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Prevalence, Associated Risk Factors, and Transmission Risk Scoring of Classical Swine Fever in Smallhold Farms in the Philippines

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Abstract

Background: Classical swine fever (CSF) is a notifiable disease, and the limited epidemiological data in the Philippines underscores the need for effective disease surveillance. **Methods:** This study aimed to determine the prevalence of antibodies against CSF virus (CSFv) using enzyme-linked immunosorbent assay and CSFv RNA using real-time reverse transcription polymerase chain reaction in pigs from smallhold farms in 21 Philippine provinces. The association between seropositivity and factors from interviews of abattoir officers was analyzed using the least absolute shrinkage selection operator regression. A semi-quantitative method was also adapted to estimate the transmission risk. **Results:** Our study found an overall seroprevalence of 36.0% (153/425, 95% Confidence Interval: 31.5%-40.8%), while all 423 samples tested negative for CSFv. A positive association was found in water treatment, swill feeding, CSF vaccination, and keeping vaccination records, while CSF history, proximity to residential areas, and raising native pigs negatively impacted seropositivity. Nueva Ecija was considered high-risk for CSF transmission, while others fell within the moderate, low, and very low risk categories. **Conclusions:** Our findings highlight the CSF seroprevalence and factors to consider for improved prevention and control. Classifying the provinces according to transmission risk also provided insights on future targeted surveillance and efficient resource allocation.

Keywords

Philippines, Prevalence, Risk scoring, Transmission

1. Introduction

Classical swine fever (CSF), also known as hog cholera, is a notifiable animal disease affecting all pig species [1]. As a highly contagious disease with the potential to cause severe economic consequences, it is also recognized as a transboundary animal disease [2]. The causative agent, CSF virus (CSFv), is a single-

stranded, positive-sense RNA virus from the genus *Pestivirus* of the *Flaviviridae* family, and it is taxonomically related to the bovine viral diarrhea and border disease viruses [3]. While there is only one known CSFv serotype, this virus has three genotypes, with genotypes 2 and 3 causing recent outbreaks in Europe and Asia [4,5]. Despite these genetic differences, the CSFv does not produce a clinically distinct disease in all age groups of pigs [1]. Following infection, the acute disease starts with leukopenia and immunosuppression, predisposing pigs to microbial co-infections [1]. As the disease progresses, pigs may exhibit fever, inappetence, constipation, lethargy, and petechial hemorrhages on the ears, abdomen, and thighs, and mortalities often occur 1 to 4 weeks after acute infection [1]. Similarly, chronic and persistent infections, which are common in piglets infected *in utero,* also result in delayed mortalities [1]. Aside from vertical transmission, CSFv also spreads directly between infected and healthy pigs through saliva, urine, and feces [6] as well as indirectly through contaminated feeds, swills, water, clothing, and farm equipment [7].

In the 1990s, the Netherlands reported one of the largest CSF occurrences, where 10 million pigs across 429 farms were culled, resulting in USD 2.3 billion in economic losses [8]. Around USD 12 million was also lost in Belgium in 1997 from eight affected farms with low pig density [9]. Given these serious economic impacts, investigating risk factors linked to outbreaks can benefit CSF control and prevention. Among the identified factors in global occurrences were increased herd size, denser pig population, increased pig transportation, proximity of infected and susceptible herds, swill feeding, a longer interval between disease onset and reporting, the introduction of diseased pigs, and having no CSF vaccination [10–12]. The disease also reemerged in Japan in 2018, 26 years after its last reported case, and intervention strategies such as stamping out, movement control, disinfection protocols, and active surveillance in both domestic and wild pigs were implemented [13]. In the absence of effective treatment, the World Organization for Animal Health (WOAH) also recommends a robust reporting system, decontamination of swill or prohibition of its feeding, compartmentalization strategies in affected areas, vaccination, proper handling and treatment of pig products and by-products, and surveillance programs as CSF control and prevention [14].

Given the non-specific CSF symptoms, surveillance and monitoring are based on a combination of clinical observation, viral detection, and serology tests [15,16], such as enzyme-linked immunosorbent assay (ELISA) and real-time reverse transcription polymerase chain reaction (qRT-PCR). ELISA is a reliable diagnostic tool for detecting anti-CSFv antibodies, and discrimination between infected and vaccinated animals has been possible with modifications in the coated antigens and improvement in vaccine development [17,18]. It is commonly applied to large-scale epidemiology studies for seroconversion and post-vaccination surveillance [19]. On the other hand, qRT-PCR offers precise viral detection by targeting the highly conserved region within the 5′ UTR of the CSFv genome in experimentally infected pigs. With around two hours of turnaround time, it is considered a rapid tool for CSF diagnosis [20]. Integrating these two diagnostic methods into the CSF surveillance program is, therefore, crucial in undertaking and enhancing disease monitoring and control.

Currently, the Philippine swine industry faces significant production and economic losses due to the ongoing African swine fever (ASF) crisis [21]. Disease prevention and control remain challenging, especially in smallhold farms, due to biosecurity lapses and limited access to vaccines for economically important animal diseases [22]. With the focus on ASF, other swine diseases such as CSF appear to be neglected. In the 2000s, prevalence in 14 provinces ranged from 30 to 40%, based on the laboratory tests conducted by the Philippine Animal Health Center [23]. Bulacan, Pampanga, and provinces in Mindanao were also affected in the following years [24,25], highlighting the national challenge in CSF control. With the potential enzootic distribution of CSF in the Philippines [26], disease surveillance, along with examining risk factors and assessing transmission risk, will enable a better understanding of its local epidemiology to prevent future outbreaks and ensure food security. Hence, this study aimed to determine the distribution of CSF in the Philippines using ELISA and qRT-PCR, identify associated risk factors, and evaluate the transmission risk in selected provinces to aid policymakers in designing evidence-based prevention and control strategies.

2. Materials and Methods

2.1 Ethical Statement

The existing Institutional Animal Care and Use Committee of the University of the Philippines Los

Baños carefully reviewed and approved all animal procedures in this study under approval number UPLB-2022-001, in full compliance with the national guidelines and policies governing the ethical use of animals in scientific research.

2.2 Study Sites

A total of 21 provinces (Benguet, Ilocos Sur, Pangasinan, Nueva Ecija, Pampanga, Batangas, Cavite, Laguna, Occidental Mindoro, Palawan, Camarines Sur, Aklan, Negros Occidental, Bohol, Cebu, Samar, Zamboanga del Sur, Bukidnon, Davao del Sur, North Cotabato, and Surigao del Norte) were purposively chosen based on the following criteria: geographic representatives from different administrative regions of the Philippines, with high swine population size, and willingness of the local government units to participate. The term "municipality" used in this study refers to both municipalities and cities. A maximum of four municipalities were selected from each province based on these same criteria, and sample collection was conducted in slaughterhouses that primarily served smallhold pig farms within a municipality. Overall, samples were collected from 43 municipalities from January 2022 to November 2023.

2.3 Sample Size Determination

A sample size of 384 was computed using the formula [27] for estimating disease prevalence in an infinite population with 50% expected prevalence, 5% desired margin of error, and 95% confidence level. In this cross-sectional study, a total of 425 blood samples were collected, higher than the computed sample size to improve the precision of the sample estimate.

2.4 Sample Collection

Aseptic blood sampling (3–5 mL) was performed via the external jugular vein from a total of 13 to 30 domestic pigs, regardless of sex and breed, aged at least 5 months, in pig holding pens of selected slaughterhouses. The collected blood samples were transferred to a properly labeled VacugenTM tube (BioSpectra Marketing, Iloilo City, Philippines) and allowed to clot at room temperature for approximately 30 to 45 minutes. The samples were centrifuged at 2,000 x *g* for 10 min to collect the sera and transferred to a properly labeled Labopette cryovial tube (Labotech Trading, Las Piñas City, Philippines). All samples were transported to the laboratory in an insulated specimen transport box and kept at -80°C for further analysis.

2.5 Enzyme-linked Immunosorbent Assay (ELISA)

The serum samples were thawed under controlled cold temperature and tested in duplicate using the Classical Swine Fever Antibody Test kit (IDEXX, Montpellier, France) following the manufacturer's competitive ELISA protocol. Optical density (OD) was recorded at 450 nm with the MultiskanTM Go Microplate Spectrophotometer (Thermo Fisher Scientific Corporation, Vantaa, Finland). The assay was considered valid if the mean OD of the negative control exceeded 0.50 and the blocking percentage of the positive control was greater than 50%. The presence of anti-CSFv antibodies was determined by calculating the percentage of the test samples' absorbance relative to the negative control. The absorbance value of the sample was subtracted from that of the negative control, then divided by the absorbance of the negative control, and then multiplied by 100 to express it as a percentage. A sample was classified as positive if its blocking percentage was at least 40% and negative if it was at most 30%.

2.6 Real-Time Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

All samples were handled in a Biosafety Level 2 (BSL-2) laboratory, adhering to standard BSL-2 procedures. The samples were thawed in a cold, controlled environment, and the total RNA was consequently extracted using the QIAamp® Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The purity and quantity of extracted RNA were checked spectrophotometrically using a NanodropTM 2000/2000c (Thermo Fisher Scientific, DE, USA) prior to further testing.

The total RNA was reverse transcribed using random hexamer primers with the cDNA synthesis kit (HiScript® III RT SuperMix, Vazyme International LLC, Nanjing, China) following the manufacturer's instructions with minor modifications. Sixteen (16) µl of the gDNA wiper-treated total RNA and 4 µl of the mastermix were incubated at 37 °C for 15 minutes and 65 °C for 5 seconds. The qRT-PCR assay was conducted using the Toptical Gradient 96 (Analytic Jena, Göttingen,

Germany) with the Primerdesign™ Classical Swine Fever virus genesig Advanced kit in tandem with Oasig lyophilized 2x qPCR standard mastermix. For CSFv detection, a mixture was prepared using 10 µl of 2x qPCR Mastermix, 1 µl of CSFv specific primer/probe mix, 3 µl RNAse-free water, and 5 µl cDNA. The primers were designed to target the 5' UTR of the CSFv genome, based on the reference sequence with GenBank accession number HQ148062.1, producing an amplicon of 106 bp anchored at nucleotide position 158. Amplification was carried out under the following thermal conditions: enzyme activation at 95 °C for 2 minutes, followed by 50 cycles of denaturation at 95 °C for 10 seconds and annealing/extension at 60 °C for 60 seconds with signal acquisition. Results were interpreted based on the detected FAM-labeled channel amplification as cycle threshold (Ct) values, wherein samples with a Ct value of at most 35 were classified as positive.

2.7 Interview of Abattoir Officers

Using a structured questionnaire, an interview with meat inspectors and veterinarians in each slaughterhouse and local government unit, respectively, was conducted. Farm demographics, common farm practices, history of CSF vaccination and outbreak, and details of the CSF surveillance program were obtained for the association test with seropositivity. A written consent form was provided only to those who agreed to the interview, and demographic data were treated with the utmost confidentiality.

2.8 Data Analysis

All data were recorded and organized in Excel (Microsoft, WA, USA). To calculate the positivity rates, the number of positive samples was divided by the number of tested samples and multiplied by 100. Statistical analyses were conducted in R (Posit Software, MA, USA). Using the *imputeMCA* function of the *missMDA* package [28] with default settings, imputation of missing information under 14 factors from the survey data was undertaken, except for Benguet with one sampled municipality due to the absence of interview responses. The association between these factors and seropositivity was assessed using the least absolute shrinkage and selection operator (LASSO) regression analysis [29] with the *glmnet* package [30]. The open-access Quantum Geographic Information System version 3.40 was also used to generate the geographical distribution maps of the positivity rates and risk classifications.

2.9 Semi-quantitative Risk Scoring

The risk of CSF transmission was assessed semi-quantitatively, adapting the methods of the European Food Safety Authority in ASF risk assessment with minor modifications in assigning risk scores [31]. Specifically, the probability of transmission as measured by eight factors—four from this study and four from published databases—was estimated for each sampled province. A greater weight in the final risk score was allocated to factors that were directly linked to CSF occurrence.

Seropositivity was given a maximum risk score of "5.0". The range between the highest and lowest positivity rates was split into five equal intervals, and the risk score was inversely proportional to seropositivity (i.e., 0-20%=5.0, 21-40%=4.0, 41-60%=3.0, 61-80%=2.0, 81-100%=1.0). Swill feeding, vehicle disinfection, and water treatment from interviews were factors influencing seropositivity in this study, resulting in their inclusion in the risk scoring. Municipalities were scored based on the percentage of farms engaging in swill feeding (i.e., 0%=0.0, 1-25%=1.0, 26-50%=2.0, 51-75%=3.0, and 76-100%=4.0). Under vehicle disinfection, a municipality received a grade of "0" if it was practiced both before entry and after leaving the farm, "1.0" if it was done either before or after, and "2.0" if not done at all. Those employing water treatment methods in farms were given a "0" score; otherwise, a grade of "1.0" was assigned. For each of these three factors, the mean of the risk scores of all municipalities reflected the risk of their respective provinces.

For the succeeding four factors, data covering the sampling period in each province were obtained from online databases. A large herd size was associated with CSF occurrence [10], so the percentage of pigs in commercial farms was computed by dividing the number of pigs raised in commercial farms by the total number of pigs raised in a province, then multiplied by 100 [32]. The swine population density (heads/km^2), which reflected the closeness of pigs linked to CSF transmission [10], was also estimated by getting the ratio between the total number of pigs in a province [32] and the most recent provincial data in total land size [33]. The assignment of risk scores in these two factors followed the method employed in seropositivity, except for the direct relationship between factors and risk score and the assignment of

a "0" score for those who had "0" raw data. As the CSFv survives in and can be transmitted through pork and pork products [7], the regional data on total frozen pork inventory (metric tons) [34] was also used to estimate its contribution to transmission at the provincial level. Similar to swill feeding, a maximum score of "4.0" was assigned, and four equal intervals were derived from the range of the pork inventory. The role of increased human interactions in the potential mechanical transmission of CSFv [7] was considered minimally. As represented by the human population density (heads/km^2) in a province, the human population size [33] was divided by the total land size [34]. Provinces with a population density greater than the median received a score of "1.0"; otherwise, a "0" score was assigned.

The scores across these eight factors were summed for each province. The range between the maximum and minimum possible values of the overall risk scores was partitioned into six equal intervals, and each province was categorized into one of the following risk bands: extreme, very high, high, moderate, low, and very low [35].

3. Results

3.1 Serological and Molecular Detection of CSF

Out of 425 samples subjected to competitive ELISA, 153 (36.0%, 95% Confidence Interval: 31.5-40.8%) tested positive (Table 1). The highest

Table 1. Positivity rates in classical swine fever from smallhold farms in 21 Philippine provinces.

REGION	PROVINCE	ELISA		qRT-PCR	
		TESTED SAMPLES	POSITIVE SAMPLES (%)	TESTED SAMPLES	POSITIVE SAMPLES (%)
CAR	Benguet	17	17 (100.0)	17	0 (0.0)
I	Ilocos Sur	20	3 (15.0)	20	0 (0.0)
	Pangasinan	16	0 (0.0)	16	0 (0.0)
III	Nueva Ecija	15	6 (40.3)	15	0 (0.0)
	Pampanga	15	9 (60.0)	15	0 (0.0)
IV-A	Batangas	13	4 (30.8)	13	0 (0.0)
	Cavite	21	11 (52.4)	21	0 (0.0)
	Laguna	16	6 (37.5)	16	0 (0.0)
IV-B	Occidental Mindoro	16	0 (0.0)	16	0 (0.0)
	Palawan	16	0 (0.0)	14	0 (0.0)
V	Camarines Sur	15	5 (33.3)	15	0 (0.0)
VI	Aklan	27	0 (0.0)	27	0 (0.0)
	Negros Occidental	15	8 (53.3)	15	0 (0.0)
VII	Bohol	13	2 (15.4)	13	0 (0.0)
	Cebu	22	15 (68.2)	22	0 (0.0)
VIII	Samar	28	7 (25.0)	28	0 (0.0)
IX	Zamboanga del Sur	30	1 (3.33)	30	0 (0.0)
X	Bukidnon	20	12 (60.0)	20	0 (0.0)
XI	Davao del Sur	30	19 (63.3)	30	0 (0.0)
XII	North Cotabato	30	4 (13.3)	30	0 (0.0)
XIII	Surigao del Norte	30	24 (80.0)	30	0 (0.0)
	TOTAL	***425***	***153 (36.0)***	***423***	***0 (0.0)***

ELISA: enzyme-linked immunosorbent assay; qRT-PCR: real-time reverse transcription polymerase chain reaction

seropositivity was observed in Benguet (100.0%, 17/17), followed by Surigao del Norte (80.0%, 24/30), Cebu (68.2%, 15/22), and Davao del Sur (63.3%, 19/30) (Fig. 1). Conversely, all samples in Pangasinan (n=16), Occidental Mindoro (n=16), Palawan (n=16), and Aklan (n=27) were seronegative (Fig.1). In the qRT-PCR assay, all of the 423 tested samples were negative for CSFv RNA (Table 1).

3.2 Factors Associated with Seropositivity in CSF

Around 38.1% of the municipalities (16/42) had farms with 6-10 pigs, while a majority (59.5%, 25/42) reported that native pigs were raised in about 1-25% of farms (Table 2). Most municipalities (54.8%, 23/42) also indicated that 50% of swine farms were close to residential areas.

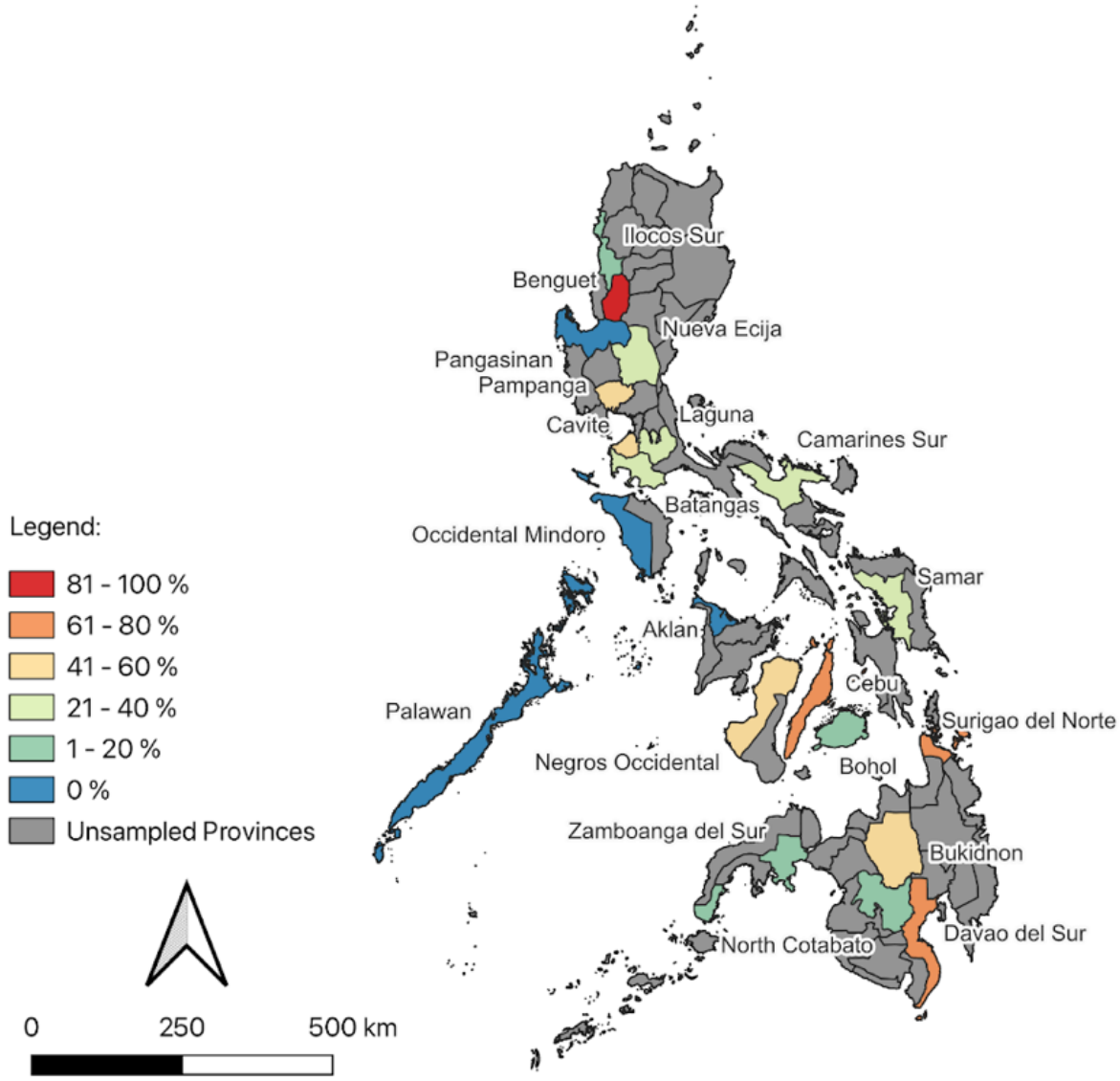

Figure 1. Geographic distribution of classical swine fever seropositive pigs in smallhold farms across 21 sampled provinces in the Philippines. The map was generated using Quantum Geographic Information System 3.40.

Table 2. Distribution of interview responses from 42 municipalities across 14 variables.

Variables	Number of municipalities (%)	Variables	Number of municipalities (%)
Average herd size		*Percentage of farms keeping vaccination records*	
1-5 pigs	14 (33.3)	0 %	14 (33.3)
6-10 pigs	16 (38.1)	1-25%	16 (38.1)
11-15 pigs	7 (16.7)	26-50%	1 (2.4)
16-20 pigs	5 (11.9)	51-75%	3 (7.1)
Percentage of farms raising native pigs		76-100%	8 (19.0)
0 %	9 (21.4)	*Housing system*	
1-25%	25 (59.5)	Pen-type	38 (90.5)
26-50%	7 (16.7)	Free range	1 (2.4)
51-75%	1 (2.4)	Mixed	3 (7.1)
76-100%	0 (0)	*Water treatment*	
Percentage of farms proximal to residential areas		No	23 (54.8)
0 %	4 (9.5)	Yes	19 (45.2)
1-25%	9 (21.4)	*Disinfection of transport vehicles in the farm*	
26-50%	10 (23.8)	No	12 (28.6)
51-75%	10 (23.8)	Before entry or after exit	7 (16.7)
76-100%	9 (21.4)	Before entry and after exit	23 (54.8)
Percentage of farms with biosecurity measures		*Disease reporting*	
0 %	0 (0)	No	4 (9.5)
1-25%	16 (38.1)	Yes	38 (90.5)
26-50%	12 (28.6)	*Surveillance program for classical swine fever*	
51-75%	6 (14.3)	No	24 (57.1)
76-100%	8 (19.0)	Yes	18 (42.9)
Percentage of farms engaging in swill feeding		*Vaccination against classical swine fever*	
0 %	15 (35.7)	No	31 (73.8)
1-25%	18 (42.9)	Yes	11 (26.2)
26-50%	5 (11.9)	*History of classical swine fever*	
51-75%	2 (4.8)	No	41 (97.6)
76-100%	2 (4.8)	Yes	1 (2.4)
Percentage of farms raising animals other than pigs			
0 %	1 (2.4)		
1-25%	21 (50.0)		
26-50%	11 (26.2)		
51-75%	3 (7.1)		
76-100%	6 (14.3)		

A majority of the municipalities (38.1%, 16/42) also had 1-25% of farms with biosecurity measures. Approximately 35.7% (15/42) did not engage in swill feeding. Half of the municipalities (21/42) responded that less than a quarter of their swine farms raised other animals aside from pigs. Fourteen (14) out of 42 municipalities (33.3%) had farms not keeping their vaccination records, while a majority (38.1%, 16/42) had an estimated 1-25% of farms that did. The most frequent housing type was pens (90.5%, 38/42), while water in farms in around 54.8% of the municipalities (23/42) was not treated. The majority of municipalities (54.8%, 23/42) noted that disinfection of transport vehicles was also practiced before entry and after leaving the farm. Most municipalities (90.5%, 38/42) also stated that farms reported swine disease occurrences, while around 57.1% (24/42) indicated the absence of CSF surveillance programs. Thirty-one (31) municipalities (73.8%) responded that farms were not vaccinating against CSF, and forty-one municipalities (97.6%) had farms without CSF history.

The LASSO regression revealed the association of seven (7) factors from interviews of abattoir officers with seropositivity (Table 3). The greatest positive impact was found in water

Table 3. Least absolute shrinkage selection operator coefficient of 14 variables from survey data against seropositivity in classical swine fever.

Variable	Beta Coefficient
Intercept	27.1
Average herd size	0.0
Percentage of farms raising native pigs	-1.7
Percentage of farms proximal to residential areas	-2.8
Percentage of farms with biosecurity measures	0.0
Percentage of farms engaging in swill feeding	6.6
Percentage of farms raising animals other than pigs	0.0
Percentage of farms keeping vaccination records	0.6
Housing system	0.0
Water treatment	10.7
Disinfection of transport vehicles in the farm	0.0
Disease reporting	0.0
Surveillance program for classical swine fever	0.0
Vaccination against classical swine fever	5.2
History of classical swine fever	-17.8

treatment (β=10.7), followed by swill feeding (β=6.6), CSF vaccination (β=5.2), and maintaining vaccination records (β=0.6). On the other hand, a history of CSF (β=-17.8), farms near residential areas (β=-2.8), and raising of native breeds (β=-1.7) were negatively associated with seropositivity.

3.3 Transmission Risk of CSF in 21 Provinces

A total of eight factors—four identified in our study and four obtained from online databases—were included in the risk scoring of CSF transmission in 21 sampled provinces. For the four factors from our study, an inverse relationship between seropositivity and risk score was employed, as our results suggest that the detected antibodies in ELISA and CSF vaccination were positively associated. The seropositivity rates across 21 provinces ranged from 0 to 100.0% (Table 4). Benguet had the highest seropositivity (100.0%) and was assigned a score of "1.0" (Table 5). On the other hand, eight provinces earned a "5.0" score due to very low seropositivity rates, falling within the 0 to 20% interval. In swill feeding, Nueva Ecija, Cavite, and Bukidnon had at least a score of "2.0", while farms in Ilocos Sur, Batangas, Zamboanga del Sur, Davao del Sur, North Cotabato, and Surigao del Norte were not engaging in swill feeding, leading to a "0" score. Vehicle disinfection was not practiced in farms from Bohol, Zamboanga del Sur, and Bukidnon, earning a score of "2.0", while Ilocos Sur, Pampanga, Batangas, Camarines Sur, Negros Occidental, Davao del Sur, and North Cotabato were given a "0" score for implementing vehicle disinfection before entry and after exit from farms. Under water treatment, all sampled municipalities in Ilocos Sur, Pangasinan, Nueva Ecija, Occidental Mindoro, Bohol, Zamboanga del Sur, and Bukidnon indicated that farms did not treat agricultural water, resulting in a score of "1.0", while Camarines Sur, Davao del Sur, North Cotabato, and Surigao del Norte received a "0" score for practicing water treatment.

From online databases, provinces were scored based on commercial farm proportion, swine population density, frozen pork inventory, and human population density. The proportion of commercial farms in sampled provinces ranged from 0 to 97.0%. Cavite had approximately 97.0% commercially raised pigs (Table 4), leading to a score of "5.0" (Table 5), while Samar and Surigao del Norte were given "0" scores due to the absence of commercial farms. For swine population density, the maximum value was observed in Batangas (250.4 heads/km^2), while Samar had the lowest density at 2.5 heads/km^2. After dividing the range into five equal intervals, Batangas had a score of "5.0" (200.9-250.4 heads/km^2), while Pampanga received a "4.0" score (151.3-200.8 heads/km^2). Eleven provinces also earned a score of "1.0" due to values falling within the 2.5 to 52.1 heads/km^2 interval. Under the frozen pork inventory, four equal intervals were derived between the range of 31,795.1 and 6.1 metric tons, resulting in a score of "4.0" in Nueva Ecija (23,848.0-31,795.1 metric tons) and "3.0" in Batangas (15,900.7-23,847.9 metric tons). Meanwhile, ten out of 21 provinces exceeded the median of the human population density (349.6 heads/km^2), receiving a score of "1.0".

The sum of the scores of each province across these eight factors was calculated to estimate the transmission risk of CSF (Table 5). No province fell within the extreme (26.8-32.0) or very high (21.4-26.7) risk bands, while Nueva Ecija was the lone high-risk province (16.1-21.3) (Fig. 2). The transmission risk of CSF was found to be moderate (10.8-16.0) and low (5.4-10.7) in nine provinces each, while two provinces had a very low risk level (0-5.3).

Table 4. Provincial data on eight factors for risk scoring of classical swin fever transmission.

Region	Province	Seropositivity Rate (%)	Swill Feeding	Vehicle Disinfection	Water Treatment	Pigs in Commercial Farms (%)	Swine Population Density (heads/km²)	Frozen Pork (metric tons)	Human Population Density (heads/km²)
CAR	Benguet	100.0	ND	ND	ND	12.9	15.6	ND	292.6
I	Ilocos Sur	15.0	0.0	0.0	1.0	4.2	24.3	272.9	272.0
	Pangasinan	0.0	0.5	1.5	1.0	55.2	25.7	310.5	580.3
III	Nueva Ecija	40.0	3.5	1.5	1.0	40.1	14.8	31,795.1	406.0
	Pampanga	60.0	1.7	0.0	0.3	73.7	151.8	8,809.1	1,404.9
IV-A	Batangas	30.8	0.0	0.0	0.5	7.7	250.4	19,733.1	933.7
	Cavite	52.4	2.7	1.0	0.3	97.0	51.3	6,835.7	2,846.7
	Laguna	37.5	0.5	0.5	0.8	70.6	40.8	7,587.1	1,754.0
IV-B	Occidental Mindoro	0.0	1.0	0.3	1.0	16.2	15.7	6.1	89.8
	Palawan	0.0	0.5	1.0	0.5	2.7	18.2	270.2	73.2
V	Camarines Sur	33.3	1.0	0.0	0.0	0.4	62.8	338.2	375.2
VI	Aklan	0.0	0.3	0.7	0.3	15.5	40.0	84.1	349.6
	Negros Occidental	53.3	1.5	0.0	0.5	10.6	61.0	176.4	400.7
VII	Bohol	15.4	1.0	2.0	1.0	34.9	53.9	4,376.0	292.2
	Cebu	68.2	1.0	1.7	0.3	67.6	107.1	8,052.0	964.3
VIII	Samar	25.0	1.0	1.0	0.5	0.0	2.5	142.5	131.1
IX	Zamboanga del Sur	3.3	0.0	2.0	1.0	10.5	61.2	76.7	343.8
X	Bukidnon	60.0	2.0	2.0	1.0	54.3	66.6	952.8	146.8
XI	Davao del Sur	63.3	0.0	0.0	0.0	59.9	78.6	1,005.8	533.3
XII	North Cotabato	13.3	0.0	0.0	0.0	15.2	24.1	374.4	136.9
XIII	Surigao del Norte	80.0	0.0	1.0	0.0	0.0	4.2	135.8	273.8

ND: No data

Table 5. Risk scores and classifications in classical swine fever transmission of 21 selected provinces across the Philippines.

Region	Province	Seropositivity Rate	Swill Feeding	Vehicle Disinfection	Water Treatment	Pigs in Commercial Farms	Swine Population Density	Frozen Pork	Human Population Density	Risk Score	Risk Level
CAR	Benguet	1.0	ND	ND	ND	1.0	1.0	ND	0.0	**3.0**	**Very Low**
I	Ilocos Sur	5.0	0.0	0.0	1.0	1.0	1.0	1.0	0.0	**9.0**	**Low**
	Pangasinan	5.0	0.5	1.5	1.0	3.0	1.0	1.0	1.0	**14.0**	**Moderate**
III	Nueva Ecija	4.0	3.5	1.5	1.0	3.0	1.0	4.0	1.0	**19.0**	**High**
	Pampanga	3.0	1.7	0.0	0.3	4.0	4.0	2.0	1.0	**16.0**	**Moderate**
IV-A	Batangas	4.0	0.0	0.0	0.5	1.0	5.0	3.0	1.0	**14.5**	**Moderate**
	Cavite	3.0	2.7	1.0	0.3	5.0	1.0	1.0	1.0	**15.0**	**Moderate**
	Laguna	4.0	0.5	0.5	0.8	4.0	1.0	1.0	1.0	**12.8**	**Moderate**
IV-B	Occidental Mindoro	5.0	1.0	0.3	1.0	1.0	1.0	1.0	0.0	**10.3**	**Low**
	Palawan	5.0	0.5	1.0	0.5	1.0	1.0	1.0	0.0	**10.0**	**Low**
V	Camarines Sur	4.0	1.0	0.0	0.0	1.0	2.0	1.0	1.0	**10.0**	**Low**
VI	Aklan	5.0	0.3	0.7	0.3	1.0	1.0	1.0	0.0	**9.3**	**Low**
	Negros Occidental	3.0	1.5	0.0	0.5	1.0	2.0	1.0	1.0	**10.0**	**Low**
VII	Bohol	5.0	1.0	2.0	1.0	2.0	2.0	1.0	0.0	**14.0**	**Moderate**
	Cebu	2.0	1.0	1.7	0.3	4.0	3.0	2.0	1.0	**15.0**	**Moderate**
VIII	Samar	4.0	1.0	1.0	0.5	0.0	1.0	1.0	0.0	**8.5**	**Low**
IX	Zamboanga del Sur	5.0	0.0	2.0	1.0	1.0	2.0	1.0	0.0	**12.0**	**Moderate**
X	Bukidnon	3.0	2.0	2.0	1.0	3.0	2.0	1.0	0.0	**14.0**	**Moderate**
XI	Davao del Sur	2.0	0.0	0.0	0.0	3.0	2.0	1.0	1.0	**9.0**	**Low**
XII	North Cotabato	5.0	0.0	0.0	0.0	1.0	1.0	1.0	0.0	**8.0**	**Low**
XIII	Surigao del Norte	2.0	0.0	1.0	0.0	0.0	1.0	1.0	0.0	**5.0**	**Very Low**

ND: No data

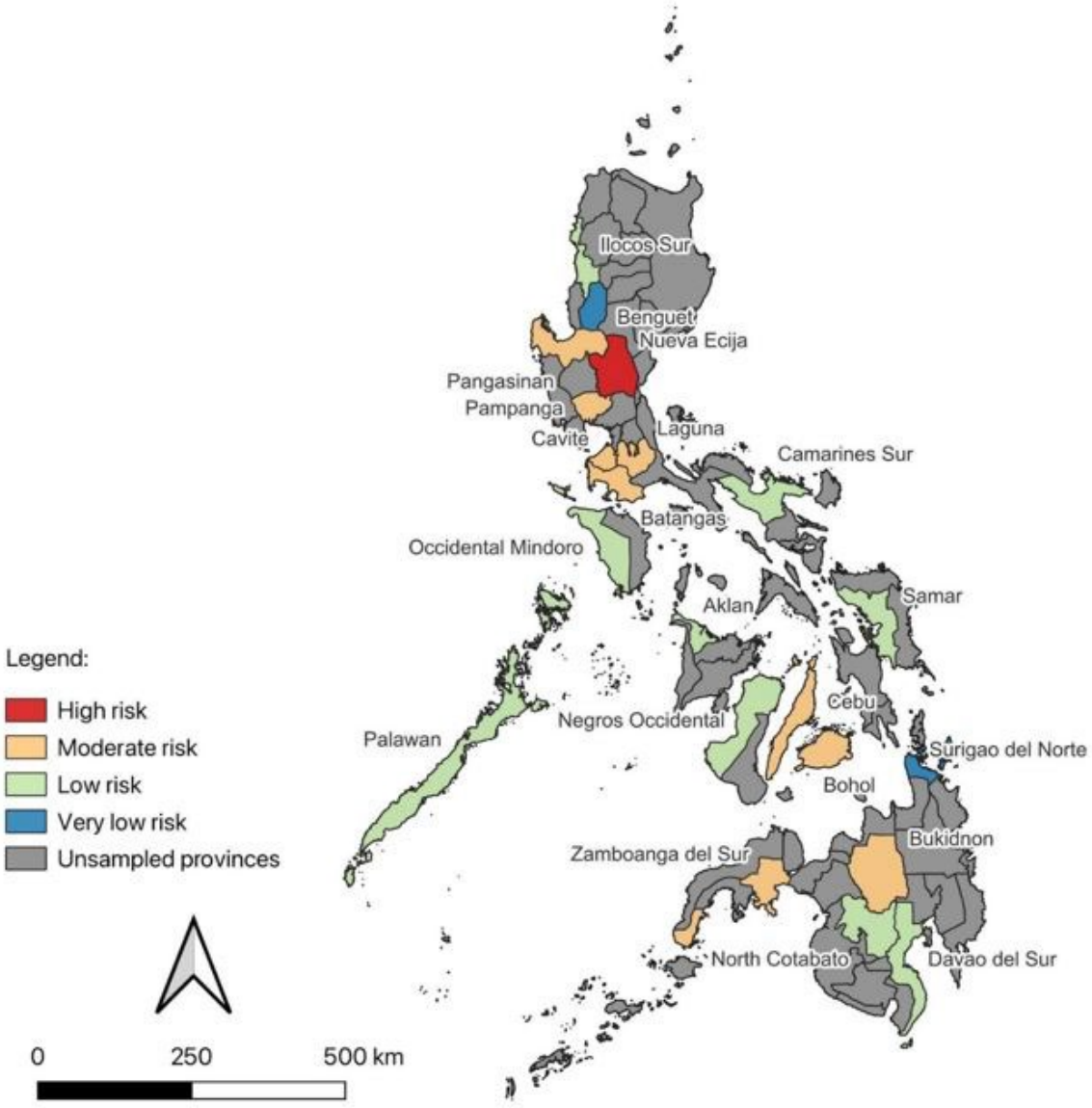

Figure 2. Geographic distribution of the risk classifications for classical swine fever transmission in 21 sampled provinces in the Philippines. The map was generated using Quantum Geographic Information System 3.40.

4. Discussion

CSF belongs to the WOAH list of notifiable diseases of domestic and wild pigs [7]. Due to its transboundary nature, it poses a serious risk to pig health, the swine industry, and both local and international trade, impacting both economic stability and food security worldwide [2]. The disease is thought to be endemic in the Philippines, with outbreaks between 2007 and 2009 affecting more than 4,000 pigs nationwide [23–25]. With the potentially severe CSF consequences and the scarce data on occurrences, surveillance using reliable and valid diagnostic methods such as ELISA and qRT-PCR can be useful in determining the CSF spread. Investigating risk factors linked to positivity and estimating transmission risk can also provide insights on how to prevent and control future occurrences while considering the local swine farming context.

Our results revealed the presence of seropositive pigs in smallhold farms across the 21 sampled provinces. This is consistent with

previous CSF research in Nueva Ecija and Pampanga, which reported seropositivity in a smaller sample size of both vaccinated and unvaccinated pigs [36]. In our study, the detected antibodies against CSFv may be attributed to passive immunity, the diagnostic kit's performance, and humoral immune response. Maternally derived antibodies (MDAs), transferred via colostrum, were found to persist for up to 10 weeks in domestic pigs [37], and these might have waned in the sampled five- to six-month-old pigs. Antibodies against other members of the genus *Pestivirus* may cross-react with those against CSFv using the kit in our study [17], potentially contributing to the seropositivity rate. Investigation of other pestiviruses, such as bovine viral diarrhea virus, causing reproductive losses in pigs and affecting sheep and goats [38,39], as well as border disease virus, which was not yet reported in the Philippines, will rule out this source of false positive samples. Additionally, examining samples from suspected CSF cases at the molecular level after a serological assay may also achieve increased specificity. For example, differentiation of CSFv from other pestiviruses was successful using qRT-PCR [20,40]. Another study developed a multiplex qRT-PCR assay for diagnosing CSF that was capable of detecting as few as 8 copies of the viral genome with exceptional specificity for CSFv, ensuring no cross-reactivity with other pestiviruses [41]. Post-infection with a wild type of CSFv also elicits antibody production, except for persistently infected pigs with immunosuppression [42]. Pigs surviving CSF were known to have prolonged and even lifelong immunity [15], and the WOAH recommends examining antibodies in probable CSF cases within a seroconversion period of 14 to 21 days post-infection [1,15]. Considering the negative association of CSF history on seropositivity from our regression analysis, it was highly likely that the detected antibodies were not derived from field infection. CSF vaccination is typically performed at five to nine weeks of age, either as a single dose or with a booster depending on vaccine type and existing CSF situation [43,44]. In one study, seroconversion takes around one to three weeks post-vaccination, and pigs remained seropositive at 45 days [45]. Other studies showed varying persistence of vaccine-induced antibodies [18,46,47], which may have lasted in the sampled pigs. Although our test kit cannot distinguish the various drivers of antibody production [17], the association of CSF vaccination with seropositivity in our study provides sufficient evidence on the increased likelihood of vaccine-derived antibodies. This is consistent with research findings in Timor-Leste and Indonesia wherein the odds of seropositivity were increased by two to three times with CSF vaccination[48,49]. For improved sensitivity and specificity, future surveillance programs may benefit from using Differentiating Infected from Vaccinated Animals (DIVA) serological tests or vaccines to reduce ambiguity in interpreting seropositivity [17,50]. On the other hand, the immunosuppressive effect of the CSFv, especially in persistently and chronically affected herds, may account for the seronegative samples, as shown previously [42,51]. Vaccination failure may also occur if the timing of administration and seroconversion coincide with the presence of MDAs [52,53]. Finally, seronegativity may also be interpreted as the complete absence of CSFv exposure.

Despite considerable seropositivity, no CSFv RNA was detected in any of the tested samples. Early viremia may lead to negative qRT-PCR outcomes due to very low and undetectable viral loads [41]. Moreover, chronic cases typically show prolonged viral shedding and would still be positive when subjected to molecular testing. These attributes demonstrate the absence of active CSF infection, whether acute or chronic, in the sampled animals. The inconsistency between our serologic and molecular findings is also similar to published studies. For instance, a study on the E2CD154 subunit vaccine showed that vaccinated pigs maintained protective immunity for at least nine months post-vaccination, with no evidence of adverse effects or prolonged viral presence [46]. The widely used live Chinese strain vaccine has also been reported to confer solid immunity within a few days after a single vaccination, with lifelong immunity and without prolonged viral shedding [18]. The Thiverval strain vaccine was also documented to offer complete protection five days after inoculation, effectively inhibiting CSFv replication post-challenge [47]. These findings underscore the efficacy of the CSF vaccines in eliciting sustained humoral immune responses without viral persistence and the significance of vaccination as part of a CSF intervention program. Also, the results of the two laboratory-based methods may also be used together in the future direction of CSF surveillance, particularly in declaring CSF-free areas.

Apart from CSF vaccination, three other factors from survey data positively impacted seropositivity. In Kenya, income level and educational attainment were substantially linked to knowledge of farm irrigators on integrated water resource management [54]. Similarly, we hypothesize that water treatment having the highest positive impact on seropositivity may be related to the smallhold farmer's capacity to afford and access vaccination, which can be confirmed by undertaking a knowledge, attitude, and practice study, along with economic analysis. Swill feeding has been implicated as a route of CSFv infection, and a study found the survival of infectious CSFv in pork sausage casing for 37 days [1,55]. This emphasizes the role of pork and pork products in CSF transmission and infection, which may result in antibody production. Furthermore, the practice of feeding untreated leftovers to pigs is common in smallhold farms as a cost-saving measure [56]. In our study, some municipalities reported both swill feeding and CSF vaccination, accounting for the association between these factors. Maintaining vaccination records was also weakly associated with seropositivity. This practice is essential in determining the proper timing of vaccination in growers, sows, and piglets farrowed from vaccinated sows to avoid vaccination failure and promote successful seroconversion [53,57].

Results of LASSO regression also showed three factors being negatively associated with seropositivity. The commonality of farrow-to-finish operations in smallhold farms in the Philippines was previously demonstrated [58]. The ongoing ASF issue may have also increased the farms employing this operation type to minimize the probability of introducing infected herds. Under this intensive system, CSF occurrence may result in a higher probability of vertical transmission, which commonly results in immunosuppression and persistent viremia in farrowed pigs [59]. This explains the inverse relationship between seropositivity and the history of CSF in smallhold farms. An increased proportion of farms near residential areas was also observed to result in lower seroprevalence. Compared to commercial farms, smallhold farms are typically found in residential backyards, have fewer biosecurity measures in place, and have less access to veterinary care [60]. These characteristics may have contributed to the lower seropositivity in the increased percentage of farms close to residential zones. Increasing native pig breed distribution also negatively impacted seropositivity, which may be attributed to the perceived resistance of these breeds to swine diseases, resulting in a lesser willingness to have these pigs vaccinated [61]. However, it is crucial to emphasize the susceptibility of all pigs to CSFv infection as shown in the comparative study between indigenous and commercial breeds in Lao's People Democratic Republic [62].

Among the sampled provinces, only Nueva Ecija was categorized as high-risk for CSF transmission due to consistently high scores across all factors, leading to an elevated overall risk. On the contrary, it is worth noting that the missing responses in Benguet influenced its very low risk classification, reinforcing the significance of complete working data for a more precise analysis. In our semi-quantitative scoring, seropositivity was inversely related to the risk score. Generally, sufficient antibody levels—whether from vaccination, field infection, or passive immunity—confer protection to pigs. For example, the Thiverval vaccine offered robust defense five days following vaccination, and protection was maintained even when vaccinated pigs were housed with CSFv-positive pigs [47]. The E2CD154 candidate vaccine also demonstrated capacity to prevent vertical transmission of CSFv [46], and MDAs were shown to be effectively transferred to piglets, providing early and short-lived immunity [37]. Antibodies in pigs surviving infection by the wild CSFv strain also persisted for long periods of time [15]. A cross-sectional study also found the lack of vaccination as a significant risk factor for CSF occurrence [12]. Collectively, these findings indicate that the lack of exposure to CSFv increases the susceptibility of pigs to contracting the disease and being a source of transmission.

Direct contact with infected pigs is a major route in CSF transmission [1]. To estimate the potential contact rate among a high volume of pigs, we considered the proportion of pigs in commercial farms and swine population density, which were among the factors found to be associated with increased odds of CSF occurrence. Commercial farms typically raise a large herd size of more than 50 pigs [63], and it was previously estimated that this farm type had a contact rate of 1.24 times a day, higher than in smallhold farms [64]. As the herd size increases, the number of susceptible pigs also rises, along with the chance of effective

contact [10]. While higher contact rates suggest greater odds of CSF occurrence, it is important to note that these farms have sufficient capacity to employ appropriate biosecurity measures and disease intervention strategies such as vaccination and disease monitoring, which can mitigate CSF transmission risk. The swine population density may also more effectively reflect the contact rate than population size. In Cuba, a denser swine population was associated with a 1.25-fold increased chance of infection [65]. Additionally, airborne transmission or "neighborhood effect" within a distance of one meter was considered during CSF outbreaks in areas with high farm density [66]. This evidence underscores the potential heightened risk of CSF transmission in areas with larger swine herd sizes and higher swine population density.

The association of farm practices with seropositivity in our study prompted an examination of these factors as contributors to CSF transmission risk. The CSFv has been shown to survive in water for 6 to 24 days at 20 °C [67]. Its infectiousness requires further assessment, and minimal studies were conducted to elucidate the role of drinking water in transmission. Given its persistence under certain conditions, disease prevention efforts may benefit from water treatment and ensuring a safe and clean water supply for pigs as a form of reducing CSFv risk. Vehicle disinfection is a critical element of farm biosecurity. In Denmark, truck disinfection at borders was mandatory due to varying persistence of CSFv and other important swine viruses in the outside environment, especially in the presence of protein and organic materials [67,68]. Moreover, trucks coming from another livestock farm were banned from entering new livestock premises for 48 hours after disinfection [68]. Model simulations found that these practices decreased the likelihood of CSFv occurrence [68] due to the susceptibility of the virus to common disinfectants such as sodium hypochlorite, quartz, and aldehydes [69]. Without vehicle disinfection practices, the risk of CSF occurrence and transmission can be higher.

The effect of frozen pork inventory and swill feeding was closely related in CSFv transmission. Studies have documented the survival of CSFv in frozen pork for years, in chilled pork for up to 85 days, and in cured or smoked pork for 17 to 188 days [67]. The volume of pork imported both legally and illegally was also estimated to pose a serious risk in Denmark because of the potential use of pork as swill [68]. Feeding kitchen leftovers to pigs was also shown to increase the chances of CSF occurrence by 8.53 times in Ecuador [12] and by 2.25 times in Bhutan [70]. These findings substantiate the inclusion of frozen pork volume and swill feeding in our risk scoring scheme. Several strategies were implemented and recommended to mitigate this risk. For instance, banning swill feeding was found to decrease the risk of CSFv introduction through frozen pork [68], though non-compliance remains an issue, particularly in smallhold farms, where it is commonly practiced to reduce production cost. It was also recommended to uniformly heat the meat for consumption to 70°C for at least 30 minutes to effectively inactivate CSFv [14]. For swills, inactivating the virus can be achieved by heating to at least 90 °C for at least 60 minutes with constant stirring or to 121 °C for at least 10 minutes [14]. The role of human population density, which reflects the increased human interaction, was also considered minimally in the risk scoring. Limited studies dealt with the role of increasing human movements in the spread of CSFv, but indirect or mechanical transmission via contaminated clothing of humans was possible [7], contributing low risk to CSFv transmission.

Considering all eight factors in assessing the risk of CSF transmission improves our understanding of its epidemiology and potential danger to the swine industry and pig health. Despite no active infection detected, the results of risk scoring provide useful information that can aid in designing future disease surveillance programs while considering efficient use of resources [66]. While a similar study including all provinces in the Philippines may be done, we recommend focusing on the identified moderate- to high-risk provinces in terms of conducting CSF vaccination programs, enforcing strict biosecurity measures, and undertaking other CSF intervention strategies to prevent disease occurrence and mitigate related impacts.

5. Conclusions

This study provides critical information on the epidemiological status of CSF in smallhold farms across 21 provinces in the Philippines. The seroprevalence implies prior CSFv exposure, which was likely due to vaccination. On the other hand, the absence of detected CSFv RNA suggests

no active infection in sampled pigs from the sampled areas. The combined results of the two laboratory methods may also be used in future disease surveillance frameworks in declaring CSF-free areas. Factors associated with seropositivity were related to on-farm practices, farm demographics, and human-driven practices. Furthermore, the 21 sampled provinces were classified into six risk levels, with no province having extreme and very high risk classifications. Overall, these findings highlight the need for strategic disease monitoring with a requirement for better vaccination strategies, strict biosecurity measures, and efficient resource allocation for CSF control and prevention in the Philippines.

Availability of Data and Materials

All data may be provided upon reasonable request to the corresponding author.

Author Contributions

Conceptualization, B.R.G.M. and M.S.E.G.L.; Methodology, B.R.G.M. and M.S.E.G.L.; Formal Analysis, G.M.V.P., J.M.G.B., and M.A.O.A.; Investigation, C.M.D.P., A.P.R.S., M.A.O.A., E.N.G.L., K.A.S.D.R., B.R.G.M., A.W.B.R., and M.S.E.G.L.; Data Curation, G.M.V.P., J.M.G.B., and M.A.O.A.; Writing – original draft, C.M.D.P., J.M.G.B., C.P.F.C., and K.A.S.D.R.; Writing – review and editing, C.M.D.P., A.P.R.S., M.A.O.A., E.N.G.L., K.A.S.D.R., B.R.G.M., A.W.B.R., J.M.G.B., G.M.V.P., C.P.F.C., and M.S.G.L.; Supervision, M.S.E.G.L., B.R.G.M., and C.P.F.C.; Project Administration, M.S.E.G.L. and B.R.G.M.; Funding Acquisition, M.S.E.G.L., B.R.G.M., and C.P.F.C.

Ethics Approval and Consent to Participate

Only abattoir workers who signed a written consent form were included in the interview.

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Conflict of Interest

The authors declare the absence of competing interests.

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