Pig flow in farrowing rooms	
All-in all-out	32 (62)
Continuous flow	20 (38)
Distance from public road (m)	
>300	13 (25)
≤300	39 (75)
Continuous variables	Median (Q1–Q3)
Total number of sows[a]	233 (160–405)
Total number of animals[b]	1269 (824–2288)
Heat producing unit (HPU)[c]	261 (167–422)
Distance from closest pig site (km)	2.5 (0.9–4.0)

[a] Unbred gilts excluded.
[b] Total of gilts, sows, piglets and finishing pigs.
[c] 1 HPU = 1000 W at 20 °C; calculated using the following equation, HPU = 0.17 × (weaners and finishers) + 0.30 × (gilts and sows) (Flori et al., 1995).

by the Ethics board on animal use of the Faculty of Veterinary Medicine (certificate number: 09-rech-1291).

2.3.1. On sites with lifelong herd clinical history of PRRS or using commercial vaccination

For sites with clinical signs compatible with PRRS at the time of sampling or using commercial vaccination, animals were sampled in order to confirm their positive status to PRRSV infection and also to maximize the probability of identifying a PRRS viral strain. A pool of lungs, tonsils and tracheobronchial lymph nodes was collected at time of necropsy of 1–3 suckling piglets, weaners or finishers, according to the stage of production being most clinically affected by dyspnea. At the onset of an outbreak with presence of abortions, sera were drawn from sows. In absence of clinical signs compatible with PRRS at the time of sampling, 10 samples were drawn from animals at higher risk of viremia (gilts recently introduced into the breeding site or piglets in mid-nursery) and one pool (maximum 5 samples per pool) was sent for further analyses.

RNA was extracted from a homogenate of lungs, tonsils and lymph nodes or from serum with QIAamp viral RNA mini kit according to the manufacturer's instructions (Qiagen Inc., Mississauga, Ontario, Canada). Subsequently, RT-PCR was accomplished using Qiagen OneStep RT-PCR Kit and primers 5FN and 5DN under PCR conditions for detection of viral RNA as described by Larochelle et al. (2003). In the presence of a RT-PCR positive sample, ORF5 sequencing was performed. PCR products were purified before sequencing with Qiaquick spin kit (Qiagen). Sequencing of ORF5 was done on both directions of PCR products using amplification primers with BigDye terminator on ABI PRISM 310 Genetic analyzer (Applied Biosystems Canada, Streetsville, Ontario, Canada). In the presence of a RT-PCR negative result, additional samples were submitted to RT-PCR when pigs showing clinical signs were available for necropsy. If RT-PCR results were again negative, the strategy described below was used.

2.3.2. On sites with no lifelong herd clinical history of PRRS

When no clinical history of PRRS was reported by the producer and no commercial vaccination was performed on the site, samples were taken to confirm absence of infection. To that purpose, 30 sows of different parities were sampled. This strategy allowed the detection of infection in large sites assuming a 10% seroprevalence with a 95% herd confidence level. Sera were tested for presence of antibodies by enzyme-linked immunosorbent assay (ELISA) commercially available (IDEXX HerdChek-PRRS 2XR) as originally described by Albina et al. (1992) and performed as recommended by the manufacturer. Seropositivity threshold was fixed to a sample-to-positive (S/P) ratio ≥0.4. When ≤2 sample(s) out of 30 were positive, these samples were retested by ELISA. The site was classified as positive when at least one sample was ELISA positive after retest. In the presence of a positive ELISA, all positive sera were pooled up to a maximum of 5, sent for RT-PCR and submitted to the same procedure as described above.

2.4. Statistical analysis

Information obtained from the questionnaire and diagnostic procedures was coded, entered into a spreadsheet and entries were double checked. The number of heat producing units (1 HPU = 1000 W at 20 °C) was calculated to allow comparison of pig inventory among farrow-to-wean, farrow-to-grow and farrow-to-finish sites using the following equation, HPU = 0.17 × (weaners and/or finishers) + 0.30 × (gilts or sows) (Flori et al., 1995).

Apparent prevalence of PRRSV infected sites was calculated as the proportion of sites housing at least one pig presenting PRRS antibodies or having RT-PCR positive results. Confidence interval (95% CI) based on an exact method was computed around the estimated apparent prevalence using the SAS version 9.1 software (SAS Institute Inc., Cary, NC, USA).

Predictors associated with PRRSV status were identified using univariable logistic regression models with robust standard errors computed in the Stata version 8.0 software (StataCorp., College Station, TX, USA), adjusted for potential clustering of sites managed by the same owner (independent producer or integrated pig company). Sites having one or more missing value for predictors investigated were excluded. Continuous variables were dichotomized around median of the distribution due to violation of linearity assumption based on graphical exploration of the log odds. Univariable associations with a *P*-value ≤0.15 were selected for inclusion in full multivariable logistic regression models using robust standard errors. Multicolinearity between these latter variables was assessed using multiple linear regression model in SAS seeking for tolerance greater than 0.4. Two different multivariable logistic regression models were then built. The first model

Table 2
Descriptive statistics for predictors tested for association with PRRS positive status for breeding sites in Estrie, Quebec, Canada (52 sites).

Description of predictors	Category	Number of sites	Number of positive sites (%)
Herd and neighbourhood characteristics of sites			
Heat producing unit (HPU)[a]	≤300	31	19 (61)
	>300	21	19 (90)
All-in all-out pig flow in farrowing rooms	Yes	32	24 (75)
	No	20	14 (70)
Distance from public road (m)	>300	13	7 (54)
	≤300	39	31 (79)
Distance from closest pig site (km)	>2.5	25	15 (60)
	≤2.5	27	23 (85)
Specific biosecurity practices			
Layout of the site			
Barrier at the entrance of the site, closed at any time	Yes	1	0 (0)
	No	51	38 (75)
Distance of parking from closest barn (m)	>30	4	2 (50)
	≤30	48	36 (75)
Fence surrounding the site perimeter	Yes	1	1 (100)
	No	51	37 (73)
Entrance protocol for people			
No-entry sign on the entrance door	Yes	35	27 (77)
	No	17	11 (65)
Locked doors at all times	Yes	17	10 (59)
	No	35	28 (80)
Doorbell at the entrance	Yes	20	13 (65)
	No	32	25 (78)
Shower at the entrance	Yes	10	4 (40)
	No	42	34 (81)
Separation between clean and contaminated areas (shower, line, bench)	Yes	16	8 (50)
	No	36	30 (83)
Downtime for visitors (h)	≥24	30	20 (67)
	<24	22	18 (82)
No employee in contact with pigs from other sites, in transport or slaughterhouses	Yes	9	9 (100)
	No	43	29 (67)
Transportation of animals			
Truck washed between loads of pigs	Yes	15	9 (60)
	No	37	29 (78)
Own transport (absence of commercial transport)	Yes	2	1 (50)
	No	50	37 (74)
Access to barn(s) by the driver of commercial company	Yes	6	4 (67)
	No	46	34 (74)
Pigs re-entering the barn after loading	Yes	7	5 (71)
	No	45	33 (73)
Dead pig, pest and manure management			
Incinerator, burying or composting (no rendering)	Yes	19	11 (58)
	No	33	27 (82)
Access to the site by rendering truck	Yes	26	23 (88)
	No	26	15 (58)
Rodent control performed by exterminator	Yes	35	28 (80)
	No	17	10 (59)
Bird-proofed wire screens in barn(s)	Yes	35	27 (77)
	No	17	11 (65)
Access of dog and cat within the barn(s)	Yes	5	4 (80)
	No	47	34 (72)
Manure provided from other sites spread on the site	Yes	3	2 (67)
	No	49	36 (73)
Feed, semen and gilt deliveries			
Access to barn by feed delivery personnel	Yes	14	12 (86)
	No	38	26 (68)
Purchase of semen	Yes	47	33 (70)
	No	5	5 (100)
Access to barn by semen delivery personnel	Yes	11	8 (73)
	No	41	30 (73)
Self-replacement (no purchase of gilts)	Yes	13	10 (77)
	No	39	28 (72)

Table 2 (*Continued*)

Description of predictors	Category	Number of sites	Number of positive sites (%)
Self-replacement or purchase of gilt with quarantine	Yes	14	11 (79)
	No	38	27 (71)
Purchase of PRRS positive gilts	Yes	1	1 (100)
	No	51	37 (72)
Global biosecurity score			
Higher biosecurity pattern	Yes	22	12 (55)
	No	30	26 (87)

[a] 1 HPU = 1000 W at 20 °C; calculated using the following equation, HPU = 0.17 × (weaners and finishers) + 0.30 × (gilts and sows) (Flori et al., 1995).

considered specific biosecurity practices described in Table 2. The second model used a biosecurity score similar to the one described by Lambert et al. (2010), which was obtained using two-step clustering procedure available in SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA) to group sites in different biosecurity patterns according to 21 specific biosecurity measures. A level of biosecurity, low or high, was subsequently attributed to the two patterns obtained based on the frequency of specific biosecurity measures observed in these latter patterns (see article for details). Backward model building strategy was used to select variables, with a $P > 0.05$ as criterion for variable removal. At each step, changes of more than 30% in coefficient estimates were assessed as a sign of confounding. For each final model, Hosmer–Lemeshow goodness-of-fit test was computed and deviance residuals were observed for presence of extreme observations. Predicted probabilities of infection with 95% confidence interval were computed for covariate patterns observed in our dataset. The sensitivity of results to potential misclassification bias of PRRSV status resulting from the vaccination process was evaluated from final models. This was done by recomputing the models without sites performing vaccination on which a field virus strain (defined as a virus with <98% ORF5 pairwise homology with commercial vaccine strains) was not identified and a PRRS outbreak had not been reported in the sow herd within the last 5-year period.

Adjusted population attributable fractions (PAF) were generated in Stata using the aflogit function for cross-sectional data (Brady, 1998). The function recovers PAF estimates for each of the exposure term specified in the final multivariable logistic regression model using robust standard errors. Thus, the procedure enables confounders to be taken into account in the computation. This statistic provides an estimate of the impact of the risk factor on the disease occurrence in the population accounting for both the strength of the association on the outcome and the prevalence of the risk factor in the population (Dohoo et al., 2003). The principle behind using logistic regression to estimate PAF is to fit the multivariable model and then calculate the number of case predicted by the model. Then, the exposure effect is removed from the dataset by resetting exposure covariates to zero. The predicted probability of infection by PRRSV for each site with the new covariate values but under the same multivariable logistic model are recomputed and summing these probabilities gives the number of positive sites one would expect if the exposure was absent from the population.

3. Results

A total of 87 owners of sow operations listed into the FPPQ database were contacted for participation in the study. Nineteen (19) producers were excluded due to halt of production or to the absence of sows on the site, 6 producers refused to participate for lack of interest or time and 11 were unreachable after several calls. This gave an overall participation on an ownership basis of 75%, and resulted in the inclusion of 54 production sites. All questionnaires were filled out during live interviews with the exception of 5 sites (phone interviews). Most of the time (85%), a single barn was present on the site. Of 19 sites suspected PRRSV negative, 14 were confirmed negative, the remaining being classified as PRRSV positive. The 35 sites suspected PRRSV positive were confirmed either by RT-PCR ($n = 29$) or ELISA ($n = 6$). Consequently, the apparent prevalence of PRRSV positive status was estimated to 74.0% (95% CI, 60.3–85.0) for the 54 sites.

Two of the 54 sites were excluded from the risk factor analysis because of missing data from the questionnaire. Commercial vaccination was performed on 14 of the 52 (26.9%) sites. Herd and neighbourhood characteristics of the participating sites are described in Table 1. Table 2 shows the proportion of PRRSV positive sites according to predictors investigated. All negative sites purchased semen from an accredited insemination center negative for PRRSV. When rendering trucks had access to the main entrance of the site, carcasses were picked-up at a mean distance of 35 m from the closest barn. Five variables had to be excluded from regression analyses due to absence of variation in the values for positive or negative sites: barrier at entrance of the site, fence surrounding perimeter, contact of employees with other pigs, purchase of semen and purchase of PRRSV positive gilts. Furthermore, clear separation between clean and contaminated areas and absence of rendering (incinerator, burying or composting) on the site were also excluded for strong colinearity with shower and absence of access to the main entrance of the site by the rendering truck, respectively.

After these exclusions, 24 variables were considered for analyses. Predictors having univariable associations with PRRSV status with P-value ≤ 0.15 and thus included in full multivariable models are presented in Table 3. Four variables were significantly associated with PRRSV status in the first final multivariable model (Table 4). According to this model, sites housing ≤ 300 HPU and located at ≤ 2.5 km from the closest pig site had a predicted probability of being

Table 3
Predictors associated ($P \leq 0.15$) with PRRS positive status using univariable logistic regression with robust SE on ownership (52 sites).

Description of predictors	Odds ratio	95% (CI)	*P*-value
Herd and neighbourhood characteristics			
Heat producing unit >300 (HPU)[a]	6.0	1.3–27.2	0.02
Distance from public road ≤300 (m)	3.3	0.7–16.9	0.15
Distance from closest pig site ≤2.5 (km)	3.8	1.1–12.9	0.03
Specific biosecurity practices			
No locked doors at any time	2.8	0.8–9.5	0.1
No shower at the entrance	6.4	1.7–23.9	<0.01
Rendering with access to the site by rendering truck	5.6	1.5–21.7	0.01
Global biosecurity score			
Lower biosecurity pattern	5.4	1.3–21.8	0.02

[a] 1 HPU = 1000 W at 20 °C; calculated using the following equation, HPU = 0.17 × (weaners and finishers) + 0.30 × (gilts and sows) (Flori et al., 1995).

positive for PRRSV of 32.9% (95% CI, 11.5–65.1) if they were requiring a shower and were not allowing access to the main entrance of the site by the rendering truck. However, their probability of being positive increased to 77.6% (95% CI, 38.2–95.1) in absence of precautions regarding the rendering truck, to 81.0% (95% CI, 57.3–93.1) in absence of shower and to 96.8% (81.4–99.5) in absence of the two latter biosecurity practices. For the second multivariable model, two main effects remained significant when specific biosecurity practices were substituted by a global score: pig inventory and distance from closest pig site. The two final multivariable regression models did not show any lack of goodness-of-fit or influential observations (all deviance residuals <0.7). No evidence of confounding was found during model building between variables dropped and kept in models.

Final models were recomputed excluding the 5 sites for which it was not possible to determine whether the site was infected by a field virus strain or only by a vaccine strain. Similar estimates of main effects were obtained for most variables of model 1: HPU > 300 (OR: 9.8; $P = 0.02$), distance from the closest pig site ≤2.5 km (OR: 7.3; $P < 0.01$), and absence of shower at the entrance (OR: 7.4; $P = 0.01$). However, the access to the main entrance of the site by the rendering truck was marginally not statistically significant (OR: 5.4; $P = 0.06$). In model 2, estimates of main effects were similar (i.e., less than 12% change in odds ratio) and remained statistically significant.

Adjusted population attributable fractions obtained for final model 1 (using herd characteristics and specific biosecurity variables) are presented in Table 5. The PAF estimate for each risk factor takes into account the other three risk factors present in the multivariable model. Due to large confidence intervals, PAF of specific exposures should be compared with caution. Nevertheless, the estimate of PAF generated for absence of shower was twice the size of those obtained for the other risk factors included into the model. Considering quite similar OR among risk factors of final model 1 (Table 4), the relative importance of the prevalence of this latter risk factor suggests that producers could benefit from enhancement of this measure in the field.

4. Discussion

The apparent prevalence of PRRSV positive site was estimated to 74% (CI: 60–85). Of the 103 farms surveyed in Illinois in United States, 49% had been diagnosed with PRRSV infection between 1992 and 1997 (Weigel et al., 2000). Our result was rather similar to the seroprevalence reported in Korea between 1995–1996 for farrow-to-finish herds (69%, $n = 160$) and slightly lower to the one obtained in Spain in 2002–2003 (91%, $n = 44$), both studies being conducted on non vaccinated herds (Cheon et al., 1997; Lopez-Soria et al., 2010). The apparent prevalence we observed raises several concerns regarding feasibility of PRRSV regional control or elimination in this area.

Results showed that PRRSV infected sites were associated with higher odds of having >300 HPU pig inventory than non infected sites. Heat producing unit was used to

Table 4
Predictors associated ($P \leq 0.05$) with PRRS positive status using two different multivariable logistic regression models with robust SE on ownership, models 1 and 2 (52 sites).

Description of predictors	*b*	SE (*b*)	Odds ratio	95% (CI)	Wald test	*P*-value
Model 1[a]	**(using herd/neighbourhood characteristics of sites and specific biosecurity variables)**					
Intercept	−2.69					
Heat producing unit >300 (HPU)[c]	2.37	0.98	10.7	1.6–72.6	2.43	0.02
Distance from closest pig site ≤2.5 (km)	1.98	0.73	7.3	1.7–30.6	2.70	<0.01
No shower at the entrance	2.16	0.78	8.7	1.9–39.6	2.78	<0.01
Access to the site by rendering truck	1.95	0.90	7.0	1.2–41.0	2.18	0.03
Model 2[b]	**(using herd/neighbourhood characteristics of sites and global biosecurity score)**					
Intercept	−0.38					
Heat producing unit > 300 (HPU)[c]	2.10	0.78	8.2	1.8–38.0	2.68	<0.01
Distance from closest pig site ≤2.5 (km)	1.67	0.67	5.3	1.4–19.8	2.50	0.01

[a] Hosmer–Lemeshow goodness-of-fit test, $P = 0.88$.
[b] Hosmer–Lemeshow goodness-of-fit test, $P = 0.49$.
[c] 1 HPU = 1000 W at 20 °C; calculated using the following equation, HPU = 0.17 × (weaners and finishers) + 0.30 × (gilts and sows) (Flori et al., 1995).

Table 5
Population attributable fraction (PAF) obtained from model 1 (52 sites).

Description of predictors	PAF	SE (PAF)	95% (CI)[a]
Model 1 (using herd/neighbourhood characteristics of sites and specific biosecurity variables)			
Heat producing unit >300 (HPU)[b]	0.08	0.05	0.01–0.17
Distance from closest pig site ≤2.5 (km)	0.16	0.07	0.01–0.29
Absence of shower at the entrance	0.27	0.10	0.06–0.43
Access to the site by rendering truck	0.10	0.05	0.00–0.20

[a] CI calculated on log (1-PAF) scale.
[b] 1 HPU = 1000 W at 20 °C; calculated using the following equation, HPU = 0.17 × (weaners and finishers) + 0.30 × (gilts and sows) (Flori et al., 1995).

combine the effect of number and type of animals allowing comparison between sites having different production types, but also to limit the number of variables, a major concern considering our small sample size. Heat producing unit has been previously used in studies on PRRS and other swine respiratory diseases (Flori et al., 1995; Mousing et al., 1997; Mortensen et al., 2002). Larger pig inventories are in general associated with a greater risk of being PRRSV positive (Weigel et al., 2000; Mortensen et al., 2002; Evans et al., 2008; Holtkamp et al., 2010a). Several reasons could explain the impact of pig inventory on PRRSV status, including the expected number of direct (animal to animal) or indirect (person or vehicle to animals) contacts at the herd level. In fact, an increased number of replacement gilts to be introduced into the breeding herd and a higher number of dead pigs or animals that have to be slaughtered or culled could be expected in larger herds. Number of transports could therefore be higher as a consequence of these increased animal movements (Gardner et al., 2002; Ribbens et al., 2009). Furthermore, more contacts from employees and staff from technical services could be expected in larger herds, increasing traffic on the site (Bates et al., 2001; Boklund et al., 2004; Ribbens et al., 2009). Differences could also exist regarding some biosecurity or management practices (Siegel and Weigel, 1999; Gardner et al., 2002; Boklund et al., 2004; Costard et al., 2009) and studies also reveal that larger herds are at higher risk for introduction of pathogens transmitted through aerosols (Christensen et al., 1990; Flori et al., 1995). Furthermore, simulation-based studies showed that the probability of within-herd persistence of the virus increased with herd size, leading to greater time-to-extinction of the disease (Nodelijk et al., 2000).

PRRSV infected sites were associated with higher odds of being located at ≤2.5 km from the closest pig site compared to non infected sites. The effect of proximity or of exposure to infected neighbouring sites is corroborated by others (Mortensen et al., 2002; Evans et al., 2008; Holtkamp et al., 2010a). This effect could be linked to area spread of the virus, a concept introduced to summarize all short-distance processes that could be involved into transmission of PRRSV between sites, without being able to identify the exact mechanism (Lager et al., 2002; Larochelle et al., 2003). Aerosol transmission might be involved since no participating site was under air filtration. In fact, experimental studies showed that PRRSV could be transmitted to susceptible pigs by aerosols on short distances (Kristensen et al., 2004; Dee et al., 2005) and recently, live virus was identified up to 9.1 km from an infected population (Otake et al., 2010). Flying insects could also explain the transmission of the virus between sites during warm season. Mosquitoes (*Aedex vexans*) and houseflies (*Musca domestica*) can serve as mechanical vectors and certainly can contribute to the transmission of the virus between pigs within a site (Otake et al., 2002b, 2004) and between sites considering that infectious virus was identified in flies collected up to 1.7 km from an infected facility (Schurrer et al., 2004). Moreover, a herd located close to another could be more susceptible to higher traffic of vehicles or people that relates to swine industry or to potential transmission through wild-life mechanical vectors (Ribbens et al., 2009). Results showed that proximity from the closest pig site was associated to a high probability for a site to be infected even in absence of other risk factors and was also potentially associated to more than 15% of the infected sites within the study population.

PRRSV infected sites were associated with higher odds of not having a shower for people entering the barn. It was shown that the virus can survive on boots, coveralls and hands of personnel for short period of time and could result in mechanical transmission of the infection to susceptible pigs (Otake et al., 2002a; Pitkin et al., 2009). Even if some elements regarding the entrance protocol were evaluated experimentally for PRRS or for other viral diseases (Otake et al., 2002a; Amass et al., 2004; Dee et al., 2004), the relationships between the different biosecurity requirements and PRRSV status had not been quantified nor widely discussed in field studies (Mortensen et al., 2002; Evans et al., 2008; Holtkamp et al., 2010a,b). However, a positive effect of sanitation procedure for employees and visitors entering the site on the length of time the herd remains free from PRRSV infection is reported (Holtkamp et al., 2010a). Furthermore, mechanical transmission of PRRSV through fomites/personnel was also prevented for a barn requiring shower – in/out and changing clothes/boots in a 4-year regional production model experiment (Pitkin et al., 2010). Therefore, producers should restrict access to the barn to employees essential to maintenance of production and enhance their entrance protocol since it represents the last physical barrier over which the producer can still have some control to limit PRRSV introduction. Furthermore, our results showed that implementation of a shower at the entrance could help considerably in the prevention of the disease, since a quarter of the infected sites in the study population could be associated to the absence of shower.

PRRSV infected sites were associated with higher odds of allowing access to their main entrance by the rendering truck. Limited documentation was found regarding its association with health status of site for PRRS or other diseases. A positive association with the number of rendering lorries entering the farm was reported in a study investigating the occurrence of respiratory outbreaks in fattening pigs (Rose and Madec, 2002). Several findings corroborate the likelihood for PRRSV introduction through this mechanism. In fact, rendering trucks have a high potential of contacts with pathogens from a large number of farms visited each day over a large territory (Bates et al., 2001) and vehicles can carry PRRSV on their wheels up to 50 km, especially in cold weather which enhances virus survival (Dee et al., 2002; Hermann et al., 2007). In addition, the pick-up of carcasses by rendering truck was often very close to the barn which could increase the probability of a farm to be exposed to pathogens conveyed by the truck (Boklund et al., 2004). The effect of the rendering truck could be mediated through contamination of the site followed by a breach in the entrance protocol or through particles conveyed by aerosols or insects in contact with carcasses that are carried by the truck. Excluding the five sites for which it was impossible to distinguish if the site was infected by a field virus strain or only by a vaccine strain, the effect of the rendering truck turned out to be marginally not statistically significant ($P = 0.06$) but with similar size effect.

Multivariate classification has been previously used to describe and resume biosecurity or other management practices that are often correlated with each other (Hurnik et al., 1994b; Boklund et al., 2004; Ribbens et al., 2008; Costard et al., 2009). However, the association between disease status of production sites and a "global" variable resulting from the classification is seldom assessed. Using factor analysis, Hurnik et al. (1994a) classified herds according to management and environmental characteristics and found that some combinations of farm characteristics were associated with the risk of enzootic pneumonia. Baptista et al. (2010) obtained similar findings regarding biosecurity and *Salmonella* status of breeding sites. Regarding PRRSV, Mortensen et al. (2002) grouped variables related to entrance protocol and transport requirements into a biosecurity score and found no significant association with risk of infection. However, Holtkamp et al. (2010b) recently identified that higher external biosecurity risk score was associated with a greater risk of becoming PRRSV positive. In our study, no association between PRRSV status and biosecurity was identified when using a global score for biosecurity practices. Consequently, it does not support the use of a global score to assess the risk of PRRSV infection, especially if the classification is not based on quantitative estimates of the effect of single measures. Few reasons might explain the lack of association. First, specific biosecurity practices were not weighted according to their relative importance against PRRSV introduction. Also, the global score included 21 variables and most of them were not individually associated with PRRSV status of production sites. Moreover, the higher biosecurity pattern did not represent sites applying all biosecurity practices so it could not be interpreted as the combined effect of all biosecurity measures. Also, PRRSV status was defined in terms of prevalence and the score was only based on external biosecurity measures. Consequently, that score may have represented only a part of the potential effect of biosecurity on PRRSV status of production sites.

Some producers did not want to participate in the survey whereas others could not be contacted. The fairly good participation (75%) supported the internal validity of the study, but total absence of selection bias was impossible to assess. Moreover, underestimation of the participation is possible if some unreached producers were out of business. The FPPQ list from which the sample of producers was selected only records those having more than 5000$ CA of annual income from agriculture; thus very small production sites were unlikely to be included in our study. Despite a low biosecurity level observed on small or hobby farms investigated in another country (Costard et al., 2009), it is not documented whether these types of farms are more likely to be PRRSV positive. Our study was also mainly based on commercial sites. Results should not be extrapolated to multipliers or nucleus sites; better observance of biosecurity practices in this type of production could potentially lead to a greater effect of measures compared to commercial sites.

A single interviewer performed all the questionnaires with people working directly on the site to decrease the information bias. Furthermore, most questions referred to the current period of time, decreasing recall bias, and the questionnaire was administrated prior to divulgation of the results pertaining to PRRSV status assessment. However, results relied on practices as they were reported by the workers and not on direct observations raising a misclassification bias concern. People could be tempted to give the answer they were assuming to be the correct one. To that purpose, confidentiality of results was first insured. Also, the degree of compliance with the measure reported in time and by different people was not monitored and could contribute to bias the results toward a higher biosecurity level than what is really observed in reality, especially for some measures difficult to implement on the site. Imperfect diagnostic procedures could also represent a source of information bias. However, considering the high sensitivity and specificity of serological testing, which are over 97% and 99%, respectively, incorrect classification of PRRSV status should be limited (Mateu et al., 2006). Sites for which it was impossible to distinguish between infection by a field virus strain or only by a vaccine strain were kept as positive sites in the analyses in order to avoid a reduction in study power, based on the hypothesis that commercial vaccination was mainly performed to control clinical signs of disease in positive sites.

Due to expense and time limitations, a cross-sectional study was performed. Consequently, it cannot ascertain whether exposure to risk factor was prior to or after the production site became positive to PRRSV. Thus, factors identified could be associated to the risk of new virus introduction, but also to an enhancement of disease persistence within the site and even to a procedure implemented following an outbreak of the disease. Also, the PAF attempts to quantify the proportion of disease incidence which is due to a particular exposure (Last, 2001). Consequently, PAF are only valid for populations with similar distribution of exposures and here might have been overestimated because we used prevalence data.

Residual confounding in the final models could have occurred for variables not included through the selection strategy or not investigated. As an example, neither the density of farms in the neighbourhood nor their respective PRRSV status was included in the analysis. These variables might have played a role in the area spread as some authors found impact of density of farms on probability to be PRRSV positive (or other respiratory diseases) or on survival time of PRRSV negative herds (Boelaert et al., 1999; Mortensen et al., 2002; Holtkamp et al., 2010a). Due to sample size limitations, interactions between main effects were not tested. This could have clarified mechanisms involved through the main effects. It would be interesting, for example, to assess if the effect of shower was different according to distance with closest neighbour. Due to the small number of sites included, the study could also suffer from a certain lack of power to detect risk factors with smaller effect.

5. Conclusion

This study revealed a high prevalence of PRRSV infection among breeding sites in a moderate density area rising concern regarding potential regional control and elimination of the disease. PRRSV infected sites were associated with higher odds of large pig inventory and proximity to the closest pig site. Other factors having significant impact on PRRSV infection were identified and these could be relatively easily modified by producers: absence of shower and allowing access to the main entrance of the site by the rendering truck. Our study results support the use of specific biosecurity measures instead of a global score in order to evaluate the risk of PRRSV infection. Further studies should be designed to identify the mechanisms underlying the associations with large pig inventory and proximity of site, to investigate interactions between risk factors, and to assess how compliance in these practices may be enhanced to facilitate regional control and/or elimination of the disease.

Conflict of interest

None.

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