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Early-phase risk assessments during the first epidemic year of African swine fever outbreaks in Vietnamese pigs

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Abstract

Background: African swine fever (ASF) is a notifiable viral disease of pigs and wild boars that could lead to serious economic losses for the swine industry.

Objectives: The aim of this study was to identify risk factors in the early phase of ASF outbreaks in Vietnamese swine herds during the first epidemic year.

Methods: The period of interest for this case–control study was February to July 2019. A questionnaire was administered in northern Vietnam where all early cases of ASF were reported. Producers of herds with reported cases were asked to provide information starting from the day of onset of clinical signs as well as 30 days prior to that day. The period of interest for controls was within the 6 months of the first outbreak in Vietnam (February 2019). Questionnaires included 55 questions; responses were received from 67 cases and 115 controls. Logistic regression analysis was used to identify factors associated with ASF status.

Results: Thirty-seven variables of interest (among a total of 55 variables) were associated with ASF status in univariate analysis ($p < 0.05$). These 37 variables were assessed for inclusion in the multivariate analysis by backward stepwise selection. Six variables remained significant as ASF risk factors in the final model: distance to farm within 500 m, distance of irrigation systems within 200 m, total number of pigs (≤500), absence of dressing rooms for workers/visitors before entering the farm, poor hygienic practices for people within the farm, and poor hygienic practices at pig loading/unloading locations.

Conclusions: These results may help in understanding the epidemiology of ASF in Vietnam and provide a scientific basis for optimization of current interventions and development of new tools and strategies to reduce transmission of ASF.

KEYWORDS
ASF, case–control study, epidemic, risk factors, Vietnam

1 | INTRODUCTION

African swine fever (ASF) is one of the most complex viral diseases affecting pigs and imposes a significant socio-economic impact because of its high mortality rates approaching 100% (Galindo & Alonso, 2017). The causative agent of ASF is the ASF virus (ASFV), a large, double-stranded DNA virus and the sole member of the *Asfarviridae* family (Dixon et al., 2005). ASF was first described in 1909 when the virus infected domestic pigs of European origin in Kenya (Eustace Montgomery, 1921). ASF has since spread to the Iberian Peninsula as well as several European and American countries, mainly through the movement of contaminated meat products (Sánchez-Vizcaíno et al., 2015). More recently, in August 2018, ASFV first affected the world's largest pig producer, China. The virus is now spreading in several Asian countries (Blome et al., 2020). No vaccines or therapeutics against ASFV are available. Currently, disease control measures typically include culling large numbers of pigs and strict sanitary measures (Blome et al., 2020). The continuing spread of ASF and ineffective control measures pose a serious threat to the global pig industry and food security. Each farming system has its own distinct risk factors (Dixon et al., 2019). Therefore, efforts to combat the spread of ASFV will require international cooperation.

Pigs of all ages are susceptible to virulent strains of ASFV (Arias et al., 2018). The virus is present in urine, nasal secretions, saliva, and other excreta. ASFV has complex and diverse transmission modes. The virus can be spread through direct and indirect contact or via short-distance aerosols. Feed contaminated with the biological fluids of infected pigs has been identified as an efficient mode of viral transmission (Yuan et al., 2020). In addition, direct contact with contaminated surfaces, feed, faeces, or water can indirectly result in pig infection (Olesen et al., 2018). Pigs that recover from ASF infection become clinically healthy virus carriers and may represent sources of new acute infections (Olesen et al., 2018). The role of ticks widely distributed in South America in transmitting the disease to Europe has been confirmed (Pereira De Oliveira et al., 2020). Epidemiological studies of ASF outbreaks in China found that trans-regional transportation of live pigs and pork products, movement of vehicles and people, swill feeding, and human activities all play important roles in the transmission of ASFV (Wu et al., 2020). Poor management and husbandry practices significantly increase the risk of ASFV transmission (Dione et al., 2017).

Pig production is one of the main industries in Vietnam. There are approximately three million households, of which more than 70% were smallholders by small- and medium-sized farms with production type of farrow-to-finish. The sow numbers account for about 14% that are raised in large commercial farms for breeding. The 2019 ASF outbreaks in Vietnam had considerable effects on the pig sector in Vietnam and impacted the domestic supply and demand for pork. It caused severe direct and indirect economic losses among farmers, particularly whose livelihoods are largely derived from pig production. In Vietnam, the first case of ASF was diagnosed in early February 2019 on a small farm located in Hung Yen province (Le et al., 2019). Since that time, ASFV has spread rapidly in northern Vietnam (Figure 1). Seven months later, ASF was detected in all 63 provinces of Vietnam (Tran et al., 2021). Following the first report of the ASF epidemic, the Vietnamese Department of Animal Health immediately launched an emergency response and implemented various preventive and control measures in a timely fashion. Unfortunately, these measures were unable to control the spread of ASF. In the initial outbreak, movement of contaminated pigs, pig products, and infected fomites were suspected to be the main sources of ASFV transmission in Vietnam (Tran et al., 2021). However, no studies have assessed the risk of ASF transmission at early phases during the first epidemic year. The reasons why ASF quickly spread across Vietnam remain unclear. The objective of this study was to evaluate potential risk factors in the early phase of the ASF outbreak in Vietnamese pigs during the first epidemic year using a case–control approach.

2 | MATERIALS AND METHODS

2.1 | Study population

All cases of ASF during the early phase in Vietnam were reported in the northern part of the country. Most piglets are produced there and transported to southern Vietnam (Dietze, 2011). Therefore, we targeted our study population to northern Vietnam (Figure 2). The map of Vietnam including farm locations was depicted using the free, open-source Quantum Geographic Information System (QGIS) version 2.14.14 (https://www.qgis.org/en/site/).

2.2 | Sample size calculation

EpiInfo version 3.5.3 (CDC, Atlanta, USA) was used to calculate the sample size required for this unmatched case–control study. We considered the hypothetical proportion of controls assuming an exposure prevalence of 30% and an odds ratio (OR) of 3.3 based on previous studies at small farms in Uganda (Bisimwa et al., 2021) and based on the number of animals (Cappai et al., 2018). The sample size was calculated as 110 based on assumptions of the 95% confidence interval (CI), 80% power, and a case:control ratio of 1:1. After increasing sample size by 10% to reduce the bias arising from non-response and missing data, a sample size of 120 farms was determined (60 control and 60 case farms).

2.3 | Case herd definition

An ASF case farm was defined as any swine herd in northern Vietnam with at least one confirmed ASFV by real-time polymerase chain reaction (PCR) conducted at designated laboratories of the Vietnamese Department of Animal Health according to the recommendation of the World Organization for Animal Health (OIE) (OIE, 2012). In addition, pigs in the herd had to exhibit the typical clinical signs of ASF by high fever, depression, anorexia and loss of appetite, and haemorrhages in

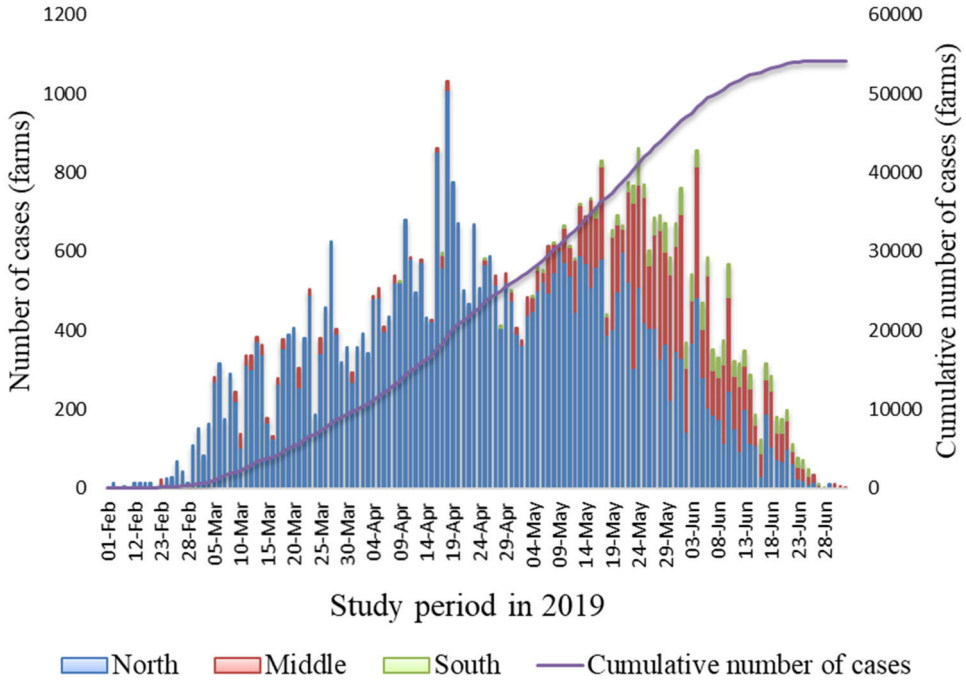

FIGURE 1 Daily number of farms with African swine fever (ASF) outbreaks in Vietnam, February to July 2019. The line bar shows the cumulative number of farms with ASF outbreaks. Each part of the stacked column indicates the number of affected farms in each of the North, Middle, and South regions

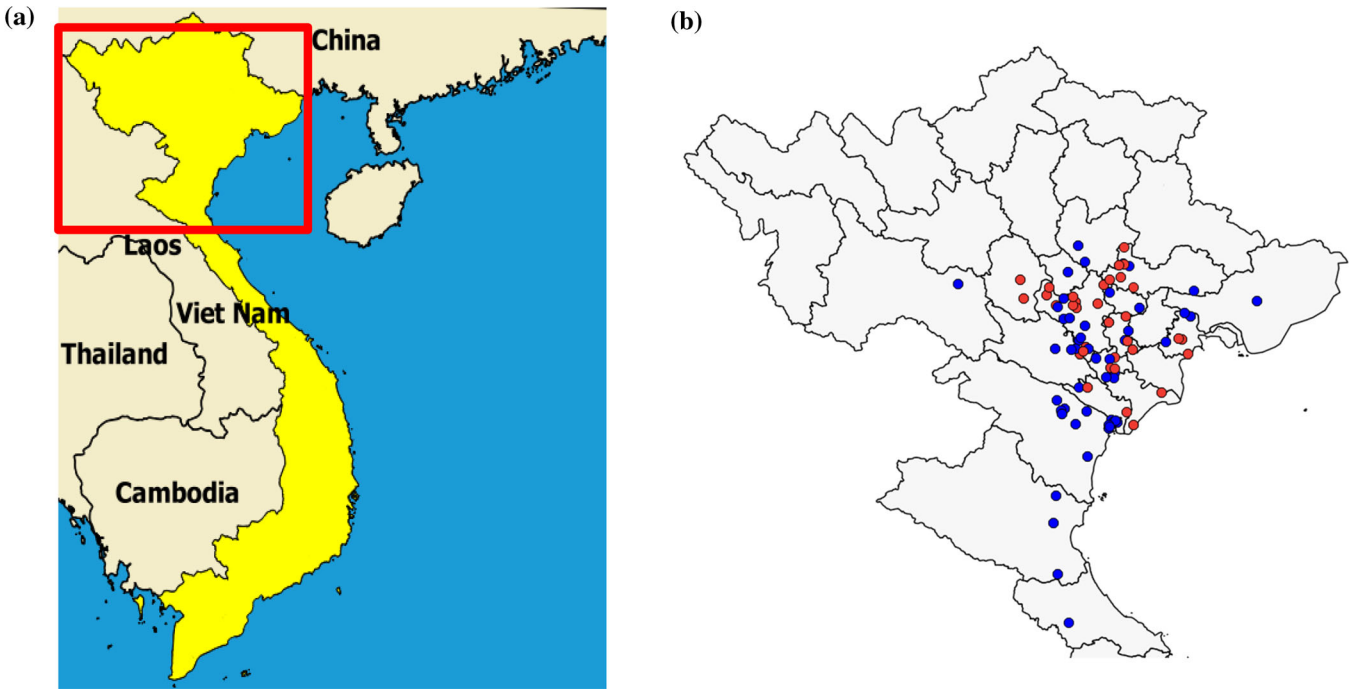

FIGURE 2 Map of the study area. (a) Map of Vietnam is shown in yellow along with neighbouring countries. The red square indicates the study area. (b) Locations of the 182 pig farms in northern Vietnam involved in this study. Red dots indicate African swine fever (ASF) case farms and blue dots indicate ASF control farms

the skin (redness of skin on ears, abdomen, and legs) during the study period from February to July 2019.

In addition, a control farm was defined as all animals tested by real-time PCR for AFSV were negative, or there were not animals showing any clinical sings of ASF within the 6 months from February to July 2019.

2.4 | Questionnaire

A questionnaire was developed based on known risk factors for ASF and other infectious diseases of pigs described in previous studies (Bisimwa et al., 2021; Cappai et al., 2018; Dione et al., 2017; Mai et al., 2020; Martínez-López et al., 2015; Olesen et al., 2018; Wu et al., 2020) and in consultation with veterinarians involved in the 2019 ASF outbreaks. The questionnaire was pre-tested with five experience veterinarians in the veterinary epidemiology field for the clarity and appropriateness of its content, questions, and responses in terms of the local situation, before the start of the study to ensure that risk factors were appropriate; based on these validation efforts, an additional risk factor (use of transit trucks at the farm gate) was added. Questionnaires were distributed to veterinarians who were present during the 2019 outbreaks and were involved in the pre-testing. The final questionnaire was used by veterinarians in direct interviews. The period of interest for this case–control study was from February to July, 2019. Case herd producers were asked to provide information from the day of onset of clinical signs as well as 30 days prior to that day. The period of interest for controls was within the 6 months of the first outbreak in Vietnam (February 2019) to ensure that farm management practices had not changed during the early phase of ASF outbreaks from February to July 2019. A letter of invitation and a consent form were sent to pig owners prior to the interview to explain the purpose and benefits of the study. Answers were fully anonymous. An unmatched case–control design was applied to swine farms in northern Vietnam because some variables such as production type and farm status may contribute to explaining the impact on the risk of ASFV infection in northern Vietnam. Therefore, these variables were treated as explanatory variables. The questionnaire was written in Vietnamese to ensure consistency (Supporting Information). The final version of the questionnaire included 55 questions in five major categories: (1) demographic characteristics of pig keepers, (2) farm location, (3) farm management practices, (4) biosecurity practices and health management, and (5) variables related to contact with people, animals, and vehicles. Each category is described in more detail below.

The category of demographic characteristics of pig keepers included four questions regarding number of years spent pig raising, pig husbandry training, primary activity, and veterinary responsibility.

The category of farm location included seven questions related to the farm's proximity to other farms, irrigation systems, main roads, residential area, distances from the barn to the pig loading/unloading location and to the living room, and distance from the isolation barn to the living room.

The category of farm management included 25 questions on farm information such as farm production system (farrow-to-finish; nucleus; farrow-to-wean; and wean-to-finish), breed, total number of pigs, farm status (company or private farm), pig movements (all-in/all-out in each barn; type of pig movement; and separate location for pig movement), type of barn, water source, swill feeding, workers, equipment sharing, manure application, insect nets, fence surrounding the premises, dressing rooms for workers, visitor quarantine for 24 h before entering the farm, wearing of work clothes outside the piggery, and sources of human food.

The category of biosecurity practices and health management included seven questions assessing the disinfection items used by workers (six items), visitors (seven items), and vehicles (11 items); the approval time of vehicles after disinfection for farm entry; the time for moving in/out of pigs and feed from vehicles; and the list of diseases occurring and vaccinations administered on the farm.

The category of contact with people, animals, and vehicles contained three parts and 12 questions focusing on contacts with people, animals, and vehicles.

2.5 | Statistical analysis

The questionnaire was administered by nine experienced veterinarians. The collected data were scrutinized for completeness, consistency, and clarity. Data were entered into Microsoft Excel and exported to R version 3.4.3 for statistical analyses. The outcome was the binary response variable ASF status which took the value 1 if the farm was ASFV positive and 0 if the farm was ASFV negative. Univariate logistic regression was used to assess the relationship between the outcome and explanatory variables, and multivariable logistic regression was used to identify independent predictors of the presence of ASF. The significance of each explanatory variable was assessed using the Wald test. ORs and their 95% CIs were calculated to measure the strength of association between explanatory variables and the outcome. Any variables significantly associated with ASF status at the $p < 0.05$ level were considered for inclusion in the multivariable logistic regression model using backward stepwise selection to control for confounding and observe changes in Akaike's information criterion (AIC). To measure the degree of multicollinearity of multiple regression variables, collinearity was assessed using the variance inflation factor (VIF) and two variables were considered correlated if VIF >10 (O'brien, 2007). The final model included all remaining variables with $p < 0.05$ and the lowest AIC. Confounding was evaluated throughout model building. A confounding variable was defined as one that, when removed from the model, changed the coefficient of any predictor variable by more than 20%. Following the identification of the main effects, all possible two-way interactions were investigated. The contribution of each variable in the final model to risk of ASF infection was quantified as the population attributable fraction (PAF) (Whittemore, 1982). Because controls were not matched to cases by location in this study, farm location was treated as a random effect.

TABLE 1 Demographic characteristics of pig keepers and associations with African swine fever (ASF) outbreak

Variables	Category	Number of cases	Number of controls	OR (95% CI)	*p*-Value
The number of years in pig raising	<5 years	15	32	1.19 (0.54–2.62)	0.66
	5–10 years	22	56	Ref	
	>10 years	30	27	2.83 (1.38–5.79)	0.004*
Having trained in pig husbandry	Yes	51	76	0.61 (0.31–1.21)	0.155
	No	16	39		
Primary activity	Animal keeping	45	63	1.69 (0.90–3.16)	0.101
	Others (business/trade/unemployed salary/crop farming)	22	52		
Veterinary responsibility person	Farmer	16	9	5.33 (2.15–13.24)	<0.001*
	Agro-vet drug shop	19	10	5.70 (2.40–13.52)	<0.001*
Veterinarians	32	96	Ref		

Abbreviations: CI, confidence interval; OR, odds ratio.
*$p < 0.05$. These variables were evaluated in multivariate analyses.

3 | RESULTS

3.1 | Univariate associations

In fact, we got responses more than expected in the study design (60 cases and 60 control farms). Therefore, we included all in the analysis to reduce the bias. A total of 182 swine farms in north Vietnam (115 controls and 67 case farms) were included in this study. Some variables were excluded because we were unable to collect the required data from all respondents. A total of 55 variables were considered as potential risk factors for ASF status. Univariate associations for the five major categories of variables are presented in Tables 1–5.

3.1.1 | Demographic characteristics of pig keepers

Table 1 shows the relationships between ASF-infected farms and the demographic characteristics of pig keepers. Farms whose operators had more than 10 years of pig raising experience had 2.83 times greater odds of ASF outbreak than those with 5 to 10 years of experience. Farms where farmers or agro-vet drug store employees were responsible for veterinary care had 5.33 and 5.70 times greater odds of ASF outbreak, respectively, compared with farms where a veterinarian was in charge.

3.1.2 | Risks associated with farm location

ASF was associated with farms located less than 500, 200, and 200 m from the nearest farm, residential area, and irrigation system, respectively ($p < 0.05$). We could not get the data from respondents regarding distances from the farm to the nearest farm with an ASF outbreak, forest, slaughterhouse, local market, and refuse dumpsite. Therefore, these variables were excluded from the analysis. In addition, barns that were less than 10 m from the living room were associated with 11.14 times increased odds of ASF outbreak compared with barns more than 50 m from the living room (Table 2). The living room of the workers had a kitchen area that could be related to the risk of ASFV contamination through near distance to the living room by food for workers may include the pork products.

3.1.3 | Risks associated with farm management

Table 3 shows the relationships between ASF outbreaks and farm management variables. Seventeen of the 25 variables assessed were associated with ASF status at the $p < 0.05$ level: farm status, production type, total numbers of pigs (for farms of less than 1100 pigs), all-in-out policy, separate location of pig removal, farmer-owned place of pig removal, insect nets, truck for pig transport from slaughterhouse, open barns, use of water directly from irrigation systems, swill feeding, presence of workers, absence of dressing rooms, wearing of work clothes outside, manure application, human food, and quarantine of visitors for 24 h prior to farm entry (Table 3).

3.1.4 | Risks associated with biosecurity practices and health management

Table 4 shows interactions between ASF status and variables related to biosecurity practices and health management. Six of the seven variables showed a correlation with ASF status at the $p < 0.05$. The only exception was the waiting time for vehicles after disinfection to enter the farm.

TABLE 2 Associations between African swine fever (ASF) outbreak and location variables

Variables	Category	Number of cases	Number of controls	OR (95% CI)	*p*-Value
Distance from farm to the closest farm	≤500 m	51	48	4.45 (2.27–8.72)	<0.001*
	>500 m	16	67		
Distance from farm to the main road	≤500 m	45	77	0.75 (0.34–1.65)	0.477
	501–1000 m	8	20	0.51 (0.18–1.51)	0.224
	>1000 m	14	18	Ref	
Distance from farm to the residential area	≤200 m	19	14	2.83 (1.07–7.49)	0.035*
	201–500 m	14	24	1.22 (0.47–3.15)	0.688
	501–1000 m	22	52	0.88 (0.38–2.06)	0.771
	>1000 m	12	25	Ref	
Distance from farm to the irrigation system	≤200 m	43	44	3.83 (1.79–8.19)	<0.001*
	201–500 m	12	24	1.96 (0.77–5.01)	0.157
	>500 m	12	47	Ref	
Distance from barn to living room	<10 m	12	2	11.14 (2.33–3.34)	<0.001*
	10–20 m	6	20	0.56 (0.20–1.55)	0.258
	21–50 m	21	41	0.95 (0.47–1.91)	0.888
	>50 m	28	52	Ref	
Distance from barn to the pig loading/unloading place	≤50 m	45	69	1.36 (0.72–2.56)	0.335
	>50 m	22	46		
Distance from isolation barn to living room	≤100 m	36	48	1.62 (0.88–2.97)	0.118
	>100 m	31	67		

Abbreviations: CI, confidence interval; OR, odds ratio.
*$p < 0.05$. These variables were evaluated in multivariate analyses.

3.1.5 | Risks associated with people, animal, and vehicle contact

Table 5 shows the univariate relationships between ASF-infected farms and people, animal, and vehicle contacts. In farms with ASF outbreaks, the presence of rodents, other animals, and ticks was strongly associated with the presence of ASF ($p < 0.001$). Vehicle visits to another farm on the same day or trips and high contacts with vehicles (more than six times per month) were also associated with ASF outbreaks ($p < 0.05$).

3.2 | Risk factors for ASF outbreaks in multivariate analysis

Among a total of 55 variables, 37 variables of interest were associated with ASF status ($p < 0.05$) (Tables 1–5). These variables were included in the multivariate model, and backward stepwise selection was performed until the *p*-values for all remaining variables (by Wald test) were less than 0.05. The results for the final model are shown in Table 6. The multivariate analysis indicated that six variables (two each from the categories of farm location, farm management practices, and biosecurity practices and health management) were strongly associated with ASF outbreaks ($p < 0.05$) (AIC = 95.4). No multicollinearity and no interactions between variables were detected.

4 | DISCUSSION

In Vietnam, pig production occurs mainly on small-scale farms with unique sociological and cultural practices in the northern part of the country. This study concluded that distance to farm within 500 m, distance of irrigation systems within 200 m, total number of pigs (≤500), dressing rooms for workers/visitors before entering the farm, and poor hygienic practices for people within the farm as well as at pig loading/unloading locations were risk factors for ASFV transmission during the early phases of the Vietnamese outbreak. These data help to explain why ASFV spread rapidly and locally in the northern part of the country. Knowledge of these ASF transmission routes in the Vietnamese production system may help to identify and implement control measures to restrict the spread of ASFV in other locations.

The most notable aspect of this study was the strong relationship between ASF outbreaks and distance from one farm to the nearest neighbouring farm and to irrigation systems. Proximity to the nearest farm (<500 m) increased the odds of ASF outbreak by 4.45-fold compared with farms with greater distances from their neighbours ($p < 0.01$). It was proved that exposing pigs to an ASFV-contaminated environment can result in infection (Olesen et al., 2018). Furthermore, pigs infected with ASFV can excrete virus into the air (de Carvalho Ferreira et al., 2013). All of these factors may increase the risk of ASFV infection for farms closer to their neighbours because

TABLE 3 Associations between African swine fever (ASF) outbreak and farm management variables

Variables	Category	Number of cases	Number of controls	OR (95% CI)	*p*-Value
Farm status	Private	41	43	2.64 (1.42–4.91)	0.002*
	Company	26	72		
Production type	FF	22	23	3.61 (1.65–7.90)	<0.001*
	Nucleus	12	6	7.56 (2.49–22.91)	<0.001*
	FW	15	18	3.15 (1.33–7.44)	0.007*
	WF	18	68	Ref	
Breed	Local	4	2	4.32 (0.75–24.87)	0.078
	Crossed	32	46	1.50 (0.81–2.80)	0.197
	Exotic	31	67	Ref	
Total pigs	≤500	27	21	4.86 (2.25–10.51)	<0.001*
	550–1100	22	26	3.20 (1.48–6.90)	0.003*
	>1100	18	68	Ref	
All-in/all-out policy in each barn	Yes	12	2	0.08 (0.02–0.38)	<0.001*
	No	55	113		
Source of pigs	Unknown	10	14	1.45 (0.59–3.59)	0.421
	Your farm	22	30	1.49 (0.75–2.95)	0.253
	Known	35	71	Ref	
Separate place for pig movement	Yes	12	2	0.08 (0.02–0.38)	<0.001*
	No	55	113		
Pig movement place is located on farm's property	Yes	18	8	0.20 (0.08–0.50)	<0.001*
	No	49	107		
Truck through the same route at entrance and exit	Yes	61	101	1.41 (0.51–3.86)	0.503
	No	6	14		
Transit trucks at the farm gate	Yes	49	83	0.95 (0.48–1.87)	0.889
	No	18	32		
Insect nets	Yes	27	21	0.33 (0.17–0.65)	0.001*
	No	40	94		
Fence around the premises	Yes	4	8	1.18 (0.34–4.07)	0.796
	No	63	107		
Source of trucks for the pig transport to the slaughterhouse	Slaughter house trucks	41	37	3.32 (1.77–6.23)	<0.001*
	Business operator trucks	26	78		
Having a separate worker in isolation barn	Yes	41	61	0.72 (0.39–1.32)	0.285
	No	26	54		
Opened barn type	Yes	16	4	8.71 (2.77–27.35)	<0.001*
	No	51	111		
Water source	Direct	34	28	3.20 (1.69–6.08)	<0.001*
	Indirect	33	87		
Feeding swill to pigs	Yes	8	0	Inf	<0.001*
	No	59	115		
Having workers in farm	Yes	7	2	0.15 (0.03–0.75)	0.009*
	No	60	113		
Dressing rooms for workers and visitors before entering the farm	Yes	11	1	0.04 (0.01–0.35)	<0.001*
	No	56	114		

(Continues)

TABLE 3 (Continued)

Variables	Category	Number of cases	Number of controls	OR (95% CI)	*p*-Value
Wearing work clothes outside of the piggery premises	Yes	24	66	0.41 (0.22-0.77)	0.005*
	No	43	49		
Having isolation barn	Yes	32	62	1.28 (0.70–2.34)	0.423
	No	35	53		
Sharing of equipment with other pig farms	Yes	0	2	0.00	0.278
	No	67	113		
Manure application	Feed for fish	4	8	3.06 (0.76–12.29)	0.104
	Mixed type	40	34	7.19 (3.10–16.65)	<0.001*
	Sold	14	18	4.75 (1.76–12.82)	0.001*
	Applied on land inside farm	9	55	Ref	
Source of human food	Local market	51	47	4.61 (2.35–9.04)	<0.001*
	Inside farm	16	68		
Isolating visitors 24 h before entering the farm	Yes	39	42	0.41 (0.22–0.76)	0.005*
	No	28	73		

CI, confidence interval; FF, farrow-to-finish; FW, farrow-to-wean; OR, odds ratio; WF, wean-to-finish.
*$p < 0.05$. These variables were evaluated in multivariate analyses.

TABLE 4 Associations between African swine fever (ASF) outbreak and biosecurity practice and health management variables

Variables	Category	Number of cases	Number of controls	OR (95% CI)	*p*-Value
Biosecurity practices apply to people inside farm	High (six items)	32	95	Ref	
	Intermediate (four, five items)	27	14	5.73 (2.68–2.24)	<0.001*
	Low (≤3 items)	8	6	3.96 (1.28–2.28)	0.012*
Biosecurity practices apply to visitors	High (seven items)	32	93	Ref	
	Intermediate (five, six items)	14	14	2.91 (1.25–6.75)	0.011*
	Low (≤4 items)	21	8	7.63 (3.08–8.91)	<0.001*
Biosecurity practices apply at pig loading/unloading place	High (11 items)	30	95	Ref	
	Intermediate (8–10 items)	23	18	4.05 (1.93–8.49)	<0.001*
	Low (≤7 items)	14	2	22.17 (4.76–102.13)	<0.001*
Time that vehicles have to wait after disinfection to get into the farm	≤2 h	44	89	0.56 (0.29–0.09)	0.086
	>2 h	23	26		
Time for moving in/out pig/feed from vehicles	<30 min	10	7	Ref	
	30–60 min	37	84	0.31 (0.11–0.87)	0.021*
	>60 min	20	24	0.58 (0.19–1.81)	0.349
Diseases happen in farm	High (≥8 diseases)	20	12	2.60 (1.01–6.75)	0.047*
	Intermediate (3–7 diseases)	31	78	0.62 (0.29–1.32)	0.213
	Low (≤2 diseases)	16	25	Ref	
Vaccination applying in farm	High (≥9 vaccines)	45	35	Ref	
	Intermediate (7–8 vaccines)	4	8	0.39 (0.11–1.40)	0.138
	Low (≤6 vaccines)	18	66	0.21 (0.11–42)	<0.001*

Abbreviations: CI, confidence interval; OR, odds ratio.
*$p < 0.05$. These variables were evaluated in multivariate analyses.

TABLE 5 Associations between African swine fever (ASF) outbreak and people, animal and vehicle contact variables

Variables	Category	Number of cases	Number of controls	OR (95% CI)	*p*-Value
Visiting of vet	Yes	41	83	0.61 (0.32–1.15)	0.125
	No	26	32		
Other visitors	Yes	23	31	1.42 (0.74–2.72)	0.294
	No	44	84		
Presence of wild birds inside farm	Yes	38	48	1.83 (0.99–3.36)	0.051
	No	29	67		
Presence of rodents inside farm	Yes	61	83	3.92 (1.54–9.96)	0.003*
	No	6	32		
Presence of chicken in farm	Yes	49	36	5.97 (3.06–11.66)	<0.001*
	No	18	79		
Presence of ducks in farm	Yes	28	18	3.87 (1.92–7.78)	<0.001*
	No	39	97		
Presence of dog in farm	Yes	55	67	3.28 (1.59–6.79)	<0.001*
	No	12	48		
Presence of cat in farm	Yes	25	10	6.25 (2.76–14.13)	<0.001*
	No	42	105		
Presence of ticks in farm	Yes	36	32	3.01(1.60–5.66)	<0.001*
	No	31	83		
Presence of wild boar near farm	Yes	0	2	0.00	0.278
	No	67	115		
Vehicles visit another farm on the same day/trip	Yes	21	16	3.39 (1.59–7.22)	0.001*
	Unknown	10	6	4.31 (1.46–12.71)	0.005*
	No	36	93	Ref	
Number of truck vehicles visit to farm/month	High (≥11)	30	25	4.53 (2.16–9.53)	<0.001*
	Intermediate (6–10)	19	22	3.26 (1.46–7.29)	0.003*
	Low (≤5)	18	68	Ref	

Abbreviations: CI, confidence interval; OR, odds ratio.
*$p < 0.05$. These variables were evaluated in multivariate analyses.

of cross-contamination from animals, vehicles, and human contacts and movements as well as aerosol transmission. Proximity of farms to irrigation systems also showed a significant positive relationship with ASF outbreak (OR = 2.89). This finding could relate to typical farming practices in Vietnam that takes advantage of proximity to irrigation systems; some farmers have built fishponds next to irrigation systems to simplify adding or draining water for fish culture as required. During the epidemic period, because of a lack of biosecurity awareness, some farmers discarded dead pigs into the irrigation system. This practice likely incurred a significant risk of introducing ASFV into the irrigation system or groundwater, where it could then be spread by water flow. In addition, water for pig rearing could be driven from the irrigation systems surrounding the farms, drill wells, or fish ponds (Mai et al., 2020). In this study, water source (direct from drill well or irrigation system) was not included in the final model although this variable showed an association with ASF outbreak in the univariate analysis ($p < 0.001$). The use of irrigation systems was identified as a significant risk factor for porcine reproductive and respiratory syndrome in Vietnam (V. M. Truong & Gummow, 2014). Use of contaminated water from the Danube River was implicated in introducing ASF to farms (Houghton, 2018). Water can easily become contaminated and play a role in disease transmission. The level of virus required for infection in liquid is lower than in feed (e.g., minimum infectious dose of ASFV in liquid 10^2 50% tissue culture infective dose [TCID50] compared with 10^4 TCID50 in feed) (Niederwerder, 2021). Eliminating the effects of these two location variables, the PAF was reduced by 59.01% and 41.98% for ASF status.

In this study, the final model included two management category variables: total number of pigs (≤500) and absence of dressing rooms for workers/visitors (ORs of 3.02 and 22.39, respectively). In Vietnam, most large farms are commercialized and operated by companies (company farm status), while medium and small farms are mainly operated by farmers (private farm status). The first case of ASF in Vietnam was reported on a small farm in early February 2019 (Le et al., 2019) and the

TABLE 6 Multivariate analysis of risk factors associated with African swine fever (ASF) outbreak in a case–control study of northern Vietnamese pig farms in 2019

Variables	OR	95% CI	PAR	*p*-Value
Distance from farm to the irrigation system (≤200 m)	2.89	1.55–5.40	41.98	0.001
Distance from farm to the closest farm (≤500 m)	4.45	2.27–8.72	59.01	0.004
Dressing rooms for workers and visitors before entering the farm (no)	22.39	2.82–177.81	15.68	0.002
Total pig (≤500)	3.02	1.53–5.96	26.96	0.015
Biosecurity practices apply to people inside farm (< 6 items)	5.20	2.63–10.25	42.18	0.043
Biosecurity practices apply at pig loading/unloading place (<11 items)	5.86	2.96–11.58	45.80	0.004

Abbreviations: CI, confidence interval; OR, odds ratio; PAR, population attributable fraction.

disease quickly spread to all 63 provinces (Tran et al., 2021). ASF was mostly reported in small private farms during the early phases of ASF in Vietnam. Although farm status was not included in the final model, it was strongly associated with ASF outbreak in univariate analysis ($p = 0.002$). This could be because private farms are highly connected to one another and it is exceedingly rare for pigs from private farms to move to company farms (A. D. Truong et al., 2020). Previous studies indicate that farms in northern provinces were highly connected through pig movements by pig purchases, especially in small farms (Baudon et al., 2017; Lee et al., 2021). In addition, boar hiring is the most common in these farms (Baudon et al., 2017). As a result of high connectivity between private farms through animal, vehicle, and human contacts, there was a higher risk of cross-contamination for private farms than for company farms. Moreover, biosecurity measures were less strictly applied in small farms compared with the large-scale industrial farms. A previous study indicated that ASF control measures mainly rely on strict sanitary interventions (Blome et al., 2020). In this study, 11/12 farms that did not have dressing rooms were case farms; all of 12 these farms were private farms. Two variables related to biosecurity practices were strongly associated with ASF outbreak. Applying less than six and 11 practice items to individuals inside the farm and at pig movement locations increased the odds of ASF outbreak by 5.20 and 5.86 times, respectively, compared with farms applying all practice items. Absence of dressing rooms for workers or visitors and lower biosecurity practices could be the potential risks for spreading ASF because of the high environmental resistance of ASFV. ASFV is able to persist for long periods in contaminated fomites or meat (Mazur-Panasiuk et al., 2019). Poor management and biosecurity practices were identified as crucial factors in increasing the risk of transmission of ASF in Uganda (Dione et al., 2017). According to a previous study, the limited biosecurity level of small domestic farms had the most significant contribution to the introduction of ASF into domestic farms (Boklund et al., 2018). Low biosecurity at pig movement locations, along with high environmental resistance of ASFV, could lead to rapid ASFV spread via transport vehicles. This finding may partially explain why ASF quickly spread to all 63 provinces in Vietnam and mainly to private farms. The spread of ASF may be associated with small private farms, which generally have lower biosecurity standards than larger commercial operations.

The major limitation of the study was bias introduced by the selection of control farms, which may have affected the risk factor analysis. During the study period, more control farms culled their entire herds in the early stages of the outbreak. In addition, control farms were not geographically matched with case farms. Therefore, a location variable was included in the model as a random effect. A strength of this study was that study farms represented areas with high levels of pig production, and the questionnaires were administered by experienced veterinarians. In addition, there is a possibility that our results were affected by recall bias which is one of the most important problems for the case–control study. In this study, only eight of 182 responded farms did not have any written records, so that veterinarians questioned carefully as much as possible to reduce recall bias.

In conclusion, proximity to other farms and irrigation systems, poor hygienic management, and lower levels of biosecurity practices were identified as risk factors during the early stages of ASF outbreaks in Vietnamese pigs during the first epidemic year. These assessments could contribute to understanding the epidemiology of ASF in Vietnam and provide a scientific basis for optimization of current interventions and development of new tools and strategies to reduce transmission of ASF.

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ETHICS STATEMENT

Informed consent and signed documents of agreement for this study was obtained from all individual participants involved in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of University of Miyazaki review board.

AUTHOR CONTRIBUTIONS

Data curation, investigation, writing—review and editing: Le Anh Tuyen. *Investigation and writing—review and editing*: Le Van Truong. *Investigation*

and writing—review and editing: Pham Thi Lan Huong. *Investigation and writing—review and editing*: Vu Duc Hanh. *Investigation and writing—review and editing*: Vu Viet Anh. *Investigation and writing—review and editing*: Nguyen Xuan Hoa. *Conceptualization, data curation, methodology, supervision, validation, and writing—review and editing*: Satoshi Sekiguchi.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are included in the article and Supporting Information.

PEER REVIEW

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