

EPIDEMIOLOGICAL INVESTIGATION AND TRANSMISSION RISK SCORING OF SWINE INFLUENZA IN PHILIPPINE SMALLHOLD PIG FARMS USING LABORATORY AND FARM-LEVEL INDICATORS

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ABSTRACT

This study investigated the prevalence, risk factors, and transmission risk of swine influenza (SI) in pigs from smallhold farms in the Philippines due to its possible risks to animal and public health. A total of 425 blood and 407 nasal swab samples were collected from slaughterhouses in 21 provinces and analyzed using competitive enzyme-linked immunosorbent assay and real-time reverse transcription polymerase chain reaction (qRT-PCR) assay to detect SI antibodies and viral RNA, respectively. Results revealed an overall seropositivity of 22.8% (97/425) and qRT-PCR positivity of 0.5% (2/407). Water treatment, vaccination, and swill feeding positively influenced seropositivity, while the distribution of native pigs and proximity of farms to residential areas had negative impacts. The semi-quantitative risk scoring of SI transmission demonstrated that Cebu (16.2/19.1) was considered an extreme-risk area, while Davao del Sur (15.2/19.1) was classified as a very high-risk area. The remaining 19 provinces had moderate (n=5), low (n=12), and very low (n=2) risks. These findings provide local epidemiological data on SI and emphasize the significance of continued surveillance and strict biosecurity to mitigate the potential risks of SI.

Keywords: ELISA, PCR, Philippines, risk factors, swine influenza, transmission

INTRODUCTION

Swine influenza (SI) is caused by influenza A viruses (IAVs), which are enveloped, single-stranded RNA viruses from the family *Orthomyxoviridae* (Ma *et al.*, 2009). Currently, 18 hemagglutinin (HA) and 11 neuraminidase (NA) subtypes of IAVs have been identified, with H1N1, H1N2, and H3N2 as the predominant subtypes in swine (Li and Robertson, 2021; US Centers for Disease Control and Prevention (CDC), 2024b). The entry of swine influenza virus (SIV) in a swine population is known to be a persistent challenge, and various studies provided evidence of pigs serving as a significant host for IAV reassortment (Li and Robertson, 2021). For example, a cohort study documented the coexistence of different subtypes at the individual pig, herd, and farm levels (Rose *et al.*, 2013). Additionally, pigs

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are susceptible to both avian and human influenza viruses due to the similarity in their tracheal sialic acid linkage (Ito *et al.*, 1998), with avian influenza viruses being isolated from pigs with and without clinical disease (Karasin *et al.*, 2000; He *et al.*, 2013; Meseko *et al.*, 2018). The swine H1N1 influenza virus also caused a flu pandemic in 2009, which led to more than 622,000 cases and 12,220 deaths globally (Hajjar and McIntosh, 2010). These documented occurrences emphasize the SIV's enzootic, zoonotic, and pandemic potential.

SIVs are commonly transmitted via direct contact, aerosols, and contaminated fomites, and infection in pigs typically results in almost 100% morbidity but relatively low mortality (Li and Robertson, 2021). Respiratory and subclinical disease are usually observed, but co-morbidities and infection with new SIV subtypes were reported to aggravate respiratory symptoms and produce reproductive signs such as stillbirths and abortions (Deblanc *et al.*, 2012; Gumbert *et al.*, 2020). Production parameters, specifically average daily gain and feed conversion ratio, were also reduced. For instance, stochastic modeling in Norway found that SI-positive pigs needed 770 to 1,470 kg of additional feed to attain the required body weight (Er *et al.*, 2016), and the inefficiency increases production time and costs (Kothalawala *et al.*, 2006). Pig trade restrictions and the low pork demand during the 2009 swine flu pandemic also cost the US swine industry approximately USD 1.3 billion in losses (Butler, 2009 as cited in Pappaioanou and Gramer, 2010). Given the various threats and impacts of SI, increasing surveillance efforts is a step to safeguarding public health and avoiding economic repercussions (Li and Robertson, 2021).

Co-infections of SI with other bacterial and viral respiratory pathogens are common; therefore, laboratory diagnostic tests are required to monitor and confirm occurrences (World Organization for Animal Health (WOAH), 2009). Real-time reverse transcription polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA) are among the WOAHO-recommended methods in determining SI prevalence as part of surveillance programs (WOAH, 2023). The qRT-PCR amplifies and detects SIV RNA in samples such as nasal swabs and oral fluids (Li and Robertson, 2021), and the PCR product can be sequenced for subtyping and phylogenetic analysis (WOAH, 2023). On the other hand, ELISA assesses the presence of antibodies against influenza A structures, commonly the highly conserved nucleoprotein A, in the serum of pigs (Li and Robertson, 2021). The results of these diagnostic tests, along with survey data, have also been used in cross-sectional studies to determine factors associated with SI occurrences (Suriya *et al.*, 2008; Souza *et al.*, 2019). Risk factors in various studies included commercial farms, high swine population density, the presence of neighbors raising pigs, the presence of other animals in swine farms, and a longer contact time with pigs (Suriya *et al.*, 2008; Adeola *et al.*, 2017; Souza *et al.*, 2019; Ding *et al.*, 2021). Disease monitoring and investigating risk factors are crucial in preventing the spread and mitigating the potential serious consequences of SI (Ding *et al.*, 2021). This information can also help identify at-risk areas through qualitative, semi-quantitative, or quantitative methods, enabling efficient response and resource allocation (Horigan *et al.*, 2023).

As of January 2025, the Philippine swine population was at 8.75 million heads, with an 8.55% reduction from last year's 9.57 million heads. Pigs raised in smallhold farms account for around 69.58% of this inventory (Philippine Statistics Authority (PSA), 2025). These farms are typically situated in low-resource settings, limiting strict biosecurity implementation, proper diagnosis, and adequate treatments (Hsu *et al.*, 2023). Over the last six years, African swine fever (ASF), an economically important disease, has caused an

unstable growth trend in the national swine population (Bureau of Animal Industry (BAI), 2025). Although both smallhold and commercial farms were affected, the former remains particularly more vulnerable due to limited capacity to implement preventive and control measures.

While ASF has been the primary focus of disease management efforts to minimize production and economic losses in the Philippines, other swine diseases, such as SI, may also pose a comparable risk with potential implications for both animal and human health (Hajjar and McIntosh, 2010; Li and Robertson, 2021). Currently, local epidemiological data on SI, a priority zoonotic disease in the Philippines (BAI, 2024), remains limited. Given the vulnerability of smallhold farms, potential underreporting of SI, and related impacts, this study aimed to determine SI prevalence using qRT-PCR and ELISA, identify risk factors, and conduct a semi-quantitative risk scoring of transmission in smallhold pig farms in the Philippines.

MATERIALS AND METHODS

Ethical Statement

All animal procedures in this study were reviewed and approved by the existing Institutional Animal Care and Use Committee of the University of the Philippines Los Baños, with approval number UPLB-2022-001 in compliance with the national guidelines and policies on the use of animals in scientific studies. In addition, only those who provided written consent were included in the interview, and demographic information was handled with the highest confidentiality.

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 selected as geographic representatives of different administrative regions in the Philippines. In this paper, the term “municipality” was used to refer to both municipality and city. Sample collection was conducted in slaughterhouses to represent smallhold pig farms in a municipality due to the ongoing ASF issue in the country and farm restrictions. From each province, at most four municipalities with an operational Class A slaughterhouse were included for sample collection. Overall, 43 municipalities were sampled from January 2022 to November 2023.

Sample size estimation

In this cross-sectional study, a sample size of 384 was derived using the formula for estimating disease prevalence in an infinite population (Thrusfield and Brown, 2018), with an expected SI prevalence of 0.50, 5% desired margin of error, and a 95% confidence level. To improve the precision of results, samples were collected from 425 blood and 407 nasal swab samples, both of which were collected from the same individual pigs. The slight discrepancy between the sample sizes was due to logistic and operational factors. Only nasal swabs were collected from some pigs in Batangas (n=15), Palawan (n=21), and Negros Occidental (n=20) to avoid potential disruption of the regular operations of the slaughterhouse during blood collection. In addition, the 30 swab samples from Surigao del Norte were excluded

due to insufficient volume (Table 1).

Table 1. Positivity rates in swine influenza using serum and nasal swab samples from pigs in smallhold farms in 21 provinces in the Philippines

REGION	PROVINCE	ELISA ¹		qRT-PCR ²	
		TESTED SAMPLES	POSITIVE SAMPLES (%)	TESTED SAMPLES	POSITIVE SAMPLES (%)
CAR	Benguet	17	11 (64.7)	17	0 (0.0)
I	Ilocos Sur	20	0 (0.0)	20	0 (0.0)
	Pangasinan	16	0 (0.0)	16	0 (0.0)
III	Nueva Ecija	15	2 (13.3)	15	0 (0.0)
	Pampanga	15	12 (80.0)	15	0 (0.0)
IV-A	Batangas	13	0 (0.0)	15	0 (0.0)
	Cavite	21	7 (33.3)	21	0 (0.0)
	Laguna	16	0 (0.0)	16	0 (0.0)
IV-B	Occidental Mindoro	16	0 (0.0)	16	0 (0.0)
	Palawan	16	0 (0.0)	21	0 (0.0)
V	Camarines Sur	15	3 (20.0)	15	0 (0.0)
VI	Aklan	27	2 (7.4)	27	0 (0.0)
	Negros Occidental	15	2 (13.3)	20	0 (0.0)
VII	Bohol	13	0 (0.0)	13	0 (0.0)
	Cebu	22	7 (31.8)	22	1 (4.5)
VIII	Samar	28	1 (3.6)	28	0 (0.0)
IX	Zamboanga del Sur	30	0 (0.0)	30	0 (0.0)
X	Bukidnon	20	9 (45.0)	20	0 (0.0)
XI	Davao del Sur	30	16 (53.3)	30	1 (3.3)
XII	North Cotabato	30	2 (6.7)	30	0 (0.0)
XIII	Surigao del Norte	30	23 (76.7)	NT ³	NT ³
Total		425	97 (22.8)	407	2 (0.5)
95% confidence interval of proportion		[19.0-27.2]		[0.09-2.0]	

¹ ELISA, enzyme-linked immunosorbent assay using serum samples
² qRT-PCR, real-time reverse transcription polymerase chain reaction using nasal swab samples
³ NT, not tested due to insufficient sample volume

Sample collection

About 13 to 30 pigs, regardless of sex and breed, were randomly selected for sample collection in the piggens of slaughterhouses. At least 3 mL of blood was aseptically collected from the external jugular vein. The sample was transferred to a properly labeled red-capped tube (Vacugen™ BioSpectra Marketing, Iloilo City, Philippines), where the blood was allowed to clot at room temperature for approximately 30 min. Serum was collected after centrifugation at 2,000 x g for 10 min and transferred into a properly labeled Labopette

cryovial tube (Labotech Trading, Las Piñas City, Philippines). In addition, nasal swab samples were carefully collected using a commercially available nasopharyngeal sample collection kit (eNATR, Brescia, Italy) from the same pigs. A sterile swab was inserted to a depth of 3–4 cm into the nasopharyngeal opening and was rotated up to 5 times and held in place for 5 sec successively for both nasal cavities using the same swab. The swab sample was placed in a properly labeled, kit-supplied container with guanidine thiocyanate. All collected samples were brought to the laboratory in an insulated biological specimen transport box and were kept at -80°C until use.

Enzyme-linked immunosorbent assay (ELISA)

The serum samples were thawed under controlled cold temperature and tested in duplicate using the Influenza A Virus Antibody Test Kit (IDEXX, Montpellier, France) following the manufacturer's instructions for competitive ELISA detection of anti-IAV antibodies. Optical density (OD) was measured at 650 nm using the Multiskan™ Go Microplate Spectrophotometer (Thermo Fisher Scientific Corporation, Vantaa, Finland). The assay was deemed valid if the negative control mean was at least 0.60 OD and the positive control mean was less than 0.50 OD. The detection of antibodies was based on the mean of the sample-to-negative (S/N) ratios from duplicate tests for each test sample. Samples with a mean S/N ratio of less than 0.60 were classified as seropositive, while those with a mean S/N ratio of at least 0.60 were considered negative.

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

Swab samples were processed in a Biosafety Level 2 (BSL-2) laboratory following standard BSL-2 protocols. After thawing the nasal swab samples in a controlled cold environment, RNA extraction was performed using the QIAamp® Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality and quantity of the extracted total RNA were assessed using a Nanodrop™ 2000/2000c spectrophotometer (Thermo Fisher Scientific, DE, USA) before proceeding with further testing.

The one-step qRT-PCR was conducted using the commercial BIO-T Kit® Avian and Swine Influenza Virus V2 (Biosellal, Dardilly, France) for detection of viral RNA of H1N1, H1N2, and H3N2 SIV subtypes. Each reaction included an external positive control, an internal positive control, and a negative control (distilled water). The reaction mixture had a total volume of 20 µL, consisting of 15 µL ready-to-use master mix and either 5 µL extracted total RNA (at least 10ng/reaction) or 5 µL of controls. Amplification was carried out in duplicate and run on TOptical Real-time PCR Thermocycler (Analytik Jena AG, Göttingen, Germany) under the following temperature conditions: an initial preheating step at 50°C, followed by 40 cycles of two-stage amplification-denaturation for 10 sec at 95°C and annealing/extension for 45 sec at 60°C with signal acquisition in the FAM channel. The quantification cycle (Cq) values were determined using the automatic baseline threshold option, and qRT-PCR results were analyzed using the instrument software (qPCRsoft version 3.2.8.0). Samples with Cq values of at least 35 were classified as positive.

Interview Data

Meat inspectors in each slaughterhouse or veterinarians of local government units were interviewed using a structured questionnaire. Data regarding 14 potential risk factors

were gathered to determine the significant association with ELISA results. These factors included: (1) average herd size; percentage of farms (2) raising native pigs (or local, black pigs, which where hypothesized to arise from interbreeding with wild pigs (Eusebio, 1980; Bondoc, 1998, 2008 as cited in Banayo *et al.*, 2023) (3) near the residential areas, (4) practicing biosecurity measures, (5) practicing swill feeding, (6) raising animals other than pigs, and (7) keeping vaccination records; (8) housing system; (9) water treatment; (10) disinfection of transport vehicles; (11) disease reporting; (12) disease surveillance; (13) swine influenza vaccination; and (14) history of swine influenza (Table 2).

Table 2. Distribution of responses of the 42 municipalities in 14 variables from interviews.

Variables	Number of municipalities (%)	Variables	Number of municipalities (%)
<i>Average herd size</i>		<i>Percentage of farms keeping vaccination records</i>	
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)
		76-100%	8 (19.0)
<i>Percentage of farms raising native pigs</i>		<i>Housing system</i>	
0 %	9 (21.4)	Pen-type	38 (90.5)
1-25%	25 (59.5)	Freerange	1 (2.4)
26-50%	7 (16.7)	Mixed	3 (7.1)
51-75%	1 (2.4)		
76-100%	0 (0.0)		
<i>Percentage of farms near the residential areas</i>		<i>Water treatment</i>	
0 %	4 (9.5)	No	23 (54.8)
1-25%	9 (21.4)	Yes	19 (45.2)
26-50%	10 (23.8)	<i>Disinfection of transport vehicles in the farm</i>	
51-75%	10 (23.8)	No	12 (28.6)
76-100%	9 (21.4)	Before entry or after exit	7 (16.7)
		Before entry and after exit	23 (54.8)

Table 2 Continuation...

Variables	Number of municipalities (%)	Variables	Number of municipalities (%)
<i>Percentage of farms practicing biosecurity measures</i>		<i>Disease reporting</i>	
0 %	0 (0.0)	No	4 (9.5)
1-25%	16 (38.1)	Yes	38 (90.5)
26-50%	12 (28.6)	<i>Disease surveillance</i>	
51-75%	6 (14.3)	No	30 (71.4)
76-100%	8 (19.0)	Yes	12 (28.6)
<i>Percentage of farms practicing swill feeding</i>		<i>Swine influenza vaccination</i>	
0 %	15 (35.7)	No	39 (92.9)
1-25%	18 (42.9)	Yes	3 (7.1)
26-50%	5 (11.9)	<i>History of swine influenza</i>	
51-75%	2 (4.8)	No	2 (4.8)
76-100%	2 (4.8)	Yes	40 (95.2)
<i>Percentage of farms raising animals other than pigs</i>			
0 %	1 (2.4)		
1-25%	21 (50.0)		
26-50%	11 (26.2)		
51-75%	3 (7.1)		
76-100%	6 (14.3)		

Data Analysis

All data were recorded and organized in Excel (Microsoft, WA, USA). The positivity rates were computed by dividing the number of positive samples by the number of tested samples, multiplied by 100. Statistical analyses were also carried out using R (Posit Software, MA, USA). Missing information in the interview data was treated with imputation using the *imputeMCA* function of the *missMDA* package (Josse and Husson, 2016) with default settings. To determine the association between 14 factors from interview data and seropositivity, the least absolute shrinkage and selection operator (LASSO) regression analysis (Tibshirani, 1996) was employed with the *glmnet* package (Friedman *et al.*, 2010). A 10-fold cross-validation was also performed to determine the optimal lambda value, which minimized the mean squared error (MSE) in the LASSO model. Geographical distribution maps of the positivity rates and risk classifications were also constructed using the open-access Quantum Geographic Information System version 3.40.

Risk Scoring

A semi-quantitative approach based on the Influenza Risk Assessment Tool (IRAT) with modifications was adapted for the transmission risk classification of provinces in SI (CDC, 2024a) as employed in Trock *et al.* (2015). The risk question was the likelihood of disease transmission, whether animal-to-animal or zoonotic. Each province was scored across 9 factors: four based on our results (qRT-PCR and ELISA positivity, practice of SI vaccination, and vehicle disinfection) and five from online databases (swine population size, percent of pigs in commercial farms, swine, chicken, and human population density).

The qRT-PCR and ELISA positivity rates were used directly as raw data. For SI vaccination, municipalities practicing this strategy were assigned a score of “1,” while those not practicing were given a “0.” Vehicle disinfection was graded as follows: “2” if disinfection was not practiced; “1” if conducted either before entry or after exit from the farm; and “0” if practiced both before entry and after exit. The mean scores of municipalities within each province were used as the provincial raw data for SI vaccination and vehicle disinfection. For the remaining five factors, data corresponding to the sampling period were obtained from online databases. If the time-specific data were unavailable, the most recent published information was used. The swine population size (heads) was directly retrieved from the PSA database, while the proportion of pigs in commercial farms (%) was computed by dividing the number of pigs raised in commercial farms by the total number of pigs regardless of farm type (PSA, 2025). The population densities (human, swine, chicken) were also estimated by dividing the population size (heads) by the recent estimate of land size (km²) per province (PSA, 2021; PSA, 2025).

Based on the raw data, provinces were ranked per factor, with a higher rank indicating a higher risk. Provinces with no available data for a given factor were excluded from the ranking procedure for that factor, while those with a raw score of zero were assigned a rank of “0”. Among the remaining provinces with non-zero values, ranks were given in descending order. For provinces with tied raw data, the initial ranks were averaged to determine the final rank assignment.

To measure the weights of each factor, the formula described in Trock *et al.* (2015) was also used in this study, and the computed values are shown in Supplementary Table 1. Briefly, the nine factors were ranked based on the impact on SI transmission, with the qRT-PCR and ELISA positivity having the top two ranks and vehicle disinfection as the lowest-ranked factor. The reciprocal of each rank was computed and summed across all factors. The weight of each factor was then determined by dividing the reciprocal of a factor by the total of the reciprocals. The rank of each province in each factor was multiplied by the weight of each factor to compute the risk score. The transmission risk in a province based on the sum of the risk scores across all nine factors was classified into six bands: extreme (16.0-19.1), high (12.8-15.9), very high (9.7-12.7), moderate (6.5-9.6), low (3.3-6.4), and very low (0-3.2) (Oliveira *et al.*, 2014). Almost equal intervals per risk level were assigned based on minimum and maximum total risk scores, with differences in precision due to rounding off values.

RESULTS

Positivity Rates in ELISA and qRT-PCR

A total of 425 serum samples from domestic pigs in slaughterhouses from 21

provinces across the Philippines were tested for the presence of antibodies against the nucleoprotein A of the SIV using competitive ELISA. The overall seropositivity was 22.8% [95% confidence interval (CI): 19.0-27.2%] (97/425) (Table 1), and the highest positivity rates were recorded in Pampanga (80.0%), Surigao del Norte (76.7%), and Benguet (64.7%) (Figure 1). On the contrary, eight provinces, namely Ilocos Sur, Pangasinan, Batangas, Laguna, Occidental Mindoro, Palawan, Bohol, and Zamboanga del Sur, exhibited zero positivity rates. In addition, the qRT-PCR assay was performed on a total of 407 nasal swab samples from the same pigs that underwent ELISA testing. The qRT-PCR results revealed that most of the provinces (19/21) had negative samples (Table 1). Positive samples were only detected in Cebu (4.5%, 1/22) and Davao del Sur (3.3%, 1/30), which accounted for the overall qRT-PCR positivity rate of 0.5% [95% CI: 0.09-2.0%].

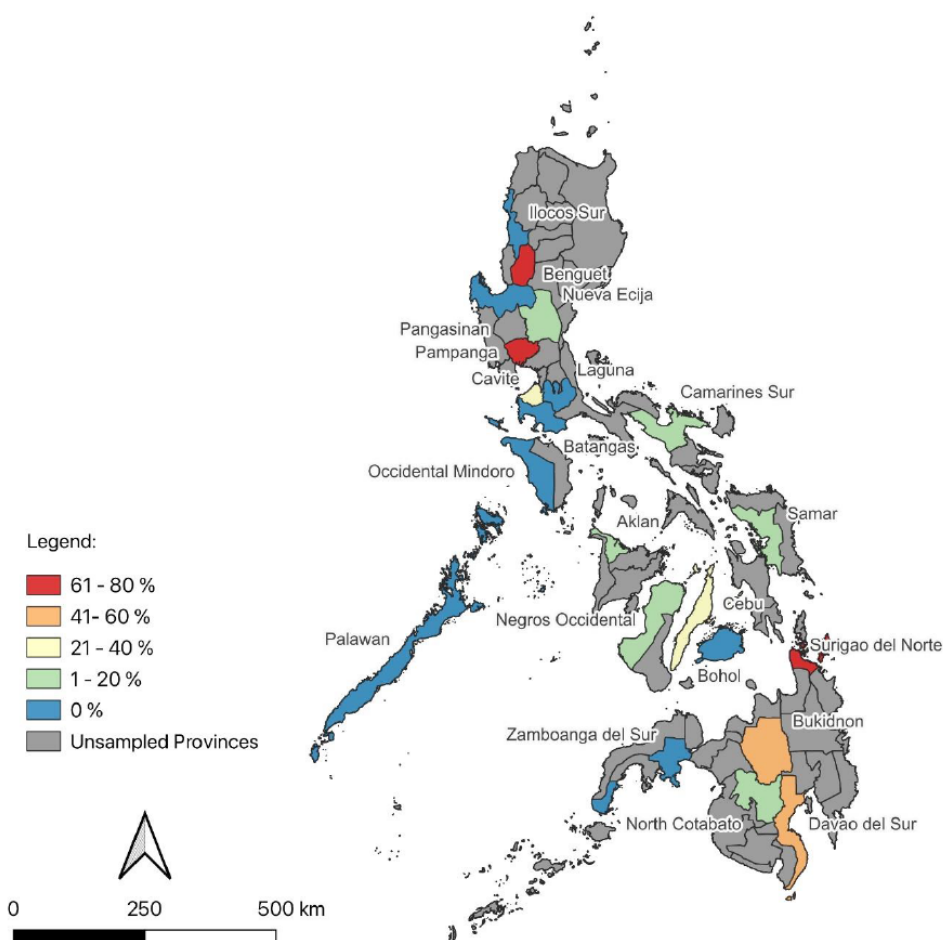


Figure 1. Geographical distribution map of the seropositivity in swine influenza of pigs from smallhold farms in 21 sampled provinces in the Philippines. The map was generated using Quantum Geographic Information System 3.40.

Factors Associated with Seropositivity

Data on 14 factors were gathered through a personal interview with an officer of the slaughterhouse or with a veterinarian in each municipality, to represent the smallhold swine farms. Most of the municipalities (38.1%, 16/42) had farms with 6-10 pigs, while around 59.5% (25/42) of the municipalities had 1-25% of farms raising native pigs (Table 2). In terms of the proportion of farms adjacent to residential areas, the 26-50% and 51-75% choices had 10 responses (23.8%) each. Approximately 38.1% of the municipalities also had 1-25% of farms adhering to biosecurity measures. Fifteen municipalities (35.7%) were also not practicing swill feeding, while 18 municipalities (42.9%) responded that around 1-25% of their farms engaged in this practice. The majority of municipalities (50%, 21/42) also reported that an estimated 1-25% of their swine farms were raising animals other than pigs. Most of the municipalities (38.1%, 16/42) only had 1-25% of farms keeping vaccination records, while approximately 33.3% (14/42) had no farms maintaining these records. It is noteworthy that this variable referred to general farm practice and not specifically to SI. The pen-type (90.5%, 38/42) was the most common housing system, and water treatment was not commonly employed in the farms of most municipalities (54.8%, 23/42). Disinfection of transport vehicles before entry to and after exit from the farm was also the most common practice in most municipalities (54.8%, 23/42). Thirty-eight out of 42 municipalities (90.5%) had farms reporting disease occurrence, but thirty municipalities (71.4%) had farms not conducting disease surveillance. Around 92.9% (39/42) of the municipalities also reported the absence of SI vaccination, despite the majority (95.2%, 40/42) also reporting SI history.

The LASSO regression was carried out to assess the significant association of these factors with seropositivity. The province of Benguet was not included in this analysis due to the complete absence of interview data. Using the cross-validation procedure, the optimal lambda was identified as 3.01, which minimized the MSE to 622.2. This outperformed the more conservative lambda of 8.37 (within one standard error), which yielded a slightly higher MSE of 626.7. Using the optimal lambda, five predictors were retained in the final model. Water treatment ($\beta=11.56$) had the greatest positive impact on seropositivity, followed by SI vaccination ($\beta=6.31$), and swill feeding ($\beta=4.48$) (Table 3). On the contrary, the percentage of farms raising native pigs ($\beta=-1.35$) and in proximity to residential areas ($\beta=-1.47$) had negative impacts on the outcome. The other nine factors had zero coefficients, implying non-significant effects on seropositivity.

Risk Scoring of SI Transmission

Provinces were ranked in each factor based on their raw data (Supplementary Tables 2 and 3), and the risk score was computed by multiplying each rank by the weight of the factor (Table 4). For qRT-PCR positivity, Surigao del Norte was excluded from the ranking procedure due to the absence of viable samples, while eighteen provinces also had “0” ranks. Given these conditions, Cebu and Davao del Sur received risk scores of 7.1 and 6.8, respectively, due to the greater weight given to qRT-PCR positivity. Under ELISA positivity, eight provinces were given “0” scores, while 13 provinces recorded non-zero scores, with Pampanga (3.7), Surigao del Norte (3.6), and Benguet (3.4) receiving the highest scores. For SI vaccination, eight provinces had no data, while three provinces had zero scores. The remaining ten provinces had a score of “1”, indicating the absence of SI vaccination. For vehicle disinfection, Bohol and Bukidnon had the highest scores (0.8) due to the absence of this practice at the farm level.

Table 3. Least absolute shrinkage selection operator coefficients of the 14 variables from interview data with seropositivity in swine influenza as the outcome.

Variables	Beta Coefficients
Intercept	12.60
Average herd size	0
Percentage of farms raising native pigs	-1.35
Percentage of farms near the residential areas	-1.47
Percentage of farms practicing biosecurity measures	0
Percentage of farms practicing swill feeding	4.48
Percentage of farms raising animals other than pigs	0
Percentage of farms keeping vaccination records	0
Housing system	0
Water treatment	11.56
Disinfection of transport vehicles in the farm	0
Disease reporting	0
Disease surveillance	0
Swine influenza vaccination	6.31
History of swine influenza	0

Depending on the time of sampling, the raw data of each province in the next five factors for risk scoring were collected from various online databases (Supplementary Table 2). For the swine population size, Batangas and Bukidnon had the greatest risk scores due to the high number of pigs. On the other hand, Cavite had the highest proportion of commercial farms, resulting in the highest risk score under this factor. The greatest estimated swine population density was computed for Batangas, gaining the score of “1.5”. Pampanga and Batangas had the densest chicken population, while Cavite had the highest human population density. These three provinces had the highest risk scores in the corresponding factors.

The sum of the risk scores in nine factors was computed to semi-quantitatively estimate the transmission risk in a province (Table 4). Cebu (16.2/19.1) had an extreme risk level, followed by the very high-risk category of Davao del Sur (15.2/19.1) (Figure 2). While no province was categorized as high risk, five provinces were considered moderate risk (9.7-12.7). The majority of the provinces (12/21) were also considered low-risk (3.3-6.4), while two provinces, Ilocos Sur (2.8/19.1) and Palawan (2.4/19.1), had a very low risk of SI transmission.

Table 4. Risk scores and classifications of 21 selected Philippine provinces for swine influenza transmission.

Region	Province	qRT-PCR Positivity ¹	ELISA Positivity ²	Swine Influenza Vaccination	Swine Population Size	% Pigs in Commercial Farms	Swine Population Density	Chicken Population Density	Human Population Density	Vehicle Disinfection	Total Risk Score	Risk Level
CAR	Benguet	0.0	3.4	ND ³	0.2	0.6	0.3	0.2	0.4	ND ³	5.1	Low
I	Ilocos Sur	0.0	0.0	1.0	0.3	0.4	0.6	0.4	0.3	0.0	2.8	Very Low
	Pangasinan	0.0	0.0	0.0	0.7	1.1	0.6	0.7	0.7	0.6	4.5	Low
III	Nueva Ecija	0.0	2.2	ND ³	0.6	1.0	0.2	0.8	0.6	0.6	6.0	Low
	Pampanga	0.0	3.7	ND ³	1.0	1.4	1.4	1.1	0.8	0.0	9.5	Moderate
IV-A	Batangas	0.0	0.0	1.0	1.5	0.4	1.5	1.0	0.8	0.0	6.2	Low
	Cavite	0.0	2.8	1.0	0.4	1.5	0.9	0.8	0.9	0.5	8.9	Moderate
	Laguna	0.0	0.0	1.0	0.5	1.4	0.8	0.6	0.9	0.4	5.5	Low
IV-B	Occidental Mindoro	0.0	0.0	1.0	0.6	0.9	0.4	0.2	0.1	0.3	3.5	Low
	Palawan	0.0	0.0	ND ³	0.9	0.3	0.4	0.3	0.0	0.5	2.4	Very Low
V	Camarines Sur	0.0	2.5	1.0	1.1	0.2	1.1	0.5	0.5	0.0	6.9	Moderate
VI	Aklan	0.0	2.0	1.0	0.4	0.8	0.7	0.6	0.5	0.4	6.3	Low
	Negros Occidental	0.0	2.2	1.0	1.3	0.6	1.0	0.7	0.6	0.0	7.3	Moderate
VII	Bohol	0.0	0.0	ND ³	0.9	0.9	0.9	0.4	0.4	0.8	4.2	Low
	Cebu	7.1	2.7	0.0	1.4	1.3	1.4	1.0	0.8	0.7	16.2	Extreme
VIII	Samar	0.0	1.6	1.0	0.1	0.0	0.1	0.1	0.1	0.5	3.5	Low
IX	Zamboanga del Sur	0.0	0.0	ND ³	1.1	0.5	1.1	0.5	0.4	0.7	4.4	Low
X	Bukidnon	0.0	3.0	ND ³	1.4	1.1	1.2	0.9	0.2	0.8	8.6	Moderate
XI	Davao del Sur	6.8	3.2	ND ³	1.2	1.2	1.3	0.9	0.7	0.0	15.2	Very High
XII	North Cotabato	0.0	1.8	1.0	0.8	0.7	0.5	0.3	0.2	0.0	5.3	Low
XIII	Surigao del Norte	NT ⁴	3.6	0.0	0.1	0.0	0.1	0.1	0.3	0.5	4.7	Low

¹ qRT-PCR, real-time reverse transcription polymerase chain reaction; ² ELISA, enzyme-linked immunosorbent assay; ³ ND, no gathered data;

⁴ NT, not tested due to insufficient sample volume

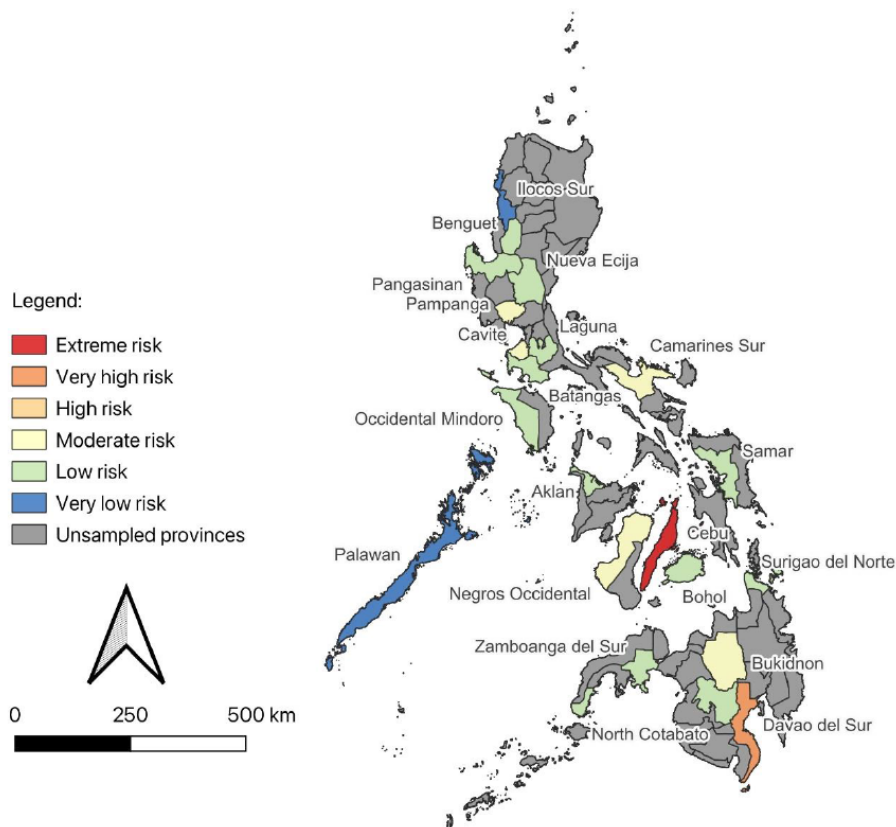


Figure 2. Geographical distribution map of the classification of swine influenza transmission risk in the 21 sampled provinces in the Philippines. The map was generated using Quantum Geographic Information System 3.40.

DISCUSSION

SI remains a global issue due to IAVs' multi-species transmission and genetic reassortment, particularly in pigs, posing serious challenges to animal and public health (Li and Robertson, 2021). Investigating its prevalence and associated factors in local swine farms and assessing transmission risk in provinces are necessary to understand disease spread, to choose the appropriate intervention strategies, and to avoid its significant consequences (Ding *et al.*, 2021; Horigan *et al.*, 2023). This study used competitive ELISA to detect SI antibodies in serum samples and qRT-PCR assay for viral RNA in nasal swab samples. The factors associated with seropositivity were analyzed using LASSO regression, and a semi-quantitative approach was employed to assess the transmission risk in each province.

Our study reported a seropositivity of 22.8% in the 21 sampled provinces in the Philippines. A lower seropositivity in backyard farms in Bhutan was documented, with rates in gilts and finishing pigs ranging from 4 to 10% (Monger *et al.*, 2014). The same study also showed that small herd farms generally yielded lower seropositivity than large farms. Additionally, larger herds were significantly associated with higher seropositivity, as seen in

Malaysia (Suriya *et al.*, 2008). The choice of smallhold pigs as subjects may have influenced the relatively low seropositivity in our study, and undertaking surveillance in commercial farms with high pig population density will also provide valuable data for surveillance purposes.

Seropositivity in SI suggests prior SIV exposure of pigs, either through field infection or vaccination. A study demonstrated that antibodies post-infection in sows were still detected more than 28 months after virus clearance (Desrosiers *et al.*, 2004). While similar studies on finishers need to be conducted, this finding supports the persistence of antibodies from natural infection for an extended period. However, our results found no significant association between history of SI occurrence and seropositivity, indicating the absence of a humoral response in sampled pigs from infection. Immunizing breeding sows and gilts is another key control measure to attain more efficient antibody production and transfer to the offspring, preventing infections or minimizing clinical disease via passive immunity (Chamba Pardo *et al.*, 2019; Lopez-Moreno *et al.*, 2022). Maternally-derived antibodies in the offspring typically persist for around 11 weeks (Rose *et al.*, 2013), and these likely waned from the sampled pigs, which were around 20 to 24 weeks old upon reaching market weight (DA-BAR, 2022). Vaccination in younger pigs also provides varying durations of immunity depending on vaccine type and age at vaccination. For instance, a commercial vaccine confers protection for 4 to 6 months, with older pigs having a longer duration of immunity (Ceva, 2025). In our study, SI vaccination was linked to seropositivity, suggesting successful seroconversion of sampled pigs after vaccination. However, a low response in SI vaccination history was recorded, which implies that the statistical association may be a result of data imputation. Smallhold swine farmers commonly rely on government vaccination programs focused on more economically important diseases such as ASF, leaving the farms vulnerable to SI infection and transmission. Therefore, there is a need for stronger SI vaccination initiatives and financial support for smallhold farmers.

The qRT-PCR assay of nasal swab samples yielded an extremely low positivity rate, despite the kit's capacity to detect the three common swine subtypes and SI's high transmissibility, with reproductive numbers varying from 2.5 to 10.7 (Romagosa *et al.*, 2011; Rose *et al.*, 2013). Similar to ELISA, the low population density in smallhold farms may have impacted the low detection rate. Positive results in this assay may indicate active infection, recent vaccination, or the mere presence of viral RNA. Asymptomatic or subclinical SI infections are typically observed in pigs (Li and Robertson, 2021). Moreover, viral shedding starts early, at one day post-infection, and may continue for at least five days (Canini *et al.*, 2020), signifying the potential contribution of actively, yet subclinically, infected pigs to qRT-PCR positivity. Recently vaccinated pigs were also shown to test PCR-positive in the study of López-Valiñas *et al.* (2023), and the timing of vaccination and specific vaccine used may offer additional insights. Although qRT-PCR assay cannot determine the stage of infection, the positive samples were submitted for subtyping, which will provide information on relevant primers for diagnosis and surveillance and appropriate vaccine choices (Li and Robertson, 2021). Expanding the detectable subtypes in swine to the less common H3N1 and H2N3 may also increase the detection rates in surveillance studies (WOAH, 2009). Our assay results, therefore, highlight the prevention of SI occurrence and spread through the combination of vaccination and surveillance (Li and Robertson, 2021).

Aside from immunization, factors related to demographics and farm practices were also found to be statistically associated with seropositivity in this study. Surprisingly, water

treatment had the highest positive impact on ELISA positivity, despite the susceptibility of the virus to detergents and disinfectants, such as 0.1 mol/L NaOH, 70% ethanol, and 70% 1-propanol (Jeong *et al.*, 2010). Specific treatment methods were not gathered in the interview, and the possibility of inappropriate water treatment, especially in resource-scarce sites, should be considered. Although waterborne transmission of SIV has not yet been documented, the H1N1 subtype survived variably in water, with lower temperatures favoring prolonged survival (Desrosiers, 2021). While the absence of biological association should not be neglected, further research is required to elucidate the role of agricultural water in SI transmission among pigs.

Swill feeding, which was commonly implicated in ASF and hog cholera transmission (Dame-Korevaar *et al.*, 2021), also positively impacted seropositivity, although its link and biological association with SI occurrence have not been established (Monger *et al.*, 2014). SI transmission usually occurs through direct contact and droplets (Li and Robertson, 2021), and the tropism of SIV was limited to the respiratory tract, not the muscle tissues (WOAH, 2009). The common practice of swill feeding in smallhold pig farms, as a way of reducing production costs, may explain this unusual finding (Li *et al.*, 2024). While swill feeding does not have direct implications for SI occurrence, its continued practice could undermine the effectiveness of existing national ASF and hog cholera control strategies.

Native pig breeds were believed to be resistant to a wide range of diseases (Kasprzyk and Walenia, 2023). Consequently, smallhold farmers may not be inclined to vaccinate their native pigs due to additional costs, accounting for the negative impact on seropositivity. Previous studies also showed the significant association of seropositivity with exposure of humans to swine and having neighbors raising pigs (López-Robles *et al.*, 2012; Souza *et al.*, 2019), but our findings contradict this association. A higher percentage of farms near the residential areas reduced the seropositivity of pigs in our study, which can be attributed to the challenges in acquiring SI vaccines in low-resource settings such as smallhold farms, which were commonly near the residential areas. Information on the frequency and duration of exposure at the individual farm level will also facilitate analysis on the effects of human-pig contact on swine seropositivity, as the proximity data was a subjective judgment of the respondents.

In our risk scoring, the potential transmission risk of SI in each province was assessed using a modified IRAT, which is a sound approach designed for influenza viruses with pandemic potential like swine influenza (CDC, 2024a). Ilocos Sur and Palawan had a very low risk of SI transmission due to consistently low scores across all factors considered. On the other hand, Cebu and Davao del Sur were identified as areas with extreme and high risk of SI transmission, respectively. A substantial portion of their risk scores was explained by the qRT-PCR positivity, which needs further assessment to determine the infectiousness and specific subtype of detected SI viral RNA. These two provinces also scored high in the population densities of pigs, humans, and chickens, and these factors demonstrate the potential for frequent contact among these species sharing a common landmass. Although it was demonstrated that increasing the number of pigs by 1000 has significantly higher odds of seropositivity (Poljak *et al.*, 2008), the impact of a highly dense population on the direct transmission of SI appears to be more important than a high population size, which may occur in both large and small land masses. A study has shown that the greater distance between swine farms served as a protective factor against H3N2 and H1N1 occurrences (Suriya *et al.*, 2008). Similarly, pig and pig farm density increase the odds of seropositivity

(Poljak *et al.*, 2008). Given the transmission route of SI, the close contact in a highly dense pig population may facilitate the maintenance and mutation of SIV. A distance below 1 km between swine and poultry farms was also found to increase the chances of SI seropositivity in pigs (Nurhayati *et al.*, 2020). Furthermore, the presence of poultry populations in swine farms, the entry of wild birds, and even the presence of dogs and cats were found to positively affect the seropositivity in pigs (Li *et al.*, 2019). While a distance of at least 1 km between swine and poultry farms in the Philippines is required to disallow disease transmission (BAI, 2004), regulating the smallhold poultry and swine farms appears to be less feasible. This may allow other IAVs from poultry to infect pigs and undergo reassortment to give rise to new subtypes or strains. The exposure of workers to swine was also associated with seropositivity in pigs (López-Robles *et al.*, 2012). Additionally, those who lacked awareness of the zoonotic risk of SI and worked with mild flu symptoms were also known to increase the odds of ELISA positivity in pigs (Li *et al.*, 2020). This shows that human-driven practices and knowledge may increase the transmission risk of SI, which may further lead to outbreaks both in humans and animals.

Although transport vehicles are not commonly implicated in SIV transmission, their role in carrying other pathogens that may cause co-infections with SI should be acknowledged (WOAH, 2009). A previous study hypothesized that transportation and mixing of pigs from various farms may facilitate cross-infections with SIV, but more distant transport appears to be protective (Cheung *et al.*, 2022). In Cambodia, another study also suspected that IAV exposure may occur at the farm level, during transport, and at the slaughterhouse, as pigs that stayed in the abattoir for at most 6 days still tested PCR-positive for the virus (Hidano *et al.*, 2024). While SI can be inactivated using common disinfectants (Jeong *et al.*, 2010), the inappropriate practice of vehicle disinfection may increase the risk of SI transmission and spread.

In conclusion, our study provides evidence for the presence of antibodies against SIV and the presence of viral RNA in the Philippines. Five factors related to farm characteristics and practices were found to be statistically associated with seropositivity, but not necessarily biologically linked to SI occurrence. Through a risk scoring scheme, provinces were also categorized into extreme, very high, moderate, low, and very low risk categories based on the likelihood of SI transmission at the human-pig interface. However, it is important to note that the risk model employed in this study carries limitations. The IRAT only assesses the potential risk associated with IAVs using the factors with available data, but other significant variables, such as genomic properties of IAV, infections in wild animals, and disease severity in affected populations included in the original IRAT, need to be considered in future works. Also, the incomplete data may result in imprecise measures or data and risk estimation. Overall, this study added epidemiological data on SI in the Philippines, emphasizing the need for vaccination, strict biosecurity measures, and surveillance efforts.

ACKNOWLEDGEMENTS

This study is part of a research project entitled “Molecular Detection and Serological Profiling of Swine Influenza and Classical Swine Fever in Backyard Farms in the Philippines” under the program “Surveillance and Molecular Epidemiology of Economically and Public Health Important Animal Diseases in the Philippines”, which was supported in funding by the Department of Science and Technology—Philippine Council for Agriculture, Aquatic,

and Natural Resources Research and Development. The authors also extend their gratitude to the participating local government units, provincial and municipal/city veterinary offices, municipal/city agriculture offices, slaughterhouse officers, and meat inspectors of the sampled provinces.

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SUPPLEMENTARY DATA

Supplementary Table 1. Weights of the nine factors used in the risk scoring of swine influenza transmission in 21 Philippine provinces.

Factor	Rank	Reciprocal of rank	Weight (reciprocal/total of reciprocal)
qRT-PCR positivity ¹	1	1.00	0.356
ELISA positivity ²	2	0.50	0.178
Swine influenza vaccination	3	0.33	0.119
Swine population size	5	0.20	0.071
Percent of pigs in commercial farms	5	0.20	0.071
Swine population density	5	0.20	0.071
Chicken population density	7	0.14	0.051
Human population density	8	0.13	0.044
Vehicle disinfection	9	0.11	0.039
Total		2.81	1

¹qRT-PCR, real-time reverse transcription polymerase chain reaction; ² ELISA, enzyme-linked immunosorbent assay

Supplementary Table 2. Raw data of 21 Philippine provinces regarding nine variables associated with swine influenza transmission.

Region	Province	qRT-PCR Positivity (%) ¹	ELISA Positivity (%) ²	Swine Influenza Vaccination	Swine Population Size (heads)	% Pigs in Commercial Farms	Swine Population Density (heads/km ²)	Chicken Population Density (heads/km ²)	Human Population Density (heads/km ²)	Vehicle Disinfection
CAR	Benguet	0.0	64.7	ND ³	44,095	12.9	15.6	89.5	292.6	ND ³
I	Ilocos Sur	0.0	0.0	1	63,077	4.2	24.3	370.1	272.0	0.0
	Pangasinan	0.0	0.0	0	140,346	55.2	25.7	1,137.8	580.3	1.5
III	Nueva Ecija	0.0	13.3	ND ³	84,425	40.1	14.8	1,284.6	406.0	1.5
	Pampanga	0.0	80.0	ND ³	313,406	73.7	151.8	5,230.8	1,404.9	0.0
IV-A	Batangas	0.0	0.0	1	780,158	7.7	250.4	5,192.4	933.7	0.0
	Cavite	0.0	33.3	1	78,324	97.0	51.3	1,305.9	2,846.7	1.0
IV-B	Laguna	0.0	0.0	1	78,602	70.6	40.8	1,068.0	1,754.0	0.5
	Occidental Mindoro	0.0	0.0	1	91,871	16.2	15.7	102.1	89.8	0.3
V	Palawan	0.0	0.0	ND ³	309,632	2.7	18.2	115.9	73.2	1.0
	Camarines Sur	0.0	20.0	1	346,399	0.4	62.8	743.2	375.2	0.0
VI	Aklan	0.0	7.4	1	70,371	15.5	40.0	1,061.2	349.6	0.7
	Negros Occidental	0.0	13.3	1	491,066	10.6	61.0	1,086.9	400.7	0.0
VII	Bohol	0.0	0.0	ND ³	257,038	34.9	53.9	676.4	292.2	2.0
	Cebu	4.5	31.8	0	572,018	67.6	107.1	2,184.3	964.3	1.7
VIII	Samar	0.0	3.6	1	14,955	0.0	2.5	55.2	131.1	1.0
IX	Zamboanga del Sur	0.0	0.0	ND ³	361,078	10.5	61.2	843.2	343.8	2.0
X	Bukidnon	0.0	45.0	ND ³	699,679	54.3	66.6	1,626.6	146.8	2.0
XI	Davao del Sur	3.3	53.3	ND ³	362,340	59.9	78.6	1,521.7	533.3	0.0
XII	North Cotabato	0.0	6.7	1	224,785	15.2	24.1	305.5	136.9	0.0
XIII	Surigao del Norte	NT ⁴	76.7	0	8,163	0.0	4.2	57.9	273.8	1.0

¹qRT-PCR, real-time reverse transcription polymerase chain reaction; ²ELISA, enzyme-linked immunosorbent assay; ³ND, no gathered data;

⁴NT, not tested due to insufficient sample volume

Supplementary Table 3. Rank of each province based on raw data in nine variables for swine influenza transmission.

Region	Province	qRT-PCR Positivity ¹	ELISA Positivity ²	Swine Influenza Vaccination	Swine Population Size	% Pigs in Commercial Farms	Swine Population Density	Chicken Population Density	Human Population Density	Vehicle Disinfection
CAR	Benguet	0	19	ND ³	3	9	4	3	9	ND ³
I	Ilocos Sur	0	0	8.5	4	5	8	7	6	0
	Pangasinan	0	0	0	10	16	9	14	16	15.5
III	Nueva Ecija	0	12.5	ND ³	8	14	3	15	14	15.5
	Pampanga	0	21	ND ³	14	20	20	21	19	0
IV-A	Batangas	0	0	8.5	21	6	21	20	17	0
	Cavite	0	16	8.5	6	21	12	16	21	12.5
IV-B	Laguna	0	0	8.5	7	19	11	12	20	9
	Occidental Mindoro	0	0	8.5	9	12	5	4	2	8
V	Palawan	0	0	ND ³	13	4	6	5	1	12.5
	Camarines Sur	0	14	8.5	15	3	16	9	12	0
VI	Aklan	0	11	8.5	5	11	10	11	11	10
	Negros Occidental	0	12.5	8.5	18	8	14	13	13	0
VII	Bohol	0	0	ND ³	12	13	13	8	8	19
	Cebu	20	15	0	19	18	19	19	18	17
VIII	Samar	0	9	8.5	2	0	1	1	3	12.5
IX	Zamboanga del Sur	0	0	ND ³	16	7	15	10	10	18
X	Bukidnon	0	17	ND ³	20	15	17	18	5	20
XI	Davao del Sur	19	18	ND ³	17	17	18	17	15	0
XII	North Cotabato	0	10	8.5	11	10	7	6	4	0
XIII	Surigao del Norte	NT ⁴	20	0	1	0	2	2	7	12.5

¹qRT-PCR, real-time reverse transcription polymerase chain reaction; ²ELISA, enzyme-linked immunosorbent assay; ³ND, no gathered data;

⁴NT, not tested due to insufficient sample volume