Scientific Article

Risk factors for porcine reproductive and respiratory syndrome outbreaks in Vietnamese small stock farms

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Abstract

AIM: To examine risk factors that could have played a role in the 2010 porcine reproductive and respiratory syndrome (PRRS) outbreak in Yenhung district, Quangninh province, North-Vietnam, with the purpose of establishing why existing control measures implemented after previous outbreaks had failed to prevent further outbreaks.

METHODS: A case-control study was carried out in Yenhung district. Data were obtained by an interview-based questionnaire survey. The sampling unit was households, which equated to small-scale pig farms. A total of 150 case and 150 control households were selected at communes affected by the 2010 PRRS epidemic during April to June. Risk factors were analysed using binary logistic regression and unconditional multiple logistic regression.

RESULTS: Households infected with PRRS were significantly associated with multiple variables belonging to three main groups: (1) location of the farms: i.e. farms positioned <1,000 m from a pig abattoir or within 500 m of local markets or 100 m of main roads; (2) farm management: i.e. where there was non-application of weekly farm disinfection, feeding uncooked swill, new introduction of purchased pigs without isolation, or usage of water from irrigation systems for raising pigs; (3) people and animal contact: i.e. where households kept animals with either no confinement or partial confinement, had visits by family members to other affected farms or had frequent visits by neighbours. The use of water from irrigation systems was found to be the risk factor most strongly associated with infected households in the 2010 outbreak (OR=22; 95% CI=12–42).

CONCLUSIONS: The results show that the epidemiology of PRRS in Quangninh province was linked to sociological and cultural practices, and that effective PRRS control needs an integrated approach coupled with behavioural changes in the pig raising practices of the general public. Failure to recognise this could explain why further outbreaks have occurred.

KEY WORDS: *South East Asia, Vietnam, Quangninh province, porcine reproductive and respiratory syndrome, PRRS, risk factors, epidemiology, husbandry practices, pigs, case-control study*

Introduction

In 2009, Vietnam had nearly 27 million pigs, and pork made up more than 70% of the total livestock production (Anonymous 2009). Although there has been a considerable transformation in pig production towards commercial farming, Vietnam's supply of pork depends mainly on smallholder pig production (Lemke and Valle Zárate 2008; Tisdell, 2009). Household smallholders accounted for 90% of Vietnamese pig stocks in 2006, with 83.4% of households having five pigs or less (Tisdell 2009). In the period 1996 to 2006, Vietnam's volume of pork production showed a steady upward trend with a slight tapering off in growth in 2006. In this period, almost all of Vietnam's supply of pork came from domestic producers and the per capita consumption of pork in Vietnam almost doubled. Because the bulk of pig production comes from domestic producers in Vietnam, any major pig disease outbreak is likely to have a marked impact on pork supplies.

Porcine reproductive and respiratory syndrome (PRRS), characterised by abortion, premature parturition in sows and fetal losses or pneumonia in young pigs, has caused major economic losses for the swine production industry worldwide (Pejsak and Markowska-Daniel 1997; Pejsak *et al.* 1997). The disease was first documented in the United States of America and Europe in the late 1980s as being caused by a distinct subtype Arterivirus (Wensvoort *et al.* 1992) and has subsequently spread to all continents (Anonymous 2010a), except Australia and New Zealand.

Direct contact between infected and naïve pigs among pen-mates, as well as airborne transmission are the main routes described for PRRS virus transmission (Bierk *et al.* 2001; Otake *et al.* 2002a; Kristensen *et al.* 2004). Biological vectors like people, ducks and houseflies may also play a role in the transmission of PRRS (Amass *et al.* 2000; Otake *et al.* 2004; Trincado *et al.* 2004). The virus can persist in semen for up to 90 days post infection (Christopher-Hennings *et al.* 1995) and can be introduced into herds via artificial insemination and sharing boars with sows from different households (Gradil *et al.* 1996; Mortensen *et al.*

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2002; Lawrence and Ronald, 2003). Vaccination using live attenuated vaccines against PRRS strains is another potential source of disease (Botner *et al.* 1997; Madsen *et al.* 1998). The virus can also survive in both fresh and frozen muscle, and susceptible pigs can become infected by eating infected meat (Larochelle and Magar 1997; Van der Linden *et al.* 2003; Magar and Larochelle, 2004).

Porcine reproductive and respiratory syndrome virus can be differentiated into two genotypes based on phylogenetic analysis of various isolates worldwide, namely type I (prototype Lelystad) represented by the European (EU) prototype, and type II (prototype VR-2332) represented by the Northern American (NA) strain (Dokland 2010). PRRS was introduced into Vietnam in 1998 and subsequently both the NA strain and EU strain were detected in Southern Vietnam by serological survey (Tran and Tran 2007), but the impacts of these strains on pig performance were minor or invisible. Since 2007, outbreaks caused by variant NA genotype virus with high pathogenicity have been reported throughout the country, with the agent most likely originating from China (Guang-Zhi Tong *et al.* 2007; Feng *et al.* 2008; Metwally *et al.* 2010). This syndrome is sometimes referred to as Porcine High Fever Disease (Le *et al.* 2012) and it affects animals of all ages, with clinical signs characterised by high fever (≥40.5°C), red discoloration of the skin, anorexia, respiratory difficulty and lethargy. During 2007 to 2010, Quangninh was one of the provinces affected by PRRS outbreaks. The disease caused heavy losses to 829 famers belonging to 20 communes of Dongtrieu district in 2007, and continued to re-emerge in subsequent years (Anonymous 2010b, c). Several measures, such as pig movement control, partial culling of diseased animals, disinfecting of infected premises, and raising public awareness were implemented to control PRRS in Vietnam after the 2007 outbreaks and in Quangninh province in particular. Nonetheless, the disease continued to spread widely in 2010, with epidemics occurring in 46 of the 63 provinces of Vietnam (Anonymous 2010a) despite control measures implemented by the government after the 2007 outbreak. The reasons why the control measures failing to prevent further outbreaks were unclear, therefore the objective of this study was to examine the risk factors that could have played a role in the 2010 PPRS outbreak in Yenhung district, Quangninh province of Northern Vietnam, with the purpose of establishing why the control measures were not working.

Materials and methods

Study design

A case-control study was conducted in Yenhung district of Quangninh province (Supplementary Figure 1), where an outbreak of PRRS occurred during April–June 2010. The unit of interest in this study was the individual pig farm, defined as any household where at least one pig was reared. Because no register of households was available, households were selected for the study using a multi-stage sampling approach.

Stage 1. Selection of communes

Affected villages were identified using case reports from the Yenhung district Veterinary Station submitted to the provincial sub-department of animal health during April to June 2010, and a new database was created that included more detailed information on location, time, animal population, number of cases and number of deaths during that period. A total of 10 communes, out of 19 in the district, were then identified based on one or more infected villages being present, and included in the survey (Supplementary Table 1)[1]. A commune is a cluster of centrally managed villages and can be loosely equated to a municipality with villages representing suburbs. Data were then sorted into infected villages and uninfected villages, in each of the 10 PRRS-affected communes.

Stage 2. Selection of villages

Within a selected commune, infected villages (defined as one or more households affected with PRRS) were stratified and ranked according to the number of pig owners in the village. Infected villages (Case villages) were then matched within the same commune as closely as possible to villages where no outbreaks had occurred (Control villages), on the basis of similar numbers of pig owners (Table 1).

Stage 3. Selection of households in a village

The required number of households selected from a village was then proportionally weighted based on the number of households affected in the village so that more households were surveyed in villages and communes where the disease was more extensive (Table 1). This number was then matched in control villages in the same commune. A required number of case households in an infected village were randomly selected from the list of affected households (Table 1). In the majority of infected villages almost all the infected households were selected (Table 1). Households had been previously classified by state veterinarians as infected (case) households during the epidemic period, from April to June of 2010, if their pigs exhibited two or more of the following clinical signs: high fever (≥40.5°C), anorexia, red discolorations or blood spot in the bodies, blue ears, lethargy, and severe reproductive failure in sows such as abortion, stillbirths and weak piglets.

Stage 4. Stratification and matching of households

The selected case households were sorted by the number of pigs the infected households owned and grouped into owners with 1–10, 11–20 and 21–70 pigs (Table 1). When the control households were selected in a control village they were matched on the basis of the number of pigs owned to ensure an equal weighting with respect to number of pigs in a particular farming enterprise. The selection of control households was done by the local communal veterinary officers and was based on their knowledge of the village. Control households had no history of the PRRS signs described during the period of the outbreak. The classification of the households was confirmed as being correct during the course of the interviews with farmers for ascertaining risk factors.

Sample size for case-control study

The sample size for the case-control study was calculated using Epi Info version 3.5.3 (CDC, Atlanta, USA) software. The proportion of cases and controls exposed to each postulated risk factor (Supplementary Table 1)[1] was obtained from the literature where possible. Uncertain values were obtained from expert opinion based on a modified Delphi method (Van Der Fels-Klerx *et al.* 2002) using a questionnaire requesting their estimation of the proportion of cases and controls likely to be exposed to each risk factor (Supplementary Table 1)[1]. The experts comprised two eligible veterinarians who understood both PRRS epidemiology and the characteristics of pig raising practices in Vietnam between 2007 and 2010. The sample size was thus calculated based on the anticipated proportion of household controls exposed to each risk factor obtained from the

Table 1. Demographic data and the process for estimating the sample size and selecting case and control households for a case-control study of communes affected by the porcine reproductive and respiratory syndrome epidemic in Quangninh province, Vietnam in 2010.

Communes	Affected villages	Number of pig owners	Number of affected owners	Pig population	Sample size	Unaffected villages	Number of pig owners	Pig population	Sample size
Lienvi	Xombac	18	5	274	4	Xomquan	19	145	4
	Xomdong 1	38	1	209	1	Xomnam 1	39	193	1
	Xomhan	32	4	220	3	Xombau	28	231	3
Yenhai	Thon 8	52	10	201	7	Thon 3	45	167	7
	Thon 7	32	4	217	3	Thon 4	32	160	3
Dongmai	Traithanh 3	41	5	311	4	Tanmai	43	525	4
	Maihoa	47	2	477	1	Traithap	46	135	1
	Bieunghi 2	49	6	452	4	Traithanh 1	48	276	4
Minhthanh	Duongngang	34	2	510	1	Donglinh	31	296	1
Songkhoai	Thon 8	64	35	617	26	Thon 2	68	722	26
	Thon 10	75	52	596	38	Thon 5	74	1,238	38
	Thon 11	47	46	569	34	Thon 4	50	476	34
Conghoa	Dongvong	82	1	295	1	Donglui	85	165	1
	Congbac	120	1	325	1	Giengmui	125	250	1
	Khenuoc	142	1	450	1	Thondinh	145	230	1
Haan	Xom 2B	18	9	1,112	7	Xom 1A	16	66	7
	Xom 2A	11	4	87	3	Xom 4A	11	54	3
	Xom 3A	22	1	96	1	Xom 1B	19	54	1
Tienan	Thanhgien	28	5	161	4	Xomdanh	29	138	4
	Cokhe	16	3	103	2	Caysam	16	57	2
	Xomchua	19	4	69	3	Xomdinh	20	103	3
Phongcoc	Thon 4	23	5	151	4	Thon 5	23	222	4
Quangyen	Khu 8	14	1	81	1	Giengthanh	18	135	1
Total	23	1,024	204	7,583	154	23	1,030	6,038	154

literature or feedback from the experts, with a minimum OR set at 2 and a case:control ratio set at 1:1 (Supplementary Table 1)[1]. The feasibility, cost and frequency of variables were also incorporated into the sample size determination (Meydrech and Kupper 1978). Consequently, a sample size of 154 households was decided upon, which gave a good balance between obtaining a meaningful result and being practically achievable (Table 1). This was then stratified and matched as described above and the final number of households used in the study is shown in Table 2. Four case households were excluded because they could not be appropriately matched with control households in terms of size or selected villages.

Questionnaire

A standardised questionnaire was developed based on known risk factors for PRRS described in the literature (Truong 2011), consultation with state veterinarians involved in the 2010 outbreak, and the characteristics of farming practices in Yenhung district. This was again reviewed in the field when the study commenced to ensure the risk factors remained relevant, and an additional risk factor (use of water from irrigation canals) was added at that point. The questionnaire comprised 44 basic questions, the majority of which consisted of possible multiple responses resulting in 72 possible response variables (Supplementary Information – Questionnaire).[1] For the purposes of analysis, the questions were grouped into four groups of related questions, namely location of the farms, farm management and sanitary practices, people and animal contact, and health management.

[1]http://dx.doi.org/10.1080/00480169.2014.888640

The questionnaire was developed in English and a Vietnamese version (translated from the English version by the author (VMT) who is Vietnamese speaking) was distributed to veterinary officers who interviewed the farmers in the villages in December 2010. The farmers were therefore interviewed within 6 months of

Table 2. Structures of small farms (households) selected for the case-control study of porcine reproductive and respiratory syndrome in Yenhung district, Vietnam, 2010.

	Household cases (n=150)			Household controls (n=150)		
Variables	1–10 pigs	11–20 pigs	21–70 pigs	1–10 pigs	11–20 pigs	21–70 pigs
Number of households	88	48	14	88	48	14
Mean herd size[a]	4.84	13.41	35.14	5.46	13.73	34.46
Standard deviation	2.24	2.55	11.5	2.35	2.69	10.76
Flooring						
Concreted (%)	90.9	98	100	93.2	100	100
Partially concreted (%)	9.1	2.0	0	6.8	0	0
Type of farm						
Farrow-to-finish unit (%)	70.5	83.3	71.4	57.9	77.1	71.4
Finishing unit (%)	29.5	16.7	28.6	42.1	22.9	22.9

[a] p (t≤t) two tail=0.06, 0.54 and 0.82 for 1–10, 11–20 and 21–70 pig group, respectively.

the last outbreak. Before initiation of the interview, a letter of invitation and a consent form was posted to the selected pig owners to describe the purpose and benefits arising from the study. Furthermore, an information session on PRRS disease and the case-control study was held in the People's Committee of Yenhung district for district veterinary officers who participated in the questionnaire-based survey. The trainees were taught how to conduct a questionnaire interview at the farmers' homes. The questionnaire was pre-tested between trainees during the information session and questions were refined according to feedback from trainees. The final questionnaire was administered by 12 interviewers in 10 communes. It was emphasised that the answers were processed anonymously and that farmers needed to be truthful for the study results to be meaningful.

Ethical considerations

The study was carried out for partial fulfilment of the degree of Master of Tropical Veterinary Science in the School of Veterinary and Biomedical Sciences, James Cook University, Townsville, Queensland, Australia and was an approved research project. Consent in written form was obtained from the farmers that agreed to participate after they had received written information about the study. The study was approved by the Human Research Ethics Committee at James Cook University (H3932).

Statistical analysis

Data entry, manipulation and descriptive statistics were performed using Microsoft Excel 2007 (Excel for Office 2007, www.microsoft.com). Univariable and Multivariable analyses were conducted with the statistical software SPSS 16 (SPSS Inc; Chicago, IL, USA) and NCSS 2007 (www.ncss.com).

Univariable logistic regression with categorical predictors was carried out according to Dohoo *et al.* (2009). Categories were converted to a series of "dummy" variables with $j-1$ variables put into the model. The coefficient of each indicator variable represents the effect of that level compared with the baseline category not included in the model (referred to as reference values in our tables). The p-values indicate whether the chosen level is significantly different from the baseline level. The relationship between response variables and the presence or absence of PRRS was analysed using univariable logistic regression followed by a multivariable regression. Forty one of the 72 response variables were selected for the univariable logistic regression. The remaining variables were not selected because they did not have a direct association with PRSS and were used as part of a retrospective descriptive study whose results are not shown in this paper. These were variables such as waste treatment, disease treatment, vaccination against other infectious diseases.

Hierarchical multivariable models were built using logistic regression with the dependent variable being households with PRRS signs. Variables with a significance of $p<0.05$ in the univariable analysis were entered into the full model, and then each non-significant variable was dropped step-by-step using a backward elimination process (Thrusfield 2005; Dohoo *et al.* 2009). The criteria for eliminating a variable were the largest p-value of Wald's test and the confirmation of the elimination process was conducted using a likelihood ratio test. A backward elimination process was used because the statistical significance of terms was assessed after adjustment for the potential confounding effect of other variables in the model (Dohoo *et al.* 2009) thus accounting for any confounding that may occur between variables. To test the robustness of each model a forward selection model with switching was also conducted that included the same variables as the backward elimination models. The results of these models are not shown because they gave almost identical results. The final model was checked by considering residuals and outliers.

For comparison of means obtained from quantitative variables, a Fisher's exact test or a Student *t*-test was used. A value of $p<0.05$ was considered significant; p-values between 0.06 and 0.1 were considered numerically reportable as potential trends. Estimates of OR and accompanying 95% CI were calculated and using SPSS 16 software.

Results

Descriptive statistics

The structure of the 300 households used in the study is shown in Table 2. There was no difference in mean value of herd size between household cases and household controls ($t=-0.36$; df=298; $p=0.72$). Overall, farrow-to-finish units were more dominant in the study than finishing units, and accounted for more than 70% of all herd groups, except for the "1–10 pigs" group in control households. More than 90% of the case and control households confined their pigs in shelters that had concrete or brick floors (Table 2).

Univariable logistic regression

The results of the univariable logistic regression models are shown in Tables 3–5.

Porcine reproductive and respiratory syndrome was found to be associated with households located less than 100, 1,000 and 500 m from main roads, slaughterhouses and local markets, respectively (Table 3). Households that were less than 500 m from a pig abattoir had 5.96 (95% CI=2.49–14.28) times greater odds of being infected with PRRS compared to households that were more than 2000 m from a pig abattoir; households located <500 m from a local market or livestock markets had 4.71 (95% CI 1.54–14.35) times greater odds of having PRRS than housholds >2000 m away; and households located <100 m of a main road had 2.12 (95% CI 1.23–3.63) greater odds of having PRRS than households >200 m from a main road (Table 3).

Table 4 shows the relationship of PRRS-infected households and variables related to farm management and hygiene practices. Using water from irrigation systems for pig husbandry was the most important risk factor with an OR of 22.4 (95% CI=12.9–41.5). Also associated with case households were households that never practiced disinfection, that introduced newly purchased pigs directly into the herd without isolation, and that used swill as a source of feed, with the risk increasing if the swill was uncooked (Table 4).

The relationship of PRRS-infected households and variables belonging to the category "people and animal contact" are shown in Table 5. Visits by family members to other infected farms was strongly associated with the presence of PRRS in case households. Similarly, if farms were visited either daily or weekly by neighbours this was associated with the presence of PRRS in case households. Farms that never confined their non-pig animals or only partially confined them were also more likely to have PRRS (Table 5).

Table 3. Logistic regression results showing the effect of a series of location specific categorical predictors on the risk of a household being affected by porcine reproductive and respiratory syndrome in a case-control study of 300 small stock pig farms in Yenhung district, Quangninh province, Vietnam, 2010.

Explanatory variables	Category	Number of household cases (%)	Number of household controls (%)	OR (95% CI)	p-value
Distance from the pig pen to living room	<10 m	92 (61.3)	61 (40.7)	1.32 (0.46–3.83)	0.61
	11–20 m	50 (33.3)	82 (54.7)	0.53 (0.19–1.56)	0.25
	>20 m	8 (5.3)	7 (4.7)	Reference	
Distance from the pen to the main roads	<100 m	53 (35.3)	33 (22)	2.12 (1.23–3.63)	0.01
	101–200	31 (20.7)	30 (20)	1.36 (0.75–2.47)	0.31
	>200 m	66 (44)	87 (58)	Reference	
Distance from the pen to a pig abattoir	<500 m	31 (20.7)	7 (4.7)	5.96 (2.49–14.28)	<0.001
	501–1000 m	27 (18)	16 (10.7)	2.27 (1.14–4.52)	0.02
	1001–2000 m	17 (11.3)	26 (17.3)	0.88 (0.45–1.74)	0.71
	>2000 m	75 (50)	101 (67.3)	Reference	
Distance from the pen to a local market or livestock market	<500 m	18 (12)	4 (2.7)	4.71 (1.54–14.360	0.01
	501–1000 m	8 (5.3)	14 (9.3)	0.60 (0.24–1.48)	0.27
	1001–2000 m	16 (10.7)	19 (12.7)	0.88 (0.43–1.80)	0.73
	>2000 m	108 (72)	113 (75.3)	Reference	

Table 4. Logistic regression results showing the effect of a series of sanitary and management practice specific categorical predictors on the risk of a household being affected by porcine reproductive and respiratory syndrome in a case-control study of 300 small stock pig farms in Yenhung district, Quangninh province, Vietnam, 2010.

Explanatory variables	Category	Number of household cases (%)	Number of household controls (%)	OR (95% CI)	p-value
Disinfection of environment on premises	Never	118 (78.7)	89 (59.3)	2.53 (1.52–4.20)	<0.001
	Weekly disinfection	32 (21.3)	61 (40.7)	Reference	–
All-in/all-out policy	No	134 (89.3)	121 (80.7)	1.80 (0.94–3.44)	0.08
	Yes	16 (10.7)	29 (19.3)	Reference	–
Introduction of newly purchased pigs without isolation	Yes	27 (18)	14 (9.3)	2.13 (1.07–4.25)	0.04
	No	123 (82)	136 (90.7)	Reference	–
Water usage for pig raising	From irrigation system	113 (75.3)	18 (12.0)	22.40 (12.09–41.50)	<0.001
	Pipe & underground water	37 (24.7)	132 (88.0)	Reference	–
Feeding swill to pigs	Yes	129 (86)	109 (72.7)	2.31 (1.29–4.15)	0.005
	Cooked	98 (65.3)	99 (66.0)	1.60 (0.87–2.95)	0.13
	Uncooked	31 (20.7)	10 (6.7)	2.95 (1.32–6.59)	0.008
	No swill	21 (14.0)	41 (27.3)	Reference	–

Households with dogs were twice as likely to have PRRS compared to households without animals (Table 5). The difference between case and control households in the frequency of responses to questions about health management and biosecurity are shown in Table 6.

Multivariable logistic regression

Covariates that were significant by univariable logistic regression were included in three separate multivariable models (Supplementary Table 2).[1] Only six out of 14 variables in these three models remained significant as PRRS risk factors once confounding had been controlled for (Supplementary Table 2).[1] These variables were retained in the subsequent models until the final model was formed that contained only variables that were significant on the Wald test ($p<0.05$).

[1]http://dx.doi.org/10.1080/00480169.2014.888640

Three variables remained significantly associated with PRRS-infected households in the final model (Table 7). These were the use of water from irrigation systems, visiting other PRRS-affected households, and farms located less than 500 m from a pig abattoir or slaughtering point. The other three variables, introduction of newly bought pigs into the herd without isolation, households between 501–1000 m from an abattoir and no confinement of other animals were not significant when they were entered into the model individually. The elimination of such variables did not considerably change the OR of other independent risk factors in the final model. The significance level of the likelihood ratio test ($p<0.001$) and goodness of fit ($p=0.716$) indicated that the three predictors as a group were highly significant and the regression model gave a good fit for the data. The use of irrigation water still remained the variable most strongly associated with case households with an OR of 30.6 (95% CI=15.1–61.9) (Table 7).

Table 5. Logistic regression results showing the effect of a series of sanitary and management practice specific categorical predictors on the risk of a household affected by porcine reproductive and respiratory syndrome (PRRS) in a case-control study of 300 small stock pig farms in Yenhung, Quangninh province, Vietnam, 2010.

Explanatory variables	Category	Number of household cases (%)	Number of household controls (%)	OR (95% CI)	p-value
Family member visited other PRRS affected farms	Yes	60 (40.0)	21 (14.0)	4.10 (2.32–7.21)	<0.001
	No	90 (60.0)	129 (86.0)	Reference	
Farm visited by pig traders or butchers	Visited daily	4 (2.7)	2 (1.3)	1.62 (0.29–9.06)	0.58
	Visited weekly	13 (8.7)	15 (10.0)	0.70 (0.32–1.56)	0.38
	Visited monthly	22 (14.7)	43 (28.7)	0.45 (0.23–0.74)	0.42
	No	110 (73.3)	90 (60.0)	Reference	
Farm visited by veterinary practitioners[a]	Visited daily	52 (34.7)	5 (3.3)	–	–
	Visited weekly	37 (24.7)	11 (7.3)	–	–
	Visited monthly	56 (37.3)	81 (54.0)	–	–
	No	5 (3.3)	53 (35.3)	–	–
Farm visited by neighbours					
	Visited daily	38 (25.3)	26 (17.3)	2.05 (1.12–3.72)	0.02
	Visited weekly	52 (34.7)	40 (26.7)	1.82 (1.07–3.09)	0.03
	Visited monthly	60 (40.0)	84 (56)	Reference	
Farm visited by family members	Visited daily	150 (100)	150 (100)	–	–
Presence of wild animals	Rodents	150 (100)	150 (100)	–	–
	Wild birds	20 (13.3)	21 (14.0)	–	–
Presence of animals in the premises	Dogs	134 (89.3)	121 (80.7)	2.02 (0.98–4.15)	0.06
	Cats	91 (60.7)	87 (58.0)	1.06 (0.60–1.85)	0.86
	Chicken	106 (70.7)	106 (70.7)	0.83 (0.47–1.49)	0.54
	Ducks	9 (6.0)	4 (2.7)	2.17(0.63–7.43)	0.22
	Cattle	3 (2.0)	3 (2.0)	0.99 (0.19–5.14)	0.98
	No animal	6 (4.0)	15 (10.0)	Reference	
Confinement of pet animals	No confinement	115 (76.7)	105 (70.0)	2.49 (1.17–5.29)	0.02
	Partially confined	24 (16.0)	21 (14.0)	2.53 (1.11–6.31)	0.04
	Totally confined	5 (3.3)	8 (5.3)	Reference	

[a] Information bias was determined because veterinary practitioners were consulted to treat diseased pigs during the 2010 PRRS epidemic. Thus, this risk factor was removed from data analysis.

Table 6. Variables associated with porcine health management of households used in a case-control study of porcine reproductive and respiratory syndrome (PRRS) in Yenhung district, Quangninh province, Vietnam, 2010.

Variables	Frequency (%)	
	Household cases (n=150)	Household controls (n=150)
Usage of semen for artificial insemination	111/111 (100%)	106/106 (100%)
Share boars with other households	0	0
Vaccination against infectious diseases		
Classical swine fever	87 (58.0)	114 (76.0)
Pasteurellosis	50 (33.3)	75 (50.0)
Salmonellosis	84 (56.0)	107 (71.3)
Head oedema	12 (8.0)	24 (16.0)
PRRS	0	0
Deal with PRRS-infected pigs		
Report to local veterinary authority	135 (90.0)	–
Treat sick pigs	119 (79.3)	–
Throw the dead pigs away	4 (2.7)	–
Sell sick pigs to a butcher	3 (2.0)	–

Table 7. Results of the final multivariable model of risk factors associated with households being infected with porcine reproductive and respiratory syndrome in a case-control study of 300 small stock pig farms in Yenhung district, Quangninh province, Vietnam, between April and June 2010.

Term	OR (95% CI)	Coefficient	SE	Z-Statistic	p-value
Distance from the pen to a pig abattoir <500 m (Yes/No)	10.92 (3.88–30.79)	2.39	0.53	4.52	<0.001
Family member visited other PRRS-affected farms (Yes/No)	5.25 (2.45–11.25)	1.66	0.39	4.27	<0.001
Using water from irrigation system for pig raising (Yes/No)	30.62 (15.15–61.89)	3.42	0.36	9.53	<0.001
CONSTANT	–	–2.15	0.28	–7.80	<0.001

Discussion

The univariable logistic regression modelling showed that proximity to main roads (<100 m), local markets (<500 m) and pig slaughterhouses (<1,000 m) were strongly associated with PRRS case households These results are comparable with those of an earlier study implicating local markets and main roads as risk factors in Quangnam province in Central Vietnam (Ky and Hung 2010). However, Ky and Hung failed to identify pig abattoirs as a source of PRRS, probably because of their study's small sample size (35 case households and 56 control households). In Yenhung district, there is no central abattoir, but commonly pigs are slaughtered at a slaughtering point near their home by butchers or pig traders to cater for traditional markets selling unrefrigerated meat. This practice is common in South East Asian countries, particularly in rural areas (Heinz 2008). According to local veterinarians, approximately three pigs were slaughtered daily at each slaughtering point in the villages surveyed. This took place under poor hygienic conditions without meat inspection and sanitary control. Thus, the probability of slaughtering PRRS-virus-infected pigs was high and anecdotal reports suggest that it was compounded by the intentional behaviour of butchers to purchase sick pigs at a cheap price during the epidemic. Understanding this social dynamic may help to explain why pig farms within a radius of less than 1,000 m of slaughtering points faced a higher risk of getting the infection compared to farms further away. The risk increased with increased proximity to pig abattoirs (slaughtering points) and the association still remained strong in the multivariable analysis. Additionally, village-to-village movement of pigs and pig products within a district by motorbike (an open form of transport) was very common in this district as well as other localities. Knowing that pigs are transported in this manner may explain why proximity to main roads could be a problem, as the virus can be disseminated by aerosol transmission, people, vehicles and fomites (Amass *et al.* 2000; Otake *et al.* 2002b; Dee *et al.* 2009).

Quarantine of newly bought pigs was not applied in most of the PRRS-affected households. Thus, disease risks from the introduction of pigs into the herd were inevitable. This finding is in-line with the findings of others (Mortensen *et al.* 2002; Lawrence and Ronald 2003; Le *et al.* 2012). In the 2010 outbreak, the disease broke out in many farms after purchase of new feeder pigs from livestock markets, where the origin of the breeding pigs was unknown (Anonymous, 2010b). Our data show that the percentage of new introductions was 33.7%, 8.3% and 20.4% of household cases keeping 21–70, 11–20 and 1–10 pigs, respectively. One possible explanation is that demand of replacement and repopulation of pigs could be higher in large farms which have greater resources, whereas the majority of medium size farms were farrow-to-finish swine units with lower funding or limited space. Pig flow in medium size farms was thus prone to exportation rather than importation of pigs and therefore these farms were less likely to be infected. The role of isolating pigs before integration into the herd was not looked at in our study.

After data stratification, our results indicated that cooked swill did not appear to significantly increase risk of on farm infection, but feeding uncooked swill from kitchens or restaurants was significantly associated with PRSS risk (OR=2.95; 95% CI=1.32–6.95). The practice of feeding waste food from the human food chain back to pigs has been common in rural areas of Vietnam and no regulation is imposed on feeding waste to pigs. Bloemraad *et al.* (1994) found that the virus might persist in various tissues for up to 48 hours post-mortem. If swill contains uncooked pork or edible viscera, as was probably the case in the area of our study, it could potentially act as a source of infection. Various studies looked at the presence of PRRS virus at slaughter. The results of these studies were variable but it was shown that the virus can persist in fresh pork meat slaughtered at commercial abattoirs in the USA (Pearson 2006). It is likely that pork from Quangninh province poses a greater risk due to the abundance of private slaughtering points and the absence of meat inspection.

The most outstanding feature in this study is the strong relationship of the use of irrigation water for pig rearing to PRRS case households (OR=22.40; 95% CI=12.09–41.50). The potential importance of this risk factor was only realised during interviews with farmers in the early stages of the investigation and a question related to pig drinking water sources was then added to the questionnaire. Households that had been interviewed previously were then retrospectively questioned about this risk factor in the same manner as the rest of the households. This variable was in turn a corollary of farming practices linked to irrigation systems. Geographically, Yenhung district is adjacent to the East Sea (Supplementary Figure 1)[1]. Thus, agricultural production in the river delta area of Yenhung district is dependent on water reservoirs and fresh water canals, because the river water has high salinity, particularly near the river mouth. Starting at Yenlap dam, fresh water goes through an earthen canal system to the villages and to the fields to serve for daily consumption and production. Taking advantage of this, some farmers have made fish ponds next to the canal so that water for culturing fish was supplemented or drained when necessary. Pig pens were also built in parallel to the fish ponds in order to reuse pig waste for fish farming via recycling manure into phytoplankton (Muller 1980). Coupled with this, during the epidemic period, poor awareness of biosecurity resulted in some farmers throwing out carcasses into the canal. Therefore, there was a high risk of introducing the pathogen into the irrigation system, which could then be dispersed via water flow or small boat movement. People at this location did not have access to pipe (municipal) water. Thus, the majority of households where the irrigation system was available used irrigated water for their pigs. As a result, 75% of households belonging to the case group used water from the irrigation system, while those from the control group were located far away from that system or used alternatives such as bore well or rain water which was kept in concreted tanks. Although no evidence of PRRS virus was found in the canal at that time, Pirtle and Beran (1996) pointed out that PRRS virus can survive in the water for up to 11 days, and Le *et al.* (2012) found an indirect association in the 2007 outbreaks with water through the use of water green crop, such as water spinach (*Ipomoea aquatic*), as pig feed. Our study shows for the first time that use of irrigated water for pig husbandry instead of tap well water was the main risk factor in the 2010 outbreaks.

Evidence of classical swine fever virus infection through people contact has been previously described (Ribbens *et al.* 2007). A similar mechanism for PRRS virus infection has been difficult to prove under experimental conditions, though people have

[1] http://dx.doi.org/10.1080/00480169.2014.888640

been shown to transiently carry PRRS virus and at least have the potential to act as a transmission vector (Amass *et al.* 2000). Our study showed that visits by family members to PRRS-affected farms remained an explanatory risk factor after adjustment for possible confounders. Possibly, curiosity by farmers about symptoms of PRRS tempted them to visit affected households of their neighbours or relatives. Furthermore, family members are also caretakers and come into contact with their pigs daily. Our study therefore serves to highlight the importance of understanding cultural practices in order to identify and explain the transmission of the virus and ultimately how to prevent further outbreaks occurring in these societies.

Some of the limitations of our study were firstly, that the sample size and power was only sufficient to detect the association between exposure and the disease based on an OR of 2 at 95% confidence. Hence, our results remain conservative. Secondly, simple random sampling was not possible in Yenhung district, as there was no registration system of pig farms at all levels, forcing a multi-stage sampling approach. Selection bias was limited by use of a two stage selection process; this involved matching control villages to case villages in terms of number of households and herd size. Thirdly, data collection about farm management, health management, location of farms and sanitary practices mainly relied on local veterinary practitioners whose knowledge about the epidemiology of the disease was limited. The accuracy of information therefore depended partly on the professional ability of each veterinarian. Questions involving sensitive issues that could put farmers in a bad light may have been glossed over or answered incorrectly. The behaviour of farmers towards dealing with sick pigs and disposal of dead animals or sale to butchers were typical examples. Fourthly, recall bias is an unavoidable limitation of a retrospective study. To reduce such bias, interviewers were trained to acquire information from farmers without biasing it. The study was also carried out within six months of the last outbreak. Fifthly, case households and control households were determined based on clinical signs, and control households could potentially have been misclassified if the disease was subclinical, due to a lack of serological tests. Alternatively case households could potentially be misclassified if pigs had other diseases with similar symptoms, such as classical swine fever, salmonellosis or porcine dermatitis and nephropathy syndrome. To improve the sensitivity and specificity of the study a case household or control household was clearly defined based on typical symptoms of PRRS; although in this study the use of a combination of only two or more signs may have impacted on this. In addition, the fact that PRSS is a highly contagious disease and the outbreak was in a naive pig population leads us to consider that the case and control households (the unit of study), were unlikely to have been misclassified.

During the outbreak veterinarians were more frequently present at case households than control households (Table 5) because the majority of infected pigs in the study area were treated by local veterinarians. For this reason this variable was not included in the study. In hindsight, the risk of PRRS virus transmission by such veterinarians could have been high as these veterinarians were deprived of knowledge about biosecurity as well as personal protective kits, including dispensable coveralls, rubber boots, face masks, gloves, etc., which could have prevented transmission of the virus.

Finally, the results of the study are likely to be a conservative estimate of association given that the analysis used an unconditional logistic regression rather than a conditional logistic regression approach (Dohoo *et al.* 2009).

This study is one of the first to quantify the importance of sociological and anthropological risk factors within Vietnamese society that have an association with transmission of PRRS virus. It is also the first to establish the important role that irrigation water can play in transmitting PRRS virus within these societies and to show this was the principle risk factor associated with the 2010 outbreak. This goes some way to explain why previous control programmes may have failed to prevent further outbreaks of PRRS in the region.

Our study found that sociological and cultural practices related to animal contact, herd management and use of irrigation systems are important risk factors for PRRS in Quangninh province, Vietnam. To address this issue, maximum effort must be focused on raising public awareness about biosecurity measures and the level of risk carried by pig producers, service providers and individuals involved in the market chain such as pig traders, transporters, butchers, etc. Education through mass media is likely to be important for controlling future outbreaks and this study has provided a basis upon which to focus this education.

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Supplementary Table 1. Sample size estimates and risk factors for an unmatched case control study in Quangninh province, Vietnam based on literature review and expert opinions about estimated proportion of PRRS unaffected households exposed to each potential risk factor with 95 confidence level, power 80, case: control ratio set at 1:1 and minimum OR detected set at 2.

Risk factors	Estimated proportion of household cases with exposure to risk factors				Estimated proportion of PRRS unaffected households with exposure to risk factors				Sample size
	Expert 1	Expert 2	Literature	Mean	Expert 1	Expert 2	Literature	Mean	
1. Pig movement									
- Farm located close to the main roads			65.7	65.7			42.9	42.9	576
- Farm located close to the local market or livestock market			28.6	28.6			7.1	7.1	658
- Farm located close to a pig slaughterhouse			2.9	2.9			8.6	8.6	694
2. People contact									
- Pig traders			25.7	25.7			3.6	3.6	1,512
- Veterinary practitioners	65.0	30.0		47.5	20.0	40.0		30.0	306
- Neighbors	75.0	70.0		72.5	50.0	50.0		50.0	238
- Feed suppliers	40.0	65.0		52.5	30.0	45.0		37.5	290
- Family member visit other PRRS affected farms	35.0	70.0		52.5	25.0	45.0		35.0	234
3. Animal contact									
- Households keep pigs with other animals			57.1	57.1			46.4	46.4	234
- Presence of wild animals (bird, rodent)			91.0	91.0				82.0	618
4. Herd management									
- New introduction without quarantine	80.0	70.0		75.0	70.0	20.0		45.0	288
- Purchase of pigs from other places	10.0	35.0		22.5	20.0	55.0		37.5	232
- Purchase of pigs from a livestock market			62.9	62.9			30.4	30.4	304
- Purchase of semen for AI	25.0	40.0		32.5	20.0	55.0		37.5	290
- Share boar with other sows	10.0	55.0		32.5	10.0	45.0		27.5	316
- All in/all out policy	10.0	30.0		20.0	10.0	50.0		30.0	246
5. Usage of swill as a source of feed									

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Risk factors	Estimated proportion of household cases with exposure to risk factors				Estimated proportion of PRRS unaffected households with exposure to risk factors				Sample size
	Expert 1	Expert 2	Literature	Mean	Expert 1	Expert 2	Literature	Mean	
- Cooked	20.0	40.0		30.0	80.0	50.0		65.0	360
- Uncooked	80.0	55.0		67.5	20.0	45.0		32.5	298
6. Hygiene									
- Poor hygiene	80.0	70.0		75.0	75.0	30.0		52.5	302
- Good hygiene	5.0	20.0		12.5	10.0	60.0		35.0	294
- Effluent treatment	2.0	35.0		18.5	5.0	60.0		32.5	298
- No effluent treatment	75.0	45.0		60.0	70.0	30.0		50.0	296
- Disinfecting periodically	2.0	30.0		16.0	10.0	35.0		22.5	350
- No disinfection	90.0	70.0		80.0	10.0	55.0		32.5	298
Mean									397
Median									298
Mode									298

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Supplementary Table 2. Hierarchical models constructed to select risk factors associated with PRSS infected households in Yenhung district, Quangninh province, Vietnam, 2010 based on results from the univariate logistic regressions (Number of households =300).

Explanatory variables	Category	Odds Ratio	95% C.I. LCI	95% C.I. HCI	Coefficient	S.E.	Z-Statistic	P-Value
Model 1: Location of the farms								
Distance from the pen to a pig abattoir	< 500 m (DP1)	4.22	1.68	10.57	1.44	0.47	3.08	0.0021
	501 – 1000 m (DP2)	2.28	1.15	4.50	0.83	0.35	2.38	0.017
	> 1000 m	Reference						
Model 2: Farm management								
Introduction of newly purchased pigs without isolation (IP)	Yes (IPY)	2.60	1.08	6.26	0.96	0.45	2.14	0.032
	No (IPN)	Reference						
Using water from irrigation system for pig raising	Yes (WIRRY)	21.46 (-)	11.26	40.88	3.06	0.33	9.32	<0.0001
	No (WIRRN)	Reference						
Model 3: People and animal contact								
No confinement of pet animals (CONFN)	Yes	2.51	1.13	5.58	0.92	0.41	2.27	0.023
	No							
Family member visited other PRRS affected farms	Yes (OUTBY)	3.93	2.20	7.00	1.37	0.30	4.64	<0.0001
	No (OUTBN)	Reference						

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Supplementary Figure 1. Location of Quangninh province and Yenhung district in Northern Vietnam.

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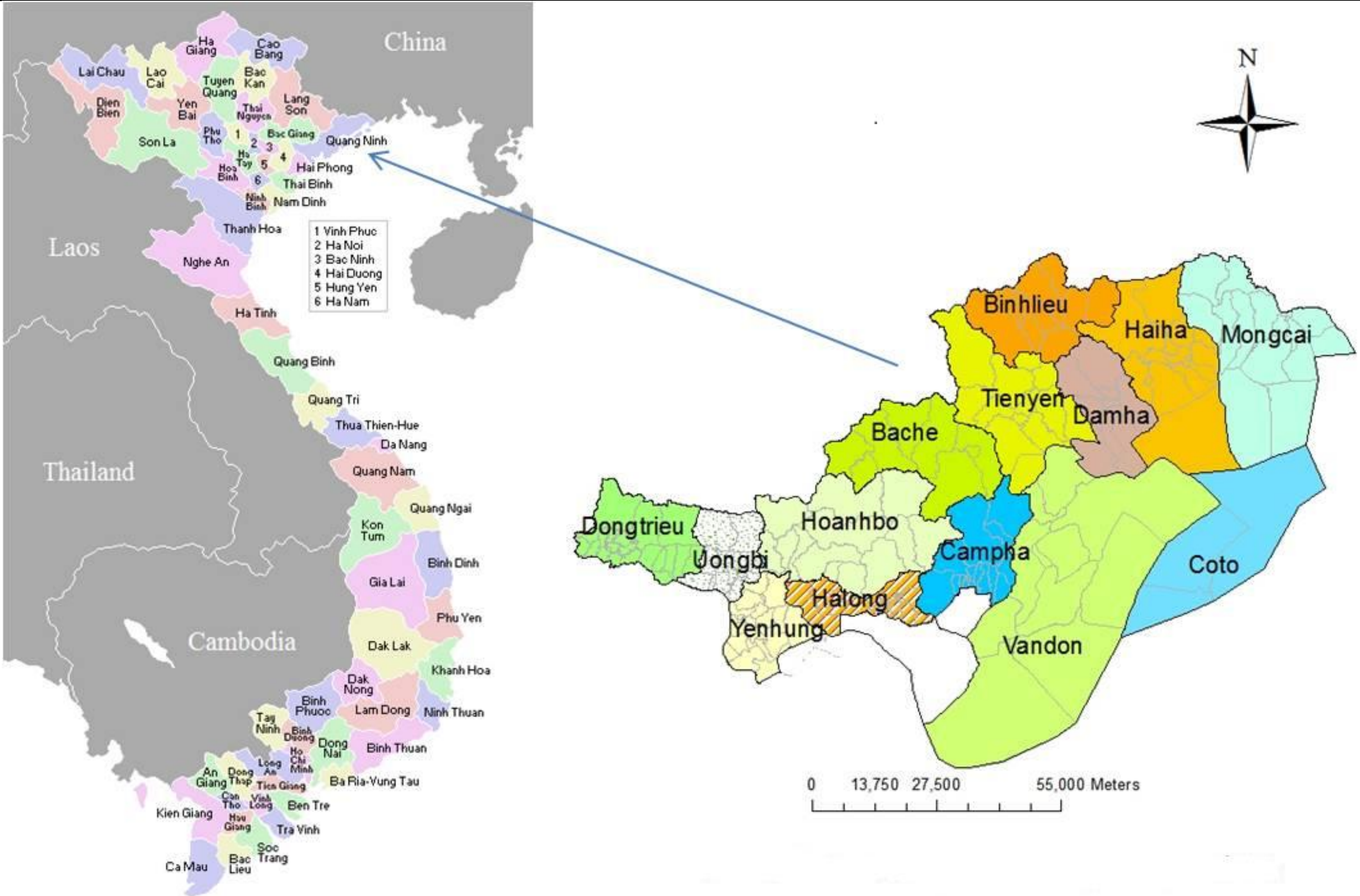

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Supplementary Questionnaire

NB! This questionnaire applies only to April-June period, 2010!

1.	**General information**	
1.1	Date *(dd-mm-yyyy)*	- -
1.2	Full name of pig owner *(Capital letter)*	
1.3	Address *(House No, village)*	
1.4	Phone number (if available)	
1.5	Village *(Capital letter)*	
1.6	Commune *(Capital letter)*	

1.7 District *(Capital letter)*

1.8 Full name of interviewer *(Capital letter)*

2. Herd information *(Please tick in the appropriate box)*

2.1. Farm type

Commercial farm

Small scale (<100 total pigs)

2.2. What kind of pigs did you raise?

		Number			Number
Young pig (10 to 20 kgs)		....	Sow		....
Finishing (over 20 kg)		....	Boar		....

2.3. Herd size

How many pigs in total did you raise? ....

3. Information about premise.

		Min	Most likely	Max
3.1. How many pens?				
3.2. What kind of floor is it?	3.3. How large is each pen (m^2)?	....	....	....
Concrete	3.4. How many pigs per pen?	....	....	....
Soil	3.5. How many feeders per pen?	....	....	....
Partially slated	3.6. How many drinkers per pen?	....	....	....

3.7. How far is the pen from your living room?

< 10 m
From 11 to 20 m
Over 20 m (details) ....

3.8. How far is the pen from the main roads (*)?

<100 m
From 101 to 200 m
Over 200 m (details) ....

(*) Main roads are defined as national roads, district roads or commune roads

3.9. What kind of breed?			Min	Most likely	Max
Local breed		How many?	....	....	....

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Hybrid breed *(localxforeign breed)*		How many?	....	....	....
Foreign breed		How many?	....	....	....

Notes: - *Local breed is defined as Mongcai breed*
- *Hybrid breed is formed by crossing local breed with foreign breed*
- *Foreign breed is defined as Landrace, Duroc, Large White, Yorkshire or crossbred between them.*

3.3. Gender

No of males .... No of females ....

3.4. Is there an abattoire in your farm?		3.14. Is there local market near your farm?	
Yes	No	Yes	No
3.5. If yes, what animal are slaughtered?		3.15. If yes, how far is from your home?	
3.6. How far from the pen?		Within 500 m	From 500 to1000 m
Within 500 m	From 500 to1000 m	From 1000 to 2000m	Over 2000m ....
From 1000m to 2000m	Over 2000m.............		

4. Herd management, hygiene, feed.

4.1. Is the pen solid and liquid wastes treated by		4.2. How often is the pen/farm disinfected?	
Biogas plant		Daily	
Taken far away from the pen		2-3 times/week	
Untreated		Never	
Other (details)	....		
4.3. How often do you clean the pen, feeder, drinker		4.4. How do you process the feed?	
Daily		Buy commercial feed	
2-3 times/week		Self process from agri-products	
Sometimes		Swill from kitchen, restaurant?	
Never		Cooked	
		Not cooked	
4.5. Do you apply all in/all out policy in your farm?		4.6. Do you seperate newly bought pigs in an isolation zone before intergrating into the herd?	
Yes		Yes	
No		If yes, for how long?	....

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4.7. Your pigs were totally confined?	Yes	No	
	No		
4.8. Where did you buy your breeding pigs?			
From the livestock market		From village where you live	
Homebred raising pig		From other places (details)	

5. Health management

5.1. During April-June, did your pigs exhibit the following clinical signs:							
	<33%	34-66%	>66%		<33%	34-66%	>66%
High fever(≥40.5^{0}C)				Abortion			
Anorexia				Stillbirths			
Red discolorations				Weak piglets			
Blood spot in the body				Other (details).............			
Blue ears				Number of death			
Lethargy							

5.2. How did you deal with your sick pigs?	Report to vet. authority	
	Treatment?	
	If yes, what medicine?	....
	Sell to the butcher	
	Other (details)	....

			Number
5.3	During April to June period did you buy in from livestock markets or other places?	Pig	....
		Other animals..........	....
5.4	If yes, did you separate the newly bought animal from the herd in an isolate pen?	Yes	
		No	
		How long? Details	....

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5.5.	Did you take your live animals to livestock market or other places during that time?	Pig	....
		Other animals..........	....

5.6	Has your pigs been vaccinated against any the following diseases?	PRRS	
		Which type? NA EU	
		When were they vaccinated? .... By who? Yourself veterinarian	
		CSF How long?	
		Pasteurellosis How long?	
		Salmonellosis How long?	
		Others.............	

5.7	Which ways were your sows impregnated?	Purchase of semen for AI
		Fresh semen frozen semen
		Sharing boars with other sows
5.8	Do you know about the health status of boars in the AI centre?	Yes
		No

5.9. During the April-June period, who visited your pig pen or your pig farm?			
Family member		Veterinary practitioner	
How often: Daily weekly monthly		How often: Daily weekly monthly	
Pig trader		Neighbours	
How often: Daily weekly monthly		How often: Daily weekly monthly	
Feed supplier		Other (details)	....
How often: Daily weekly monthly		How often: Daily weekly monthly	
5.10. During April-June period, Did you keep other animals? (dog, cat, chicken, duck)		5.11. During that time, did you see the wild animals like rat, wild birds	
Yes		Yes	
Which animal?	....	Which animal?	
Were they totally confined?		Rat	
Yes		Wild bird	
No		Which bird? Details	....

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Partially confined			

5.12. Were any of these animals sick during this period?		
Yes		If yes, which clinical signs did they show?
No		....

5.13. During April-June period, Did you go to the place where outbreak was occurring?		5.14. During that time, did you sell your pig to the butcher or dealer?	
Yes		Yes	
Which place?	......	When?	....

5.15. During April-June period, Did you see anybody buy diseased pigs in your residence?		5.16. During that time, did you see anybody in your residence sell the diseased pigs?	
Yes		Yes	
No		No	
No idea		No idea	

An additional question was supplement to the questionnaire during survey.
Which source of water did you use for raising pig?

Pipe water

Underground

water From

irrigation system

THANK YOU FOR YOUR CO-OPERATION IN THIS INVESTIGATION.

INTERVIEWER