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Epidemiology of the transboundary swine diseases in Asia & Pacific

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Epidemiology of the transboundary swine diseases in Asia & Pacific

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Editorial: Epidemiology of the transboundary swine diseases in Asia & Pacific

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Editorial on the Research Topic
Epidemiology of the transboundary swine diseases in Asia & Pacific

Asia is a leading market for pork production and consumption, contributing to over half of the world's pork supply. Smallholder-based swine management systems, which are sensitive to infectious diseases, are still prevalent in many Asian countries and directly linked to the vulnerability of the pig value chain at the national level. This is a major characteristic of the swine production in many Asian countries. For these traditional pig farming systems, the industry is now facing a critical turning point. In recent years, several transboundary swine diseases such as African Swine Fever (ASF), Classical Swine Fever, Foot and Mouth Disease, and Porcine Reproductive and Respiratory Syndrome (PRRS) have been reported across Asia, posing a potential catastrophic impact on swine production and raising significant concerns.

As evident from our Research Topic, in which 70% of the published papers are related to ASF research, ASF has received significant attention in Asia. Since its first detection in China in 2018, ASF has continued to have a severe impact on pig farming across Asia. Ito, Kawaguchi et al. conducted a comprehensive epidemiological analysis based on existing literature data and publicly available open databases to understand the ASF epidemic in Asia. Although there has been a decline in the official reports of ASF recently, this trend suggests not so much an end of the epidemic, but rather its possible endemicity in the region. As a first step toward control under these circumstances, identifying the overall pattern of the epidemic and the risk factors for its spread are critical.

Lee et al. conducted a spatiotemporal analysis and assessment of potential risk factors along the pig value chain in Lao Cai province, Vietnam. They identified spatiotemporal clusters and potential risk factors attributed to geographical features. Farmers recognized a high ASF transmission risk from visitors to farms, highlighting the importance of biosecurity across the entire pig value chain. In Oudomxay province, northern Laos, a case study of smallholder pig farming systems was conducted by Matsumoto et al.. The study investigated the management of pigs in villages affected by ASF and analyzed the frequency of risk factors and stock losses at the household level. It identified swill feeding and free-ranging as risk factors for ASF, as well as inadequate biosecurity measures leading to contamination of the environment. These studies emphasize the need for improved knowledge, awareness, and understanding of ASF infection and risk at the community level, along with the need for enhanced disease management resources from local to governmental levels.

On individual farm scales, the development and swift implementation of evidence-based measures are crucial. Understanding the mechanisms of virus transmission plays a significant role as the first step in infectious disease control strategies at the site level. Li et al. reported for the first time the evidence of ASF virus (ASFV) aerosol transmission under field conditions, previously documented only in experimental settings. In general, a time lag exists between infection occurrence and detection on farms, which significantly influences the scale of outbreak spread. Yoon et al. estimated a median interval of 9.0 days (Q1–Q3, 7.8–10.5 days) from infection to detection in South Korean ASF outbreak farms, noting variations by breeding stage and farm type. This finding is essential for early control of infectious disease outbreaks. In infected farms, culling all animals is a standard containment strategy, but challenges such as lack of human resources and inadequate facilities can hinder its execution. In Vietnam, where continuous large-scale outbreaks occur, a spot removal approach—rapidly identifying and eliminating infected individuals considering economic situation—is permitted. While this method reduces the economic burden on farmers, it risks missing potentially infected individuals. Mai et al. evaluated the effectiveness of this approach by calculating the basic reproduction number (R_0) for the in-farm spread of ASF in two midsize commercial pig farms in Vietnam.

The unprecedented outbreak of ASF underscores the need for fundamental changes in Asia's traditional pig farming management systems and may indirectly contribute to the prevention of other diseases. Zhao et al. conducted risk factors and spatiotemporal analysis of PRRS seroprevalence in China before and after the ASF outbreak, finding that the likelihood of farms being PRRSV antibody-positive was 3.1 times higher before the ASF outbreak, likely due to enhanced biosecurity measures post-ASF. Similarly, Fan et al. revealed the prevalence of Porcine Circovirus type 2 (PCV2) across China, a virus classified as an emerging infectious disease causing significant economic losses in the global pig industry. The study found variability in PCV2 positivity rates based on farm type and breeding stages, along with regional differences. These patterns suggest that the variations in protocols implemented since 2018 for ASF containment might have influenced these trends.

The recent ASF outbreaks have significantly impacted the swine industry in Asia, yet it is crucial not to overlook the severe damage caused by other swine diseases, such as PRRS and PCV2 mentioned earlier. The emergence of the Porcine Deltacorona virus (PDCoV), spreading globally with notable outbreaks in Asian countries, raises widespread concerns. Through systematic review and meta-analysis, Sun et al. calculated the estimated prevalence of PDCoV infection in pig populations in mainland China, revealing a high prevalence of 12.4%, highlighting the urgent need for enhanced biosecurity prevention and control measures.

Research on swine diseases in Asia predominantly targets domestic populations. However, the potential contribution of wildlife to disease transmission and maintenance deserves attention. Thoroughly elucidating this role is critical for holistic disease management strategies. In Asia, where wildlife surveillance systems are not fully established, further research in this field is also encouraged.

As emphasized by Ito, Bosch et al., the risk of global spread of the lower virulent ASFV reported in China highlights a scenario where Asia's current challenges could escalate into a worldwide problem. Asia stands as a major hub for swine production, with strong connections to countries worldwide. To prevent potential worldwide infectious disease outbreaks, continuous monitoring of the livestock industry within the Asia-Pacific region is essential.

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Spatiotemporal Analysis and Assessment of Risk Factors in Transmission of African Swine Fever Along the Major Pig Value Chain in Lao Cai Province, Vietnam

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African swine fever (ASF) is a contagious and lethal hemorrhagic disease with a case fatality rate approaching 100% in domestic pigs. The main objectives of this study were to describe the spatiotemporal analysis as well as to assess the potential risk factors along the pig value chain in Lao Cai province, Vietnam. A total of 925 outbreaks were reported from 2019 to 2020. The three clusters (primary, secondary and 5th) were observed near the Chinese border. The most temporal clusters were detected between May and August during the study period. In addition, we evaluated the association between ASF outbreak locations to the nearest main roads and elevation. For ASF outbreak locations to the nearest main roads, compared with the reference (<5,000 m), <1,000 m (10.22 times) and 1,000–2,000 m (1.98 times) were significantly higher occurrences of ASF. For elevation, compared to the reference (>1,500 m), the farm locations with <500 m (55.31 times) showed a significantly increased risk of ASF outbreaks. Farmers perceived that the highest risk of ASF transmission may come from collectors and slaughterers, intermediaries inside and outside the commune, feed agents and maize agents in the commune, and pig retailers. Both commercial and household pig producers considered minimizing the number of people going in and out of pig stables and improving healthcare and husbandry procedures to be both very important and feasible. There is a need for compliance by all pig producers and other actors in the pig value chain to adopt biosecurity practices. Therefore, awareness, knowledge and understanding of infection and risks of ASF need to be improved. Veterinary officials at the provincial and district levels need to improve capacity and resources to perform laboratory analysis for ASF and need to coordinate with local actors on the control and prevention of ASF in the community.

Keywords: spatiotemporal analysis, risk factors, Vietnam, value chain assessment, African swine fever (ASF)

INTRODUCTION

African swine fever (ASF) is a contagious and lethal hemorrhagic disease with a case fatality rate approaching 100% in domestic pigs (1). The disease causes huge economic losses to the pig industry and threatens food security around the world, and is classified as a notifiable disease by the World Organization for Animal Health (OIE). (2, 3). ASF has been endemic in most sub-Saharan African countries and has emerged in the Caucasus and some areas of Europe (4, 5). In Asia, the first case was reported in the northeast of China in August 2018, and then the virus was quickly spread to other countries in Asia, including Vietnam (6–8). In Vietnam, ASF outbreaks were first reported in February 2019 in Hung Yen province (250 km from the Chinese border and 50 km from Hanoi) and have rapidly spread across the entire county within a short period of time. As of Dec 2021, more than 6 million (>20% of pig production) have died and been culled across the country (7). Poor biosecurity (mainly smallholders, accounting for 65–70%) was one of the main risk factors to the rapid spread of ASF at the farm level, resulting in a huge economic impact for the pig industry in Vietnam (9, 10).

Cluster analysis is an important analytic technique in spatial or spatial-temporal epidemiology. It can detect space, time and space-time clusters of disease cases resulting from disease outbreaks. Together with remote sensing data, it is becoming popular to address the research questions in veterinary medicine (11, 12). In Vietnam, some studies have been carried out to identify the space-time clusters of Porcine reproductive and respiratory syndrome (PRRS) and Foot-and-mouth disease (FMD) (13, 14), and to estimate the spatial distribution of *Culex* mosquito abundance using remote sensing data (15). In addition, value chain assessment (VCA) is a systematic framework for describing and analyzing the inter-connected activities that transport a raw product from the producers to the final consumer (16, 17). The VCA can help us to develop cost-effective intervention strategies for the pig production system. In Vietnam, a few studies have been conducted in the livestock sector (mainly pig) (18–20).

To our knowledge, no studies have been conducted to evaluate the occurrence of ASF in space and time and to identify the potential risk factors. Therefore, the main objectives of this study were to describe the spatiotemporal analysis as well as to assess the potential risk factors along the pig value chain in Lao Cai province, Vietnam.

MATERIALS AND METHODS

Study Area and Demonstration of Data

Lao Cai is a highland province located in the northwest region of Vietnam, on the border with Yunnan province of China. There are estimated human and pig populations of 7,33,300 and 3,75,647 in 2019, respectively (21, 22). The annual average temperature is 23°C, and ranges between 18°C and 28°C in the mountainous region, and between 20°C and 22°C in the lowlands. The local surveillance data of ASF from 2019 to 2020 was obtained from the sub-Department of Animal Health (DAH). This surveillance data included the number of estimated cases, outbreak dates, and locations (commune, district and provincial level including GPS locations). All reported cases have been confirmed by the National Center for Veterinary Diagnostics (NCVD). The Bao Thang district, which has been highly damaged by ASF outbreaks, was selected for the value chain assessment (**Figure 1**). It is a lowland district with an area of 652 km², and a relatively large population of 1,03,262 people in 2019. It is an important district in livestock production (especially pig and poultry) for Lao Cai province, accounting for 25–36% of the total number of pigs and over 40% of the total number of poultry.

Data Analysis

Lao Cai province is officially divided into 17 commune-levels, and the first ASF outbreak was reported in domestic pigs in 2019. Space-time cluster analysis was carried out using the SaTScan (version 9.6 free available, http://www.satscan.org), which is commonly used to detect space and space-time clusters in public health sectors (23, 24). A space-time permutation model was selected to assess the space-time cluster occurrence as only reported cases were available, but not the pig-at-risk population data for ASF was available (24). The scan statistic executes a cylindrical window with a circular geographic base and height corresponding to time, indicating a space-time cluster. This circular window moves across each farm location and then calculates the expected cases within the window under the assumption that they are randomly dispersed in space. Clusters were identified by observed/expected cases under the null hypothesis of no clustering. For our analysis, the spatial and temporal window sizes were set to a maximum of 50%. The test statistic of the identified clusters was computed by a maximum likelihood ratio function, and the p-value was obtained by Monte-Carlo simulation with 999 replications of the dataset under the null hypothesis. The primary cluster can be defined as the most likely cluster, and the secondary cluster can be defined as non-overlapping clusters with less likelihood than the primary cluster. In addition, the associations between ASF outbreak locations and the nearest main road/elevation were evaluated during the study period as there have been a number of studies, which were conducted on this (25, 26). The road data were extracted from https://www.openstreetmap.org/ and transformed into shapefile. The various buffer zones (1 km, 2 km, and 5 km) were created around roads (**Figure 2**). The digital elevation data were obtained from NASA's Shuttle Radar Topography Mission (SRTM). The SRTM product is at a resolution of 1 arc-second (approximately 30 m). Since the outbreak locations were recorded, the number of infected farms by buffer zone along roads was calculated, and the elevation values of infected farms were extracted from the raster layer using the raster package in R (27). The number of infected farms was categorized based on distance (<1,000 m, 1,000–5,000 m, and 5,000 m>) and elevation (<500 m, 500–1,000 m, 1,000–1,500 m and >1,500 m). The negative binomial regression (NBR) model was constructed to assess the risk differences for two variables by category while <1,000 m (distance to road) and <500 m (elevation) sub-categories were used as reference, respectively. Our results were expressed as incidence

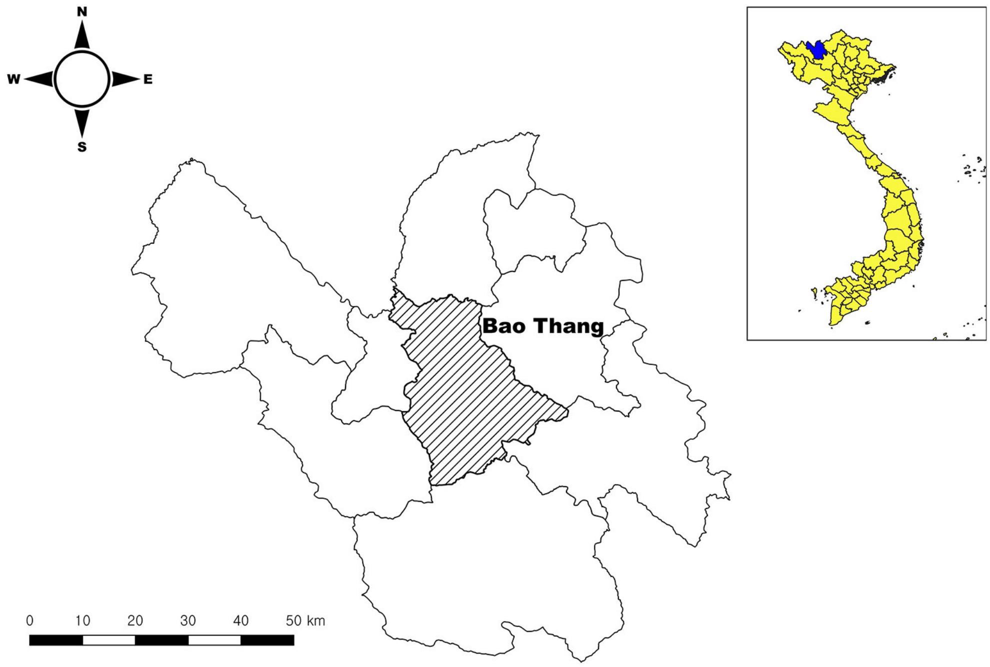

FIGURE 1 | Bao Thang district where value chain assessment was conducted for our study.

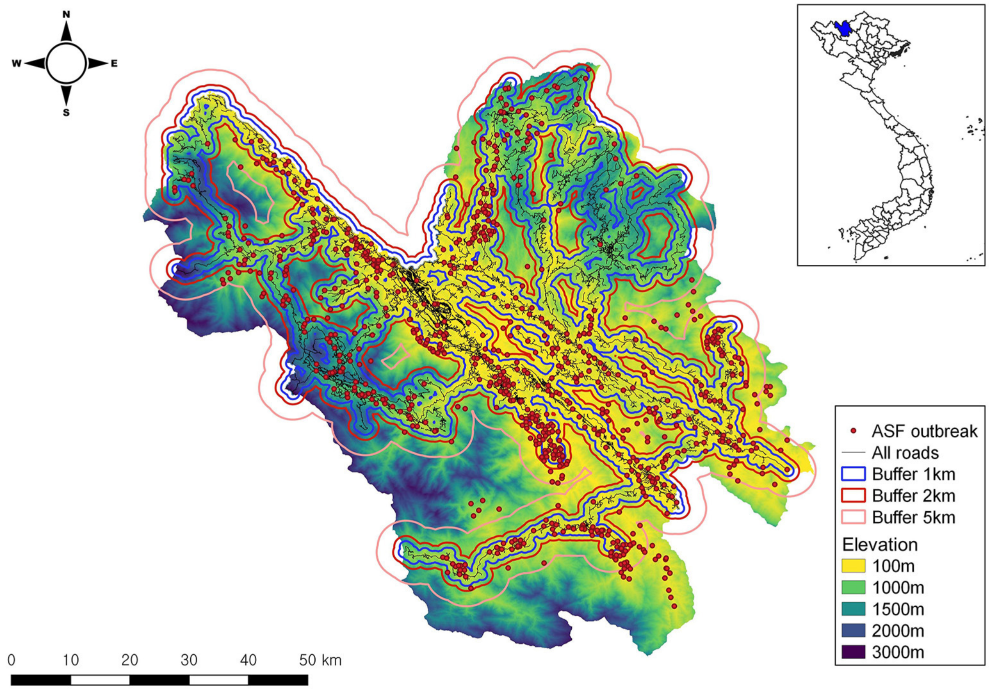

FIGURE 2 | The map was created with elevation and 4 buffers by distance to the all roads in Lao Cai province.

rate ratio ($\frac{\textit{Incidence rate in the exposed group}}{\textit{Incidence rate in the unexposed group}}$) and 95% confidence interval (CI). Although Poisson models are commonly used for the analysis of count data, the number of cases showed evidence of overdispersion (variance is more than the mean) so NBR models that embraced an overdispersion term (alpha [α]) were preferred to Poisson models (28). All data were entered

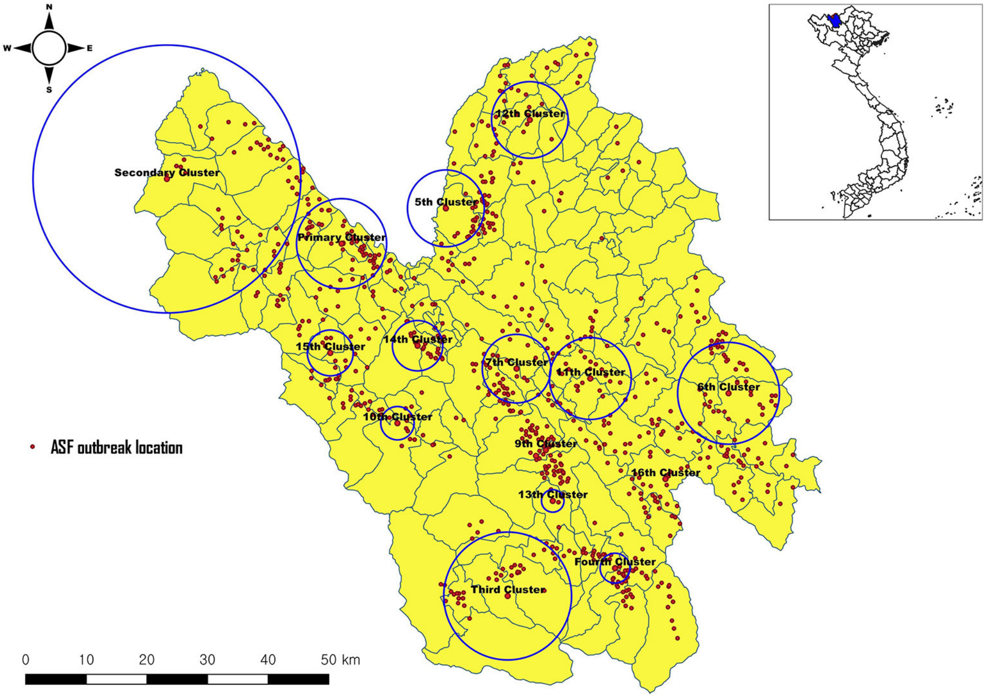

FIGURE 3 | Space-time cluster analysis of ASF outbreaks from 2019 to 2020 in Lao Cai (50% at risk).

into Microsoft Excel and analyzed using STATA version 17.0 (StataCorp, College Station, TX). A p-value of less than 0.05 was considered statistically significant.

For the assessment of risk factors along the pig value chain, data collection was conducted from 10 to 24 Dec 2020 through focus group discussions (FGDs) with local authorities (e.g., livestock officers, vets and agriculture extension workers) and pig producers. FGDs are most commonly used as a qualitative method to gain an in-depth understanding of current issues. In addition, the initial findings were validated in a stakeholder feedback workshop which was organized on 13 Jan 2021. Before the interviews, the interviewees signed a consent form after either reading or listening to the text. This research was approved by the Hanoi University of Public Health Review Board (No. 186/2020/YTCC-HD3), Vietnam. All relevant questionnaires are available (**Supplementary Material 1**).

RESULTS

Spatiotemporal Analysis

A total of 925 outbreaks were only reported in domestic pigs from 2019 to 2020. More outbreaks were reported in 2019 (708) than in 2020 (217). Using the spatial window set at 50%, a total of sixteen clusters were identified (**Figure 3**). The primary cluster was observed in July 2019 near the Chinese border (radius: 8.0 km), showing a ratio $\left(\frac{observed\ cases}{expected\ cases}\right)$ of 3.55 (3,618/1,019) (**Table 1**). The secondary cluster was identified in the northwest part of the Lao Cai province (radius: 23.78 km) with a ratio of 3.44 in July 2019. The 9th cluster showed the highest ratio (53.56), followed by the 8th cluster (25.99) and the 16th cluster (21.82). Three clusters (primary, secondary and 5th) were observed near the Chinese border. Most temporal clusters were detected between May and August during the study period. In addition, we evaluated the association between ASF outbreak locations to the nearest main roads and elevation. The NBR model showed that, compared with the reference (<5,000 m), <1,000 m (3.71 times) was significantly higher occurrences of ASF while the distance with 1,000–5,000 m was significantly preventive to the ASF outbreaks (**Table 2**). For elevation, compared to the reference (>1,500 m), the farm locations with <500 m (55.31 times) showed a significantly increased risk of ASF outbreaks, followed by 500–1,000 m (10.08 times) and 1,000–1,500 m (6 times), respectively.

Assessment of Risk Factors in Transmission of ASF Along the Major Pig Value Chains

A total of three groups (7 people / group) took part in the discussion (2 groups for local authorities and 1 group for pig producers; randomly selected from a list). In general, it took 2–3 h for the FGDs.

Main Reasons for the Transmission of ASF as Assessed by Local Authority

In Lao Cai, the first outbreak was reported in May 2019 and then spread rapidly across the province. It was assumed that the main reasons were as follows:

TABLE 1 | Space-time clusters of ASF from 2019 to 2020 in Vietnam (space window: 10% at risk).

Cluster No.	Time year/month	Obs/exp = ratio	Radius (km)	*P-value*
Primary	Jul/2019-Jul/2019	3,618/1,019.32=3.55	8.00	<0.001
Secondary	Jul/2019-Jul/2019	3,342/971.57=3.44	23.78	<0.001
3rd	Aug/2020-Dec/2020	928/64.24=14.45	11.35	<0.001
4th	Aug/2009-Aug/2019	1,607/279.61=5.75	2.65	<0.001
5th	May/2019-May/2019	724/425.75=4.05	6.82	<0.001
6th	Mar/2019-May/2019	461/805.22=3.06	9.04	<0.001
7th	June/2019-June/2019	958/149.40=6.41	6.09	<0.001
8th	April/2019-April/2019	355/13.66=25.99	0	<0.001
9th	Feb/2019-Feb/2019	224/4.18=53.56	0	<0.001
10th	Oct/2019-Oct/2019	326/16.26=20.04	2.96	<0.001
11th	April/2019-May/2019	1,305/408.17=3.20	7.27	<0.001
12th	Nov/2019-Jun/2020	404/36.69=11.01	6.80	<0.001
13th	Sep/2019-Nov/2019	489/60.70=8.06	2.00	<0.001
14th	Aug/2019-Aug/2019	638/130.50=4.89	4.47	<0.001
15th	Nov/2019-Dec/2019	142/10.68=13.30	4.07	<0.001
16th	Feb/2019-Feb/2019	88/4.03=21.82	3.48	<0.001

TABLE 2 | Multivariable negative binomial regression (NBR) models for the distance to nearest road and elevation with incidence rate ratio (IRR) and 95% confidence interval (CI).

Variable (n)	Adjusted incidence rate ratios (IRRs)	95% CI
Short distance from road		
<1000m (579)	3.71*	2.84-4.85
1000-5000m (212)	0.69*	0.52-0.90
>5000m (151)	Ref	N/A
Elevation		
<500m (719)	55.31*	31.96-95.72
500-1000m (131)	10.08*	5.70-17.82
1000-1500m (78)	6.00*	3.34-10.79
>1500m (13)	Ref	N/A

*The * symbol indicates statistically significant value P < 0.05.*

- uncontrolled slaughter points in residential areas
- farmers' use of swills from restaurants and kitchens as pig feed
- keeping of livestock close to family kitchens
- people moving from households where pigs had ASF to other households
- the officials' non-compliance with anti-epidemic regulations (for example, failing to examine, take samples from and kill diseased pigs)
- direct spread through natural mating service or trading of pig semen
- the discarding of dead pig carcasses into the environment
- small households' low adoption of biosecurity practices
- announcement of ending ASF outbreak of some communes may have caused subjective psychology in disease prevention of some livestock farmers and local authorities.

In 2020, in addition to the above reasons, a number of new issues contributed to the spread of ASF. First, pig producers killed infected pigs near roads or water sources in contravention of technical procedures by not wearing biologically protective clothing and burying pigs through middlemen. Second, producers only destroyed infected pigs but not pigs in the same cage, causing diseases to spread and prolonging the outbreak. Third, the official mechanisms participating in disease prevention and control at the district and communal levels were sometimes inconsistent and erratic. Lastly, some pig producers did not report cases of ASF on their farm to officials or delayed their reports, allowing the epidemic to spread widely and making it difficult to control.

Perception of Risk Factors Along the Pig Supply Chain Among Pig Producers in the Study Area

Two FGDs were held with farmers, each with different production scales. This Venn diagram (**Figure 4**) showed linkage of producers to other actors in the pig supply and consumption chain: Important direct relationships (bold / thin arrows), frequency of contact (write frequency along arrow), far, near distance (arrow length, inside, outside locality, province, district, commune), and assessment of the potential risk of pathogen transmission through relationships (1. Danger, 2. No danger, 3. Don't know). In general, farmers perceived that the highest risk of ASF transmission may come from collectors and slaughterers, intermediaries inside and outside the commune, feed agents and maize agents in the commune, and pig retailers. Traders bought pigs from farmers and also sold them breeding piglets. Most households bought breeding piglets from traders and did not know the origin of the pigs. In reality, traders visited many places, from household to household, and farmers. The slaughterers in the commune also visited multiple farms and different places to buy pigs; they also had to go into pig stables to catch them. An intermediary was often a trader or collector in the commune who travels to many regions to find and catch pigs. Feed and maize agents come into contact with many farmers, including those with sick pigs. However, farmers thought that the agents may spread disease, but that the risk was not as high as with traders because agents did not enter the pigpens, apart from providing breeding pigs.

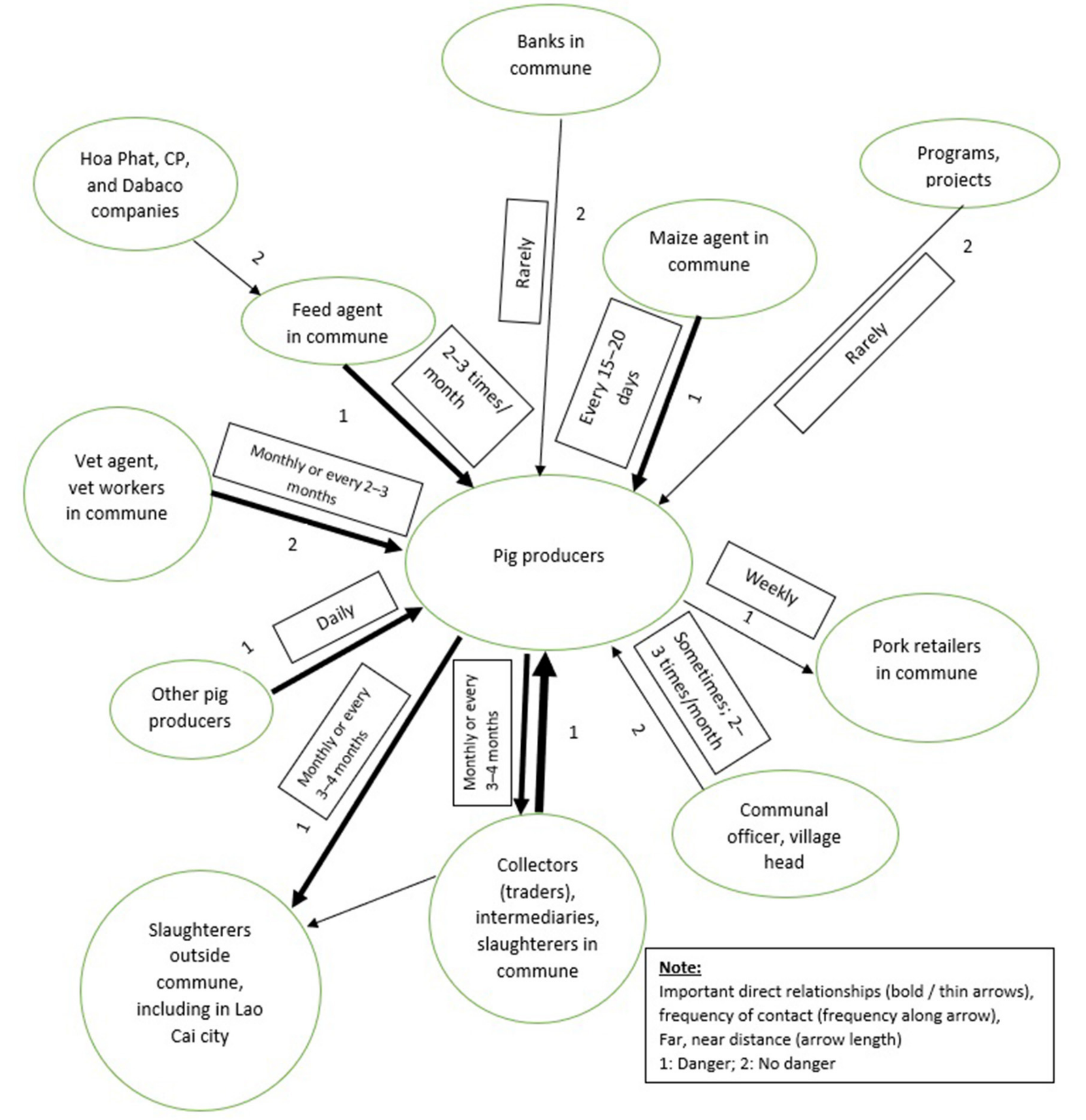

FIGURE 4 | Perceptions of danger of ASF transmission in the value chain among pig producers in the study area.

DISCUSSION

This research was the first attempt to assess the spatiotemporal analysis of ASF using local surveillance and remote sensing data, as well as to evaluate the risk factors in the pig value chain in Lao Cai, Vietnam. It was possible that ASF cases in the local surveillance system were likely to be underestimated because small-scale farms (accounting for 65–70% of the pig population in Vietnam) lacked knowledge about livestock diseases (including ASF) and were less reachable to veterinarians and animal health workers. Moreover, all pigs must be slaughtered when a new case is confirmed on the farm, which may have resulted in reluctance for reporting.

The primary, secondary and fifth clusters were observed near the Chinese border, which might be highly associated with the illegal movements between Chinese borders as the first ASF case was reported in China (6) and rapidly spread to other countries in Asia, including Vietnam (7). It was assumed that the ASF virus was transmitted through pig movements and pork products or infected fomite (29). One study found that the strain of the ASF virus in Vietnam was the same strain as the circulating virus in China (8). In addition, it showed similar outbreak patterns of the highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) in 2007 (30). The HP-PRRS outbreak was first detected in China, then quickly jumped in Vietnam and other Southeast Asian countries (31, 32).

Our study found that the distance to the road and elevation were reversely associated with the ASF outbreaks, which was consistent with previous studies (25, 26). Actually, unregulated slaughtering and the proximity of pig slaughterhouses to the main road is common in Vietnam (33). In addition, it is possible that farms at low elevation have better accessibility for human movement (e.g., traders and feed companies), resulting in an increased risk of ASF.

Since the first case of ASF was detected in northern Vietnam in February 2019, the number of affected provinces rapidly were increased within a short period of time (<4–5 months). Some potential risk factors have been proposed, such as illegal trading activities; low biosecurity; feeding of food scraps to pigs; not fully culling all pigs in infected farms, disposal of pig carcasses to public areas. Moreover, increased human and animal movements during the Tet holiday (Vietnamese New Year in late January or early February) may have played a significant role in spreading the ASF virus across the country.

It has been well recognized that wild boars and soft tickets can play a significant role in the transmission of the virus (34–36). In Asia, ASF in wild boars has been detected in China and South Korea (37–39). However, no studies have been implemented to evaluate the possible roles of wild boars and soft tickets for spreading a virus among farms in Vietnam. Especially, Lao Cai province is a mountainous region that may provide favorable conditions for wild boar habitats. Therefore, more research is necessary to assess the main transmission route of the ASF virus at the farm level.

Our research had some limitations in that it was likely that ASF surveillance data were underreported due to lack of awareness, animal health professionals and laboratory facilities in rural and mountainous areas. Especially, farmers were reluctant to report to the authorities because of low compensation rates and complicated/prolonged administrator procedures. In addition, it was assumed that the pig population did not rapidly change during the research period, which is very important for a space-time permutation model. Therefore, it was possible that our detected clusters may have been affected by the pig population at risk if the background population dramatically increased or decreased in one area compared to another.

During the stakeholder feedback workshop, participants discussed the risk factors for ASF transmission, especially the differences in risk levels between commercial farms and household farms. Although the risk of disease on any farm was tied to many factors, the human risk factors were lower on commercial farms because most of them applied high biosecurity procedures. In addition, they did not allow visitors, including traders, into the barn areas. However, for small-scale producers, the human movement was one of the most important risk factors as they applied fewer biosecurity practices and did not control their neighbors or traders who may move in and out of the production areas, which was consistent with a previous study (9). Mostly, livestock farmers considered producers to be high-risk actors as they visited many places, however, they rarely entered the pig stables of other farmers.

According to the interviewed pig producers, the greatest difficulties for restocking pig herds after ASF outbreaks on the farms and in the region were concerns about re-infection with ASF, high cost of breeding pigs and lack of capital for both household and commercial farms. In addition, for household farms, access to breeding pigs with known origin was also difficult. Restocking of breeding sows was slow due to difficulties in purchasing quality breeds, especially exotic breeds. Restocking fatteners was also slow due to the high price of commercial piglets. ASF outbreak reoccurred in the province in February 2020 and continued to spread across the province. However, large numbers of households still did not have a deep understanding of diseases and biosecurity. Currently, the majority of people believe that any sickness or death among pigs is caused by ASF, causing farmers to be hesitant about restocking.

Among the technical solutions discussed, both commercial and household pig producers considered minimizing the number of people going in and out of pig stables and improving healthcare and husbandry procedures to be both very important and feasible. The interviewees addressed the other most important practices were strengthening decontamination and disinfection (using lime and hormones with higher frequency), disinfection of transport vehicles and killing of mice, flies and mosquitoes. These solutions were also considered highly feasible for adoption on the ground. As for appropriate policies to overcome ASF, household farms suggested that they would expect compensation or support when their pigs are depopulated due to ASF, as well as support for purchasing disinfectants. In addition, the commercial farms expected support for purchasing disinfectants, farm materials and equipment. The priority for training needs in the context of ASF is quite similar between households and commercial farms. These include the detection and recognition of ASF and emerging diseases; understanding the risks of ASF infection and how to prevent it; training on breeds and controlled breeding practices; safe artificial insemination practices; and technical knowledge of sanitary and disease-free pig housing.

There was a high risk of ASF transmission from traders in the pig value chain, including collectors, slaughterers and retailers at the provincial, district and commune levels. These actors all participated in the sale and purchase of pigs that could be infected. Furthermore, live pigs and pork products were not subject to quarantine within the province, and their movement was not controlled. In addition, when an epidemic occurred, it took a long time to identify the disease due to the lack of resources and capacity among local animal husbandry actors and the inability to analyse samples locally for detecting the ASF virus. This allowed time for pathogens to spread through transportation and sales of sick pigs. Every year, Sub- DAH officers have been trained in disease prevention, control and surveillance. However, in-depth professional knowledge was still lacking. At the district level, some stations lack livestock and animal health specialized staff. In some districts, the specialized veterinary staff they have were sent to work other jobs. The veterinary officers at the provincial and district levels have limited resources and capacity for monitoring and surveillance of ASF. There is a need for compliance by all pig producers and other actors in the pig value chain to adopt biosecurity practices. Therefore, awareness, knowledge and understanding of infection and risks of ASF need to be improved. Veterinary officials at the provincial and district levels need to improve capacity and resources to perform laboratory analysis for ASF and need to coordinate with local actors on the control and prevention of ASF in the community.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hanoi University of Public Health. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Hanoi University of Public Health.

AUTHOR CONTRIBUTIONS

HL designed and ran the statistical analyses, wrote the draft of the manuscript, and prepared all the figures and tables presented. TD and LH collected the data. All authors contributed to the conception and design of this study and editing and comprehensive revision of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.853825/full#supplementary-material

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BRIEF RESEARCH REPORT
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The Evolution of African Swine Fever in China: A Global Threat?

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African swine fever (ASF) is one of the most critical diseases in the pig industry. In Asia, 15 countries have already reported an outbreak as of November 22, 2021. In 2021, China reported the genotype II lower virulent ASF virus (ASFV) and the emergence of genotype I ASFV. ASF is generally known as a contagious and lethal disease, but if chronic infection spreads, then disease control would be more difficult. In the current study, we highlighted the possibility of lower virulent virus distribution throughout China and the subsequent general risk of the virus being released from the country. The kernel density estimation showed that the two highest kernel density areas of ASF notification were located in Northeast and Midwest China. Four of the five provinces where lower virulent ASFV was isolated overlapped with areas of relatively high ASF notification density. In terms of the risk of ASFV spreading from China, eight of the 10 largest airports and three of the 10 largest seaports are located in areas of relatively high ASF notification density. There were flight flow from China to 67 countries and ship flow to 81 countries. Asia had the highest flight flow, followed by Europe, North America, Africa, and Oceania. The highest number of ship flows was also concentrated in Asia, but about 10% of ships head to Africa and South America. Chinese overseas residents were distributed in each continent in proportion to these results. Here, we highlight the potential risk of ASFV spread from China to the world.

Keywords: spatio-temporal epidemic modeling, infectious diseases, African swine fever, risk assessment, Asia, China, veterinary epidemiology, lower virulent ASFV

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INTRODUCTION

African swine fever (ASF) is one of the most feared diseases in the pig industry in recent years. This devastating transboundary disease is caused by the ASF virus (ASFV), and pig species are the only susceptible animal population. On the basis of the p72 genotyping classification, 24 genotypes have been reported worldwide to date (1, 2). Of these viruses, the ones that are currently widely spread throughout Europe and Asia are genotype II viruses, which are generally regarded as highly virulent (3). The clinical signs exhibited by infected individuals vary and are classified into four main stages based on clinical presentations and pathological lesions: Peracute, Acute, Subacute, and Chronic stage (4). Subacute and chronic forms of ASF have a low mortality rate in infected individuals. In particular, chronically infected individuals show unclear clinical symptoms, and some individuals have been reported to spread the virus for a long time (4). Susceptible individuals can become infected with the virus *via* direct or indirect contact with infected animals or contaminated materials (5, 6).

ASFV is well-known as a virus with a significantly high environmental resistance. Various studies have been conducted on the environmental resistance of viruses. The virus is shed in large quantities in the blood where the virus can survive for 15 weeks at room temperature, months at 4°C, and indefinitely when frozen (7). In the case of raw meat, it can survive for more than 3 months in meat and offal [FAO, (5, 7)]. Feces and urine are also infectious. The half-life of the virus in urine is 15 days depending on the environmental temperature (8). In feces, its half-life is reported to be 5–8 days, but viral DNA can be detected for up to 2–4 years (8). Therefore, any contaminated material, including persons, materials, or infected meat products, could represent a source of infection to ASF-free countries. Even in countries where outbreaks have already been reported, a higher level of environmental contamination could pose a higher risk of further outbreaks.

In Asia, after the first ASF outbreak was reported in Liaoning Province, China, in August 2018, 15 countries (China, Mongolia, Vietnam, Cambodia, North Korea, Laos, Myanmar, Philippines, South Korea, East Timor, Indonesia, Papua New Guinea, India, Malaysia, and Bhutan) confirmed ASF outbreaks as of November 22, 2021 (9). Although many ASF cases have been reported in wild boar in South Korea and Malaysia, ASF outbreaks in the remaining Asian countries have been mainly associated with domestic pigs at this moment (10). This is assumed to be related to the traditionally high number of backyard farms, inadequate biosecurity levels, and the non-transparent distribution network of livestock and their products (11).

Although ASFV genotype II is currently prevalent in Asia and is considered to be highly virulent, the isolation of the lower virulent ASFV genotype I and II was recently reported in China (12, 13). By deleting genes, ASFV can be artificially attenuated (14). The *EP402R* gene encodes the CD2v protein, which causes binding of red blood cells to infected cells and virus particles (3, 15). The deletion of this gene resulted in virus attenuation and induction of protection, so it is frequently targeted for ASF vaccine development (15–17). Recently, there has been a problem in China with the spread of illegal vaccines created by copying vaccines under development (14, 18). These illegal vaccines might cause chronic infection in vaccinated individuals (14). Apparently supporting this fact, Sun et al. surveyed seven Chinese provinces (Heilongjiang, Jilin, Liaoning, Shanxi, Inner Mongolia Autonomous Region, Hebei, and Hubei) with the collection of 3,660 field samples in 2020 (13), and they detected 11 different isolates of CD2v (−) ASFV of genotype II, which show lower virulence, in three provinces (seven from Heilongjiang, three from Hubei, and one from Hebei). Moreover, in June 2021, Sun et al. reported the emergence of ASFV genotype I, for the first time in Asia, from two farms in Henan and Shandong province, China (12). The results of animal experiments have shown that these isolated viruses [ASFV genotype I and CD2v (−) ASFV genotype II] can cause chronic infection and are highly transmissible (12, 13). These lower virulent viruses are characterized by unclear clinical symptoms and a long incubation period, which makes early detection of infected animals more difficult (12, 13). If these lower virulent viruses are quietly spreading throughout China and are released from the country, then this will most likely further complicate any efforts to control ASF in affected countries.

In this study, we first highlight the spatial distribution of ASF notifications across China. Second, the spatial risk of these lower virulent ASFVs [ASFV genotype I and CD2v (−) ASFV genotype II], spreading throughout the country, was assessed. To do so, we overlaid the ASF notification density map with the areas where these lower virulent viruses were found, then we indicate, in an indirect manner, the general risk of ASFV spread from China.

MATERIALS AND METHODS

Data and Sources

The whole of China was set as the study area. Epidemiological information regarding dates, coordinates, and event source (fomites, swill feeding, illegal movement of animals, etc.) including both domestic pig and wild boar for the periods from August 1, 2018, to September 4, 2021, were obtained from the World Animal Health Information System (OIE-WAHIS) database (9).

Temporal Trend and Spatial Distribution of ASF in China

On the basis of the information obtained from OIE-WAHIS (9), 3-month outbreak trends for ASF in China, including epidemic information (reported number and event sources), were described using Microsoft Excel Software. Furthermore, each outbreak was categorized by year, by event source, and its spatial distribution was depicted using ArcGIS 10.8.1 software (ESRI Inc., Redlands, CA, USA).

Risk of the Lower Virulent ASFV Spreading Nationwide

To assess the spatial risk of ASF outbreaks spread across the country, the kernel density analysis (19), a non-parametric estimation tool for assuming the continuous density distribution from a series of events, was applied using the data obtained from OIE-WAHIS (9, 20). Here, we assumed that if the lower virulent ASFV is located in an area of high kernel density, the risk of the virus being spread is considered as high. For more accurate kernel density maps, it is important to obtain the correct location of the case point and to set the search radius (bandwidth) appropriately. To explore the ideal bandwidth, we applied a multi-distance spatial cluster analysis tool in ArcGIS software version 10.8.1 according to the manufacturer's guidelines and previous studies (21, 22). A common transformation of Ripley's K function was used in the analysis. For analysis of the spatial pattern of ASF outbreaks, observed K values were compared with the Expected K values of a completely random spatial distribution of ASF outbreaks with 999 simulations, which is equal to a confidence level of 99.9%. The Diff K values contain the Observed K values minus the Expected K values. The Expected K values giving the highest Diff K values can be interpreted as the maximum distance of the relationships between ASF outbreaks in China; thus, it was set as the optimal bandwidth. The kernel densities obtained were then classified into five risk levels based on Jenks natural brakes classification (23) and defined as very high, high, medium, low, and very low as indirect risk indicators.

In general, the size of the pig production in the area is considered as one of the important factors that affect the occurrence of ASF (24). To visually understand the relationship between the kernel density of ASF notification and the scale of the swine industry, we firstly identified provinces with high pig stocks on average based on information from the Global Economic Data, Indicators, Charts & Forecasts (CEIC) database for 2017–2019 (25) and then overlaid these locations on the map. Furthermore, to investigate the possibility that the lower virulent ASFV is spreading throughout China, the results of the kernel density analysis and the location of the provinces where these viruses have been isolated (12, 13) were depicted together on the map using ArcGIS 10.8.1 software. In this study, lower virulent ASFV was defined as ASFV genotype I and CD2v (–) ASFV genotype II isolated in China.

The Risk of ASFV Spread From China

In this section, we assessed the general risk of ASFV spread from China. There are various pathways by which the virus can be released from the country. These include the movement of live pigs, pork products, and human movement such as travel or humanitarian aid (26). China has not exported live pigs to foreign countries since the first confirmation of ASF in China, and the number of destinations for pork products is very limited (27). Therefore, the most probable route of virus release is assumed to be *via* international travel by ship or airplane.

Because there was insufficient information available to conduct a quantitative risk assessment in this study, the risk was estimated indirectly by considering the flight and ship flow from China and the number of Chinese overseas residents per country.

First, to understand the geographical relationship between the ASF confirmed area and international ports, the locations of China's 10 largest airports and seaports (28, 29) were overlaid with the map of kernel density analysis using ArcGIS 10.8.1 software. Second, information on traffic volumes at ports trading with China was obtained from the World Bank database (30) and converted to national scale information using Microsoft Excel Software.

For flight flow, airports with flights coming from China were attributed with total seats from the year 2019. Regarding ship flow, we calculated the loading capacity between China and the destination countries. All seaports with reported international trade in the first quarter (Q1) of 2020 were attributed with the sum of quarterly deployed capacity twenty-foot equivalent unit (TEU) (31, 32). The obtained results were depicted on the world maps using the XY To Line tool on ArcGIS 10.8.1 software (33).

According to previous studies, Chinese tourists tend to prefer Chinese food at least once a day even when they are in a travel destination, and, even after several generations of migration abroad, they still have the habit of eating Chinese food on a daily basis (34–36). In this study, the Chinese overseas residents were defined as people of Chinese birth or ethnicity who reside outside China. We assumed that Chinese overseas residents may bring in goods from China, which could be one of the important risky behaviors for other countries in terms of ASFV introduction. At present, there is no official information on the number of Chinese overseas residents. Therefore, the available relevant information was collected on the basis of various data sources (37–41). The obtained data were depicted by overlaying a map showing the flight or ship flows from China using the Add Join tool on ArcGIS 10.8.1 software (42).

RESULTS

Temporal Trend and Spatial Distribution of ASF in China

The 3-month temporal trend of ASF outbreaks is shown in **Figure 1**. After a peak in the number of outbreaks in the last quarter of 2018, there was an overall decreasing trend in the number of notifications. In the third quarter of 2020, there were only three outbreaks, but the ongoing outbreaks continued, and since then, the number of outbreaks increased again. Focusing on the characteristics of each outbreak, the sources of outbreaks reported to the OIE show differences in 2018–2019 and in 2020–2021. About 83.6% of the event sources of ASF reported from 2018 to 2019 were "unknown or inconclusive". However, after 2020, about 87.5% of the event sources were "illegal transport of animals".

The spatial distribution of outbreaks was also remarkably different in 2018–2019 and in 2020–2021. From 2018 to 2019, outbreaks were widely distributed throughout the entire nation. Meanwhile, from 2020 to 2021, the outbreaks seem to be concentrated around the central part of the country (**Figure 2**).

Risk of Lower Virulent ASFV Spreading Nationwide

The results of the multi-distance spatial cluster analysis indicated that 625.5 km was the maximum distance of significant spatial association between ASF notifications of domestic pigs in China during the study period.

The results of the kernel density estimation analysis showed that the two highest kernel density areas were located in Northeast and Midwest China. In Midwest China, the third highest kernel density area was detected in the central coastal areas. Areas of medium kernel density were widely distributed around the two main hot spot areas (**Figure 3**).

Among the provinces of high kernel density, five of them are also high swine production area (Sichuan, Yunnan, Guangxi, Hunan, and Hubei) (**Supplementary Figure 1**).

Four of the five provinces where the lower virulent ASFV was isolated were located in areas of medium or higher kernel density. In particular, Hubei province, where the lower virulent ASFV genotype II was reported in 2020, was located between the two main hotspots. On the other hand, the kernel density in Heilongjiang province, where seven lower virulent ASFV genotype II isolates were reported, was classified as low (**Figure 3**).

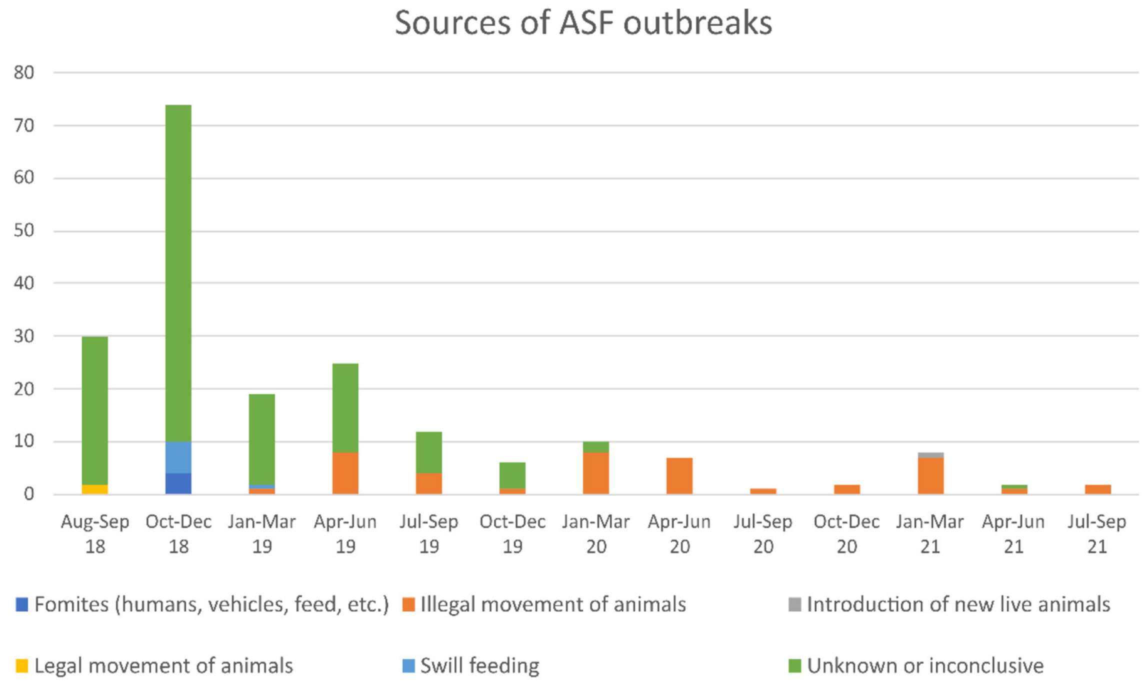

FIGURE 1 | Temporal trend of African swine fever (ASF) in China. The number of ASF outbreaks every 3 months, including the details of the event sources, is shown in a bar graph. These information sources are based on official reports to the OIE.

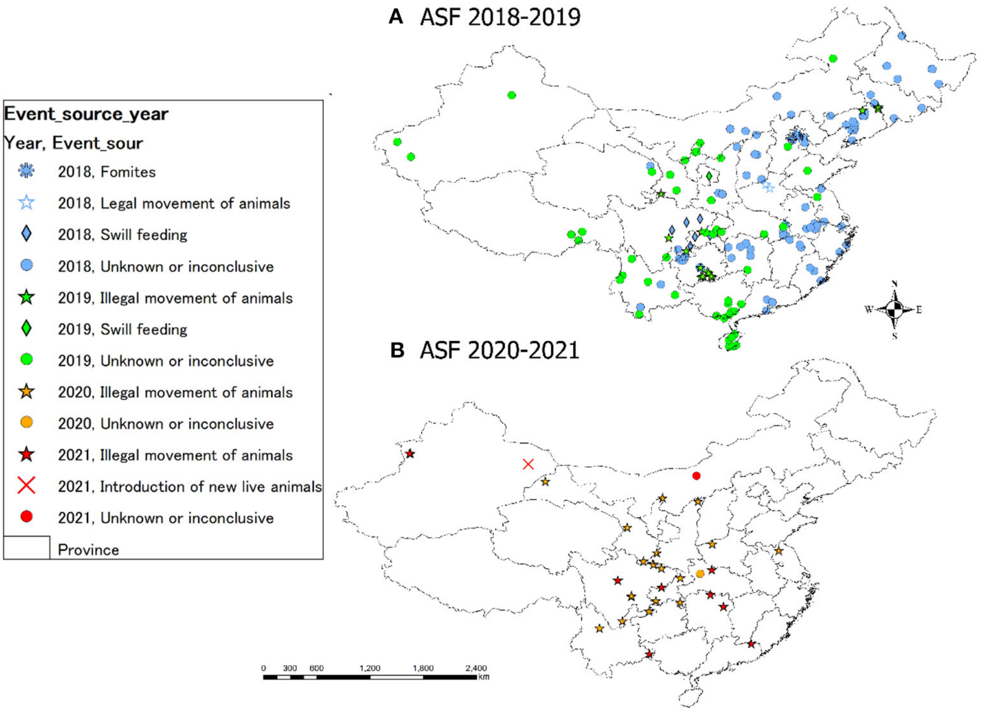

FIGURE 2 | Spatial distribution of ASF in China. Each ASF notification was classified by event source; maps **(A)** and **(B)** show the situation of outbreaks in 2018–2019 and in 2020–2021, respectively. These information sources are based on official reports to the OIE.

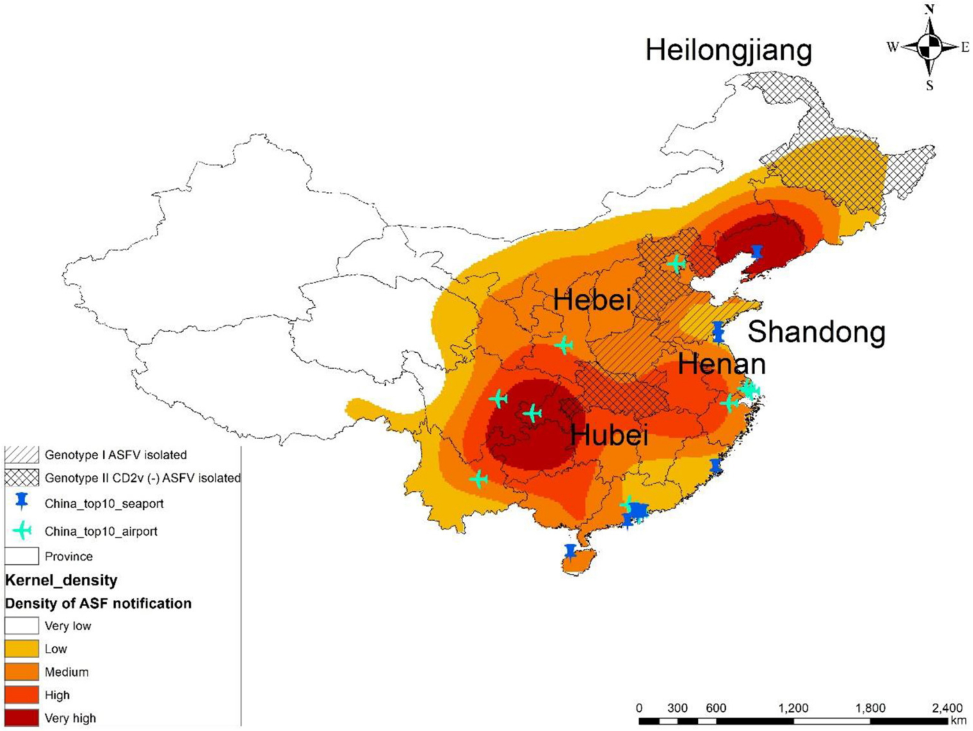

FIGURE 3 | Density of ASF notifications in China overlapped with the lower virulent ASFV reported area and China's 10 largest airports and seaports. The graduated color shades illustrate the estimated kernel density of ASF notifications (notifications/km^2). Each colored area indicates frequency of ASF notification number per >4.61 × 10^{-5} (very high), 3.11 × 10^{-5} to 4.6 × 10^{-5} (high), 1.81 × 10^{-5} to 3.1 × 10^{-5} (medium), 6.9 × 10^{-6} to 1.8 × 10^{-5} (low), and <6.9 × 10^{-6} (very low). Provinces where ASFV genotype I and CD2v (–) ASFV genotype II were isolated are represented by a diagonal line and a grid line, respectively. The locations of China's 10 largest airports and seaports are represented by airplane and pin symbols, respectively.

The Risk of ASFV Spread From China

Of China's 10 largest airports, one is located in the "very high" risk area, followed by two in the "high"-risk, five in the "medium"-risk, and two airports in the "low"-risk area. In terms of China's 10 biggest seaports, one is located in the "very high"–risk area, followed by two in the "medium", and seven seaports in the "low" kernel density area (**Figure 3**).

There are 67 countries and regions that had flight routes from China in 2019. Of these countries, 31 belonged to Asia, 22 to Europe, eight to Africa, four to North America, and two to Oceania (**Figure 4**). In terms of the amount of flight flow, 81.1% of total flight capacity is in Asia, 11.1% in Europe, 6.3% in North America, 0.9% in Africa, and 0.6% in Oceania (**Supplementary Table 1**).

In Q1 of 2020, China had ship flow to 81 countries and regions. Of those countries, 26 belonged to Asia, 19 to Europe, 15 to Africa, eight to Oceania, seven to South America, and six to North America (**Figure 4**). In terms of the amount of ship flow, 63.9% of total capacity is in Asia, 15.8% in Europe, 9.9% in North America, 5.2% in Africa, 4.4% in South America, and 1.0% in Oceania (**Supplementary Table 2**).

The number of Chinese overseas residents per country is shown in **Figure 3**, showing that 71.4% of Chinese overseas residents (29,000,000) live in other countries of Asia, followed by 16.3% (6,604,000) in North America, 5.5% (2,230,000) in Europe, 3.7% (1,500,000) in Oceania, 1.7% (700,000) in Africa, and 1.4% (572,000) in South America.

DISCUSSION

Because the first ASF outbreak was reported in 2018 in China, continuous outbreaks have been reported in the country (9). In March 2020, 10 regulations were issued by the Chinese government to further strengthen the prevention and control of ASF and to strictly enforce illegal activities in the swine industry chain (43). The regulations are designed to restrict behaviors that pose a risk of spreading ASF (e.g., concealment of the outbreak, use of illegal vaccines, illegal transport of pigs, and swill feeding). The change in the spatiotemporal trend of outbreaks observed in 2018–2019 and in 2020–2021 (see **Figures 1**, **2**) may be associated with the enforcement of this crackdown. However, there is no reliable and sufficient information on the contribution of the enforcement of this regulation to disease control and can therefore only speculate. Recent outbreaks have been confirmed mainly from the south-central area, and most of these were attributed to the illegal transport of animals (9). Considering that about 45% of this "illegal transport of animals" were reported at highway checkpoints and that the maximum distance associated between cases was calculated to be 625.5 km in this study, it is highly likely that pigs were transported from distant locations (9).

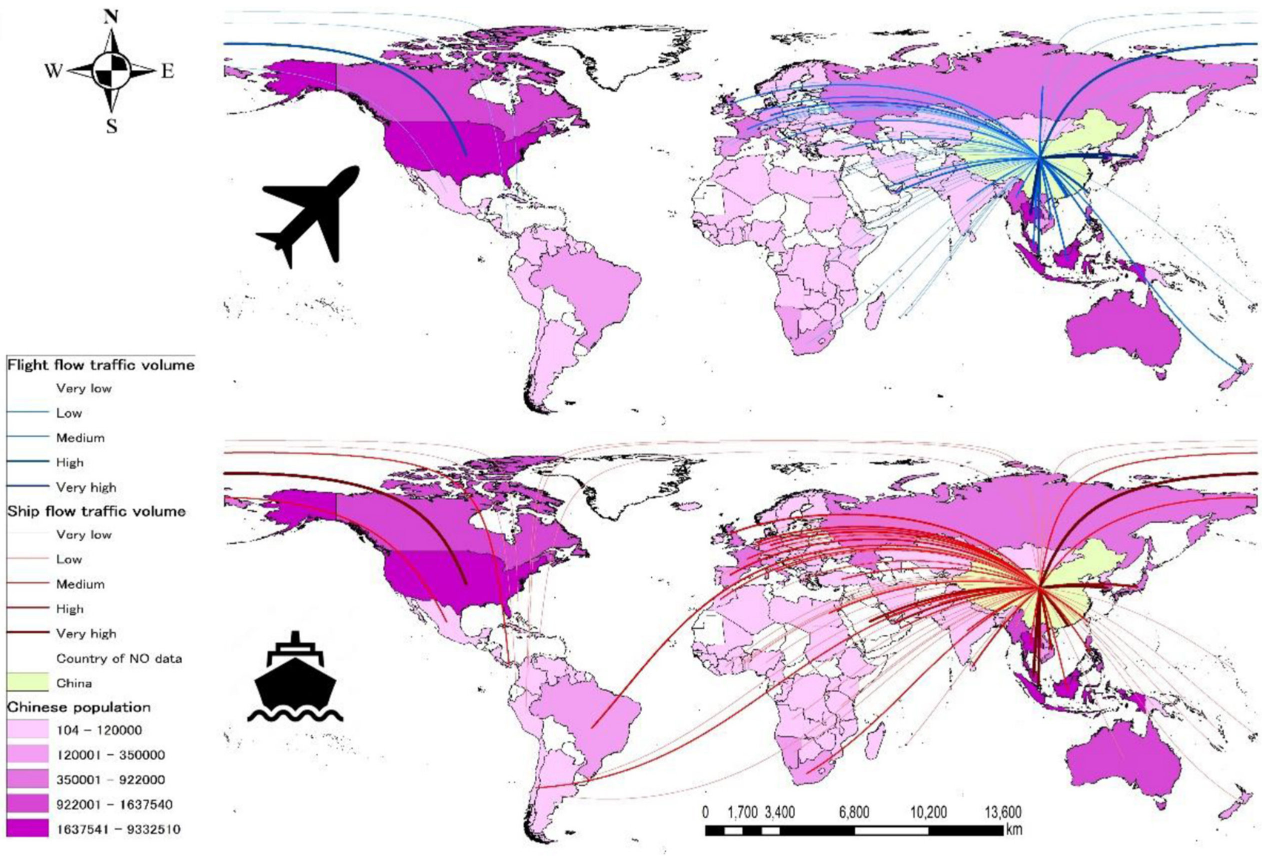

FIGURE 4 | The flight and ship flows connected to China combined with the distribution of Chinese overseas residents. The flight flow and ship flow from China are depicted above and below, respectively. The graduated color and width in the map represent the total traffic volume from the highest (darker/thicker) to the lowest (lighter/narrower). Each line indicates total traffic volume of $>1.81 \times 10^7$ (very high), 8.91×10^6 to 1.8×10^7 (high), 2.91×10^6 to 8.9×10^6 (medium), 9.51×10^5 to 2.9×10^6 (low), and $<9.5 \times 10^5$ (very low) seats in flight flow. For ship flow, each line indicates total traffic volume of $>7.51 \times 10^6$ (very high), 3.61×10^6 to 7.5×10^6 (high), 2.21×10^6 to $3.6 \times 1\ 0^6$ (medium), 7.51×10^5 to 2.2×10^6 (low), and $<7.5 \times 10^5$ (very low) per quarterly deployed capacity (TEU). In each map, the graduated color represents the number of Chinese overseas residents from the highest (darker) to the lowest (lighter).

Of the ASF outbreaks reported from China, 44 outbreaks (28, 15, and 1 cases reported in 2018, 2019, and 2020, respectively) have occurred in backyard farms, and seven cases were detected at slaughterhouses in seven different provinces (9). Before ASF emergence in China, more than 60% of pigs were produced by small-scale or backyard farmers, which generally have low biosecurity (44). Around 50% of these farmers still exist after the introduction of ASF (45, 46); hence, the potential for ASF to cause serious consequences remains high (44). Furthermore, the fact that ASF cases were reported from slaughterhouses indicates the potential risk of underreported outbreak, as well as the risk of contaminated pork being distributed. These multiple uncertainties suggest that unreported ongoing outbreaks might be present across the country.

In March 2020, the Harbin veterinary research institute released information about their ongoing ASF vaccine development (16). Around the same time, illegal vaccines, which appeared to be copied from the Harbin Institute vaccine, emerged in China (18, 47). In May 2021, the Harbin Institute reported that lower virulent ASFV of genotype II was isolated from samples collected at several locations in 2020 (13). Subsequently, the identification of ASFV strains belonging to genotype I, which were shown to cause chronic infections in affected animals, was reported from Shandong and Henan province in June 2021 (12). The potential circulation and spread of lower virulent viruses that cause chronic infections can be assumed to delay detection and thus complicate efforts to control ASF (12, 13). Under conditions where outbreaks are not effectively monitored, this could lead to further outbreaks, which could spread silently throughout the country, and also poses the risk of spreading the virus outside the country.

The results of the multi-distance spatial cluster analysis indicated that the maximum associated distance between ASF cases in China during this study period was 625.5 km. Long-distance transportation of live pigs is common in China, and the movement of infected pigs by truck is considered to be the main reason why ASF outbreaks spread quickly throughout the country in the early period (48). The reason that the maximum associated distance was extremely long can be attributed to this traditional transportation system.

The five provinces where lower virulent ASFV genotype I or genotype II was reported locate close to the hotspots of ASF notification. On the basis of the assumption of underreporting and illegal transportation across the country, it cannot be ruled out that such viruses are already spreading quietly to other parts of the country. In particular, Hubei province is in a high-risk area; therefore, it is more likely that lower virulent ASFV is prevalent in the surrounding areas. In Heilongjiang province, where the largest number of lower virulent ASFV isolates has been found, no official outbreaks have been confirmed since 2019, despite the fact that highly virulent ASFV genotype II isolates have also been reported by Sun et al. (13). Considering the geographical proximity to the hotspots of ASF notification and the fact that many outbreaks have been reported along the border with China in the adjacent Russian Far East region, it is highly likely that ASF exists in this area (49). There are still many questions concerning the factors that influence the occurrence of ASF in China. As suggested by the results of this study, it is possible that the scale of the swine industry is one of the risk factors for ASF outbreaks (**Supplementary Figure 1**). However, more extensive and comprehensive research is needed to further understand the risk factors. For example, it would be interesting to survey the pork supply routes to urban areas and the associated inter-provincial transportation networks. Differences in the surveillance effort between regions may be one of the social factors for reporting bias.

If the lower virulent virus in China is released from the country, then the control of ASF in the world would be extremely difficult. In this study, by analyzing the general risk of ASFV spreading from China, we also implied the risk of these lower virulent viruses spreading. Currently, our study showed that eight of the 10 largest airports in China locate in areas of medium to high risk. Three of the 10 largest seaports in China locate in the medium or higher risk areas. The relationship between the location of the port and the density of ASF notification of the surrounding area is not yet clear. However, it is reasonable to assume that people would use an airport close to their residence, and if the density of ASF notification in that area was high, then the risk of exposure to contaminated materials would be high (50).

Over 80% of flights from China were concentrated in Asia. In proportion to this, at airports in Asian countries, there have been many reports of ASFV detection in pork products illegally brought in from China by passengers (51–54), JAQS, (55). In view of this, the United States, where many flights arrive from China, and Central American countries, which have yet to experience an ASF outbreak, need to monitor the risk of ASF entry.

The same can be said for ship flow. Because information on the number of passengers on board was not available for this study, the flow of cargo ships was considered here. It is assumed that the more the ship is loaded, the more crew members will be on board. Stockpiling food for the long voyage is essential, and if the port is located in a high risk area, then the risk of the food being contaminated with the virus could increase. In addition, if leftover food is discarded at the destination, then the risk of ASFV introduction increases. In fact, it is believed that the ASF outbreak in Georgia in 2007 was caused by leftover food brought in from East Africa (56); therefore, this pathway of virus entry should not be ignored. However, it should be noted that there is a high degree of uncertainty in this regard. We have no information on how the pork value chain in China is organized. Investigating the domestic distribution channels for live pig and pork products (e.g., where the pork available on the market is produced and how food procurement provided to shipping companies and airlines is organized) would be an important point to more accurately assess the risk of ASF spread from the country. Compared to flights, the destinations of ships were more varied, with more routes to Africa and South America. Currently, an ASF outbreak was confirmed in the Caribbean country of the Dominican Republic in the summer of 2021 (9). Although the origin of the outbreak is still unknown, this fact once again emphasized the warning that diseases can jump to distant parts of the world. Meanwhile, the risk of the virus being reverse imported must also be considered. If there are traffic flows going to a destination, it means that there are flows coming back. These risks should also be carefully monitored, especially because there are various types of viruses circulating in Africa.

Although more than 70% of Chinese overseas residents are concentrated in Asia, it should be noted that even South America, which has the smallest number of Chinese overseas residents, has more than 500,000 people there. Those who reside there may have opportunities to bring back contaminated products from their home countries. In fact, there are already many Chinese pig farms in Africa, and plans are underway to build large-scale pig farms in South America as well (57, 58). In recent years, China has been actively investing in humanitarian aid and development projects overseas, and it is believed that many people have been going into the field from their home countries as a response (59). Because ASF has spread rapidly to other Asian countries after the disease entered China, it is essential to consider, in advance, various scenarios to prevent further outbreaks.

In this study, we highlighted the possibility of the lower virulent virus spreading throughout China and the general risk of ASFV release to the world based on the publicly available data source. However, it must be mentioned that our study was developed under several major biases and assumptions. For example, the outbreak-related data in this study are based on the information reported to the OIE-WAHIS database. This may not adequately reflect the actual situation although, because endemic countries are not obliged to report each outbreak to the OIE. Furthermore, the source of the outbreak was provided by the reported country, and the reliability of the information is not discussed. Another limitation is that the official reports did not contain any information about the lower virulent virus, so we had to refer to the only available research reports by Sun et al. (12, 13). Because of the partial coverage of the study area in these studies, potential biases existed, and thus, our approach was very limited. If a comparable study had been conducted at a nationwide level, then it might have provided a more accurate picture of the epidemic and, thus, might have provided different results. In response to the widespread of illegal vaccines, the Chinese government has tightened its crackdown on such vaccines (47). Various measures have been taken to control the outbreak, including the implementation of movement restrictions that divide China into five areas, along with unannounced inspections of slaughterhouses (60). However, as a country with a huge land area, it is quite difficult to control all activities. Accurate understanding of the situation is essential to control the outbreak. Outbreaks continue to occur because the circulation of the virus is being maintained somewhere. Although there are few cases reported from wild boar in Asia, wild boar are considered to be widely distributed and populations are also assumed to be high (61). Therefore, the trend of ASF cases in wild boar also needs to be monitored.

The most important aspect of controlling the disease is the same for both domestic pigs and wild boar: early detection. Especially in the case of the lower virulent virus reported in China, unclear clinical symptoms and a long incubation period make early detection of infected individuals more difficult (12, 13). It is important that China, as well as other countries, understands this and establishes comprehensive measures to control the disease. As an approach toward farmers, advising them on biosecurity should be a top priority. Furthermore, establishing an adequate compensation system for affected farmers would help prevent illegal trade (62). Farmers with little or no compensation could choose to hastily slaughter or sell their sick pigs at local markets (62), so regular ASFV testing at farms and slaughterhouses, as well as inspection of meat products on the market, would also help to monitor the situation.

Further research is needed to better understand the spread of ASF in China and other parts of Asia. The assessment developed here can be used as the basis for a detailed country assessment whenever accurate and complete data are available. Ultimately, we believe that it will contribute to improving the effectiveness of surveillance and control programs and epidemiological studies on this complex infectious disease.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

SI, JB, and JS-V: conceptualization. SI and JB: methodology and formal analysis. SI, JB, MM-A, and JS-V: validation and writing—review and editing. SI, JB, and MM-A: writing—original draft preparation. JS-V: supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.828498/full#supplementary-material

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Risk Factors and Spatial-Temporal Analysis of Porcine Reproductive and Respiratory Syndrome Seroprevalence in China Before and After African Swine Fever Outbreak

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Porcine reproductive and respiratory syndrome (PRRS) is an infectious viral disease that causes great harm to the pig industry. PRRS virus (PRRSV), the causative agent of PRRS, is characterized by severe reproductive failure and respiratory confusion. This study performed a cross-sectional investigation of PRRSV seroprevalence and collected 14,134 serum samples in pig farms without PRRSV vaccination from 12 provinces and two cities in China from 2017 to 2021 to detect PRRSV antibodies by enzyme-linked immunosorbent assay (ELISA). The apparent and true PRRSV antibody prevalence was estimated and compared based on the Clopper-Pearson method and Pearson chi-square test, respectively. Risk factors associated with the PRRSV serological status of pig farms were analyzed through univariate and multivariable logistic regression analysis. An automatic autoregressive integrated moving average (ARIMA) model procedure was used for time-series analysis for PRRSV seroprevalence. Spatial clusters of high PRRSV seroprevalence were detected by SaTScan software. The total true PRRSV seroprevalence of the animal level was 62.56% (95% confidence interval [CI]: 61.74–63.37%). Additionally, 286 out of 316 pig farms were positive for PRRSV antibodies at the herd level. Pig farms without pseudorabies virus (PRV) infection were 5.413 (95% CI: 1.977–17.435) times more likely to be PRRSV antibody positive than those with PRV. Identically, the possibility of pig farms being PRRSV antibody positive before an African swine fever (ASF) outbreak was 3.104 (95% CI: 1.122–10.326) times more than after ASF. The odd ratio values of medium and large pig farms with PRRSV infection are 3.076 (95% CI: 1.005–9.498) and 6.098 (95% CI: 1.814–21.290). A fluctuant decline pattern for PRRSV prevalence was observed in the temporal analysis. Three significant clusters of high PRRSV seroprevalence were first detected in China, covering a time frame from January 2018 to September 2018, which reveals high PRRSV prevalence before the outbreak of ASF. These findings show the epidemic situation and spatial-temporal distribution of PRRSV infection in China in recent years and could help develop reasonable measures to prevent PRRSV infection.

Keywords: risk factors analysis, spatial-temporal analysis, seroprevalence, PRRSV, ASF outbreak

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is an infectious viral disease that is caused by PRRSV and characterized by reproductive failure in sows and severe respiratory confusion and mortality in young pigs. It is responsible for substantial economic losses in the global swine industry (1–3). PRRSV is a single-stranded, positive-sense RNA virus from *Arteriviridae*, genus *Arterivirus*, that contains ≈15 kb nucleotides and encodes >10 open reading frames (ORFs) (4, 5). PRRSV had two main genotypes, namely European type (type 1) and North American type (type 2), of which North American type is the major epidemic genotype in China (6–9).

The first PRRSV strain of China was isolated and identified from aborted fetuses in 1996 that belonged to the North American type (8, 10). A highly pathogenic PRRSV (HP-PRRSV) strain that was featured with the discontinuous deletion of 30 amino acids in the Nsp2 gene and 20% mortality in pigs had occurred and resulted in the deaths of one million pigs in China in 2006 (11, 12). In recent years, new PRRSV field isolates with low mortality show high similarity in gene sequences with NADC30 strains isolated in America in 2008 and have been successively reported and defined as NADC30-like strains in China since 2014 (13, 14). The HP-PRRSV and NADC30-like strains are the primary epidemic strains that circulate in pig farms in China (9). Three hundred and sixty-five PRRSV strains were isolated by Jiang et al. (15) from 1996 to 2017 in China and used to analyze evolution and genome, which demonstrated that the HP-PRRSV, NADC30-like, and intermediated PRRSV were the major epidemic strains.

Infection and transmission of PRRSV from infected pigs to susceptible pigs can horizontally or vertically emerge through direct or indirect contact within the herd (6). Pitkin et al. even reported that PRRSV could be disseminated by aerosol with a distance of ≈120 m (16). The high variability in the PRRSV genome and diversity in PRRSV transmission modes causes tremendous challenges in controlling PRRSV infection in fields. Therefore, knowing the risk factors and spatial-temporal distribution of PRRSV infection is necessary to contain and eradicate PRRS illness.

Risks of PRRSV infection in pig herds increased with increased pig farm size, absence of purchased gilts quarantine, and semen purchased outside for artificial insemination (17). Fablet et al. (18) found that setting the temperature low in fattening rooms and with pigs, which coinfect with *Mycoplasma hyopneumoniae* and H1N2 swine influenza viruses, could raise herds' PRRSV seropositive possibility. Spatial analysis of PPRSV type 1 and 2 seroprevalences executed in Denmark statistically significantly detected clusters from 2007 to 2010 with higher PRRSV type 1 seroprevalence (19). However, related research on risk factors and spatial-temporal distribution of PRRSV infection are highly inadequate in China. Meanwhile, the ASF outbreak across China has caused a massive loss of pig populations, threatened the stability of the meat supply chain, and changed the feeding and management mode of pig farms, which is important for China's pig industry (20, 21). Therefore, knowing about the epidemic situation of PRRSV infection and providing reference information for policymakers related to future PRRSV control in China is urgently demanded after the ASF outbreak in 2018 (22).

MATERIALS AND METHODS

Sampling Region and Population

This study included 14,134 serum samples from 316 farms without PRRSV vaccination located in 12 provinces and two cities in China from 2017 to 2021. Collated serum samples were tested for PRRSV antibodies using an enzyme-linked immunosorbent assay (ELISA). Then, attained data were used to seek risk factors associated with the PRRSV serological status of pig farms and investigate spatial-temporal PRRSV seroprevalence clusters in China before and after the ASF outbreak. The sampling regions successively covered all seven geographical areas of China, namely central (Henan, Hubei, and Hunan provinces), eastern (Shandong, Jiangsu, Anhui, Jiangxi, Fujian provinces, and Shanghai city), northeast China (Liaoning province), south (Guangdong province), southwest (Sichuan province), northwest (Shaanxi province), and northern (Tianjin city) districts. These areas are ≈2.3 million square kilometers located in longitudes of 97°20′ E to 126°00′ E and latitudes of 18°10′ N to 43°30′ N with high pig feeding density. There are various monsoon climates with 3–28°C of annual average temperature and multiple geographic patterns, including plateaus, mountains, plains, hills, and basins. Pigs were divided into six categories according to different age and usage as follows: piglets (from birth to 21 days), weaned piglets (age of 22–70 days), growing-finishing pigs (above 70 days), replacement gilts, multiparous sows (at least one party), and boars (23). All the selected pig farms were without PRRSV vaccine immunization. Moreover, location coordinates of pig farms were acquired from Baidu Map (https://map.baidu.com/).

Sampling Design

Biosecurity measures were enhanced in pig farms after the ASF outbreak, thereby increasing the difficulty of collecting serum. Meanwhile, an accurate estimate for the number of pigs in the study region was inadequate. Therefore, a convenience sampling plan was conducted in this investigation. Detailed variable information on animal and farm levels was obtained by face-to-face interviews with pig farm owners. The documented variable information primarily included sampling time, sampling position, number of samples, season, farm size, pig farm topography, the background of pigs, ASF outbreak, and pseudorabies virus (PRV) purification in pig farms. The ASF outbreak, PRV purification, and pig farms topography are binary variables. Farm size is defined as small (≤100 sows), medium (100–500 sows), and large (≥500 sows) according to the number of sows in herds.

Sampling Method

Randomly selected pigs had blood extracted from the precaval vein using sterile vacutainer tubes without decoagulant. The gathered whole blood was sent to a third-party laboratory, "Wuhan Keweichuang Biotechnology Co, Ltd," in a cold chain

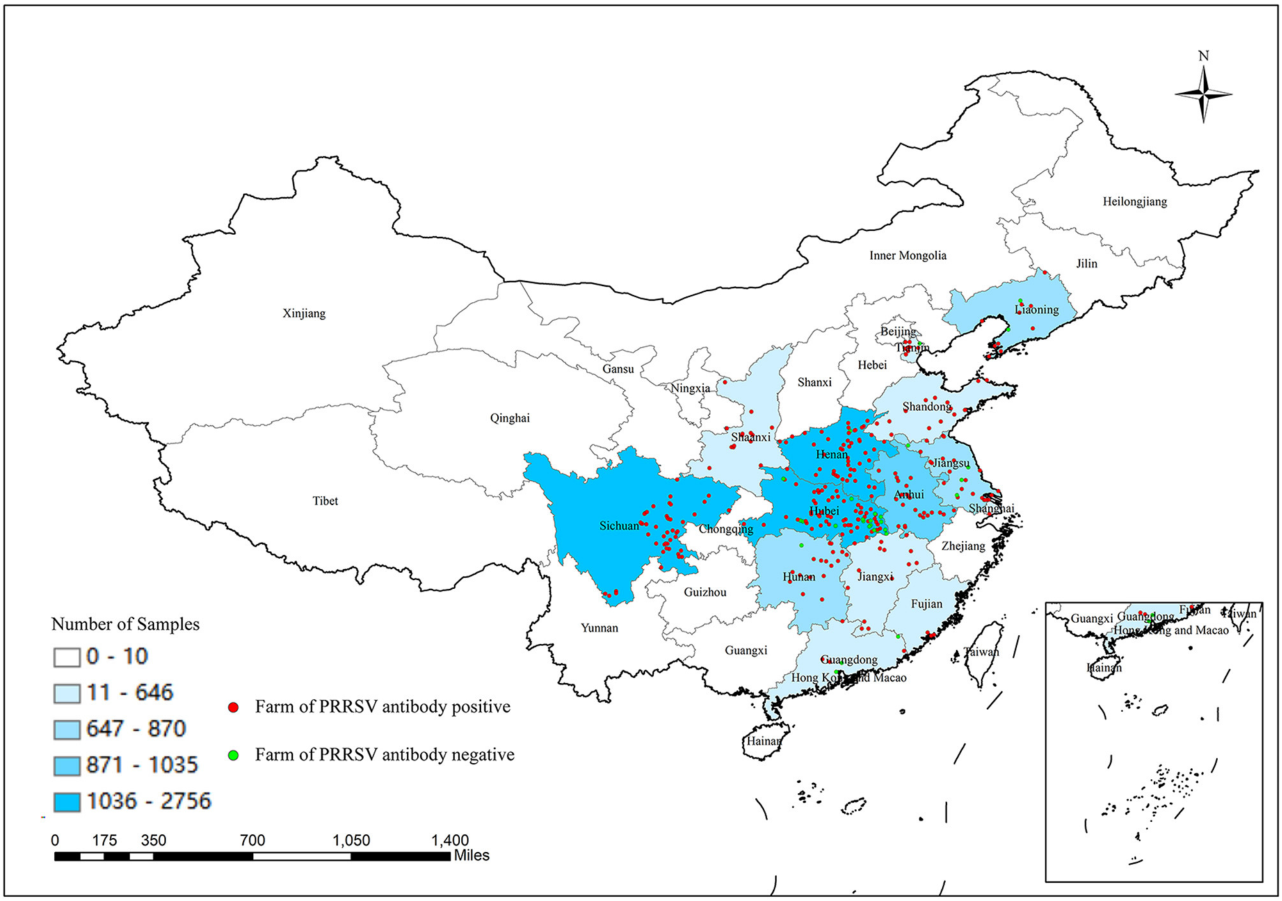

FIGURE 1 | Number of samples collected from different provinces or cities in China and location of selected pig farms.

and centrifuged to obtain serum under 3,000 rpm for 5 min. The acquired serum was stored at −20°C until use.

Detection of Serum for PRRSV Antibody by ELISA

PRRSV antibodies were detected by the PRRS X3 Ab Test kit with a sensitivity of 98.8% and specificity of 99.9% (IDEXX, USA) according to instruction (24). The operating procedures were described as follows. First, the serum was diluted in a ratio of 1:40 by sample diluent. Then 100 μl of diluted serum was added to the coated plates and incubated for 30 min at 18–26°C. Secondly, the plates' solution was discarded, and the plates were washed three times using the wash solution. Subsequently, 100 μl of the conjugate was added to plates to incubate for 30 min at 18–26°C again. The above-mentioned wash process was repeated. Thirdly, 100 μl of the substrate was dispensed to test the well for 15 min of incubation in a dark place. Then 100 μl of stop solution was added to the wells. Finally, the absorbance of each well in plates was measured at 650 nm by Multiskan FC (Thermo scientific, USA). The S/P value of PRRSV antibodies was calculated according to the formula: (absorbance of sample-absorbance of negative control)/(absorbance of positive control-absorbance of negative control). S/P values ≥ 0.4 were considered PRRSV antibody positive. Otherwise, the serum was negative. A pig farm was deemed as PRRSV infection with at least a PRRSV antibody-positive sample.

Data Analysis

Obtained data were entered and organized in Excel (Microsoft Excel 2007, USA). The apparent and true PRRSV prevalence of animal levels was estimated using the EpiR package (version 2.0.43) based on the Clopper-Pearson method (25). Simultaneously, the Pearson chi-square test was used to analyze the differences in PRRSV seroprevalence among provinces and pig categories (26).

The serological status of pig farms was registered as a dichotomous variable (positive or negative). The potential risk factors associated with the serological status of pig farms were explored among putative variables using univariate logistic regression analysis. Variables with a *p*-value of <0.1 in the univariate analysis were selected for multivariable logistic regression analysis (27). The variance inflation factor (VIF) was used to identify multicollinearity (28). Variables relevant

TABLE 1 | The apparent and true prevalence of PRRSV antibody with Pearson chi-square test in 12 provinces and two cities in China.

Provinces	No. of positive samples	No. of total samples	Apparent prevalence with 95% CI (%)	True prevalence with 95% CI (%)	χ^2 values	P value
Anhui	676	1,035	65.31 (62.33–68.22)	66.07 (63.05–69.01)	814.260	$<2.2 \times 10^{-16***}$
Fujian	135	215	62.79 (55.96–69.27)	63.52 (56.59–70.08)		
Guangdong	133	193	68.91 (61.87–75.36)	69.72 (62.58–76.26)		
Henan	1,911	2,611	73.19 (71.45–74.88)	74.05 (72.29–75.77)		
Hubei	1,249	2,756	45.32 (43.45–47.20)	45.81 (43.92–47.72)		
Hunan	450	870	51.72 (48.35–55.09)	52.30 (48.88–55.72)		
Jiangsu	486	795	61.13 (57.64–64.54)	61.84 (58.30–65.29)		
Jiangxi	327	550	59.45 (55.22–63.59)	60.14 (55.84–64.32)		
Liaoning	604	743	81.29 (78.30–84.03)	82.26 (79.23–85.04)		
Shandong	421	646	65.17 (61.36–68.85)	65.93 (62.06–69.65)		
Shanghai	92	165	55.76 (47.83–63.47)	56.39 (48.36–64.21)		
Shaanxi	235	549	42.81 (38.62–47.06)	43.27 (39.03–47.58)		
Sichuan	1,584	2,471	64.10 (62.18–66.00)	64.85 (62.89–66.77)		
Tianjin	438	535	81.87 (78.34–85.04)	82.85 (79.27–86.06)		
Total	8,741	14,134	61.84 (61.04–62.65)	62.56 (61.74–63.37)		

*CI, confidence interval. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.*

TABLE 2 | The apparent and true prevalence of PRRSV antibody with Pearson chi-square test in different stages of pigs.

Background	No. of positive samples	No. of total samples	Apparent prevalence with 95% CI (%)	True prevalence with 95% CI (%)	χ^2 values	P value
Piglets (≤21 days)	666	1,401	47.537 (44.894–50.191)	48.062 (45.384–50.751)	769.570	$<2.2 \times 10^{-16***}$
Weaned piglets (22–70 days)	1,149	2,682	42.841 (40.958–44.740)	43.304 (41.400–45.228)		
Growing-finishing pigs (≥71 days)	1,366	1,953	69.944 (67.856–71.972)	70.764 (68.648–72.818)		
Replacement gilts	989	1,326	74.585 (72.151–76.910)	75.466 (73.000–77.821)		
Multiparous sows (≥1 parity)	3,360	4,838	69.450 (68.130–70.746)	70.264 (68.927–71.577)		
Boar	1,211	1,934	62.616(60.416–64.778)	63.340(61.111–65.530)		
Total	8,741	14,134	61.84 (61.04–62.65)	62.56 (61.74–63.37)		

*CI, confidence interval. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.*

to biological meaning are retained in the model in presence of multicollinearity between variables.

Samples were not collected because of coronavirus disease 2019, which caused missed PRRSV seroprevalence data in February and March 2020. Hence, the missed data were filled using the mice package (version 3.14.0) through multiple interpolation methods (29). Time-series of PRRSV seroprevalence were analyzed using an automatic (ARIMA) procedure. The parameters p (the number of autoregressive terms), d (the number of non-seasonal differences), and q (the number of moving average terms) were determined to forecast PRRSV seroprevalence with 95 and 80% CI in the 19 months following the study period (May 2021).

SaTScan software release 9.6 version was employed to analyze spatial and temporal clusters of high PRRSV seroprevalence (30). The numbers of PRRSV seropositive and seronegative samples from each pig farm were respectively treated as case and control groups. We used the month level for time aggregation to cover all PRRSV seropositive samples from December 2017 to May 2021. All data analyses were achieved utilizing R software (31). Maps were plotted using ArcGIS 10.7 (ESRI, USA).

RESULT

PRRSV Seroprevalence of Animal and Herd Levels

The numbers of collected samples in each province and locations of pig farms are shown and labeled in **Figure 1**. A total of 14,134 samples were obtained with an overall 62.56% (95% CI: 61.74–63.37%) true prevalence of PRRSV infection. PRRSV seroprevalences of Tianjin city and Liaoning province have the highest (>80%). Hubei and Shaanxi provinces have the lowest PRRSV prevalence in all sampling regions (<50%). The Pearson chi-square test results revealed statistically significant differences in PRRSV prevalence in various provinces and pig

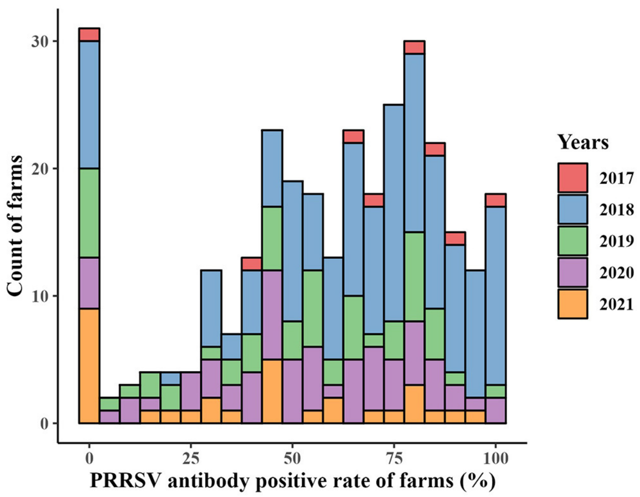

FIGURE 2 | Histogram of PRRSV prevalence of pig farms from December 2017 to May 2021.

categories (**Tables 1**, **2**). The true prevalence of replacement gilts is the highest (75.466% 95% CI: 73.000–77.821%). The lowest prevalence of PRRSV (43.304%, 95% CI: 41.400–45.228%) appears in weaned-piglets. We selected 316 pig farms to sample serum in this study, of which 286 are PRRSV antibody-positive and 30 pig farms PRRSV antibody-negative (data not shown). **Figure 2** shows the histogram of PRRSV antibody positive rate of pig farms (mean: 58.12%; median: 64.69%; range: 0–100%).

Risk Factors Analysis Related to PRRSV Serological Status of Pig Farms

Season and topography variables are not significantly associated with the PRRSV serological status of pig farms (p-value > 0.1) in the univariate logistic analysis presented in **Table 3**, which are removed from the subsequent multivariable logistic analysis. The identified risk factors associated with the PRRSV serological status of pig farms are shown in **Table 4** through multivariable logistic analysis. Medium and large pig farms are 3.076 (95% CI: 1.005–9.498) and 6.098 (95% CI: 1.814–21.290) times more likely to be PRRSV antibody-positive compared with small pig farms, respectively. Similarly, the possibility of pig farms being PRRSV antibody-positive before the ASF outbreak is 3.104 (95% CI: 1.122–10.326) times that after the ASF outbreak. The odds ratio (OR) value of pig farms with PRV purification is 5.414 (95% CI: 1.977–17.435), implying that PRV purification may decrease PRRSV infection pressure.

Temporal Analysis of PRRSV Seroprevalence

Figure 3A reveals an epidemic curve of PRRSV seroprevalence by month. The highest PRRSV prevalence appeared in February 2018 and showed a comprehensive fluctuant decline trend since November 2018. Meanwhile, an ARIMA model has been established and used to forecast PRRSV prevalence with predictive limits of 95 and 80% CI in the 19 months following the current research date (May 2021). No change pattern of autocorrelation residuals was observed, which indicated the applicability of the established model and forecasts of PRRSV prevalence in this study (**Figure 3B**).

Spatial Analysis of PRRSV Seroprevalence

Three significant clusters of high PRRSV seroprevalence are detected in China through spatial-temporal analysis from December 2017 to May 2021 (**Figure 4**, **Table 5**). The time frame of three clusters of high PRRSV seroprevalence all occurred between January and September 2018, before the ASF outbreak. The coordinate of the largest cluster is 36.657313 N, 118.008476 E, with a radius of 489.26 km. Its time frame and relative risk are from January 1, 2018, to September 30, 2018, and 1.37, respectively. The second cluster locates at 28.306173 N, 117.546305 E, with a radius of 426.62 km. This cluster's time frame and relative risk are from March 1, 2018, to March 31, 2018, and 1.58, respectively. The coordinate of the smallest cluster is 31.220567 N, 104.046397 E, with a radius of 186.47 km. The time frame and relative risk are January 1, 2018, to May 31, 2018, and 1.54, respectively.

DISCUSSION

PRRSV is the causative agent of PRRS and is an influential pig disease that causes great harm to the pig industry. Meanwhile, China is the largest pork producer and consumer worldwide (32), thus, investigating the epidemic status of PRRSV infection in China is necessary. However, most studies about PRRSV epidemiology focused more on molecular genetic evolutionary analysis in China, not on serological prevalence (15, 33–35). Consequently, we performed a widespread cross-sectional study to collect serum samples in pig farms without PRRSV vaccination from 12 provinces and two cities in China from 2017 to 2021. Implementing a complete sampling plan, including most pig farms in China, is impracticable because of the inability to know accurate numbers of pigs fed in China and the expense limit. Therefore, convenience sampling was adopted in our study.

A total of 14,134 serum samples were collected from 316 pig farms to detect antibodies by ELISA as PRRSV infection diagnosis. The PRRSV seroprevalence of animal and herd levels are, respectively, 61.84 and 90.51% (286 pig farms of PRRSV seropositive), implying a high PRRSV infection in China, which follows a previous report performed by Guo et al. (9), wherein >80% of pig farms were PRRSV seropositive. The apparent and true prevalence of PRRSV infection in different provinces and pig categories show subtle differences, which indicates our results can reflect real PRRSV infection situations. Meanwhile, a significant difference was found in PRRSV seroprevalence for diverse provinces or cities by the Pearson chi-square test, which imply a probable existence of different spatial risks of PRRSV infection. The top three highest PRRSV seroprevalences are Tianjin, Liaoning, and Henan, all located to the north of China. The reason for this might be the low temperature that contributes to PRRSV survival and increases infection risk (36). The PRRSV prevalence in growing-finishing pigs, replacement gilts, boars, and multiparous sows are all >60%, which signifies

TABLE 3 | Univariate logistic analysis of risk factors associated with PRRSV serological status of pig farm levels.

Variables	Category	OR with 95% CI	*P* value
Season	Autumn	1 (Reference)	
	Spring	0.704 (0.436–3.024)	0.767
	Summer	1.439 (0.508–5.315)	0.449
	Winter	0.793 (0.575–5.242)	0.349
Size	Small (<100 sows)	1 (Reference)	
	Medium (100–500 sows)	3.655 (1.378–9.429)	$7.576 \times 10^{-3**}$
	Large (>500 sows)	5.805 (2.037–16.911)	$9.630 \times 10^{-4***}$
Geographic location of pig farm	Eastern China	1 (Reference)	
	Central China	0.290 (0.0668–0.880)	0.051
	North China	0.455 (0.0520–9.683)	0.512
	Northeast of China	0.205 (0.0297–1.708)	0.105
	Northwest of China	1.429 (0.0366–3.145)	0.996
	South China	0.756 (0.0101–0.496)	0.006**
	Southwest of China	1.429 (0.0716–1.741)	0.991
Pig farm topography	Hill or mountain	1 (Reference)	
	Plain	2.118 (0.890–5.869)	0.113
After the ASF outbreaks	Yes	1 (Reference)	
	No	2.772 (1.113–8.407)	0.044*
PRV purification	No	1 (Reference)	
	Yes	3.475 (1.515–8.993)	0.005**

*OR, odds ratio. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.*

TABLE 4 | Multivariable logistic analysis of risk factors associated with PRRSV serological status of pig farm levels.

Variables	Category	OR with 95% CI	*P* value
Size	Small (<100 sows)	1 (Reference)	
	Medium (100–500 sows)	3.076 (1.005–9.498)	0.048*
	Large (>500 sows)	6.098 (1.814–21.290)	0.004**
Geographic location	Eastern China	1 (Reference)	
	Central China	0.496 (0.107–1.699)	0.306
	North China	0.215 (0.021–4.924)	0.226
	Northeast of China	0.137 (0.016–1.351)	0.068
	Northwest of China	3.219 (0.061–4.526)	0.995
	South China	0.113 (0.013–0.956)	0.042*
	Southwest of China	2.416 (0.019–8.758)	0.991
After the ASF outbreaks	Yes	1 (Reference)	
	No	3.104 (1.122–10.326)	0.042*
PRV purification	No	1 (Reference)	
	Yes	5.413 (1.977–17.435)	0.002**

*OR, odds ratio. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.*

that they could be a major source of PRRSV infection in herds. The replacement gilts had the highest PRRSV prevalence of 75.466% and are a dangerous signal for comprehensive control of PRRSV in herds because gradually replacing multiparous sows with negative gilts has been an important measure for PRRSV eradication (37). Previous research has also reported that quarantining new incoming sows can reduce the risks of PRRSV infection (38). Maternal antibodies of PRRSV obtained from sows can maintain for ≈2–4 weeks in piglets and start to decline at 4–10 weeks of age (39). Hence, the PRRSV prevalence of piglets and weaned-piglets might be overestimated because of the existence of maternal antibodies. Meanwhile, we can infer that the PRRSV positive antibodies of growing-finishing are induced by field PRRSV infection because the maternal antibodies have disappeared at this stage.

The possibility of pig farms being PRRSV seropositive increased with the size of pig farms, as already described by Firkins et al. (17), who revealed an association between larger

TABLE 5 | Spatial-temporal analysis of PRRSV seroprevalence in China from 2017 to 2021.

Cluster	Coordinates	Radius (km)	Time frame	Relative risk	*P* value
1	36.657313 N,118.008476 E	489.26	2018/1/1–2018/9/30	1.37	$<10^{-16***}$
2	28.306173 N,117.546305 E	426.62	2018/3/1–2018/3/31	1.58	$<10^{-16***}$
3	31.220567 N, 104.046397 E	186.47	2018/1/1–2018/5/31	1.54	$<10^{-16***}$

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

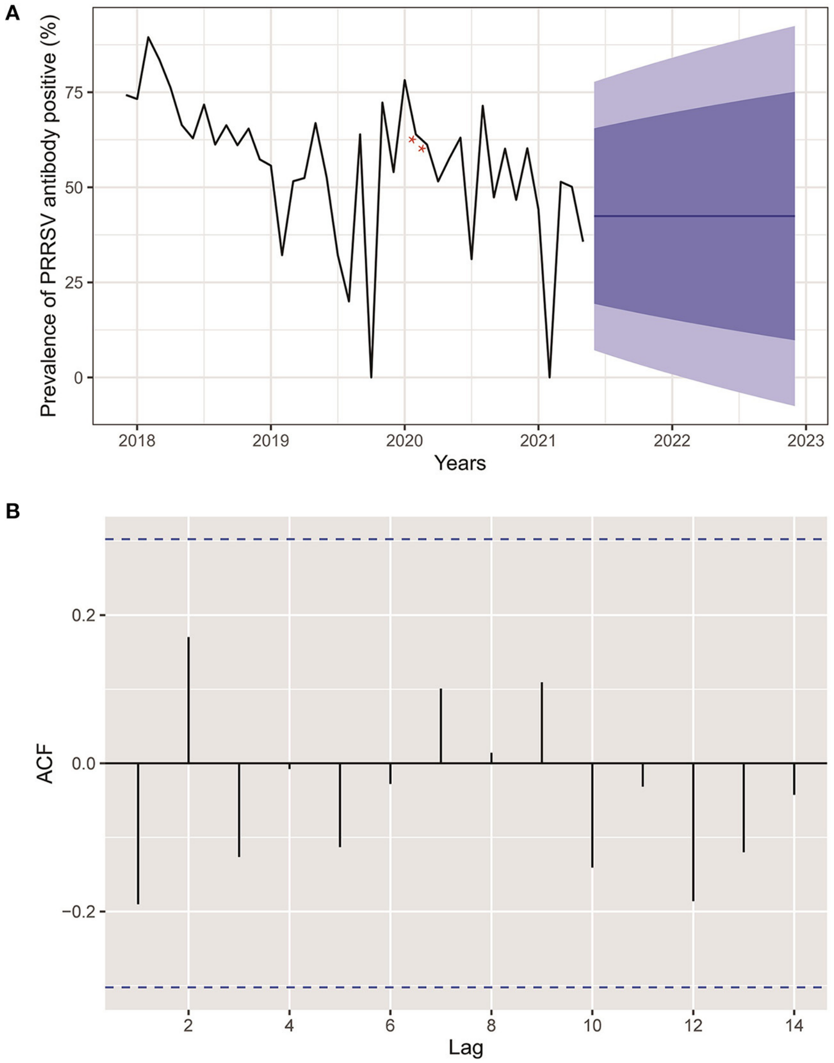

FIGURE 3 | (A) Actual and forecasted PRRSV prevalence. Black line was the actual PRRSV prevalence from December 2017 to May 2021. The blue line represented the forecasted PRRSV prevalence in the next 19 months. The dark and light blue areas were 80 and 95% CI of forecasted PRRSV prevalence, respectively. Red stars indicated interpolated values for PRRSV prevalence of February and March 2020 deficiency because of COVID-2019. **(B)** Autocorrelation plots of PRRSV prevalence.

herd size with increased risks of PRRSV infection. The farmers of pig farms without PRRSV vaccination often prefer to control disease through enhanced biosecurity measures whether on large or small farms, which implies that biosecurity levels might be similar for pig farms of different sizes (private communication). However, the high probability of PRRSV seropositivity in large pig farms might be due to the following reasons. More frequent contact between pigs in large pig farms might enhance the chances of virus spread. Furthermore, pigs on small pig farms have a simpler herd structure and can receive more care from breeders. A pig farm with PRV infection was 3.104 times more likely to be PRRSV-positive than one without PRV infection,

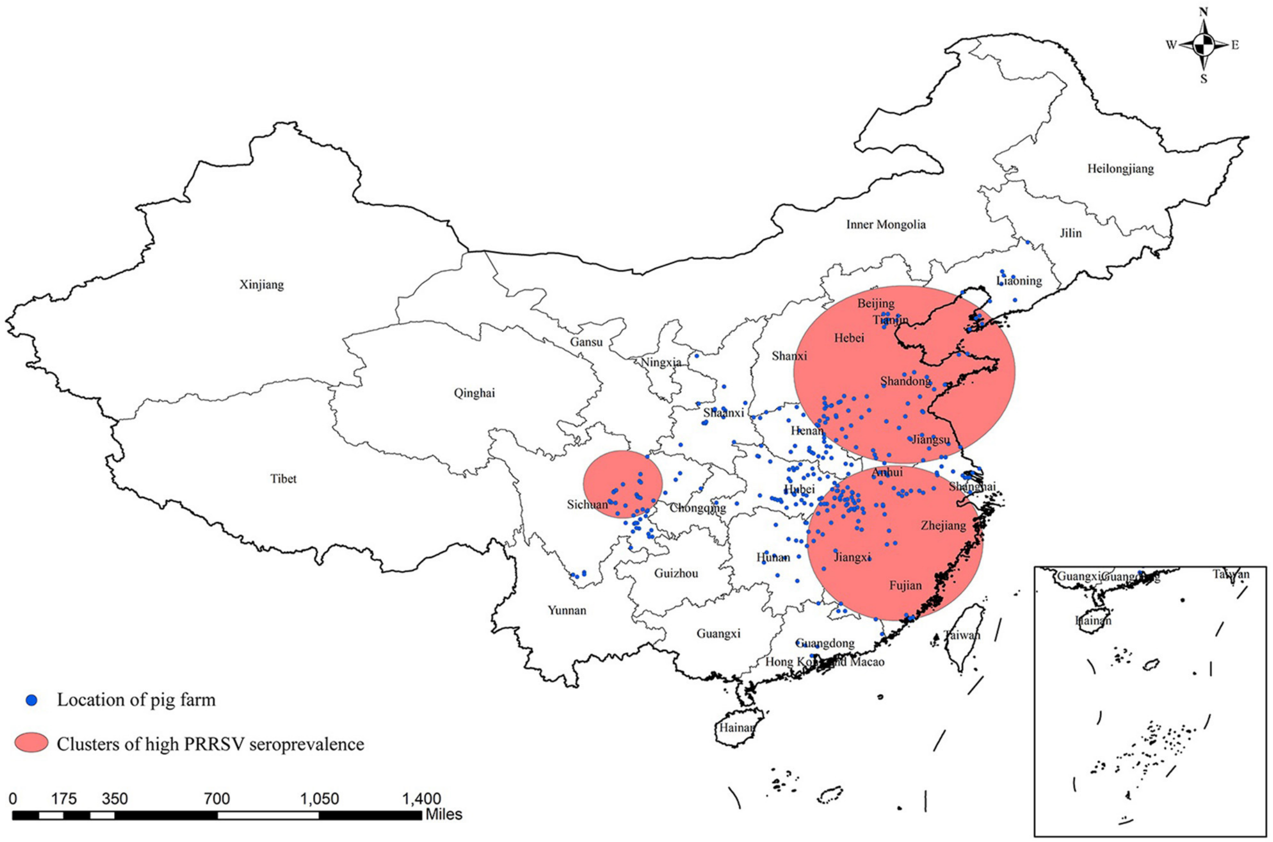

FIGURE 4 | Significant spatial clusters (p-value < 0.05) of high PRRSV seroprevalence in China from December 2017 to May 2021 with a maximum window size of 25% of the population at risk.

which implies that PRV infection in pig herds was associated with a PRRSV seropositive status, playing a similar role in PRRSV infection as *Mycoplasma hyopneumoniae* and H1N2 swine influenza A virus found by Fablet et al. (18). The underlying mechanisms and causal relationship of these viral interactions remained murky, hinting that control of PRRSV infection needed to simultaneously adopt collaborative measures for multiple viruses in herds. The OR value of pig farms being PRRSV seropositive before the ASF outbreak was remarkably higher than that after the ASF outbreak, which was potentially caused by implementation of strengthened biosafety measures in pig farms after the ASF outbreak (40). Only the OR value of pig farms located in south China was statistically significant compared to the reference pig farms located in eastern China in the geographic location variable. However, considering the large p-value (0.042) and possible sampling error, the geographic location variable might not be the risk factor related to the PRRSV serological status of farms.

Little information is available about the spatial-temporal distribution of PRRSV infection in China due to the weak development of veterinary epidemiology, not to mention the comparation of the PRRSV epidemic before and after the ASF outbreak. Additionally, spatial-temporal analysis of PRRSV infection can contribute to detecting clusters of high PRRSV prevalence and exploring variation trends of PRRSV infection helping policymakers to design more precise and cost-effective intervention policies related to future PRRSV control in China. This study was the first to perform a spatial-temporal analysis of PRRSV seroprevalence in China after the ASF outbreak. The prevalence of PRRSV began a gradual decline in November 2018, when ASF entered China. The results of temporal analysis of PRRSV prevalence in our study exhibited a fluctuant decline pattern without obvious seasonal or periodic trends, which might be caused by the disadvantage of convenience sampling method with imperfect sample representativeness, although the incidence of PRRSV infection in autumn and winter is usually higher than that in spring and summer according to expert opinions (private communication) (41). Additionally, the PRRSV prevalence by month in **Figure 3** showed a general decline after the ASF outbreak similar to the risk factor analysis described above. The forecasted values of PRRSV prevalence in the next 19 months (until December 2022) remained unchanged, which indicated that the PRRSV prevalence would tend toward a stable epidemic in China in the future. Three significant-high PRRSV seroprevalence clusters were first detected in China: two large clusters located in eastern China and a small cluster located in southwest China close to Sichuan province. Interestingly, the time frames of the three clusters of high PRRSV seroprevalence were all between January 2018 and September 2018 before the massive ASF outbreak in China (42), further demonstrating reduced PRRSV infection after the ASF outbreak. The detailed spatial epidemiology of PRRSV infection in China remained unknown, which demands another example to study, although we detected three clusters of high PRRSV prevalence in this study. We all demonstrate that PRRSV seroprevalence after the ASF outbreak displays an apparent decrease compared to that before the ASF outbreak through risk factors and spatial-temporal analysis.

We collected 14,134 samples from 316 pig farms without PRRSV vaccination located in 12 provinces and two cities in China from 2017 to 2021 to detect PRRSV antibodies using the ELISA method. The total true prevalence rate of PRRSV was 62.56% (95% CI: 61.74–63.37%) for pig level, and 286 pig farms (90.51%) were PRRSV antibody-positive, showing a widespread PRRSV epidemic in China. Additionally, we found that farm size, the ASF outbreak, and PRV purification variables were risk factors associated with the PRRSV serological status of pig herd using the multivariable logistic analysis. Temporal analysis for PRRSV seroprevalence showed a fluctuant declining trend. Three significant clusters of high PRRSV seroprevalence that occurred before the ASF outbreak were first detected in China through spatial-temporal analysis. The research findings obtained in our study fill in the knowledge gap of the epidemic situation, risk factors, and spatial-temporal distribution of PRRSV infection in China in recent years and could help form policies for PRRSV prevention in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Ethical Committee of Huazhong Agricultural University Huazhong Agricultural University. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

Conceptualization, experimental design, and project guide: JZ. Investigation, sampling, data analysis, and writing draft: PZ. Investigation and sampling: CW and WC. Supervision: RF. All authors have read and consented to publish the manuscript.

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Estimation of basic reproduction number (R_0) of African swine fever (ASF) in mid-size commercial pig farms in Vietnam

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African swine fever (ASF) is a devastating disease affecting the global swine industry. Recently, it has spread to many countries in Africa, Europe, Asia, and the Caribbean, leaving severe damage to local, regional, national, and global economies. Due to its highly complex molecular characteristics and pathogenesis, the development of a successful vaccine has been an unmet challenge. Therefore, ASF control relies solely on biosecurity, rapid detection, and elimination. Epidemiological information obtained from natural ASF outbreaks is critical for designing and implementing ASF control measures. Basic reproduction number (R_0), an epidemiological metric used to describe the contagiousness or transmissibility of infectious agents, is an important epidemiological tool. In this study, we have calculated R_0 for the in-farm spread of ASF among fattening pigs and sows in two midsize commercial pig farms, HY1 and HY2, that practice the spot removal approach in controlling ASF outbreaks in Vietnam. The R_0 values for the sows and fattening pigs were 1.78 (1.35–2.35) and 4.76 (4.18–5.38) for HY1 and 1.55 (1.08–2.18) and 3.8 (3.33–4.28) for HY2. This is the first study to evaluate the transmission potential of ASF in midsize commercial pig farms in Vietnam. Based on the R_0 values, we predict that the spot removal approach could be used to successfully control ASF outbreaks in midsize commercial sow barns but not in fattening pens.

KEYWORDS

African swine fever, African swine fever virus, basic reproduction number, epidemiology, African swine fever (ASF) decision making

Introduction

African swine fever (ASF) is one of the most dangerous infectious diseases of swine and causes nearly 100% mortality in infected animals. It was first reported in Kenya in 1921 and recognized as an endemic disease in Sub-Saharan Africa (1). In 2007, Georgia reported its first outbreak, followed by epidemics in Russian Federation, Caucasus, Belarus, and Ukraine (2). On 1 August 2018, ASF was confirmed in a pig farm in Shenbei district of Shenyang, Liaoning province, China. The outbreak killed 47 out of 383 pigs in the farm. Later, it was confirmed that the ASF virus (ASFV) responsible for the outbreak belonged to p72 genotype II, closely related to the virus circulating in Europe and the Russian Federation (3). Subsequently, ∼165 ASF outbreaks were reported in 32 provinces in China that killed over one million pigs (http://www.fao.org/ag/againfo/programmes/en/empres/ASF/situation_update.html). In early February 2019, the first outbreak of ASF in Vietnam was reported in Hung Yen province (4). Then, it quickly spread to the rest of the country and affected all 63 provinces. Over six million pigs were killed in the process to stop the disease and control the situation (FAO and Ministry of Agriculture and Rural Development, Vietnam). ASF is now considered endemic in many countries in Southeast Asia including Vietnam. There have been many attempts to develop an effective vaccine for ASF with limited success (5, 6). At present, the only viable strategy for ASF eradication is by stamping it out.

The majority of commercial pig farms in Vietnam are midsize farms that house several hundreds to thousands of pigs. They are operated independently by farmers or under contracts with private companies. At the beginning of the ASF outbreak in Vietnam, rapid detection and complete depopulation of commercial pig farms were employed. This approach, together with the high mortality associated with ASF, led to rapid depletion of the national swine population in Vietnam and severe economic burden on pig farmers. Therefore, the Department of Animal Health in Vietnam allowed practicing spot elimination, which is rapid detection and removal of only ASFV-infected animals (also called “pulling the tooth”). The success of this method depends on many factors including contagiousness of the ASF virus responsible, veterinary infrastructure, sound and readily accessible veterinary diagnostics, strong biosecurity practices, and epidemiological situation of the disease in the affected region.

Basic reproduction number (R_0), the number of secondary cases generated from a single infected individual in a susceptible population, is a critical epidemiological tool (7–10). In addition, basic reproduction number (R_0) represents the total counting of the number of generated secondary cases for the entire period of the infection of the initial case. It provides information required to understand outbreak dynamics and the scale speed of disease spread. It is useful for evaluating potential disease control strategies (11). R_0 is not a biological constant for a given pathogen, and it is affected by a number of geographical and epidemiological factors such as types of pig (domestic vs. wild boars), farm type (backyard vs. commercial), size, biosecurity, and sanitary levels of the affected farms (12–15). The aim of this study was to provide an estimated R_0 value calculated based on the information obtained from two midsize commercial pig farms in Vietnam that conduct spot elimination.

Methods

Farm design and capacity

For this study, two commercial farrow-to-finish pig farms (HY1 and HY2) located in two different districts in Hung Yen province, Vietnam were selected immediately after ASF outbreaks were confirmed in the two farms. The two farms belonged to two different private companies. The farms recorded and reported the daily status of herds since the R_0 values of commercial farms are limited in terms of epidemiology. Therefore, we chose the two farms to obtain more information on disease progression inside restricted facilities. Both farms use the close-system model and are designed according to the standard commercial swine barn layout in which sows are housed individually in single stalls and fattening pigs in groups of 30-40 per pen. Both farms are equipped with automatic cooling systems, and the sows and fattening pigs are housed 50-100 m apart from each other. The ages of the fattening pigs ranged from 10 to 22 weeks. The capacity and the total number of pigs in each farm are shown in Supplementary Table S1. Strict biosecurity measures, daily cleaning, and rapid disposal of sick/dead animals followed by thorough disinfection are practiced in both farms (Supplementary Table S2). In addition, both farms use commercial grade rations from different suppliers and practice no swill feeding; the workers are assigned to each individual barn, and no visitors are allowed in the farms.

Data source

The ASF outbreak in each farm was confirmed by real-time PCR (VDx® ASFV qPCR; Median Diagnostics Inc., Seoul, Korea) as described previously (16) using whole blood samples collected from pigs showing fever (rectal temperatures above 40°C for more than 2 days), loss of appetite, and/or cutaneous hemorrhages. Data related to each farm and the ASF outbreak were collected from the respective farm owners. Since determining the exact initial day of the ASF infection was not possible, the first day each farmer noticed the above clinical signs was considered the initial day of infection, and the day the whole herd was culled was the end of infection (15). During the study, the pigs in both affected farms were monitored daily for clinical signs. Whole blood was collected from any animal showing ASF-like clinical signs and tested for ASF by real-time PCR.

Definitions

A confirmed case of ASF was defined as pigs showing high fever, anorexia, lethargy, cutaneous hemorrhages, or death followed by a positive ASFV real-time PCR result. The serial interval was defined as the time gap between the onset of the primary and secondary cases in the chain of transmission.

Statistical analysis

In this study, we used the R programming language (version 4.0.5, https://www.r-project.org/about.html) to perform statistical analysis. We assumed that any pig showing ASF-like symptoms for the first time and was later confirmed by real-time PCR as an infected case. For each farm, the basic reproduction number (R_0/R naught) was calculated for sows and fattening pigs separately using the maximum likelihood method in "earlyR". The package "projections" was used to produce a plausible trajectory prediction of newly infected cases and cumulated cases of each outbreak in the next 14 days (17–20). The mean and standard deviation of the deaths were used to estimate R_0 and fitted in a gamma distribution. The maximum likelihood method and "get_R" function were used to calculate R_0 distribution. The likely values of R_0 were generated using the bootstrap method with 1,000 replicates and presented in a histogram format. The prediction and simulation require the existing daily incidence, a serial interval distribution, and the estimated R_0 values under the assumption that they are being fitted into the Poisson distribution and based on the daily record of infected cases.

Early R mathematical model

$$R_0 = \sum_{\mathbf{s=1}}^{\mathbf{t}} \mathbf{I\,(t-s)\,ws}$$

The ratio of the number of newly infected cases created at time step t, It, to the total infectiousness of infected cases at time t, provided by the sum of infection incidence up to time step t-1, weighted by the infectivity function ws, is used to calculate estimated R_0. If the circumstances stayed the same at time t, each sick individual would infect an average of R_0 secondary cases (18).

Projections mathematical model

We fit the data of estimated R_0, daily incidence, and a serial interval into the model, which is denoted as:

$$\lambda t = \sum_{s=1}^{t-1} ysw\,(t-s)$$

where *ys* is the incidence in the real-time event at time *S* and *w (t–s)* is the probability mass function vector of serial interval distribution. The model is based on the assumption that daily incidence carries out by approximately Poisson distribution when daily infectiousness can be determined (20).

Results

The basic reproduction number (R_0) of an infectious pathogen is the average number of infected cases directly generated by one case in a population. Previous studies have shown that the R_0 values for ASFV generated from domestic pigs and wild boars in the field were different from those measured under experimental conditions (13–15, 21, 22). In this study, we report the in-farm R_0 for sows and fattening pigs in two midsize commercial farms in Vietnam. The maximum likelihood method (Figures 1A,C,E,G) was used to produce R_0 estimates for HY1 sows (1.78) and fattening pigs (4.76) and HY2 sows (1.55) and fattening pigs (3.8). The bootstrap method (Figures 1B,D,F,H) was used to estimate R_0 values after fitting the collected data to the Poisson distribution. During each outbreak in the study, the mean infected cases of sows and fattening pigs per day were 4.5 and 13.94 for HY1 and 3.3 and 14.28 for HY2 (Table 1). The in-farm R_0 estimated with a 95% confident interval (C.I) for the sows and fattening pigs was 1.78 (1.35–2.35) and 4.76 (4.18–5.38) the HY1 and 1.55 (1.08–2.18) and 3.8 (3.33–4.28) for HY2 (Table 1 and Figures 1A,C,E,G). Using the R_0 values, the probable and plausible number of new cases in each farm for the subsequent 14 days was calculated using the "projections" package (Table 2 and Figure 2). Based on the calculation, the cumulative cases for HY1 for the subsequent 14 days were 17.45 and 51.55% for the sows and fattening pigs, respectively. For HY2, the predicted cumulative cases were 30.73% for the sows and 11.21% for the fattening pigs.

Discussion

The two farms enrolled in this study ended up eliminating their entire herd within 18 days since the first detected case. By the time of stamping out, ASF had claimed the lives of 14.06% (54/384) of the sows and 14.09% (237/1682) of the fattening pigs in HY1, and 17.19% (33/192) of the sows and 26.2% (257/981) of the fattening pigs in HY2 (Supplementary Table S1). R0 value is not a biological constant for a specific pathogen, and

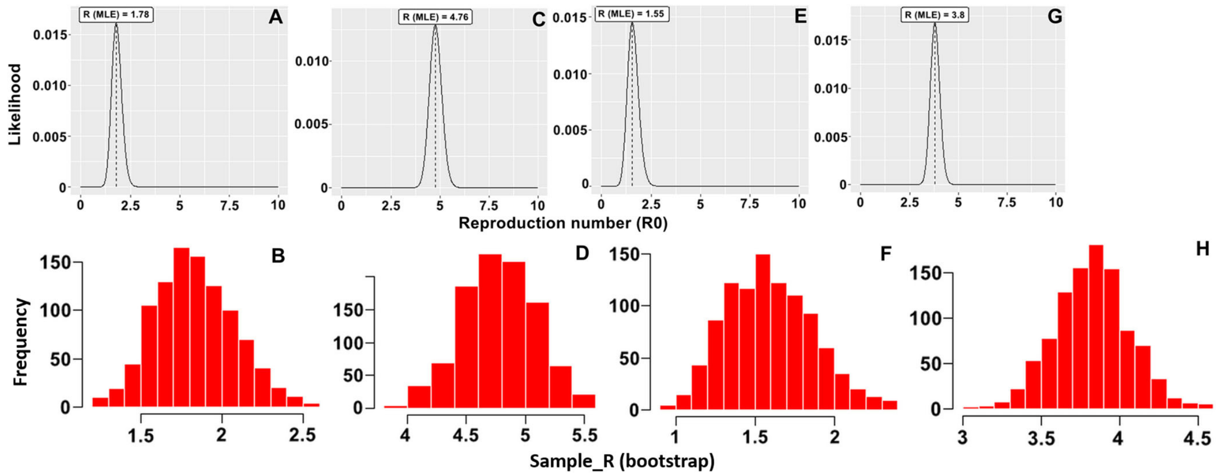

FIGURE 1
Distribution of likely R_0 value with the maximum likelihood (ML) method and histogram of 1,000 likely R_0 values using the bootstrap method for **(A,B)** sows and **(C,D)** fattening pigs in the HY1 farm and **(E,F)** sows and **(G,H)** fattening pigs in the HY2 farm.

TABLE 1 Mean, the standard deviation of infected cases per day, and R_0 values.

Farm	Type of pig	Actual pig population	Mean	Standard deviation	Basic reproduction number (R_0) (95% C.I)
HY1	Sow	384	4.5	2.78	1.78 (1.35–2.35)
	Fattening	1682	13.94	15.98	4.76 (4.18–5.38)
HY2	Sow	192	3.3	2.54	1.55 (1.08–2.18)
	Fattening	981	14.28	10.25	3.80 (3.33–4.28)

CI, Confident interval.

TABLE 2 Prediction of daily and cumulative cases for the next 14 days based on the obtained R_0.

Day	HY1				HY2			
	Cases per day		Cumulated cases		Cases per day		Cumulated cases	
	Sow	Fattening	Sow	Fattening	Sow	Fattening	Sow	Fattening
1	2 (0–4)	4 (1–8)	2 (0–5)	4 (1–8)	1 (0–4)	3 (0–7)	1 (0–4)	3 (0–7)
2	2 (0–5)	5 (1–11)	4 (0–8)	9 (3–18)	2 (0–5)	3 (0–7)	3 (0–8)	6 (2–12)
3	2 (0–6)	7 (2–14)	6 (1–12)	17 (6–31)	2 (0–6)	4 (1–8)	5 (0–12)	10 (4–18)
4	2 (0–7)	10 (4–19)	8 (2–17)	27 (11–49)	2 (0–7)	4 (0–9)	7 (1–18)	14 (6–24)
5	3 (0–7)	14 (5–26)	11 (3–22)	42 (18–72)	2 (0–8)	4 (1–9)	10 (1–23)	19 (10–30)
6	3 (0–8)	19 (7–36)	14 (3–29)	61 (28–106)	3 (0–9)	5 (1–10)	13 (2–31)	24 (13–37)
7	4 (0–10)	26 (10–46)	18 (5–38)	88 (40–151)	3 (0–11)	6 (1–12)	16 (2–40)	30 (16–47)
8	4 (0–11)	36 (14–64)	22 (6–47)	124 (57–214)	4 (0–13)	7 (2–14)	20 (3–52)	37 (21–58)
9	5 (0–13)	48 (20–84)	28 (7–59)	173 (78–300)	5 (0–14)	8 (3–15)	24 (3–65)	45 (26–69)
10	5 (0–14)	65 (27–114)	33 (8–72)	241 (110–421)	5 (0–17)	9 (3–17)	30 (4–79)	55 (31–83)
11	6 (1–17)	89 (38–160)	40 (9–88)	334 (149–571)	6 (0–19)	11 (4–20)	36 (4–95)	66 (37–99)
12	7 (0–18)	123 (53–217)	47 (10–106)	461 (212–787)	7 (0–22)	13 (5–22)	42 (4–113)	78 (44–118)
13	8 (1–20)	167 (72–295)	56 (12–128)	633 (290–1073)	8 (0–26)	14 (6–25)	50 (5–140)	93 (52–140)
14	10 (1–24)	227 (99–401)	67 (14–156)	867 (398–1476)	9 (0–29)	17 (7–29)	59 (5–166)	110 (62–165)

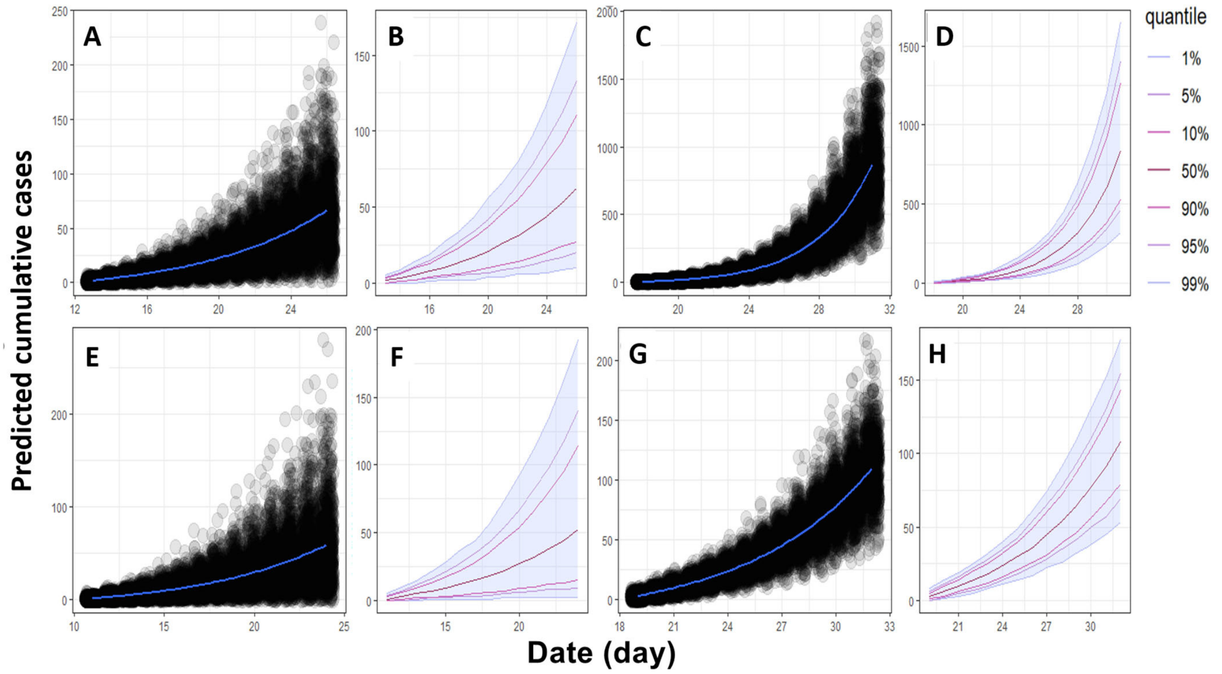

FIGURE 2
Epidemiological trajectories of expected new cumulative cases of ASF in both farms in the next 14 days. **(A,B)** new cumulative cases in sows and **(C,D)** new cumulative cases in fattening pigs in the HY1 farm. **(E,F)** new cumulative cases in sows and **(G,H)** new cumulative cases in fattening pigs in the HY2 farm.

it is affected by many factors such as infectiousness of the ASFV strain, duration of infectivity of affected pigs, number of susceptible pigs in the farm, types of pig (domestic vs. wild boars), type of farm (backyard vs. commercial), biosecurity, and sanitary levels of the affected farm (12–15). The infection status of ASF-infected pigs is generally unknown in the field, and R_0 values are calculated based on the specific group of pigs under consideration. Therefore, R_0 estimates are also dependent on how the population at risk is defined and how large it is. Therefore, a comparison of R_0 estimated from different studies is challenging. Within farm R_0, the values calculated in our study for fattening pigs (4.76 for HY1 and 3.8 for the HY2) were lower than what was reported for fattening pigs under experimental conditions and in some natural outbreaks. Within farm R_0, the values calculated for a historic outbreak of ASF genotype I in Ukraine in 1977 ranged from 5.68 to 9.21 (21). The R_0 values calculated under experimental conditions and using a moderately virulent strain ASFV Malta 78 ranged from 6.9 to 46.9 (22). The lower R_0 values observed in our study for fattening pigs could be due to many factors including the strict biosecurity measures when the first case was reported, daily cleaning, and spot removal followed by thorough disinfection deployed in both ASF-infected farms.

The calculated R_0 values of the sows in both farms in our study (1.78 for HY1 and 1.55 for HY2) were significantly lower. This could be due to many factors including better management, sanitation, biosecurity conditions in the midsize farms, and the spot removal approach that quickly removed infected animals from the farms. The closest match for our R_0 values for the sows is the R_0 values calculated for wild boars in the Czech Republic ($R_0 = 1.95$) and Belgium ($R_0 = 1.65$). In both of these studies, R_0 was calculated based on the identification of fresh carcasses of dead wild boars recovered in infected zones (15). Moreover, the accuracy of the R_0 value relies mostly on whether all infected cases have been identified. All the pigs showing symptoms underwent a real-time PCR test. Therefore, the percentage of unidentified cases is considered low. We suspect that the low R_0 values in our study may be highly affected by the improved biosecurity of the farms and the fact that all the herds were culled, which may not represent the true progression of an outbreak.

ASF has been endemic in the domestic pig population of Vietnam, and under the current ASF situation and its control strategy in Vietnam, almost all commercial pig farms apply higher biosecurity levels as described in Supplementary Table S2. Once ASF is confirmed in a sow farm, farmers quickly apply the spot removal strategy by removing sick/dead sows and two adjacent sows, followed by thorough cleaning and disinfection of the farm, equipment, etc. (Supplementary Table S2). At the same time, the farmer will also reduce the density of the sows on the farm by the removal of the weak, old, and reproductive impairment/failure sows. In the case of the fattening pig farms, when the first case of ASF was confirmed in the pen, the farmers quickly remove all the pigs in the infected and two adjacent pens, followed by thorough cleaning and disinfection of the farm, equipment, etc.

Despite these efforts, as to what were seen in the HY1 and HY2 farms, ASF continued to spread in some commercial farms in Vietnam. The success of spot removal depends on many factors, including the time taken to detect ASF in a given farm, the biosecurity level of the farm, the experience of the farm crew in handling infectious diseases in pigs, etc. For the fattening pig farms in Vietnam, the spot removal approach appears to only reduce the speed of transmission in the farms, but ultimately most of the farms stamped out the whole herd. This is supported by the calculated R_0 values in this study. It is accepted that an infectious disease outbreak ends if the R_0 value is < 1, and it continues if R_0 has a value > 1 (23). Therefore, for fattening pigs, with R_0 values ranging from 3.33 to 5.38, we suggest that spot removal is highly unlikely to work; therefore, culling the entire herd is the best option. In contrast, in sow farms in which pigs are individually housed and high biosecurity and management practices are implemented, R_0 can be brought under 1. In the HY1 and HY2 farms used in our study, the R_0 values calculated ranged from 1.08 to 2.35. Therefore, with further improvements in the detection and removal of sick/infected animals and additional biosecurity measures, spot removal could be performed to control ASF infections in breeding farms. This will avoid the total depopulation of highly valuable sow farms and, in turn, shortage of piglets. In line with this, observations from field veterinarians show that most midsize sow farms in Vietnam that enforce high biosecurity measures and spot removal are able to quickly and successfully eradicate ASF outbreaks (personal communication with swine veterinarians in Vietnam).

For further prediction of new cumulative cases in the next 14 days, the results were 17.45 and 51.55% for the sows and fattening pigs in the HY1 farm, respectively. For HY2, the predicted cumulative cases were 30.73% for the sows and 11.21% for the fattening pigs. The prediction of new cumulative cases of the fattening pigs in the HY2 farm was the lowest (11.21%) despite the high R0 value ($R_0 = 3.8$). The prediction model highly depends on the daily cumulative cases following an exponential trend, which is directly affected by the improved biosecurity measures implemented by the farms. The model performs best when the near-future patterns of incidence follow an exponential trend. However, cumulative cases of the HY2 fattening pigs partially followed an exponential pattern because the reported cases did not represent an entire transmission process but only its early stage. The differences between the predicted and field data were a drawback of this model, as discussed in a previous study (19).

In conclusion, in this study, we calculated within farm R_0 values for two ASF-affected midsize commercial farms in Vietnam that practiced spot removal to control the spread of the outbreak. Both farms failed to completely stop the spread of ASF and ultimately were depopulated. Based on the R_0 values calculated in this study, it was evident that spot removal of fattening pigs is highly unlikely to be successful. However, with additional improvements in the area of veterinary oversight on identification, laboratory confirmation, rapid removal, disposal of infected animals, and additional biosecurity measures, spot removal may be a practical approach for sow farmers to successfully control ASF outbreaks. The R_0 estimations calculated in this study can also be used for other ASF-related epidemiological studies on midsize commercial pig farms in Vietnam and other countries.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

AA, DD, VL, and TLN supervised and suggested experiment ideas. NL, TTHN, TL, and VN collected the field samples and daily farm data. NM and TT analyzed the data and prepared the manuscript. The final manuscript was approved by all authors.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.918438/full#supplementary-material

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Evidence of aerosol transmission of African swine fever virus between two piggeries under field conditions: a case study

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African swine fever (ASF) is a devastating and economically significant infectious disease that has caused enormous losses in the commercial pig sector in China since 2018. The primary transmission routes of the African swine fever virus (ASFV), the causative agent of ASF, are direct pig-to-pig contact or indirect contact with virus-contaminated objects. While aerosol transmission of ASFV has been previously reported under experimental conditions, no reports have described it under field conditions. In this case study, aerosol-associated samples were collected over a monitoring period of 24days in an ASFV-positive farm. A complete and clear chain of ASFV transmission through aerosols was observed: pigs in Room A on Day 0-aerosol in Room A on Day 6-dust of air outlets in Room A on Day 9-outdoor aerosols on Day 9-dust of air inlets in Room B on Day 15-aerosols/pigs in Room B on Day 21. Furthermore, a fluorescent powder experiment confirmed the transmission of dust from Room A to Room B. This study represents the first report providing evidence of aerosol transmission of ASFV under field conditions. Further research is needed to study the laws of aerosol transmission in ASFV and develop effective strategies such as air filtration or disinfection to create a low-risk environment with fresh air for pig herds.

KEYWORDS

ASFV, aerosol, air outlet, air inlet, dust

Introduction

ASF is an acute, febrile, highly contagious infectious disease listed by the World Organization for Animal Health (WOAH) as a notifiable disease (1), with a morbidity and mortality rate as high as 100% in domestic pigs when it first occurred in China (2, 3). ASFV, the causative agent of ASF, belongs to the *Asfivirus* genus within the *Asfarviridae* family. It was first reported in 1921 in East Africa, and rapidly spread to other African countries (4). ASFV outbreak was first reported in China in 2018 (5, 6), and it caused the death of 1.193 million pigs by November 2021 (7).

The major transmission routes of ASFV include direct pig-to-pig contact or indirect contact with virus-contaminated objects, such as excretory materials (8, 9), feed (10), water (10, 11), and needles (2). To prevent ASFV diffusion and maintain the health of pig populations, a partitioned approach has been developed and proven effective. This approach involves improving the biosecurity level of pig farms to reduce the risk of ASF introduction, strengthening monitoring procedures for early detection, culling and removing positive groups to eliminate the risk, and implementing strict disinfection measures to eliminate pollution sources and interrupt transmission routes (12). However, with the emergence of mutant ASFV strains, further improvements in this strategy are necessary.

Aerosol transmission is another important route for ASFV spread. Aerosol transmission occurs when susceptible animals inhale pathogen-carrying particles with a diameter of less than 5 μm (13). Aerosols typically contain suspended solid or liquid particles in the air (13). While a study in 1977 showed ASFV transmission up to a distance of 2.3 meters in a confined space, no detection of ASFV in the air was reported (14). However, since 2012, air sampling methods have proven effective in detecting ASFV particles in the air. Although only a few experimental studies (15, 16) have reported aerosol transmission of ASFV, no field studies have been conducted to date. In this study, we present evidence that aerosols carrying ASFV can be found in piggeries under field conditions.

Method

Farm description

The farm in this study is a commercial farm located in Shandong Province. It is equipped with automatic feeding systems, automatic drinking water systems, and comprehensive biosecurity measures. External biosecurity standards require that all individuals and materials entering the farm must undergo bathing or disinfection procedures and test negative for ASFV before entry. Internal biosecurity standards involve dividing the farm area into one living area and four breeding areas, each with a one-way gate at the entrance. People entering the breeding areas must take a bath and change into disinfected clothes, and all materials entering these areas must undergo high-temperature treatment or be soaked in disinfectant. Furthermore, farmers are dedicated to specific herds and do not cross between them. Therefore, there was no any intersection of feed, water, materials or farmers between rooms.

ASFV was detected on this farm in December 2021. The farm consists of two delivery rooms, Room A and Room B, each housing 60 sows. These rooms are adjacent to each other, with a distance of 10 meters between them, as shown in Figure 1. The ventilation mode during winter in Room A and B is longitudinal, as commonly used in northern Chinese pig farms during the winter season. It is worth noting that this ventilation mode is smaller than that used in the summer. The first ASFV-positive sow was detected in Room A and this day were defined as Day 0. Subsequently, whole-piggery samples were collected every 6 days, and ASFV-positive sows were removed from the herd, while the remaining sows were continuously tested. All sows in both Rooms A and B were sampled using serum and tested by qPCR, with a Cq value of <40 considered positive. Additionally, a whole-piggery-sampling was performed in Room B on Day 21.

Collection of different samples

Indoor aerosol samples were collected using the MD8 air scan sampling device (Sartorius, Nieuwegein, Netherlands) at an air speed of 50 m^3/min for 20 min. Sterile gelatine filters of a pore size of 3 mm and a diameter of 80 mm (type 17,528-80-ACD, Sartorius) were then dissolved in 5 mL of normal saline. Outdoor aerosol samples were collected using the GR1356-Microbial concentration sampler (Qingdao Guorui Liheng Environmental Protection Technology Co. LTD, Qingdao, China) at an air speed of 120 m^3/min for 6 h. All microorganisms were subsequently gathered in 5 mL of normal saline. As depicted in Figure 1, the sampler was positioned in the middle of the two rooms.

Dust samples from the surface of air outlets and air inlets were collected by wiping them with a gauze (10 cm × 10 cm), and then eluting them with 10 mL of normal saline. All samples were collected once every 3 days and tested by qPCR, with a Cq value of <40 considered positive.

qPCR

All the samples were tested using qPCR following the previously described method (17). Briefly, 300 μL of serum, aerosol solution, or dust solution were subjected to DNA extraction using the Automatic nucleic acid extractors (NPA-96E) from Bioer Technology Co., Ltd. (Hangzhou, China). Subsequently, 5 μL of the extracted DNA was utilized for qPCR detection, which was performed on a Step One Plus instrument (ABI) using the PerfectStart® II Probe qPCR SuperMix (TransGen Biotech, China) according to the manufacturer's instructions. Specific primers for the ASFV B646L gene were designed based on the ASFV isolate Pig/HLJ/18 (GenBank: MK333180.1) (5) and used for qPCR: 5'-AAAATGATACGCAGCGAAC-3′(forward), 5'-TTGTTTACCAGCTGTTTGGAT-3′ (reverse), and 5'-FAM-TTCACAGCATTTTCCCGAGAACT-BHQ1-3′ (probe) (17). The detection limit of the qPCR assay was determined to be 2.5 copies/μL of the ASFV genome. The results of qPCR were recorded as quantification cycle values (Cq values), and a Cq value of <40 was considered as a positive result.

Fluorescent powder experiment

Fluorescent powder, a dust-like substance, is commonly employed to simulate the movement and dispersion of dust or aerosols. It has been utilized in various settings, including the assessment of contamination and the effectiveness of cleaning procedures in theatres during the COVID-19 pandemic (18). In this study, fluorescent powders were placed near the four outlets in Room A. After a 3 day period, dust samples were collected from the surfaces of the air outlets in Room A and the air inlets in Room B using the previously described method. Subsequently, gauzes were spread out and photographed under dark conditions to visualize the presence and distribution of the fluorescent powder.

Results

In this field study, we monitored the detection of ASFV in aerosol-associated samples in an ASFV-positive farm over a 24 day period following the confirmation of the first case of ASFV-positive pigs.

As shown in Figure 2, pigs of whole herds in Rooms A and B have been detected throughout the monitoring period, from Day 0 to Day

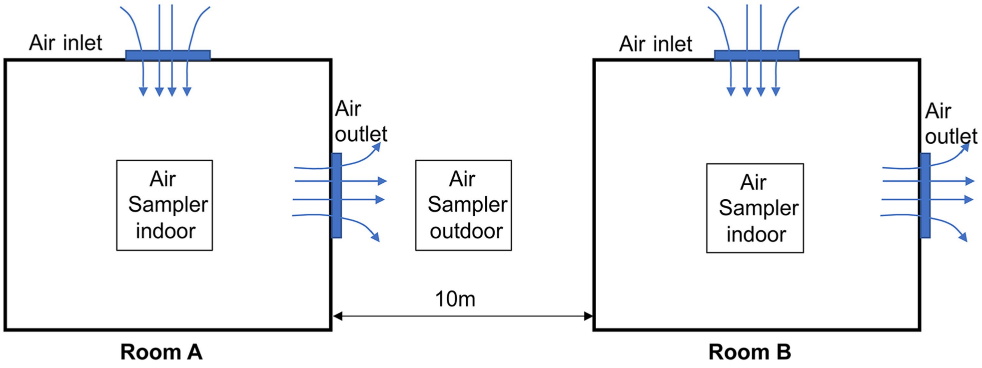
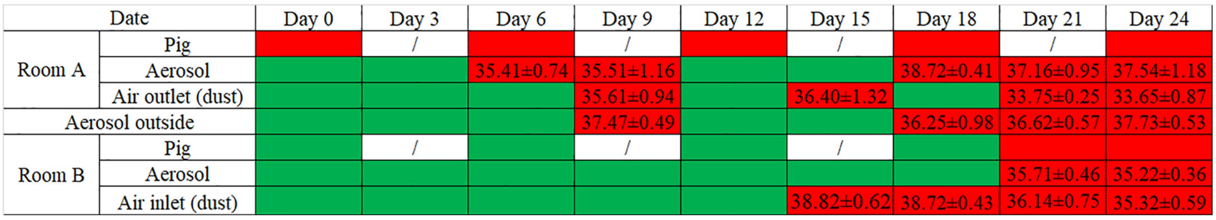


FIGURE 1
Schematic map of two ASFV-positive piggeries and areas of aerosol-associated samples.



Date		Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24
Room A	Pig	🟥	/	🟥	/	🟥	/	🟥	/	🟥
	Aerosol	🟩	🟩	35.41±0.74	35.51±1.16	🟩	🟩	38.72±0.41	37.16±0.95	37.54±1.18
	Air outlet (dust)	🟩	🟩	🟩	35.61±0.94	🟩	36.40±1.32	🟩	33.75±0.25	33.65±0.87
Aerosol outside		🟩	🟩	🟩	37.47±0.49	🟩	🟩	36.25±0.98	36.62±0.57	37.73±0.53
Room B	Pig	🟩	/	🟩	/	🟩	/	🟩	🟥	🟥
	Aerosol	🟩	🟩	🟩	🟩	🟩	🟩	🟩	35.71±0.46	35.22±0.36
	Air inlet (dust)	🟩	🟩	🟩	🟩	🟩	38.82±0.62	38.72±0.43	36.14±0.75	35.32±0.59

FIGURE 2
ASFV detection in pigs and aerosol-associated samples in Rooms A and B. Cells in red color: ASFV-positive; cells in green color: ASFV-negative; number in red cells: Cq value of qPCR (Mean±SD); "/": no detection.

24. The Cq values of positive pigs were shown in Supplementary Table S1. In Room A, aerosol samples initially tested positive on Day 6, and continued to be positive until Day 24, despite negative samples on Days 12 and 15. Interestingly, Cq values of aerosol samples on Day 6 and 9 were lower compared to those on Day 18, 21, and 24, possibly indicating the removal of most ASFV-positive pigs in the later stage. Dust samples collected from air outlets were the last to be test positive on Day 9 among all sample types, and remained positive until Day 24. Furthermore, from Day 15 on, a downward trend in Cq values was observed from Day 15 onwards, suggesting the accumulation of the virus in the dust. These findings suggest that during an ASFV outbreak, ASFV particles excreted from infected pigs can be present in suspended aerosols and settling dust.

In Room B, as shown in Figure 2, ASFV-positive dust collected from air inlets was first detected on Day 15. Notably, there was a significant drop in the Cq value on Day 21. On the same day, pigs and aerosol samples were also detected as positive for ASFV, suggesting a possible association with the presence of positive dust in the air inlets.

Figure 2 also reveals that outdoor aerosol samples first tested positive on Day 9, coinciding with the collection of dust samples from air outlets in Room A. Additionally, outdoor aerosol samples remained positive until Day 24.

To investigate whether the dust in the air inlets of Room B originated from Room A, fluorescent powder was used to trace the dust trajectory from Room A. As depicted in Figure 3, 3 days later, fluorescent spots were observed on gauzes from both the air outlets in Room A and the air inlets in Room B, indicating the potential transmission of dust from Room A to Room B.

Discussion and conclusion

Aerosol transmission of infectious agents is widely recognized as one of the most challenging routes to prevent and control (19), particularly in commercial farms. Several swine virus, including foot-and-mouth disease virus (FMDV) (20), porcine reproductive and respiratory syndrome virus (PRRSV) (21), porcine epidemic diarrhea virus (PEDV) (22), and influenza A virus (IAV) (23), have been reported to spread through aerosols. However, the aerosol transmission of ASFV has been has been a subject of debate, with limited evidence from experimental studies (15, 16). Moreover, the current prevention strategies do not specifically address aerosol transmission risks. In this case study, we have found evidence of aerosol transmission of ASFV between two piggeries under field conditions.

By analyzing the dates of the first detection of different samples, we have identified a complete and clear chain of ASFV transmission via aerosols: infected pigs in Room A release aerosols, which contaminate the dust on air outlets in Room A. Subsequently, outdoor aerosols become contaminated, leading to the deposition of contaminated dust on air inlets in Room B, resulting in the transmission of aerosols and/or infected pigs to Room B. This represents a novel transmission route of ASFV between piggeries. The source of ASFV-positive aerosols is likely the excretions and secretions of ASFV-positive pigs, including urine, sneezes and feces (7). Previous research has proven that the positive aerosols were associated with viruses in feces (15), supporting our hypothesis. Dust also plays a crucial role in spreading ASFV particles among piggeries, although it

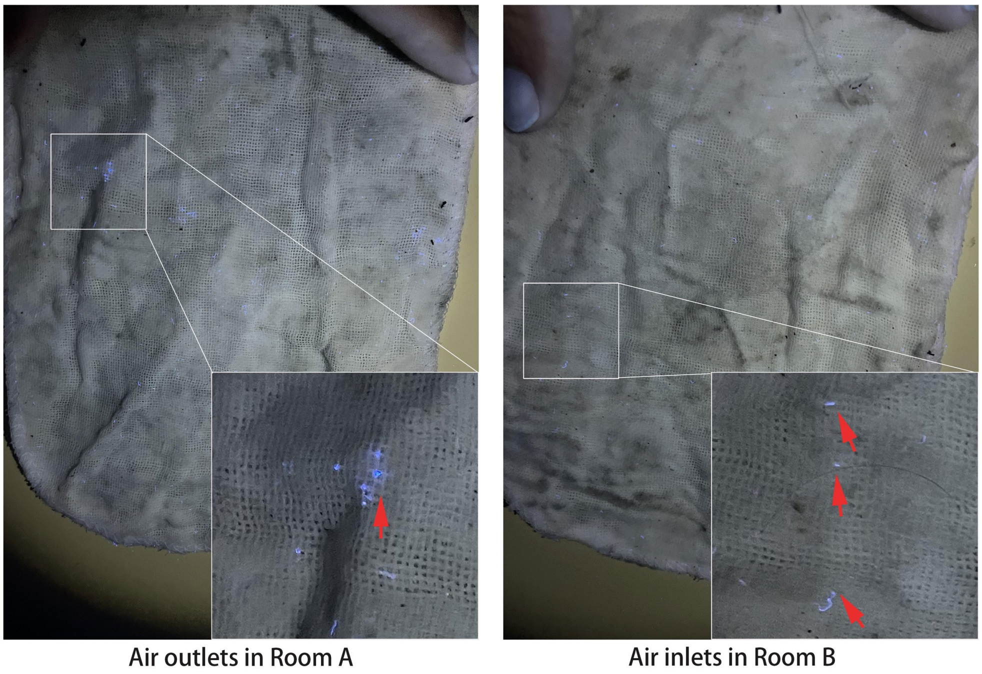


FIGURE 3
Fluorescent spots of gauzes from Rooms A and B. Upward arrows point to the blue fluorescent spots.

is often overlooked by farmers due to its presence in hard-to-reach locations.

The presence of ASFV-positive dust poses a significant risk to the entire herd, especially if it becomes agitated due to factors such as sudden changes in wind direction or feeding activities in piggeries. In addition, contaminated feed, which has been identified as a primary risk for ASFV transmission (24, 25), can contribute to the presence of ASFV in dust. Therefore, timely removal of dust in piggeries is crucial for the control and prevention of ASFV transmission.

Outdoor aerosol detection is another critical factor to consider. Due to the wide range of outdoor aerosols, detecting their presence can be challenging. In our study, we employed the GR1356-Microbial concentration sampler, which allowed continuous aerosol collection for 6 h at a time. Outdoor aerosol samples remained positive until Day 24 during the experimental period, highlighting the persistent risk of aerosol transmission. Air filtration systems have been proven effective in preventing aerosol transmission of other pathogens, such as PRRSV (26). Therefore, integrating air filtration systems into the biosecurity measures against ASFV and other pathogens is recommended, especially for small farms with poor biosecurity practices in China (7).

The travel distance of viral aerosols is a significant concern. Wilkinson et al. demonstrated that ASFV can be transmitted through the air with a maximum distance of 2.3 meters (14). In our study, the distance between Rooms A and B was 10 meters, indicating that ASFV aerosols traveled at least 10 m. The difference in transmission distance could be attributed to environmental factors such as the outdoor temperature and the wind speed, as the temperature was below 4°C and strong winds were prevalent in northern China in winter. Furthermore, the transmission distance might also be influenced by the strain of the virus. In this case study, the ASFV strain belonged to Genotype I, causing mild onset of infection and chronic disease (27), and previous research has suggested that lower virulence strains tend to be highly transmissible (28). Further research is needed to investigate the transmission distance of ASFV aerosols.

In conclusion, this case study provides evidence of aerosol transmission of ASFV under field conditions, expanding our understanding of ASFV transmission routes. We emphasize the importance of considering air inlet and outlet filtration, strengthening air disinfection measures, and reducing dust levels in pig farms to create a low-risk environment with fresh air for pig herds.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval were not required for the animal study because the manuscript is a case study of spontaneous disease. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

XL conceived and designed the analyzation method. XL, ZH, and MF collected and analyzed the data, and also wrote the original draft and reviewed and edited the manuscript. XT, WW, WG, LB, and XJ contributed to collect samples from field farms and the data of qPCR test. All authors contributed to the article and approved the submitted version.

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Conflict of interest

XL, ZH, MF, and WW were employed by Xiajin New Hope Liuhe Agriculture and Animal Husbandry Co., Ltd. XL, ZH, MF, XT, WG, LB, and XJ were employed by Shandong New Hope Liuhe Agriculture and Animal Husbandry Technology Co., Ltd. (NHLH Academy of Swine Research).

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1201503/full#supplementary-material

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Infection characteristics of porcine circovirus type 2 in different herds from intensive farms in China, 2022

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Introduction: Porcine circovirus type 2 (PCV2) is the primary etiological agent of porcine circovirus diseases (PCVD), which are widespread in most pig herds, causing huge economic losses in the global pig industry. Therefore, it is critical to assess the infection characteristics of PCV2 in different swine herds to develop effective strategies against PCVD.

Methods: In this study, routine diagnostic and monitoring protocols were used to collect 12,714 samples from intensive farms in China, and PCV2 was tested for by qPCR to determine positivity rates and viral loads in samples from different herds and materials.

Results: PCV2 was found to be prevalent throughout China, and fattening farms had higher positivity rates than breeding farms. The PCV2 positivity rates in breeding farms in Southern China were higher than those in Northern China. Growing–finishing pigs demonstrated the highest positivity rate in the tested samples, while pre-weaning piglets and adult sows had the lowest. Meanwhile, samples with viral loads exceeding 106 copies/mL in growing–finishing pigs had 27.2% positivity, compared to 1.9% and 3.3% in sows and piglets, respectively. The results of the viral loads in the serum samples followed a similar trend.

Discussion: The findings reveal that PCV2 circulates in different herds from intensive farms, with positivity increasing from pre-weaning to growing–finishing herds. It is urgent to develop effective strategies to reduce PCV2 positivity in growing–finishing herds and prevent viral circulation among pigs.

KEYWORDS

PCV2, pre-weaning piglet, nursery pig, growing–finishing pig, gilt, sow, positivity rate, viral load

Introduction

Porcine circovirus type 2 (PCV2) is a small and ubiquitous single-stranded DNA virus in the genus *Circovirus* of the family *Circoviridae* (1). It is the primary causative agent of porcine circovirus diseases (PCVD), which include subclinical (PCV2-SI), systemic (PCV2-SD), and reproductive (PCV-2-RD) diseases, in addition to porcine dermatitis and nephropathy syndrome (PDNS), resulting in significant economic losses to the swine industry (2). While commercial vaccines have been available for many years, mass vaccination of PCV2 has failed to eradicate the virus (3–5).

PCV2 can be detected in the blood, tissue, colostrum, semen, saliva, nasal, fecal, and urinary secretions of pigs, as well as in the environment (6, 7). As a result, PCV2 virus is shed through several different routes and spread between pigs both horizontally and vertically (6, 8, 9), promoting its transmission between different farms and pig herds. Therefore, comprehending the prevalence and dynamics of PCV2 infection is crucial for the efficacious prevention and control of its transmission in diverse pig herds.

A systematic review and meta-analysis study conducted in China revealed that the pooled prevalence of PCV2 was 46.0–50.1% in intensive farms and 37.5% in extensive farms during 2015–2019 (10). However, there is a lack of comprehensive information on the prevalence of PCV2 in various herds and sample types. The current study collected several clinical samples from intensive pig farms across China and analyzed the prevalence of PCV2 infection in different pig farms and herds. The findings help to inform the development of future strategies to prevent the spread of this disease.

Materials and methods

Study farms

A total of 131 breeding farms and 91 fattening farms with a two-site production system were selected for this study in China in 2022. The breeding farms, which had a herd composition consisting of pre-weaning piglets (0–21 days of age), gilts (90–230 days of age), adult sows (>230 days of age and in gestation or with a history of gestation), and boars (>300 days of age), had a breeding stock ranging from 750 to 3,000 sows. On the other hand, the fattening farms had a production scale of over 6,000 pigs and included nursery piglets (21–70 days of age) and growing–finishing pigs (70–180 days of age). These farms had similar PCV2 vaccination protocols that were reliably followed. At 14 days of age, the pre-weaning piglets were vaccinated with one dose of the inactivated PCV2 vaccine. The gilts and boars were vaccinated twice with an inactivated vaccine at 14 and 90 days of age. Other age-stage herds did not receive the PCV2 vaccine.

Sample collection

Samples were collected through routine diagnostic and monitoring procedures across various herds, as shown in Table 1. Umbilical cord blood was obtained from neonatal piglets. Following the delivery of the sows, umbilical cord blood was extracted from their piglets using a syringe and subsequently plastic tube. The collection of testicular processing fluid samples was collected during piglet castration at 5 days of age, whereby approximately 20 liters of piglet testicles were gathered in a plastic bag, and the resulting liquid was transferred into a plastic tube. To obtain oropharyngeal swab samples, a long swab was inserted into the throat and moved back and forth twice. The head of the retrieved swab was then broken off and eluted into a sealed bag with 2 mL of normal saline, and the eluent was transferred into a plastic tube. Placenta plastic tube. Placenta samples were obtained by extracting approximately 5 g of placenta tissue from aborted sows and storing it in a sealed bag. Oral fluid samples were collected by suspending a piece of cotton rope in each pen for the pigs to chew on. After 20 min, the ropes were retrieved and placed in separate plastic bags, and the liquid from each piece was

TABLE 1 Routine diagnostic or monitoring protocols used in pig farms.

Herd	Clinical signs	Materials	Minimum number sampled	Testing frequency
Pre-weaning piglet	Healthy pigs	Umbilical cord blood	10 pigs	Monthly or by batch
		Testicular processing fluid	Majority of litters from one batch of farrowing	Weekly or by batch
	Weak piglets	Serum	15 pigs	Weekly or by batch
	Weak piglets	Oropharyngeal swabs	15 pigs	Weekly or by batch
Nursery pig	Healthy pigs	Serum	30 pigs	By batch herd test
		Oral fluid	15 pens	By batch herd test
	Dead pigs	Lymph node	3–5 pigs	-
Growing–finishing pig	Healthy pigs	Serum	30 pigs	By batch herd test
		Oral fluid	15 pens	By batch herd test
	Dead pigs	Lymph node	3–5 pigs	-
Gilt	Healthy pigs	Serum	30 pigs	By batch herd test
		Oral fluid	15 pens	By batch herd test
Adult sow	Abortion sows	Placenta	3 pigs	Weekly
	Inactive or poor appetite sows	Oropharyngeal swabs	15 pigs	Weekly
		Serum	30 pigs	Monthly
Boar	Healthy pigs	Semen	All pigs	Monthly

TABLE 2 PCV2 positivity rate in different pig farms throughout China.

Geographical region	Breeding farm						Fattening farm					
	Tested sample	Positive sample	Positivity rate at the sample level	Tested farm	Positive farm	Positivity rate at the farm level	Tested sample	Positive sample	Positivity rate at the sample level	Tested farm	Positive farm	Positivity rate at the farm level
Northern China	1,752	64	3.7% CI: 2.8–4.5%	47	16	34.0% CI: 20.0–48.1%	2,739	1,156	42.2% CI: 40.4–44.1%	46	34	73.9% CI: 60.7–87.1%
Southern China	3,323	536	16.1% CI: 14.9–17.4%	84	45	53.6% CI: 42.7–64.5%	1,138	418	36.7% CI: 34.5–40.1%	45	27	60.0% CI: 45.1–74.9%
Total	5,075	600	11.8% CI: 10.9–12.7%	131	61	46.6% CI: 37.9–55.2%	3,877	1,574	40.6% CI: 39.1–42.1%	91	61	67.0% CI: 57.2–76.9%

Northern China included Liaoning, Shandong, Tianjin, Hebei, Henan, and Gansu; Southern China included Jiangsu, Zhejiang, Anhui, Jiangxi, Hubei, Hunan, Sichuan, Chongqing, Guizhou, Guangdong, Guangxi, and Hainan. A total of 5,075 clinical samples, including umbilical cord blood, testicular processing fluid, serum, and oropharyngeal swab samples, were collected from breeding farms. A total of 3,877 clinical samples, including oral fluid, lymph node, and serum samples, were collected from fattening farms. CI, 95% confidence interval for the positivity rate.

squeezed into a plastic tube. To obtain lymph node samples, the inguinal lymph node tissue of deceased pigs was excised using a scalpel, and 2 g of tissue was taken and placed in a hermetically sealed plastic bag. To obtain serum samples, blood was extracted from the anterior vena cava, left to rest at room temperature for 30 min, and then centrifuged at 1,000 g for 2 min. The serum was subsequently collected in plastic tubes. In pre-weaning piglet herds, oropharyngeal swabs and sera were collected at 20 days of age. Semen samples were exclusively collected from boars. All samples were stored at −20°C.

qPCR

The lymph node or placental tissue (0.5 g) was weighed, 1.5 mL lysis solution was added, and the tissue samples were prepared using Precellys lysing kits with the Precellys tissue homogenizer (Bertin, France). Serum and other liquid samples were oscillated and centrifuged at 5,000 g for 1 min. Total DNA was extracted from 200 μL of each sample using the Virus DNA Extraction Kit II (Geneaid, Taiwan) in accordance with the manufacturer's instructions. Extracted DNA (2.0 μL) was then amplified using real-time PCR of an ORF2 section of PCV2 as previously described (11, 12). Samples with Ct values of <40 were considered positive. The quantification of viral genome copies was performed using titrated plasmids containing the ORF2 of PCV2 (13). Viral titers inferred from the real-time PCR results were expressed as the viral copy number per milliliter of both the liquid and tissue samples (copies/mL).

Statistical analysis

Statistical analyses were performed using SPSS Statistics version 22. The positivity rates were represented as absolute and relative frequencies (%) with a 95% confidence interval. The PCV2 DNA copies of each serum sample were analyzed using a one-way ANOVA, with a *P*-value < 0.05 being considered significant.

Results

Detection rates of PCV2 in pig farms throughout China

A total of 12,714 clinical samples were collected from diverse pig farms in 18 provinces of China. Among these, 5,075 samples, comprising umbilical cord blood, testicular processing fluid, serum, and oropharyngeal swab samples, were obtained from 131 breeding farms, while 3,877 samples, including oral fluid, lymph node, and serum samples, were collected from 91 fattening farms. Based on the geographical distribution, the 18 provinces were divided into two regions, namely Northern China (Liaoning, Shandong, Tianjin, Hebei, Henan, and Gansu) and Southern China (Jiangsu, Zhejiang, Anhui, Jiangxi, Hubei, Hunan, Sichuan, Chongqing, Guizhou, Guangdong, Guangxi, and Hainan). The positivity rate for PCV2 at the farm level was 46.6% (95% CI: 37.9–55.2%) and 67.0% (95% CI: 57.2–76.9%) in breeding and fattening farms, respectively. In

TABLE 3 PCV2 positivity in different pig herds.

Materials	Sample positivity rate % (*n*) in pig herds					
	Pre-weaning piglet	Nursery pig	Growing–finishing pig	Gilt	Adult sow	Boar
Serum	1.2% (CI: 0.2–3.0%) (5/404)	13.1% (CI: 9.9–16.3%) (56/429)	37.4% (CI: 34.2–40.5%) (338/904)	44.5% (CI: 40.0–49.0%) (207/465)	3.7% (CI: 1.9–5.6%) (15/403)	/
Oral fluid	/	41.6% (CI: 37.9–45.5%) (270/648)	51.8% (CI: 49.0–54.6%) (631/1,218)	38.6% (CI: 32.3–44.8%) (91/236)	/	/
Placenta of aborted sow	/	/	/	/	1.8% (CI:−0.2–3.8%) (3/168)	/
Oropharyngeal swabs	9.0% (CI: 7.2–10.8%) (85/944)	/	/	/	5.5% (CI: 3.8–7.3%) (36/654)	/
Umbilical cord blood	3.0% (CI: 2.1–3.9%) (40/1,342)	/	/	/	/	/
Testicular processing fluid	25.7% (CI: 21.7–29.7%) (118/459)	/	/	/	/	/
Lymph node	/	39.4% (CI: 33.9–44.9%) (121/307)	42.6% (CI: 37.5–47.6%) (158/371)	/	/	/
Semen	/	/	/	/	/	0.7% (0.4–1.0%) (26/3,762)
Total	7.9% (CI: 6.9–8.8%) (248/3,149)	32.3% (CI: 29.8–34.8%) (447/1,384)	45.2% (CI: 43.3–47.2%) (1,127/2,493)	42.5% (CI: 38.8–46.2%) (298/701)	4.4% (CI: 3.3–5.6%) (54/1,225)	0.7% (CI: 0.4–1.0%) (26/3,762)

CI, 95% confidence interval for the sample positivity rate.

breeding farms, the PCV2 positivity rate was 34.0% (95% CI: 20.0–48.1%) in Northern China and 53.6% (95% CI: 42.7–64.5%) in Southern China. In the fattening farms, the PCV2 positivity rate was 73.9% (95% CI: 60.7–87.1%) in Northern China and 60.0% (95% CI: 45.1–74.9%) in Southern China. At the sample level, the positivity rate was 11.8% (95% CI: 10.9–12.7%) and 40.6% (95% CI: 39.1–42.1%) in the breeding and fattening farms, respectively. In the breeding farms, 3.7% (95% CI: 2.8–4.5%) of the samples in Northern China were PCV2 positive, while 16.1% (95% CI: 14.9–17.4%) in Southern China were positive. In the fattening farms, 42.2% (95% CI: 40.4–44.1%) of the samples were PCV2 positive in Northern China, while 36.7% (95% CI: 34.5–40.1%) were positive in Southern China (Table 2). A total of 3,762 semen samples from boars were collected from 18 of the 131 breeding farms, with only one farm testing positive at a proportion of 0.7% (95% CI: 0.4–1.0%) (Table 3). These findings suggest a potential regional variation in the PCV2 positivity rate in China and a very low positivity rate in boars.

PCV2 positivity in different pig herds by sample type

Samples were obtained from diverse pig herds encompassing all ages and genders of pigs (Table 3). The highest rates of detection were observed in growing–finishing pigs (45.2%, 95% CI: 43.3–47.2%), followed by gilt sows (42.5%, 95% CI: 38.8–46.2%) and nursery pigs (32.3%, 95% CI: 29.8–34.8%). Conversely, pre-weaning piglets (7.9%, 95% CI: 6.9–8.8%), adult sows (4.4%, 95% CI: 3.3–5.6%), and boars (0.7%, 95% CI: 0.4–1.0%) exhibited relatively low rates of detection. Regarding serum samples from the different pig herds, PCV2 positivity was highest in gilt sows (44.5%, 95% CI: 40.0–49.0%), followed by growing–finishing pigs (37.4%, 95% CI: 34.2–40.5%). Nursery pigs, adult sows, and pre-weaning piglets displayed positivity rates of 13.1% (95% CI: 9.9–16.3%), 3.7% (95% CI: 1.9–5.6%), and 1.2% (95% CI: 0.2–3.0%), respectively. The testicular processing fluid sample had a positivity rate of 25.7% (95% CI: 21.7–29.7%), indicating that these samples were the most reliable for monitoring PCV2 infection in pre-weaning piglets. In nursery pigs, oral fluid samples had the highest positivity rates (41.6%, 95% CI: 37.9–45.5%), followed by lymph nodes (39.4%, 95%CI: 33.9–44.9%). Among growing–finishing pigs, both oral fluid and lymph node samples had high positivity rates of 51.8% (95% CI: 49.0–54.6%) and 42.6% (95% CI: 37.5–47.6%), respectively, indicating a high rate of infection in these herds. In addition, all the samples in the adult sows had low positivity rates, and the positivity rate in the placenta samples was only 1.8% (95% CI: −0.2–3.8%). Boars were only sampled for semen, which exhibited a very low positivity rate of 0.7% (95% CI: 0.4–1.0%).

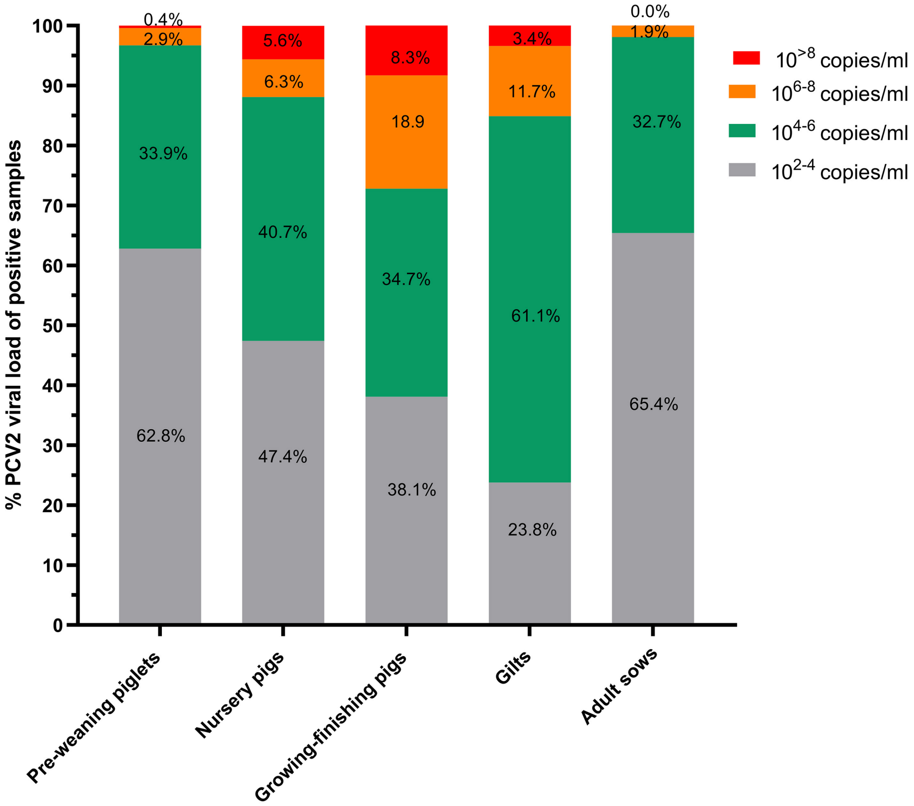

FIGURE 1
The percentage of PCV2-positive samples by PCV2 copy number in different herds. PCV2 DNA was isolated from all PCV2-positive samples, and the viral copy number was determined by qPCR using a standard curve. The results are expressed as the viral copy number per milliliter of liquid and tissue sample (copies/mL).

PCV2 viral loads in different pig herds

Viral loads were divided into four gradients, containing 10^{2-4}, 10^{4-6}, 10^{6-8}, and $10^{>8}$ viral particles, and the proportion of samples within each gradient was calculated. More than 90% of the positive samples in the adult sows and pre-weaning piglets had viral loads $<10^6$, and most were in the range of 10^{2-4} (Figure 1). More samples in the 10^{4-6} gradient were found in nursery pigs, growing–finishing pigs, and gilts than in adult sows and pre-weaning piglets. Notably, >15% of the positive samples in the growing–finishing pigs and gilts exhibited viral loads $>10^6$, with some samples exhibiting viral loads $>10^8$. Specifically, 8.3% of the samples from the growing–finishing pig herds exhibited viral loads $>10^8$, followed by 5.6% of samples from the nursery pig herds.

The analysis of the serum samples collected from the various herds was conducted independently. Viral loads were highest in the samples from the growing–finishing pigs but did not significantly differ from those of the nursery pig and gilt samples ($P > 0.05$) (Figure 2). The pre-weaning piglet herds, nursery herds, and gilt herds did not exhibit significant difference ($P > 0.05$), while adult sow herds had the lowest viral loads ($P < 0.05$). The observed trends in viral loads were consistent with the PCV2 positivity rates (Table 3).

Discussion

The expansion of intensive pig farms and the migration of diverse pig herds across China in recent years have necessitated the development of more effective prevention and control strategies for PCV2. This study found that PCV2 has spread widely in intensive pig farms, with a higher positivity rate observed in fattening farms, corroborating the findings of Liu et al. (10) and Li et al. (14). However, the positivity rates of PCV2 in breeding and fattening farms differ between Northern and Southern China (Table 2). Southern China has a higher positivity rate of PCV2 in breeding farms. This phenomenon may be attributed to differences in the geographical environment, climate, and protocols implemented since 2018 to contain African swine fever (15–17); these factors can impact the construction density, pig movement management, environmental control strategies, biosafety level, and disease prevention procedures of breeding and fattening farms.

Semen transmission is considered an important route of PCV2 transmission (6, 18, 19). However, the present study revealed that only 0.7% of the 37,62 semen samples collected were found to be positive for PCV2, and the viral loads of the positive samples were observed to be very low (Table 3). These findings suggest that semen transmission may not be a primary route for the spread of PCV2 in intensive pig farms. Unfortunately, other sample types were not

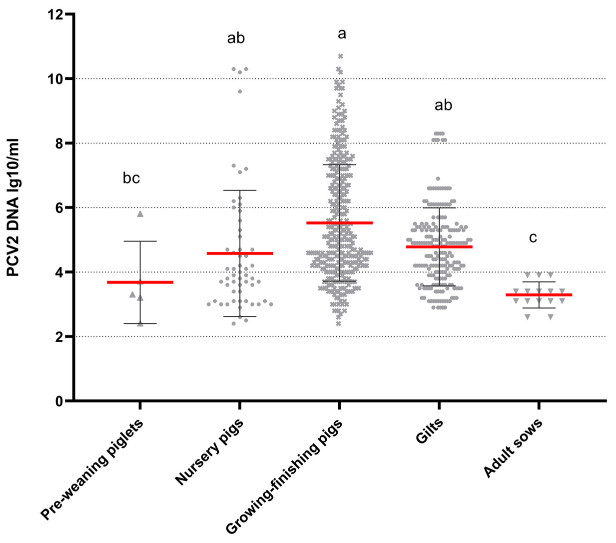

FIGURE 2
Viral loads of PCV2-positive serum samples in different herds. The results are expressed as the log10 viral copy number per milliliter of serum sample (copies/mL). Mean values with superscript letters a, b, and c are with significant differences across groups. Different letters indicate significant statistical differences ($P < 0.05$), and the same letter indicates no significant statistical differences ($P > 0.05$).

collected from the boars in this study, so additional information regarding PCV2 infection in boars is not available.

The detection rate of the PCV2 genome in serum samples from pre-weaning piglets was low at 1.2%, while the detection rate in testicular processing fluid samples was significantly higher at 25.7%. These findings align with previous research by Dieste-Pérez et al. (20), and they suggest that testicular processing fluids may be a more effective means of monitoring PCV2. In addition, the high positivity of testicular fluids indicates that infection occurs early in piglets, and the risk of PCV2-associated diseases is high after weaning (21). The results of this study also indicate a higher proportion of PCV2-positive samples in nursery and growing–finishing pigs compared to pre-weaning piglets (Table 3), which suggests that PCV2 carried by weaned piglets was being transferred from breeding to finishing farms and transmitted horizontally through continuous contact between pigs (22). The co-mingling of pigs of different ages and the exchange of animals, people, equipment, and sundries between barns or herds may contribute to the horizontal transmission of PCV2 in such intensive farming environments (6, 23). At the same time, the gilt samples had a higher PCV2 positivity rate, suggesting that gilts from the quarantine and rearing areas played a major role in the spread of PCV2 and the maintenance of infection in sow herds (24).

The serum and lymph node samples collected from the nursery and growing–finishing pigs were analyzed for positivity rates of PCV2. The results indicate that the lymph node samples had a higher positivity rate (39.4%) compared to the serum samples (13.1%) in the nursery, which may be attributed to the virus's preferential targeting of immune cells in the lymphoid tissue without causing extensive viremia (25). Furthermore, the lymph node samples from the growing–finishing pigs had a higher positivity rate (42.6%), indicating that PCV2-associated immunosuppressive diseases affected pig growth during the nursery and growing–finishing periods. These findings also suggest that oral fluid samples are good indicators of PCV2 infection in nursery and growing–finishing pigs and could serve as reliable material for PCV2 detection at the environmental and individual pig levels (26).

The main difference between subclinical and clinical PCVD is the severity of the lesion, which correlates with the viral load in samples, especially in the sera and tissues (27, 28). Viral loads $>10^6$ copies/mL are believed to strongly influence the development of PCVD (12). In adult sow herds and pre-weaning piglet herds, only 1.9% and 3.3% of PCV2-positive samples, respectively, exhibited viral loads $>10^6$, indicating that PCVD was mild during the farrowing period. There was a significant increase in samples with viral loads $>10^6$ copies/mL in both the growing–finishing pig and gilt herds, with 8.3% of growing–finishing herds having viral loads $>10^8$ copies/mL, suggesting that PCVD was severe in the two herds. In addition, fewer samples from the gilts than from the growing–finishing pig herds had viral loads $>10^6$ copies/mL, which may be because the gilts had received two PCV2 vaccine doses at 14 and 90 days of age.

A serum viral load has been established as a reliable indicator of both PCV2-associated diseases and average daily weight gain (ADWG) (27, 29, 30). The present study observed a gradient of PCV2 in serum samples from various herds, as depicted in Figure 2. This trend was consistent with the PCV2 positivity rates reported in Table 3, which is in line with the findings of López-Soria et al. (29). Furthermore, a standard was also established for viral loads in serum, with samples $>10^{5.3}$ being considered as high (29). The growing–finishing herds in this study exhibited serum samples with high viral loads, necessitating the development and implementation of corrective measures.

In summary, the present study has demonstrated that PCV2 circulates in diverse herds, with its incidence increasing from pre-weaning herds to growing–finishing herds. These findings illustrate that growing–finishing herds have the highest risk of PCVD, indicating the need for effective strategies to reduce the positivity rate of PCV2 in growing–finishing herds and prevent viral circulation among pigs.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

XL and JR conceived and designed the experiments. XL, JR, MF, LB, and XT supervised and conducted the experiment and analyzed the data. MF, LB, and XT wrote the original draft. XL reviewed and edited the manuscript. ZH, WW, LS, GY, SL, LY, LW, YiW, YoW, and ZY contributed to the samples and methods. All authors have read and approved the final manuscript.

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Conflict of interest

XL, MF, LB, XT, ZH, WW, LS, GY, SL, LY, LW, YiW, YoW, and ZY were employed by Shandong New Hope Liuhe Agriculture and Animal Husbandry Technology Co., Ltd., New Hope Liuhe Co., Ltd., and Xiajin New Hope Liuhe Agriculture and Animal Husbandry Co., Ltd.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Epidemiology of porcine deltacoronavirus among Chinese pig populations in China: systematic review and meta-analysis

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Porcine deltacoronavirus (PDCoV) is a newly emerging and important porcine enteropathogenic coronavirus that seriously threatens the swine industry in China and worldwide. We conducted a systematic review and meta-analysis to access the prevalence of PDCoV infection in pig population from mainland China. Electronic databases were reviewed for PDCoV infection in pig population, and meta-analysis was performed to calculate the overall estimated prevalence using random-effect models. Thirty-nine studies were included (including data from 31,015 pigs). The overall estimated prevalence of PDCoV infection in pigs in China was 12.2% [95% confidence interval (CI), 10.2–14.2%], and that in Central China was 24.5% (95%CI, 16.1–32.9%), which was higher than those in other regions. During 2014–2021, the estimated prevalence of PDCoV infection was the highest in 2015 at 20.5% (95%CI, 10.1–31.0%) and the lowest in 2021 at 4.8% (95%CI, 2.3–7.3%). The prevalence of PDCoV infection in sows was 23.6% (95%CI, 15.8–31.4%), which was higher than those in suckling piglets, nursery piglets, and finishing pigs. The prevalence of PDCoV infection was significantly associated with sampling region, sampling year, pig stage, and clinical signs (diarrhea). This study systematically evaluated the epidemiology of PDCoV infection in Chinese pig population. The findings provide us with a comprehensive understanding of PDCoV infection and are beneficial for establishing new controlling strategies worldwide.

KEYWORDS

PDCoV, epidemiology, systematic review, meta-analysis, Chinese pig population

Introduction

Coronaviruses (CoVs) cause respiratory and gastrointestinal diseases in humans and animals. Porcine deltacoronavirus (PDCoV) is a newly emerging and important porcine enteropathogenic coronavirus that causes severe enteritis with acute diarrhea and dehydration in pigs. PDCoV infection can occur in pigs of all ages but mainly affects suckling piglets with mortality rate as high as 30–40% (1). Different from other enteric CoVs, PDCoV causes not only extensive intestinal lesions but also significant gastric lesions and mild pulmonary lesions (2). Aminopeptidase N (APN) is considered as an entry receptor of PDCoV, which is widely distributed in various tissues of multi-species, leading to presence of cross-species transmissibility (3). PDCoV can infect calves, turkeys, poultry, and mice and has independently infected children, proving its potential cross-species transmission capacity (4–6). Its spread seriously threatens the global pig industry and public health.

CoVs belong to the subfamily *Coronavirinae*, family *Coronaviridae* of the order *Nidovirales*. These positive-sense, single-stranded RNA viruses have the largest genome size among known RNA viruses. CoVs are genetically classified into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus (DCoV) (7, 8). PDCoV belongs to the genus DCoV and has a size of approximately 25.4 kb (8, 9). Each genus of CoVs usually infects hosts in a specie-specific manner. Alphacoronavirus and Betacoronavirus infect mammals, and Gammacoronavirus primarily infect birds. DCoV can infect birds and mammals and is composed of nine avian DCoVs (*White-eye Coronavirus*; *Sparrow Coronavirus*, SpCoV; *Magpie robin Coronavirus*; *Night heron Coronavirus*; *Wigeon Coronavirus*; *Common Moorhen Coronavirus*; *Bulbul Coronavirus*; *Thrush Coronavirus*; and *Munia Coronavirus*) and three mammal DCoVs (*Asian Leopard Cats Coronavirus*, *Chinese ferretbadger Coronavirus*, and PDCoV) (9). The genome of PDCoV is similar to that of SpCoV in the same genus, indicating that the interspecific transmission of DCoV from birds to pigs may have occurred recently. The PDCoV genome organization is in the following order: 5′untranslated region (UTR), replicase open reading frame 1ab (ORF 1ab), spike (S), envelope (E), membrane (M), nucleocapsid (N), and 3′UTR, with two open reading frames encoding accessory genes nonstructural protein 6 (NS6) and nonstructural protein 7 (NS7) between M and N gene and within N gene (10–13). According to phylogenetic and comparative sequence analysis, PDCoV could be divided into four lineages: Early China, China, Thailand, USA (14). Early China and China lineages include strains from China. Thailand lineage includes strains from Laos, Vietnam and Thailand. USA lineage includes strains from USA, Mexico, Peru, Japan, Korea, and China. USA and China lineages are the major genotypes globally, and Thailand and China lineages have higher intra- and inter-lineage recombination and genetic diversity than USA lineage (14–16). Most recombination breakpoints occur in the S and ORF1ab genes, and recombination in ORF1a may result in the porcine innate immune evasion. Recombination of the S gene is a common phenomenon among CoVs; the S gene of PDCoV evolves at a lower rate than porcine epidemic diarrhea virus (PEDV) in pigs (17–22).

PDCoV was first identified in Hongkong, China in 2012. The first PDCoV strain (HKU15) was detected from rectal swabs of healthy pigs by the coronavirus diversity molecule monitoring in Hongkong (9). However, its pathogenic potential was not recognized until the first PDCoV-related diarrhea epidemic was reported in Ohio, USA in February 2014 (1). Since then, many Asian countries (Korea, China, Japan, Thailand, Laos, and Vietnam) and American countries (United States, Canada, Mexico, and Peru) have reported the PDCoV epidemic, causing a widespread concern (15, 23–28). In mainland China, since first report of PDCoV in 2015, it has quickly spread over the country. A large number of studies on PDCoV infection have been conducted in China (17, 23, 29–40). Therefore, we conducted a meta-analysis to systematically assess the prevalence and distribution characteristics of PDCoV infection in China. The findings would provide us with a comprehensive understanding of PDCoV infection and are beneficial for establishing new controlling strategies worldwide.

Materials and methods

Search strategy and selection criteria

This meta-analysis was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement (41). A search was conducted on PubMed, Web of Knowledge, CNKI, Wanfang, and Chongqing VIP databases between January 1, 2015 and October 31, 2022 for all studies that possibly contained data for PDCoV infection in pig populations. The databases were searched using MeSH terms and variants: "PDCoV," "epidemiology or incidence or prevalence or investigation or surveillance or rate," and "China or Chinese." Studies without language limitation were included.

The eligibility for inclusion of all studies identified from the database search was independently assessed and compared by two authors. All retrieved articles were manually selected based on the relevance of publication titles and abstracts to PDCoV epidemiology. The full texts of articles considered potentially relevant based on titles and abstracts were independently reviewed by two authors. Exclusion criteria were as follows: retrospective studies, repeated studies, or nonpig studies; providing final results without sample information, such as sampling time and sample size; and sample size was <60.

Data extraction and quality assessment

We extracted the following information from each study: first author, publication year, province of the study, administrative region, positive sample size/sample size, detection method, target gene, coinfection, and study design. The data were extracted by two authors independently, who reached a consensus through a discussion on the controversial information. The quality of included studies was evaluated according to the Grading of Recommendations Assessment, Development, and Evaluation method (42). We assigned a score to each publication. Study was awarded 1 point each when the research objective was defined, the detection method was described, the sampling method was described, subjects were classified into different subgroups, and the risk factors were determined. The publication quality was defined as low (1 point), moderate (2–3 points), or high (4–5 points). High scores indicated high quality.

Statistical analysis

We estimated the prevalence of PDCoV infection by pooling data from included studies. We used the DerSimonian–Laird random-effect model to analyze the data (43, 44), and compared the differences using Wilcoxon two-sample test or t-test. A forest plot was used to present combined estimates with 95% CIs.

We evaluated statistical heterogeneity using p and I^2 statistics, and it was considered insignificant only when $p > 0.1$ and $I^2 < 50\%$. The fixed-effect model was adopted in the absence of publication heterogeneity; otherwise, the random-effect model was used. Potential publication bias was assessed via a funnel plot, Egger's regression test, and Begg's test. Sensitivity analysis was conducted by modifying the inclusion criteria of this meta-analysis. The investigated factors were sampling region, sampling year, and pig stage. All the analysis was conducted using the Stata software (version 12.0, Stata Corporation, College Station, Texas).

Results

Literature search

As shown in Figure 1, the literature search yielded 539 relevant studies (226 studies in English and 313 studies in Chinese), of which 243 were duplicates. After the title and abstract of each article were carefully reviewed, 98 articles were considered potentially valuable, and their full texts were retrieved for detailed evaluation. After the full text was reviewed, 59 potentially relevant articles were excluded from this meta-analysis. Among them, 53 articles did not provide required sufficient data or did not meet the inclusion criteria; 4 articles had a sample size of <60; and two articles were review papers. Finally, 39 publications were included for our meta-analysis.

Characteristics of included studies

The characteristics of the included studies are listed in Table 1. The articles were published between January 1, 2015 and October 31, 2022 and covered 25 provinces in China. A total of 31,015 pig samples and 3,149 PDCoV-positive cases were included in the meta-analysis. In terms of epidemiological design, all 39 publications were cross-sectional studies and calculated period prevalence. Among them, 14 papers were written in English and 25 in Chinese. According to the established criteria, 25 publications were of high quality (4 or 5 points) and 14 publications were of moderate quality (2 or 3 points).

Prevalence of PDCoV infection in administrative regions of China

The estimated pooled prevalence of PDCoV infection in pig population from mainland China was 12.2% (95%CI, 10.2–14.2%; Table 1; Figure 2). The prevalence rates of PDCoV infection in Central China, North China, and South China were 24.5% (95%CI, 16.1–32.9%), 18.5% (95%CI, 9.7–27.3%), and 12.2% (95%CI, 9.0–15.3%), respectively. These rates were higher than those in other administrative regions (Figure 3; Table 2). By contrast, the PDCoV positive rates in Northeast China and Northwest China regions were low with percentages of 3.9% (95% CI, 2.4–5.3%) and 3.1% (95% CI, 1.1–5.2%), respectively (Figure 3). Among the 39 studies, 28 reported coinfections. Coinfection diarrhea viruses included PEDV, *transmissible gastroenteritis virus* (TGEV), *porcine rotavirus* (PoRV), *porcine kobuvirus*, *swine acute diarrhea syndrome coronavirus*, and *porcine astrovirus*; the coinfection rate accounted for 13.6–100% of the PDCoV infection rate (Table 1).

Subgroup analysis

All subgroup analyses included sampling region, sampling date, pig stage, and clinical signs (diarrhea). Among the seven administrative regions of China, the estimated prevalence of

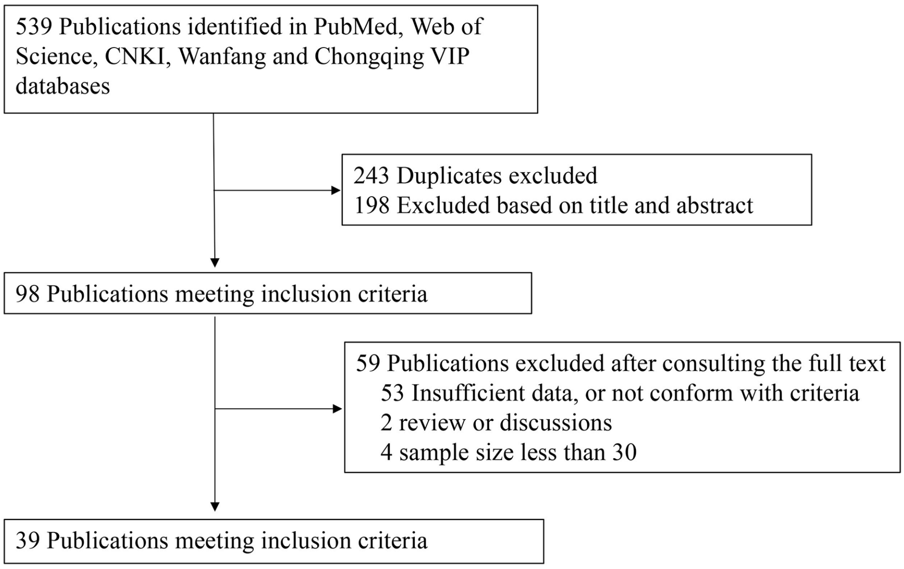

FIGURE 1
Data search and selection.

TABLE 1 Characteristics of the included studies for PDCoV infection among pigs in China.

Reference	Province	Region	No. positive/ examined	Coinfection/ rate	Detection Method/ Target Gene	Study design	Quality score
Dong et al. (29)	Anhui, Jiangsu, Hubei, Guangxi	East China/South China/Central China	14/215	PEDV/TGEV (50%)	RT-PCR/M/N gene	C-S	5
Song et al. (23)	Jiangxi	Central China	120/356	PEDV (58.3%)	RT-PCR/N gene	C-S	5
Su et al. (30)	Heilongjiang	Northeast China	30/109	-	ELISA/N protein	C-S	3
Mai et al. (31)	Guangdong	South China	55/252	PEDV/TGEV/RoRV/ PKV (49.1)	RT-PCR/M gene	C-S	5
Zhai， et al. (32)	Guangdong, Hainan, Guangxi	South China	5/390	PEDV (100%)	RT-PCR/N gene	C-S	5
Luo et al. (33)	Hebei	North China	96/871	-	ELISA/M protein	C-S	4
Wang et al. (34)	Gansu, Qinghai, Sichuan	Northwest China/ Southwest China	7/189	PEDV (42.9%)	RT-PCR/M gene	C-S	4
Jia et al. (35)	Heilongjiang, Jilin, Liaoning	Northeast China	26/672	-	rRT-PCR/N gene	C-S	3
Zhang et al. (36)	5 provinces	South China/ East China/ Central China	813/2987	PEDV/TGEV /SADS-Cov/ PoRV (90.1%)	RT-PCR/N gene	C-S	5
Zhang et al. (17)	18 provinces	7 districts	94/719	PEDV (36.2%)	rRT-PCR/M gene	C-S	3
Zhang et al. (37)	Henan	Central China	101/430	PEDV/TGEV	RT-PCR/S gene	C-S	5
Feng et al. (38)	Sichuan	Southwest China	84/634	PEDV (56.0%)	RT-PCR/–	C-S	5
Shi et al. (39)	Shanghai	East China	26/753	-	RT-PCR/M gene	C-S	3
Li et al. (40)	8 provinces	4 districts	150/7107	PEDV/PoRV (16.7%)	RT-PCR/M gene	C-S	4
Ren et al. (45)	Sichuan, Chongqing	Southwest China	6/222	-	RT-PCR/–	C-S	3
Zhang et al. (46)	Jiangxi	Central China	78/249	-	RT-PCR/N gene	C-S	4
Peng et al. (47)	Sichuan	Southwest China	20/60	PEDV/PoRV (85.0%)	RT-PCR/–	C-S	3
Zhou et al. (48)	Guangdong	South China	47/273	PEDV (91.5%)	RT-PCR/–	C-S	3
Liu et al. (49)	Sichuan	Southwest China	16/226	-	RT-PCR/M gene	C-S	3
Luo et al. (50)	Hebei	North China	22/130	PEDV (13.6%)	rRT-PCR/N gene	C-S	3
Shan et al. (51)	Zhejiang	East China	12/282	-	rRT-PCR/–	C-S	3
Song et al. (52)	Guangdong	South China	56/420	PEDV (44.6%)	RT-PCR/–	C-S	4
Xu et al. (53)	Zhejiang	East China	21/546	PEDV/TGEV/PoRV (18.2%)	RT-PCR/N gene	C-S	5
Feng et al. (54)	Sichuan	Southwest China	7/141	PEDV (57.1%)	RT-PCR/N gene	C-S	5
He et al. (55)	Guangxi	South China	70/1547	PEDV/PoRV (32.9%)	RT-PCR/–	C-S	4
Hou et al. (56)	Hebei	North China	105/570	-	ELISA/S1 protein	C-S	4
Lu et al. (57)	Tianjin	North China	417/1519	PEDV/TGEV/PoRV (–)	RT-PCR/M gene	C-S	4
Duan et al. (58)	Guangxi	South China	76/914	PEDV/PoRV (26.3%)	RT-PCR/–	C-S	4
Feng et al. (59)	Sichuan	Southwest China	51/430	-	ELISA/M protein	C-S	4
Ma et al. (60)	Shanghai	East China	25/518	PEDV/PKV/PAstV (96%)	RT-PCR/M gene	C-S	3
Shi et al. (61)	Guangxi	South China	46/792	PEDV/TGEV/PoRV (54.3%)	RT-PCR/N gene	C-S	3

(Continued)

TABLE 1 (Continued)

Reference	Province	Region	No. positive/ examined	Coinfection/ rate	Detection Method/ Target Gene	Study design	Quality score
Yan et al. (62)	Guangxi	South China	83/1463	PEDV/TGEV/PoRV (79.5%)	RT-PCR/N gene	C-S	4
Chang et al. (63)	5 provinces	East China	166/594	PEDV/TGEV/PoRV (58.3%)	RT-PCR/N gene	C-S	5
Li et al. (64)	Henan	Central China	54/154	PEDV (68.5%)	RT-PCR/M gene	C-S	3
Duan et al. (65)	Guangxi	South China	92/1206	PEDV/PoRV (13.6%)	rRT-PCR/−	C-S	5
Li et al. (66)	Xinjiang	Northwest China	11/1388	PEDV (36.4%)	RT-PCR/S gene	C-S	5
Zhu et al. (67)	Shanxi	Northwest China	12/184	-	RT-PCR/N gene	C-S	3
Wang et al. (68)	Hunan	Central China	0/303	PEDV/TGEV/PoRV (−)	rRT-PCR/−	C-S	5
Wang et al. (69)	Xinjiang	Northwest China	35/1200	PEDV/TGEV/PoRV (97.1%)	RT-PCR/−	C-S	4

C-S, cross-sectional study; rRT-PCR, real-time reverse transcription-polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; PEDV, porcine epidemic diarrhea virus; TGEV, transmissible gastroenteritis virus; PoRV, porcine rotavirus; PKV, porcine kobuvirus; PAstV, porcine astrovirus; SADS-CoV, swine acute diarrhea syndrome coronavirus.

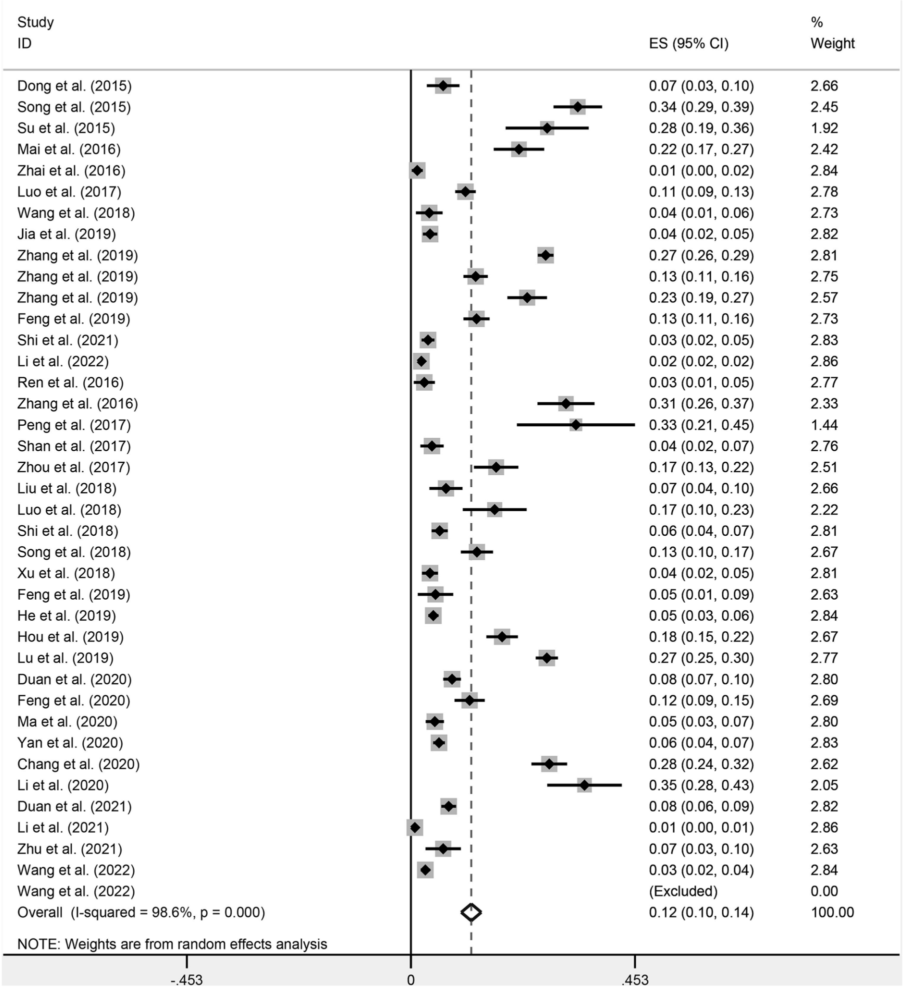

FIGURE 2
Meta-analysis of PDCoV infection among pigs in China with random-effect analysis.

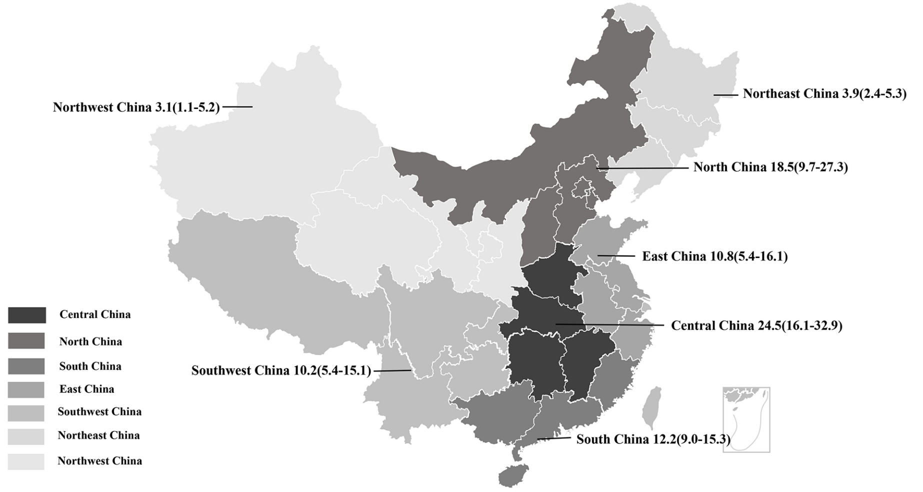

FIGURE 3
Geographical distribution of PDCoV infection among pigs in China. Pooled prevalence rate (%) and 95%CI are shown for each district.

TABLE 2 Results of the subgroup analyses on PDCoV infection among pigs in China.

category	Subgroup	No. studies	No. examined	No. positive	Prevalence (%) (95%CI)	Heterogeneity		
						I^2(%)	p-value	X^2
Region	Central China	5	3,703	991	24.5 (16.1–32.9)	95.6	0.000	90.02
	North China	4	3,090	640	18.5 (9.7–27.3)	97. 3	0.000	111.03
	South China	11	7,773	691	12.2 (9.0–15.3)	96.9	0.000	322.64
	East China	6	2,774	272	10.8 (5.4–16.1)	97.3	0.000	182.69
	Southwest China	6	1713	184	10.2 (5.4–15.1)	92.3	0.000	65.28
	Northeast China	1	672	26	3.9 (2.4–5.3)	-	-	0.00
	Northwest China	4	2,916	65	3.1 (1.1–5.2)	89.2	0.000	27.79
Sampling year	Before 2014	5	2008	411	15.6 (4.5–26.8)	98.3	0.000	237.32
	2015	4	1,269	253	20.5 (10.1–31.0)	94.8	0.000	57.69
	2016	9	2,490	454	18.2 (12.3–24.0)	93.9	0.000	130.54
	2017	13	4,084	621	12.7 (8.0–17.4%)	97.4	0.000	469.30
	2018	13	6,385	559	11.3 (7.6–14.9)	97.7	0.000	523.71
	2019	6	2,648	129	8.8 (5.1–12.6)	90.2	0.000	50.92
	2020	2	2,318	46	2.0 (1.3–2.6)	2.3	0.312	1.02
	2021	3	2,677	103	4.8 (2.3–7.3)	88.5	0.000	17.34
Pig stage	Sow	9	1,685	418	23.6 (15.8–31.4)	93.1	0.000	115.42
	Suckling piglet	9	4,381	927	20.4 (11.5–29.4)	98.6	0.000	552.45
	Nursery piglet	3	857	107	10.9 (2.5–19.3)	94.1	0.000	33.83
	Finishing pig	3	422	61	14.1 (9.0–19.3)	53.4	0.117	4.29

PDCoV infection in pigs in Central China was the highest at 24.5% (95%CI, 16.1–32.9%), and that of Northwest region was the lowest at 3.1% (95% CI, 1.1–5.2%; Table 2; Figure 3). During 2014–2021, the estimated prevalence of PDCoV infection was the highest in 2015 at 20.5% (95%CI, 10.1–31.0%) and the lowest in 2021 at 4.8% (95%CI, 2.3–7.3%), showing a downward trend (Table 2). The prevalence rates of PDCoV infection in sows and suckling piglets were 23.6% (95%CI, 15.8–31.4%) and 20.4% (95%CI, 11.5–29.4%), respectively, which were significantly higher than those in nursery piglets and finishing pigs (Table 2). The prevalence of PDCoV infection was significantly associated with sampling region, sampling date, pig stage, and clinical signs (diarrhea) but was insignificantly associated with detection method and target gene.

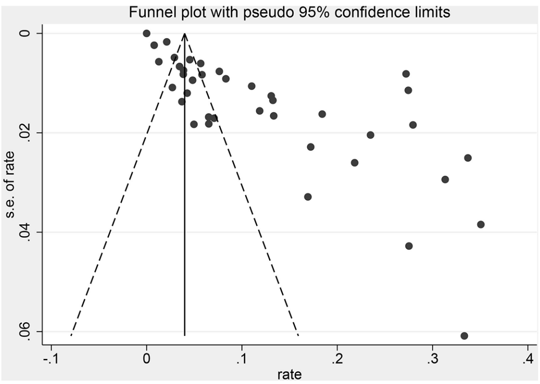

FIGURE 4
Funnel plot with pseudo 95%CIs for the examination.

Publication bias and sensitivity analysis

The funnel forest plot was used to measure and illustrate the degree of publication bias of selected studies. The funnel plot was asymmetrical to the overall prevalence (Figure 4), suggesting significant bias in the studies selected for our analysis. A sensitivity analysis was conducted by excluding one study each time to determine whether modification of the inclusion criteria for the meta-analysis would affect the final results. All results were insignificantly changed (data not shown).

Discussion

Coronavirus endangers human and animal health and thus causes serious public health problems and huge economic losses. PEDV and TGEV, which belong to the genus Alphacoronavirus, are two major diarrheal pathogens endangering the pig industry. Severe acute respiratory syndrome coronavirus (SARS), middle east respiratory syndrome coronavirus, and SARS-CoV-2, which belong to the genus *Betacoronavirus*, have caused three pandemics in human history (70–72). Infectious bronchitis virus, which belongs to the genus *Gammacoronavirus*, is the main pathogen of respiratory diseases in poultry industry. DCoV is the fourth coronavirus genus formally classified by the International Committee on Taxonomy of Viruses in 2012. PDCoV diarrhea broke out in the USA for the first time in 2014, causing significant economic losses in the American swine industry, and then spread across many countries of Asia and America.

This study is the first meta-analysis and systematic review of PDCoV infection in pig herds in China. Studies on PDCoV infection in pigs from 25 provinces in China were included, all of which were cross-sectional. The pooled prevalence of PDCoV in China reached 12.2%, which indicated that PDCoV occurs extensively in Chinese pig herds. Coinfection with other enteric pathogens was common among PDCoV-positive samples. Among these pathogens, PEDV, TGEV, and PoRV had the highest frequency of coinfection. This situation implied that the current causes of diarrhea among Chinese pig populations are complex and diverse, and coinfection may cause severe clinical symptoms.

Several molecular and immunological methods have been developed to detect PDCoV. Among the molecular methods, specific RT-PCR remains the ideal choice for detection of PDCoV. Immunological methods can determine previous exposure to PDCoV and define antibody responses to infection and vaccination. Among the included papers, 35 used RT-PCR method (5 papers used rRT-PCR) and 4 utilized ELISA method. In clinical diagnostic testing, S, M, and N genes are the most commonly used diagnostic targets for PDCoV infection.

Subgroup analyzes were performed by sampling region, sampling date, and pig stage. From the perspective of geographical distribution, PDCoV was ubiquitous in pig populations in China and has large regional differences. The prevalence rates of PDCoV infection in Northeast China and Northwest China were comparatively low. On the contrary, the prevalence rates in Central China, North China, and South China were high possibly due to the large amount of pig production, high frequency of pig transport, and high humidity of climate in these regions. From the perspective of time distribution, epidemic reports have been available every year since the first report of the epidemic in mainland China in 2015. In the 1st year of the initial outbreak of the epidemic, the prevalence of PDCoV was the highest at 20.5%. Thereafter, it gradually stabilized and reached 4.8% in 2021. This situation showed that PDCoV is still prevalent in pigs in China and remains an important pathogen of porcine diarrheal disease. In terms of infected pigs, PDCoV can infect pigs of all ages. However, the clinical condition is severe in piglets. Our review found that the prevalence of PDCoV infection was significantly higher in sows (23.6%) and suckling pigs (20.4%) than in nursery (10.9%) and finishing pigs (14.1%). These results suggested that piglets are at greater infection risk, leading to high mortality from PDCoV than those of adult pigs. Moreover, the transmission of presence of virus in sows cannot be ignored.

In summary, this review reflects the trend of PDCoV infection prevalence in swine populations in China. However, this meta-analysis has certain limitations. For example, sample sizes were low in some regions (or low sample sizes were reported in certain cases). Analysis was also limited to date of sampling, geographic location, gene of interest, pig stage, and clinical signs. Other potentially influential factors, such as farm size, breed, and sampling season of pigs, were not analyzed. All data were from pigs with diarrhea. Additional samples of healthy pigs are suggested to be included to assess the infection of PDCoV in pigs in China. The abovementioned factors should be considered when conducting epidemiological studies in the future.

Conclusion

Our meta-analysis shows a high prevalence (12.4%) of PDCoV infection in Chinese pig herds. The prevalence rate is significantly associated with sampling region, sampling year, pig stage, and clinical signs in pigs (diarrhea). Therefore, biosecurity prevention and control should be strengthened to reduce the spread of PDCoV between regions. Climate, such as humidity and temperature, correlates with the breakout of PDCoV, Thus, this study recommends to keep pig house dry and warm. Surveillance of PDCoV and detection of other diarrhea pathogens should be strengthened in suckling piglets and sows due to high morbidity in suckling piglets and high virus-carrying rate in sows. The prevalence of PDCoV shows a downward trend; however, consideration of susceptibility of coronavirus to mutation, recombination, and cross-species transmission and continuous surveillance studies in swine remain essential (including non-diarrheal swine) to monitor the geographical spread and incidence trend of PDCoV and detect the genetic evolution.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

JS conceptualized the paper and wrote the manuscript. JS, QZ and JZ collected and analyzed the data. CZ and ZL revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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What can we learn from the five-year African swine fever epidemic in Asia?

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Today's global swine industry is exposed to the unprecedented threat of African swine fever (ASF). Asia, the site of the most recent epidemics, could serve as a huge viral reservoir for the rest of the world given the severity of the damage, the huge swine industry, and the high volume of trade with other countries around the world. As the majority of ASF notifications in Asia today originate from pig farms, the movement of live pigs and associated pork products are considered critical control points for disease management. Particularly, small-scale or backyard farms with low biosecurity levels are considered major risk factors. Meanwhile, wild boars account for most notified cases in some countries and regions, which makes the epidemiological scenario different from that in other Asian countries. As such, the current epidemic situation and higher risk factors differ widely between these countries. A variety of studies on ASF control have been conducted and many valuable insights have been obtained in Asia; nevertheless, the overall picture of the epidemic is still unclear. The purpose of this review is to provide an accurate picture of the epidemic situation across Asia, focusing on each subregion to comprehensively explain the disease outbreak. The knowledge gained from the ASF epidemics experienced in Asia over the past 5 years would be useful for disease control in areas that are already infected, such as Europe, as well as for non-affected areas to address preventive measures. To this end, the review includes two aspects: a descriptive analytical review based on publicly available databases showing overall epidemic trends, and an individualized review at the subregional level based on the available literature.

KEYWORDS

African swine fever, Asia, domestic pig, wild boar, infectious disease, epidemiology, disease control, risk assessment

1. Introduction

African Swine Fever (ASF), caused by the ASF virus (ASFV), is a contagious disease of domestic and wild pigs (1) and is one of the most influential transboundary animal diseases for the livestock industry in the world today. The clinical stages can be divided into four main categories: peracute, acute, subacute, and chronic (2); however, symptoms vary according to the balance between the virulence of the virus strain and host immunity, contributing to the variety of regional epidemiological scenarios. An essential aspect of this virus is its high environmental resistance, being well known for its ability to remain infectious for long periods under various conditions (3). Susceptible animals can be infected through direct contact with infected animals or indirect contact with contaminated materials (4, 5).

Asia is one of the main epidemic areas in the current global ASF epidemic; it accounts for more than half of the world's pork production and plays an important role in world trade. Rather than controlling ASF, the epidemic situation is becoming more complex, raising fears that ASF could spread further around the world, primarily by the movement of contaminated materials. Given the history of multiple ASF jumps from Africa to Europe, it is possible that Asia could play a similar role in the near future. Asia has the potential to become the global reservoir of the virus due to its high pig farming densities and greater human and material traffic. This would pose further threats to Europe, one of the current major epidemic areas, and likewise to ASF-free countries or those in the process of eradication.

ASF was originally confined to Africa but has been spreading globally since its reintroduction into Europe in 2007. Within the same year of its entry into Georgia, outbreaks were reported in Armenia, Azerbaijan, and Russia. In 2014, the disease reached the European Union via Lithuania, Poland, Latvia, and Estonia. By the end of June 2023, ASF was confirmed in 23 European countries (6), posing a major threat to Western European countries with large pig farming populations, such as France and Spain. In Asia, ASF was first confirmed in China in 2018. Shortly afterward, a series of infections were reported in neighboring countries, and, to date, 18 countries and regions have reported ASF. In 2020, the first ASF outbreak in the Oceania region was reported in Papua New Guinea. The following year, 2021, ASF was confirmed in Haiti and the Dominican Republic, two Caribbean countries located in the middle of the North and South American continents, for the first time in about 40 years. As these epidemics demonstrate, ASF is now a global problem.

Relevant research is being conducted in Asia in a variety of fields, ranging from molecular biology to epidemiology, as well as economics. However, much remains unknown compared with Europe, where the ASF epidemic occurred earlier and several valuable studies have been carried out. Unique sociocultural and traditional practices may contribute to the maintenance and expansion of the disease, making it challenging to obtain a complete picture of the epidemic. ASF spread in Asia has been exceptionally rapid compared with Europe, where a total of 23 countries were infected in the 16 years since 2007, whereas only four countries were infected within the first 5 years. What lies behind such a rapid and extensive spread of the disease over a 5 years period? What are the differences or similarities with the epidemic in Europe, where the spread has been relatively slow compared with Asia? The answers to these questions will provide valuable information, not only for both regions but also for countries at risk of infection in the future.

This review collected nearly 5 years of information available regarding the ASF epidemic in Asia (August 1, 2018, to June 30, 2023) and summarized the epidemic status as well as relevant background knowledge across Asia. For this purpose, it includes two aspects: a descriptive analytical review based on publicly available databases to elucidate overall epidemic trends; and a literature-based individualistic review of each region. Quantitative epidemiological ASF data were obtained from the databases of two international organizations: the EMPRES Global Animal Disease Information System (EMPRES-i) of the Food and Agriculture Organization (FAO) of the United Nations (7) and the World Animal Health Information System (WAHIS) from the World Organisation for Animal Health (WOAH founded as OIE) (6). The EMPRES-i database contains information such as the date of observation, country, subregion, and geographic coordinates of where the event occurred. This database was used to elucidate the number of notifications in each country and their spatial distribution. In addition to the WOAH data, the database also includes information provided independently by each country's institution, providing a more detailed notification count. The WAHIS database contains detailed epidemiological information, including the number of susceptible animals, the number of cases, the number of animals killed, and the epidemiological unit to which animals belong. This database was used to provide the first ASF event records and the number of infected or susceptible animals in each country. Scientific articles written in English from the beginning of 2017 to the end of June 2023 were reviewed in the PubMed database to obtain insights related to the epidemiological context. Relevant country data were retrieved from national databases or reliable online media as needed.

2. Overview of the ASF temporal trend in Asia

2.1. Introduction of ASF to Asia

In early March 2017, an ASF outbreak was reported on one backyard farm in the Irkutsk region of the Russian Federation, near the border with Mongolia. Since then, subsequent ASF outbreaks have occurred in Siberia and near the border with China, raising concerns about the disease entering Asian countries (8). Around spring of 2018, animals showing clinical signs similar to ASF began to be discovered in northeastern China (9–11) and, on August 3, 2018, ASF was officially reported in the northeastern Chinese city of Shenyang (10). The results of the phylogenetic tree based on partial sequences of the *p72* gene showed that the outbreak strain ASFV-SY18 isolated in China had a 100% nucleotide identity with the strains isolated in Georgia, Russia, and Estonia (Georgia 2007/1, Krasnodar 2012, Irkutsk 2017, and Estonia 2014), suggesting that the outbreak was caused by a pan-Russian ASFV strain (10). Several sources have been suspected for the initial introduction of ASFV into Asia, however, this remains unknown (12, 13).

2.2. ASF epidemic in Asia 2018–2023

Based on the EMPRES-i database, China was the only Asian country infected with ASF in 2018, with a total of 104 outbreaks reported; the WAHIS database documented approximately at least 358,000 susceptible and 12,700 infected animals (Tables 1, 2). In 2019, the disease rapidly spread to neighboring East and Southeast Asia, reaching 11 countries and regions (Mongolia, Vietnam, Cambodia, Hong Kong, North Korea, Laos, the Philippines, Myanmar, Indonesia, Timor-Leste, and South Korea) (Figure 1) (6). A total of 695 notifications were recorded in the database that year, the majority originating from domestic pigs as well as a small number of wild boar cases (Table 2) (7). The rough distribution of ASF occurrences in 2020 was similar to that of 2019 (Figure 2), with the highest-ever number of notifications reported (1,743) due to the constant regional disease expansion in East and Southeast Asia (7). About half of these reports originated from wild boars, mainly because of the spread of ASF infection in wild boars in South Korea (7) (Table 3 and Figure 3). In the same year, India confirmed its first ASF outbreak in South Asia (6).

The overall distribution of ASF notifications was fairly similar to that of 2019 (Figure 2), however, around 65% of the notifications in 2021 involved wild boars in South Korea (Table 3 and Figure 3). New ASF outbreaks were confirmed in Malaysia, Bhutan, and Thailand in that year, of which some wild boar cases were reported in Malaysia (Figure 2). In addition to the continuous ASF spread throughout East and Southeast Asia, a certain number of ASF events in both domestic pigs and wild boars were consistently reported in the Russia Far East, along the border with China, between 2019 and 2021 (Figure 2). While ASF was newly confirmed in Nepal in 2022, official outbreak reports from China have declined significantly, with reports concentrated in Southeast Asian countries, particularly Thailand, Vietnam, and the Philippines (Figure 2). In February 2023, Singapore newly reported ASF infection in wild boars, bringing the total number of ASF-infected countries/regions in Asia to 18 (Table 1).

TABLE 1 Timeline of the first ASF notifications in affected Asian countries in domestic and wild suids.

Country	Domestic pig	Wild boar
China	2018/8	2018/11
Mongolia	2019/1	
Vietnam	2019/2	2019/5
Cambodia	2019/3	
North Korea	2019/5	
Hong Kong	2019/5	2021/9
Laos	2019/6	2019/8
Philippines	2019/7	2021/5
Myanmar	2019/8	
Indonesia	2019/9	
South Korea	2019/9	2019/10
Timor-Leste	2019/9	
India	2020/1	
Malaysia	2021/2	2021/2
Bhutan	2021/5	
Thailand	2021/11	
Nepal	2022/3	2023/3
Singapore	2023/4	2023/2

East Asia played a significant role in disease spread during the early stages of the ASF epidemic, primarily due to a nationwide outbreak in China. Subsequently, South Korean wild boar cases have accounted for most of the notifications in this subregion. On the other hand, a certain number of notifications have been continuously recorded in Southeast Asia since 2019 due to the widespread dissemination of the disease. South Asia has also continuously reported ASF notifications since 2020, with fewer than in other subregions (Figure 4). The number of notifications peaked in 2020 in the FAO EMPRES-i database; but ASF infections have been reported constantly from most of the affected countries as of the end of June 2023, suggesting that ASF is becoming endemic in Asia. At this point, a total of 4,836 notifications were recorded in the EMPRES-i database, of which 3,074 were domestic pig-related outbreaks and 1762 were wild boar cases.

The general epidemic trend over the past 5 years is that outbreaks associated with domestic pigs are observed throughout Asia, whereas wild boar cases are found mainly in certain countries/regions (Table 3). All ASF-infected countries have confirmed outbreaks in the domestic pig sector, while wild boar cases have been officially reported in 9 of the 18 infected countries/regions (Table 1). Different major transmission mechanisms have been reported in the early and late stages of epidemics in Asia. The spread of ASFV at the beginning of the outbreak, primarily in China, most likely occurred via the transportation of infected livestock, products, or fomites. In contrast, proximity to swine enterprises and direct contact may have contributed to the later stages of the epidemic in Southeast Asia (14).

3. ASF subregional update in East Asia

3.1. ASF epidemic status

China, Hong Kong, Mongolia, South Korea, and North Korea are the ASF-infected countries/regions belonging to East Asia (Figure 5). After the rapid and widespread expansion of the disease in the early epidemic stages in China, official notifications are now sporadically reported from the entire country, thus becoming an endemic situation (15). Outbreaks have been observed in vast areas, many of which geographically overlap with large pig farming areas (16). There is a clear seasonal trend in the outbreaks, with the highest frequency of reports occurring during winter and spring. This is presumably due to a surge in consumer demand for pork during the Chinese New

TABLE 2 Annual notifications of ASF in Asia.

	[a]Total notifications	[a]DP outbreak	[a]WB case	Susceptible animals	Infected animals
2018	104	102 (98.1%)	2 (1.9%)	358,309	12,700
2019	695	636 (91.1%)	59 (8.9%)	8,489,292	155,754
2020	1743	846 (48.5%)	897 (51.5%)	2,988,452	83,950
2021	1,105	389 (35.2%)	716 (64.8%)	70,617	9,980
2022	941	860 (91.4%)	81 (8.6%)	95,988	22,324
2023	248	241 (97.2%)	7 (2.8%)	375,751	37,171

DP, Domestic pig; WB, Wild boar.

[a]Total notifications are retrieved from the EMPRES-i database and susceptible/Infected animals are based on the WAHIS database.

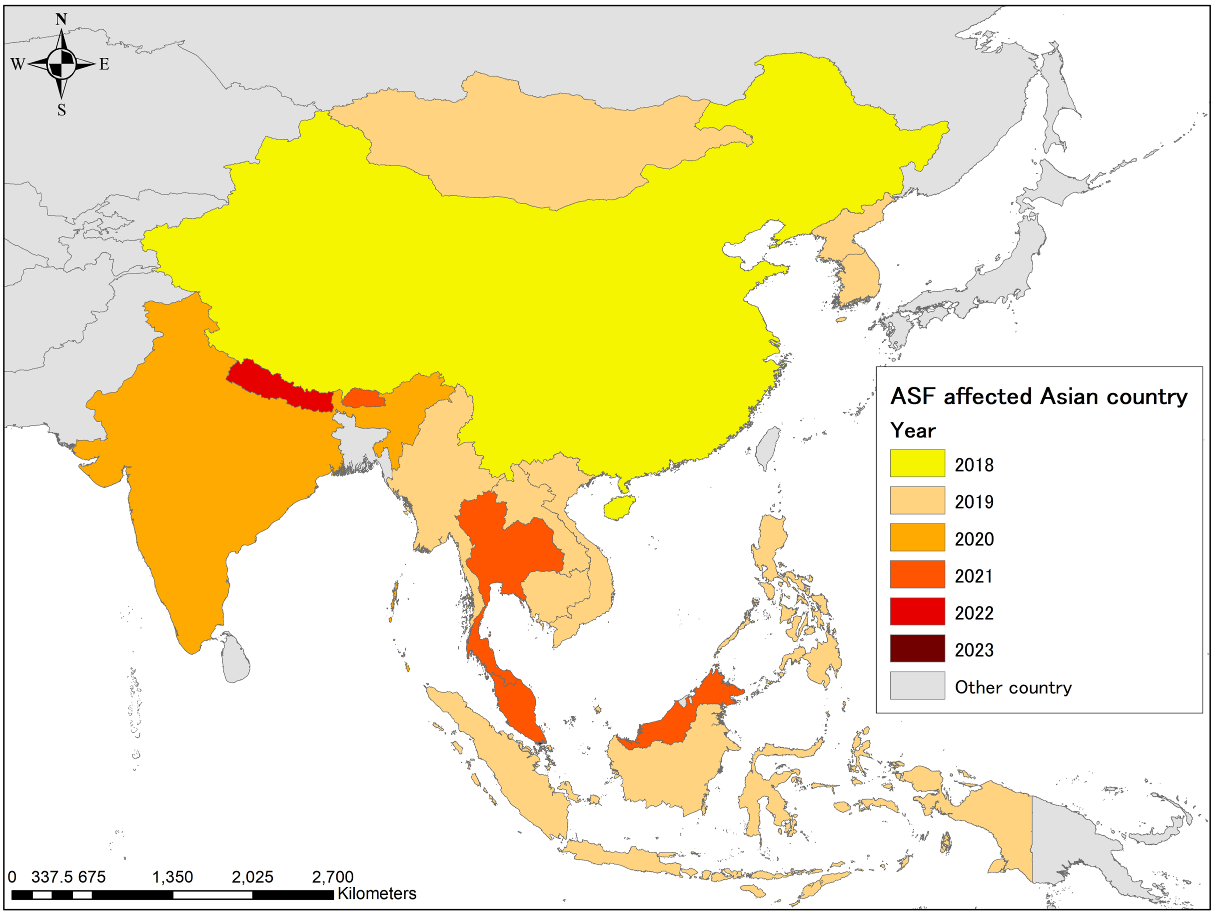


FIGURE 1
Year of the first confirmed African swine fever (ASF) case in infected Asian countries as of 30 June 2023. The map was depicted using ArcGIS 10.8.1 (ESRI, Redlands, CA, United States).

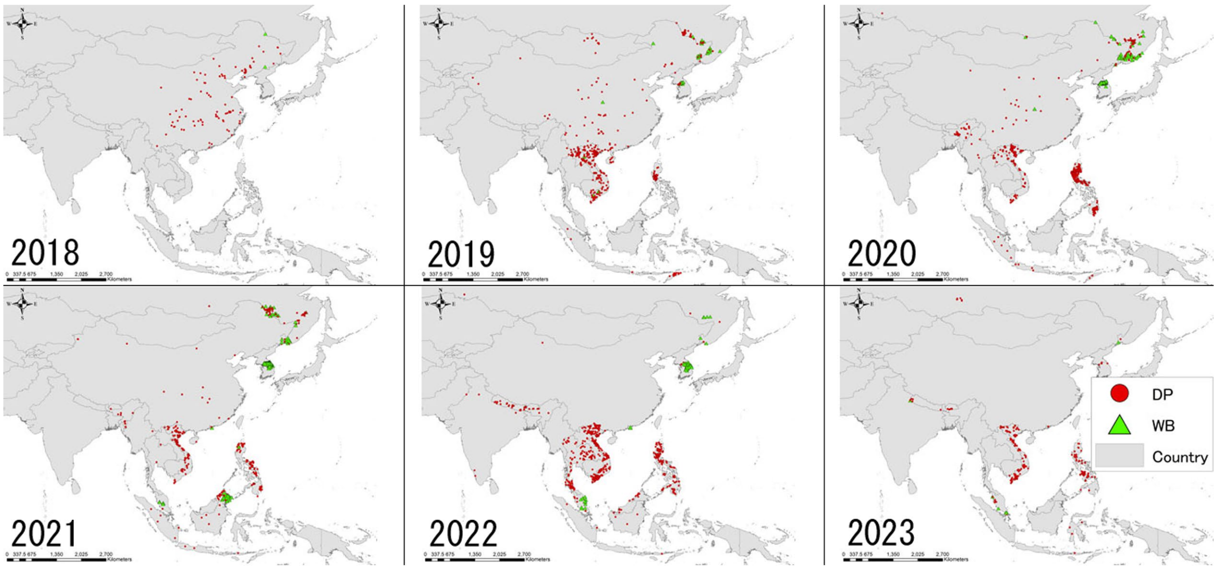


FIGURE 2
Annual trend of ASF spatial distribution in Asia including the geographically close Russian Far Eastern region as of June 30, 2023, based on the FAO EMPRES-i database. The map was depicted using ArcGIS 10.8.1 (ESRI, Redlands, CA, United States).

Year, one of China's traditional festivals (17, 18). Many of these notifications are related to domestic pigs (97.2%), however, a certain number of ASF-positive wild boar cases are also reported (2.8%). For example, Hong Kong has recorded nine notifications in the EMPRES-i database since 2019, four of which are wild boar cases. ASF notification in both domestic and wild suids has also been confirmed in neighboring border areas, particularly in the Russian Far Eastern side of the border with China (19).

TABLE 3 Total number of ASF notifications per host and country.

Country	DP outbreak	WB case	Total
South Korea	36 (2.1%)	1,690 (97.9%)	1726
Philippines	1,181 (99.9%)	1 (0.1%)	1,182
Vietnam	1,050 (99.7%)	3 (0.3%)	1,053
China	212 (97.2%)	6 (2.8%)	218
Laos	165 (98.8%)	2 (1.2%)	167
Malaysia	83 (61.9%)	51 (38.1%)	134
Thailand	118 (100%)	0 (0%)	118
India	76 (100%)	0 (0%)	76
Indonesia	43 (100%)	0 (0%)	43
Nepal	39 (97.5%)	1 (2.5%)	40
Bhutan	18 (100%)	0 (0%)	18
Timor-Leste	13 (100%)	0 (0%)	13
Cambodia	12 (100%)	0 (0%)	12
Mongolia	11 (100%)	0 (0%)	11
Myanmar	10 (100%)	0 (0%)	10
Hong Kong	5 (55.6%)	4 (44.4%)	9
Singapore	1 (20%)	4 (80%)	5
North Korea	1 (100%)	0 (0%)	1

*Notifications are based on the EMPRES-i database.

The phylogenetic analysis of ASFV isolated in China in 2018 showed great similarity to the highly virulent ASFV isolates from Eastern Europe (20). Likewise, recent ASFV isolates from East Asian countries such as Mongolia and South Korea were shown to be highly virulent genotype II ASF viruses with high homology to each other (10, 21–23). Due to this background, the highly virulent genotype II ASFV is generally considered to be predominant in this region (24) but this may not be true for China. Recent reports indicate that ASFV genotypes I and II, including lower virulent and recombinant strains, are simultaneously prevalent in Chinese swine herds, demonstrating that many different, genetically diverse ASFV strains are present (25–28). The non-hemadsorbing lower virulent genotype II and genotype I ASFVs have been repeatedly isolated in several Chinese provinces, which potentially relates to the production of illegal vaccines (29–31).

According to the EMPRES-i database, South Korea recorded the highest number of notifications in Asia as of the end of June 2023 (Figure 3). This is because the finding of one ASF-positive wild boar is frequently counted as one case, and the majority of notifications are reported in wild boars in South Korea. The number of official notifications peaked in 2020 and has been decreasing since then, however, the disease has not stopped spreading. Sporadic outbreaks have been reported on farms, spatially overlapping with the expansion of wild boar cases. Wild boars clearly play a pivotal role in the spread of ASF in South Korea. The current epidemic is most likely the result of multiple localized disease entries (32) or continuous transmission pressure along the border (33). In the early stages of the epidemic, a series of outbreaks were reported in neighboring areas following the initial ASF confirmation on a northwestern pig farm. Shortly thereafter, the first case in wild boars was officially confirmed (7), and, to date, numerous notifications, mainly from wild boar populations, have been reported. The infected areas continue to expand from the

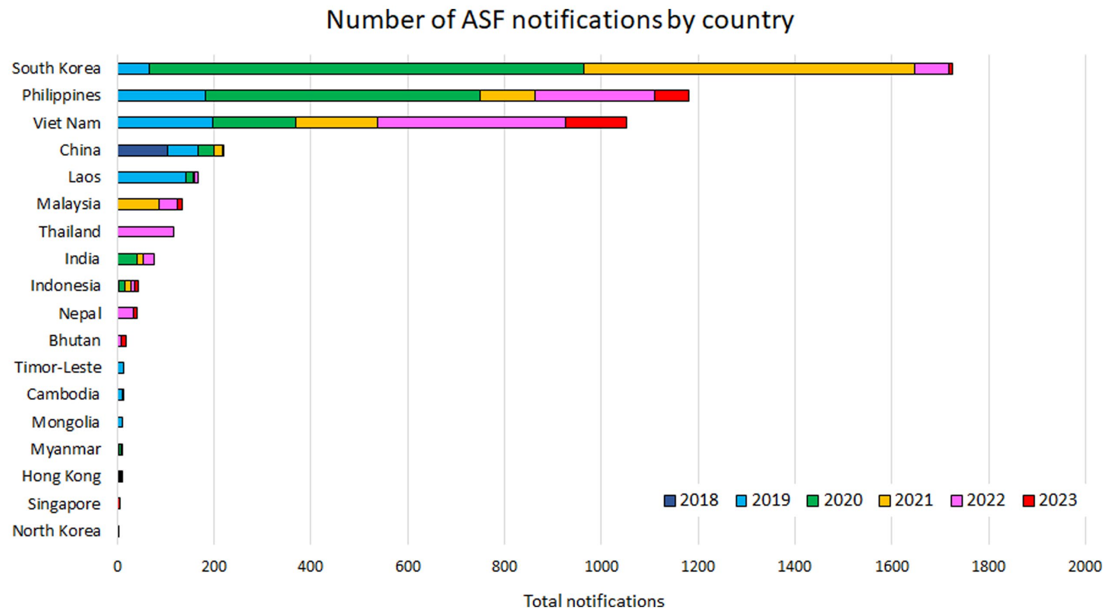


FIGURE 3
The number of annual and total ASF notifications by country as of June 30, 2023, based on the EMPRES-i database.

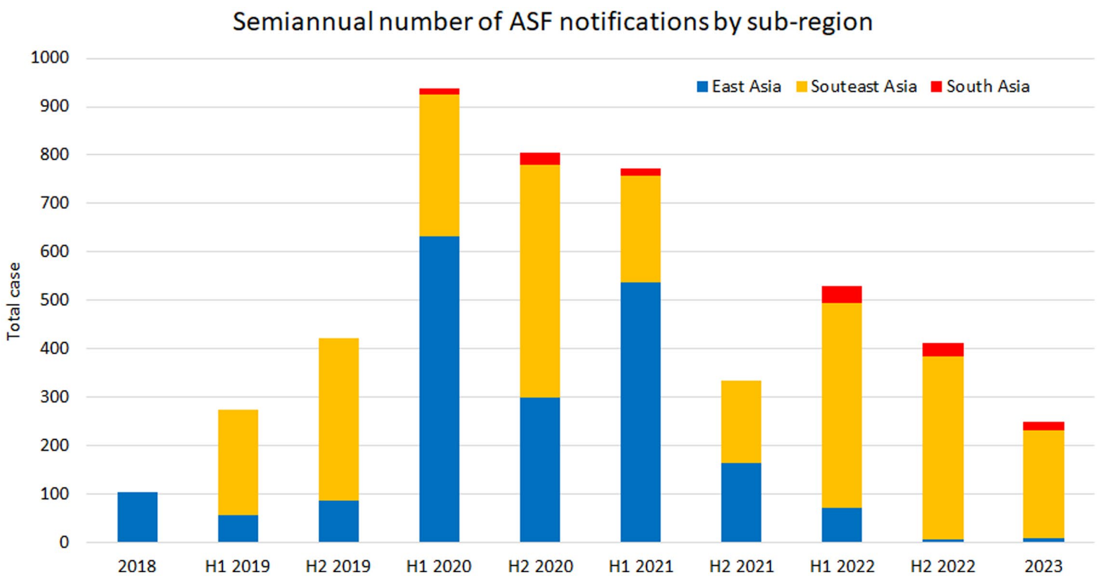


FIGURE 4
Number of semi-annual ASF notifications by subregion as of the end of June 2023, based on the FAO EMPRES-i database. H1 and H2 denote the first and second-half periods, respectively.

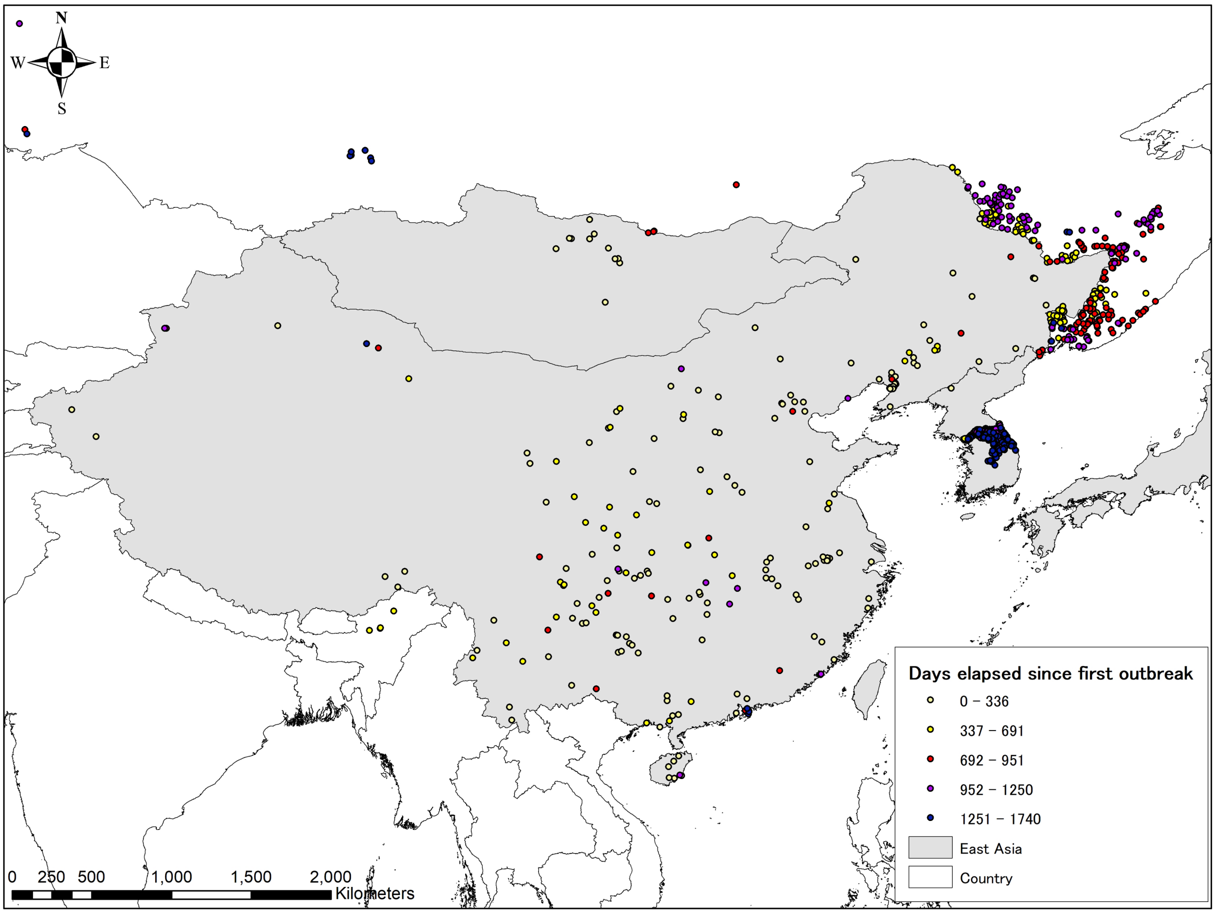


FIGURE 5
ASF evolution in Eastern Asia (including the Russian Far East) from August 1, 2018, to June 30, 2023, based on the EMPRES-i database. Lighter colors indicate earlier stages, darker colors indicate more recent occurrences. The map was depicted using ArcGIS 10.8.1 (ESRI, Redlands, CA, United States).

north to the south, serving as a corridor with the Taebaek Mountains, running north–south along the country's eastern coast, and have reached the central part of the country at this point (33–35). These infected areas are considered suitable for wild boars but harder to access for surveillance and, thus, the actual epidemic status may not be properly understood (33). There are several theories as to the detailed mechanism, however, seasonality in the number of ASF notifications has been observed, with more notifications in winter and a minimum in summer, and outbreaks on pig farms are most common in autumn (36, 37).

Mongolia was the second Asian country to be infected with ASF, which was confirmed in a pig farm on January 10, 2019. Within 1 month of the initial outbreak, the disease affected 83 pig farms in seven provinces in the country, killing approximately 2,860 animals, representing about 10% of the total pig population (38). However, there have been no new outbreaks since early February 2019, and an end to the disease was declared on April 11 of the same year (38). North Korea reported one ASF outbreak in May 2019, after which no additional information is available. Japan and Taiwan are the only countries/regions in East Asia where ASF infections have not been reported as yet. In Japan, ASF-contaminated pork products are frequently detected at international ports, and the risk of ASF entry is estimated to be high (39). Similarly, quantitative risk assessment studies conducted in Taiwan have shown a very high risk of ASFV introduction (40). A dead pig that washed ashore in the territory was recorded as an ASF-positive individual in the EMPRES-i database (7, 40).

3.2. Pig industry and wild boar distribution

3.2.1. Pig industry

East Asia is a region with a large pork industry, with China accounting for half of the world's pork production (452.6 million heads as of April 2023) and South Korea being the world's ninth-largest pork producer (41).

The rise in pork consumption driven by rapid economic development has led to an increase in the number of people seeking business opportunities in China. As a result, a complex, large trade network and value chain involving many different stakeholders has formed within the Chinese pork food system (42). The large-scale pig farming regions are mostly located in coastal areas and are divided into northern and southern regions. Pig farming is in the transition stage from bulk culture to large-scale agriculture, with 26 million households engaged in pig farming (15). The proportion of large-scale pig farming is increasing, nevertheless, the majority of farmers are small-scale for solely private consumption, where farms with less than 500 pigs account for about 99.4% of all pig farmers (15). After the ASF outbreak, guidelines for the prevention and control of ASF were issued to promote large-scale pig farming and reduce the number of small-scale farmers (43). Consequently, the number of small farmers may have decreased significantly but this pig production model is still likely to last a long time (15).

The swine industry is an indispensable part of South Korean agriculture, accounting for 30% of the livestock sector and producing more than 1 million tons of pork annually. As it is preferred over beef and chicken, pork is consumed in large quantities, thus it is also imported into the country (44). The highly intensive industry, with about 11.2 million heads divided among approximately 5,700 farms, is distributed mainly in the mid-western region of South Korea (45). The overall trend in the swine industry is toward structuring, modernization, and efficiency, with traditional small farms being closed, and larger, more modern swine farms on the rise (44). Recently, eight major on-farm quarantine facilities were established to improve the quarantine level for pig farms nationwide. These standards include the installation of internal and external fences with height criteria, the set-up of equipment essential for the disinfection and prevention of cross-contamination, and the use of nets to prevent the entry of wild animals and the storage of carcasses. The costs of installing these facilities are subsidized through the support program (46).

3.2.2. Wild boar distribution

The Chinese wild boar population, including both wild and domesticated animals, is assumed to be very large and widely distributed throughout China. In addition, free-ranging feral pigs are present in many areas (47). The spatial density distribution of wild boars is unknown but it is estimated to be 2–5 heads/km^2 in densely populated areas, with the total number reaching several million (48–50). Although reported wild boar cases are scarce, these conditions raise the possibility of their contribution to the maintenance of ASFV (15).

Before ASF introduction, the wild boar population in South Korea was growing rapidly, with an estimated population of 300,000 animals in a wide range of habitats, from forests to urban environments (32, 51, 52). Approximately 70% of the country is covered by forests and mountains, providing the optimal habitat for wild boars. Geographically, the Taebaek Mountains run north-south along the east coast of the Korean Peninsula, with two mountain ranges extending west in the central region and south-southwest in the central and southern regions, serving as a home for wild boars (53). On the border with North Korea, there is a 248 km-long and 4 km-wide barrier called the demilitarized zone (DMZ) that extends from the east to the west coast. Following this zone is a restricted civilian entry zone with a 7–15 km perimeter, which serves as a paradise for wildlife to thrive due to restricted human access (54). The average density of wild boars nationwide was reported by the government to be 4.1 heads/km^2 as of October 2020 (55, 56). However, there are large regional differences, and it was noted that the density calculated after culling and searching for carcasses was approximately 10 heads/km^2, indicating the possible underestimation of the population density (32).

3.3. Risk factors and control measures

The risk factors and countermeasures for ASF epidemics differ considerably depending on the importance of the activities associated with pigs and the role played by wild boars. In China, pig density is considered the most important risk factor, the various reasons for which are explained below. Long-distance transportation of pork and pigs was traditionally common due to the uneven distribution of pig farming industries. Measures restricting transportation to contain disease spread resulted in soaring pig prices and an increase in illegal transportation, leading to further long-distance transmission of ASFV (15). Given these considerations, the government implemented measures such as the registration and notification of pig transport vehicles, inspection of transport links as well as the detection of slaughter links (57). Furthermore, the country has been divided into five regions to restrict movement. Each region has an ASF-free zone, and only pigs from the free zones, breeding pigs, and piglets are allowed to move beyond their respective regions (58).

Distribution of contaminated pork and food waste is considered the main cause of outbreaks on small farms, while mechanical transmission of the virus by vehicles and personnel seems to be the main contributor to outbreaks on larger farms. Inadequate disinfection facilities and improper operation of cleaning and sterilization systems in slaughterhouses have been linked to several outbreaks, with a survey in 2019 reporting that, in some cities, 5% of slaughterhouses were contaminated with ASFV (59). For this purpose, the government announced a survey on the detection of ASFV in pig slaughtering and pork products distributed in January 2019 (60).

ASFV transmission via feeding leftover food to healthy pigs is known to be an important mode of viral spread (61) and is recognized as a major contributor during the early stage of the epidemic in China (62). As such, the government prohibited the feeding of food residues to pigs as of late 2018 (15, 63). These aforementioned risk factors were also raised in a previous systematic review of risk factors for ASF spread in China (64).

Wet markets play an important role in the sale of fresh meat (65, 66) and, therefore, a significant vulnerability of the pork food system in terms of managing the risk of ASFV transmission (67). Moreover, complex and large swine and pork production systems make it difficult to implement the "stamping out" tactics of complete destocking of contaminated facilities and tracing, as well as inspection of contacts (68). Possible animal disease control and prevention are influenced by those heavily involved in the value chain (traders, processors, retailers) rather than by farmers, thus complicating the implementation of ASF control measures. This makes ASF control in China more challenging compared with Europe and the current African pork food system (67).

To our knowledge, findings of ASFV in wild boars are very limited in China (69–71). Little importance has been placed on the role of wild boars in the ASF epidemic, however, it may be highly underestimated (64, 72). Despite the high density of wild boar populations and their large home range, the lack of information on their movements makes it difficult to assess the current situation (18, 70, 72). No ASF outbreaks involving tick infections have been reported in China as yet, however, more than 100 species of ticks are widespread throughout the country. While the role and mechanism of ticks in ASF transmission in China remain unknown, they have been identified as an important risk factor in various studies (15, 16, 73). Large knowledge gaps remain regarding the role of wild boars and ticks in ASFV transmission, thus underlining the need for further research (18, 64).

South Korea is considered to have implemented a relatively high level of control policy with a low ASF incidence on pig farms among Asian countries (36, 46). Contaminated vehicles and the movement of infected wild boars likely contributed to the ASFV transmission to pig farms; in particular, vehicle movement played a major role in the series of early outbreaks on farms (74). As soon as ASF is confirmed on a farm, movement restrictions and thorough disinfection are implemented for a certain period of time based on three levels of zoning (control zone, protection zone, and surveillance zone within a radius of 500 m, 3 km, and 3–10 km, respectively) (75). Persistent ASFV circulation in wild boars can be a continuous risk for pig farms. The accumulation of infected carcasses in the environment increases the risk of infectious agents flowing into farms in the summer due to natural disasters such as heavy rains and typhoons. In spring and fall, farm inspections and disinfection are intensified because of the increased risk of spatial contact with wild boars owing to increased farm work and mountain hikers, as well as the breeding season (76).

Disease containment measures among wild boars mainly consist of fencing, population control, and carcass removal. The fencing was installed in multiple stages, the first and second consisted of an electric fence enclosure of 1 to 2 km around the case report site and a semi-rigid wire mesh 1.5 m high placed approximately 5 to 10 km around it. A third fence was deployed across the country from west to east in areas 20 to 30 km away from the second fence to prevent further southward movement. Each time ASF cases were reported beyond the third fence, authorities enclosed the newly infected area (36, 75). The effectiveness of fences in preventing the spread of disease in wildlife is controversial (77), however, its role in South Korea is emphasized as a temporary measure to slow the transmission rate (78). Government-led search teams, organized nationwide at a regional scale, are constantly searching for wild boars, mainly around the infected areas (32, 79). The search was further prompted by offering a bounty for the discovery of the animal but this may have resulted in anthropogenic jumps in ASF spread. Persons without adequate biosecurity knowledge could have served as carriers of the virus by traversing infected areas during hunting and search operations (32, 34).

The Taebaek Mountains are an important pathway for the spread of ASF infection in South Korea. The high elevation of the mountains complicates consistent surveillance activities, thus making it challenging to precisely understand disease prevalence. Undetected infected carcasses may increase the concentration of virus in the environment and sustain the ASFV transmission cycle (33, 80). Recently, governments have focused on improving surveillance bias by introducing detection dogs and drones (81, 82).

In addition to the current virus strains in circulation, new ASFV introductions from abroad remain a major threat. The only land border with North Korea is fenced, so interactions are very limited. Accordingly, the quarantine framework is primarily based on border control as in island countries. While previous studies derived that the ASFV-introduction risk associated with the legal importation of live pigs and/or pork products is low (83), there are concerns regarding the risk of human-mediated pathways, such as illegal pork importation (36, 84). On the other hand, a study analyzing the distribution of ASF cases at the beginning of the epidemic identified proximity to North Korea as an important continuing risk factor (33). While wild boars are unlikely to pass through the border fence, the multiple rivers that span both countries will allow for the arrival of wild animal carcasses or portions thereof (33). The role of wildlife as vectors in the transmission dynamics of ASF in South Korea remains to be elucidated. In addition to wild boars, mammals such as raccoons, cats, and rodents, as well as birds, including vultures, are suggested to be possible spreaders of ASFV (85). In contrast, others believe that their role is limited and therefore controversial (32, 86), thus further research is required.

4. ASF subregional update in Southeast Asia

4.1. ASF epidemic status

ASF has been observed repeatedly in a wide area of Southeast Asia, with outbreaks confirmed in 10 countries (Vietnam, Cambodia, Laos, the Philippines, Myanmar, Indonesia, Timor-Leste, Malaysia, Thailand, and Singapore) (Figure 6). The first ASF notification in Southeast Asia was officially reported in February 2019, in Hung Yen province in northern Vietnam, and within the same year, six countries in the region confirmed ASF (Cambodia, Laos, the Philippines, Myanmar, Indonesia, and Timor-Leste). In that year, outbreaks were concentrated in Vietnam, Laos, and the Philippines, resulting in approximately 550 notifications in the EMPRES-i database. However, the ASFV genome was detected in the food of a traveler from Vietnam to Taiwan in February 2019, raising suspicion that ASFV was already widespread in the country before the official report (87). In 2020, the outbreak spread further, reaching 771 cases across Southeast Asia, with more than 550 outbreaks reported in the Philippines alone. In 2021, 87 new ASF outbreaks were reported in Malaysia, of which about 40% originated from wild pigs. In the same year, Thailand officially reported an ASF outbreak, and the following year 117 outbreaks were reported. The most recently infected country in this subregion is Singapore, where the disease was reported in wild boars in February 2023.

The virus strains isolated in Southeast Asian countries (Vietnam, Indonesia, and Malaysia) are highly homologous to each other and genetically similar to the genotype II ASFV isolated in China (88–90). Indeed, there are frequent reports of illegal cross-border movement of animals and meat products between China and Vietnam (11, 90, 91). Genetic analyses of ASFV isolates from domestic pigs in northern Vietnam have shown the continuous introduction of Chinese ASFV strains via illegal trade (92, 93), further highlighting that illegally attenuated vaccine strains of ASFV recently discovered in China have already spread to neighboring countries (90, 94). As many Southeast Asian countries share land borders, pig traders move across borders, and evidence of ASF infection has been found in brought-in pigs and pork products at various locations (95, 96). From the perspective of the EMPRES-i database, Vietnam and the Philippines have continuously reported numerous outbreaks since the early stages of the epidemic, with the total number of notifications exceeding 1,000 in both countries. In Vietnam, the disease had spread to all provinces within 5 months of the first ASF confirmation, killing nearly 6 million pigs, which is more than 20% of the country's pig production (97). In the Philippines, at least 300,000 pigs have been culled (98). These massive ASF epidemics not only affected farmers but also caused pork prices to soar, which greatly affected the livelihoods of consumers. The number of notifications in Indonesia recorded in EMPRES-i is small (43 notifications as of the end of June 2023), however, the outbreak was confirmed in 10 of the 34 swine industry provinces, killing over 3.5 million pigs (99).

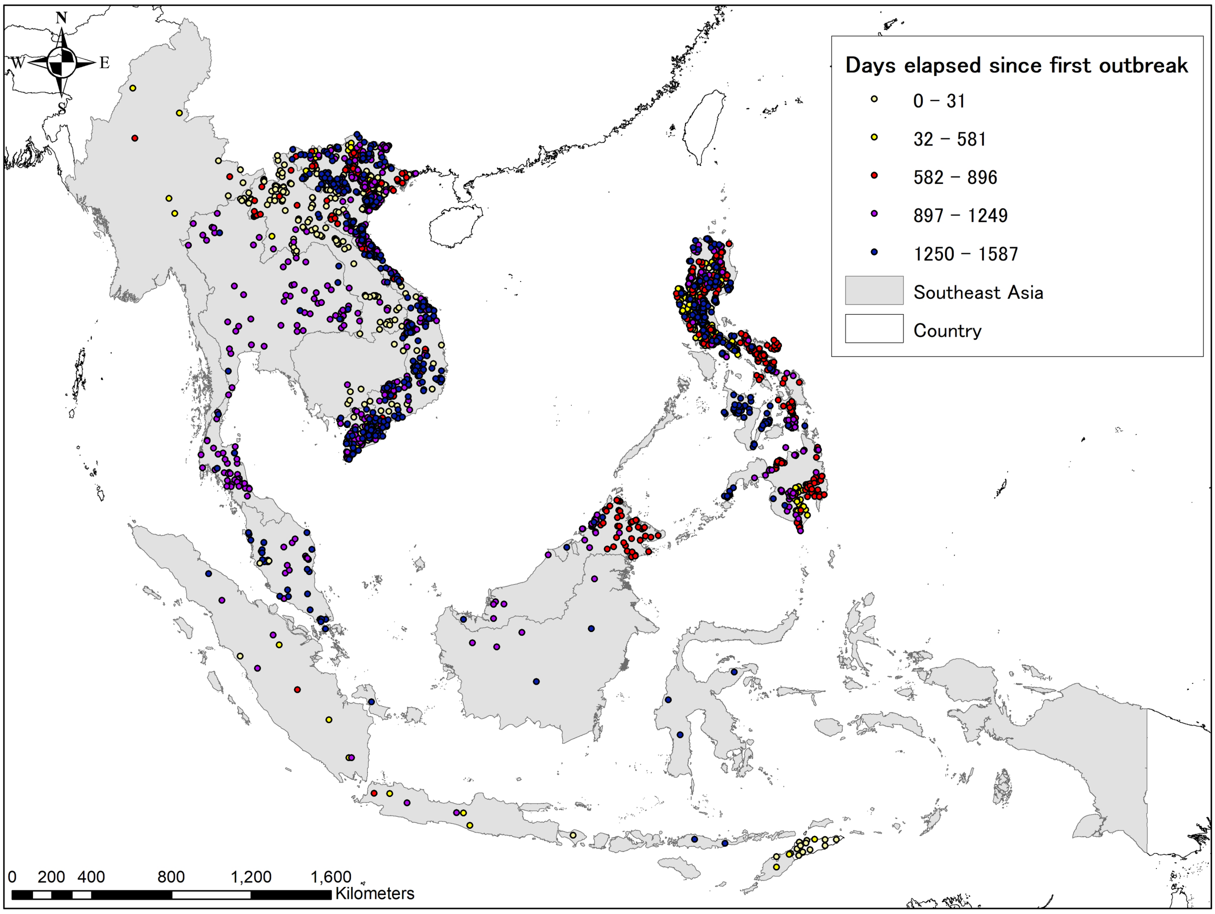


FIGURE 6
ASF evolution in Southeast Asia as of June 30, 2023, based on the EMPRES-i database. Lighter colors indicate earlier stages, darker colors indicate more recent occurrences in this region. The map was depicted using ArcGIS 10.8.1 (ESRI, Redlands, CA, United States).

4.2. Pig industry and wild boar distribution

4.2.1. Pig industry

Pigs play an important role in the lives of rural and peri-urban populations in Southeast Asia, and pork is the preferred meat in most countries. Types of pig production vary, ranging between small family units of backyard scavenging pigs, small to medium-sized semi-commercial units, and large intensive units. Like other Asian countries, the predominant practice is small-scale backyard farms with no or limited biosecurity, which are the most vulnerable to disease risks (14, 96, 100). The role of the pig industry varies among countries (101). Vietnam has a large domestic demand for pork, constituting 60% of all livestock production and raising the largest number of pigs in Southeast Asia at 30 million heads (97). In general, pig herds are very small, with about 49% being raised on small pig farms or backyard family farming units (102). Compared with the north, the south has more intensive and larger production systems (101, 103).

The overall pig stock in the Philippines was estimated at 9.49–12.7 million heads (104, 105). Of the total pig production, 70.6% are raised on private farms, while the remaining 29.4% belong to commercial farms. There are large pig farms in some areas of the country, however, backyard pig farming still accounts for 65%–83% of the total in rural areas. The average number of pigs per backyard holding is extremely limited, with many backyard families keeping one or two pigs fed on crops (104). In Thailand, the majority of pig-farming households are small-scale farmers (93.51%), with 9.5 million pigs (106). In recent years, the country has been shifting toward an intensive production system and is likely to form part of an integrated supply chain (107). Some of the live pigs and pork is exported to neighboring countries but it is primarily for domestic consumption. Large commercial pig farms are concentrated in peri-urban areas, while smaller pig producers are often found in rural and remote areas (107).

To meet the growing demand for pork in Cambodia and Laos, imports of live pigs and pork from neighboring countries such as Thailand, Vietnam, and China, along with the domestic pig farming industry, are increasing. In Laos, as of 2020, approximately 4.3 million pigs are allocated to about 580 commercial pig farms (108); in Cambodia, around 70% of pork is supplied by small-scale farmers (109). This trend of increasing pork demand is the same in Myanmar, with about 19.19 million pigs being raised in the country as of 2020 (110). Most pig farmers are small-scale farmers practicing free-range or backyard animal husbandry, and every household in the village raises at least one pig. This is not only for residual waste disposal but also for additional income (111).

About 8.9 million pigs were distributed in 34 of the 38 provinces of Indonesia before the ASF outbreak, with approximately 80% of pigs being produced by small-scale farmers holding less than 20 sows (88, 101). Although production is for domestic consumption, the pork-consuming population constitutes just 13% of the total due to the large Muslim population (101). Likewise, in Malaysia, having a large Muslim population, an estimated 1.7 million pigs are raised on 614 farms as of 2020, mainly for the country's ethnic Chinese population. The majority of pig farms in the Malay Peninsula still operate on an open-house system (112).

The pig farming situation in Timor-Leste differs slightly from other Asian countries, where almost the entire domestic pig herd is held by small-scale farmers (113). Approximately 450,000 pigs are kept in the country in both urban and rural areas, with an average of less than three pigs per household, distributed to approximately 70% of the total population (114). As in rural areas of other Asian countries, livestock tend to be perceived as part of the family or property, rather than just for commercial purposes (115). Singapore has relied on imports since pig farming was discontinued in the early 1990s (116). ASFV was detected in carcasses at slaughterhouses in Singapore after live pigs were imported from Indonesia in April 2023 (117).

4.2.2. Wild boar distribution

The Eurasian wild boar (*Sus scrofa*) is an endemic species in Southeast Asia and is widespread across forested areas (118–120). Their average density remains unknown, however, high densities of 30–40 animals/km^2 have been recorded in some areas, e.g., conservation areas in Indonesia, Malaysia, and Singapore (121). Accordingly, the potential risk of ASF infection in wild boars has been discussed (72, 122, 123). Surveys conducted in Vietnam, Laos, and Cambodia found extensive overlap between wild boar habitats and domestic pig sites around villages adjacent to forests in these countries (124). Numerous interactions between wild boars and domestic pigs have been documented owing to the common practice of free-ranging domestic pigs (96). This creates a high-risk interface for virus transmission between these groups (121, 124). While the presence of ASF in wild boars in Laos and Vietnam was confirmed, the role of wild boars in the transmission cycle of ASFV in this region was concluded to be uncertain (124). Besides the endemic wild boar (*Sus scrofa*), the disease is feared to have a potentially serious impact on 11 endemic wild pig species in Southeast Asia (125). Despite this, ASF notifications have been limited to incidental reports of mortality events in Bornean bearded pigs (*Sus barbatus*), wild boars (*Sus scrofa*) in Laos and Vietnam, and warty pigs (*Sus cebifrons*) in the Philippines (124, 126, 127).

4.3. Risk factors and control measures

The Southeast Asian swine industry faces several major problems: low biosecurity swine production systems dominated by small farmers; complex, multistage, integrated production systems; illicit transportation of pigs and/or pork products with insufficient monitoring caused by price differentials and social factors; and cross-border disease spread through long and porous borders (128).

More than 90% of outbreaks in Vietnam's early epidemics occurred on small and medium-sized farms with poor biosecurity, raising challenges for ASF prevention and control (97). As in China, the small farm sector is declining but may take time to be fully replaced by modern commercial farms (97). In these areas, people often cannot properly dispose of infected animals and dump the carcasses in rivers or roadside shrubs after slaughter, causing the disease to spread even further (102, 122, 129, 130). This can be partially explained by the limited capacity of veterinary services to deal with epidemics at the municipal level. Poor public veterinary services in the field lead to diseases not being properly diagnosed and contribute to their expansion (131). Similar practices due to the lack of biosecurity knowledge as well as the limitations of veterinary services have been observed throughout Asia (32, 96, 106, 132, 133). While these risk factors emphasize the importance of implementing strict biosecurity measures on small farms, the absence of stringent surveillance entails the risk of worsening the epidemic situation due to increased trade and consumption of infected animals (134).

Financial compensation after a disease outbreak on a farm is known to have a significant impact on farmers' behavioral patterns, including their motivation for reporting (135). Full compensation may lead to lax preventive behavior, while inadequate compensation would encourage illegal trade and underreporting of cases by farmers (67). This is a common concern for many Asian countries, where financial compensation is often inadequate (63, 64), infected meat is sent to markets and/or restaurants to hide the outbreak, and the food waste could reach another pig farm as leftovers due to swill-feeding practices (136). A study conducted in Vietnam indicated that ASF surveillance data may have been underreported due to the lack of awareness, animal health professionals, and laboratory facilities in rural areas. In particular, farmers were reluctant to report to the authorities because of low compensation rates and complicated, lengthy administrative procedures (122). An attempt to sell suspect pigs was also observed, even at a lower price before ASF was confirmed, rather than waiting for longer to obtain higher compensation (97). Note that these are problems on a regional scale, not on a farm unit basis. In the Philippines, local communities hid sick pigs to avoid culling their pigs (132).

The pork food system in Vietnam is becoming more complex and large-scale through rapid economic development (137, 138). Also, the predominance of fresh meat being sold via the wet market poses a major vulnerability in the pork marketing system from the perspective of risk management for disease spread (67, 139). The pig trade depends on market demand and price differentials; traditionally, town traders, such as slaughterhouse operators and market sellers, go to the villages to purchase pigs to supply local demand. Improvements in road infrastructures have facilitated long-distance trade from rural producers to large cities and even to foreign markets. However, the scarcity of effective tracing systems in most areas makes it hard to monitor pig movements, and unregulated movements are common (128). As a result, illegal cross-border transportation frequently occurs. In a spatial risk assessment study of ASF introduction in Thailand, distance from the border was identified as one of the highest-priority risk factors. Consequently, several ASF outbreaks are now reported in many of these land-bordering areas (140). All frequently used distribution routes, not just road transportation networks, require attention. In the island nation of Indonesia, ports have been identified as a contributing factor to ASF outbreaks because of the daily marine transportation of pigs (141). Similarly, food waste from overseas vessels is an important virus transmission pathway. A study conducted at an Indonesian port found an ASFV prevalence of 8.69% in food waste brought in by ships from China and the Philippines (141).

Southeast Asian countries also have seasonal patterns in ASF outbreaks, as in China and South Korea. The increased movement of people and/or animals during the Vietnamese New Year may have contributed significantly to the nationwide spread of the virus (122). In the Philippines, environmental factors and social practices possibly contribute to a seasonal pattern in ASF outbreaks. The third quarter, coinciding with the beginning of the academic year, is a time when small farmers tend to sell their pigs to finance education, leading to the frequent movement of livestock and pork products throughout the country. Moreover, a significant increase in precipitation during the rainy season presumably leads to the dispersal of carcasses and environmental contamination, thus contributing to the higher frequency of ASF outbreaks (98, 132).

Border controls have been tightened in many countries to prevent the entry of pigs and pork products from ASF-infected areas. In addition, the application and proper management of biosecurity on pig farms together with rigorous and intensive monitoring of high-risk areas are recommended as important strategic steps to prevent ASF. In the Philippines, the government implemented various policies and public health strategies in response to the epidemic. A series of actions, called the 1-7-10 Protocol, established the application of zoning-based culling and active surveillance activities and testing. In 2021, the National African Swine Fever Prevention and Control Program (BABay ASF) was launched to prevent and control ASF via surveillance, monitoring, and repopulation efforts (98). The Vietnamese government endorsed the "National plan for the prevention and control of African swine fever for the period 2020–2025," which defines the ASF management process from farm biosecurity adaptations to laboratory capacity development in July 2020 (142). The plan includes the application of partial culling due to the difficulty of applying this measure to all animals. This approach has the great advantage of significantly reducing livestock losses, nevertheless, it can increase the risk of a prolonged disease epidemic period unless high biosecurity levels can be maintained (143, 144). Furthermore, infected farms tend to retain and raise recovered pigs to minimize losses and shorten the time to reintroduction. Recovered pigs can progress to chronic infections and thus are a potential source of infection, contributing to the current endemic situation in Vietnam (145).

There is scant information describing the role of wild boars in ASF transmission in Southeast Asia, however, their presence throughout the region suggests the high possibility of spreading and sustaining the ASFV (127). The fewer ASF notifications in wild boars in Southeast Asia are inexplicable given their high densities, gregarious social behavior, opportunities for contact with domestic pigs, and the landscapes they occupy (146). Given reports of contact between free-ranging pigs and wild boars in rural areas, besides the lack of adequate surveillance systems, they may play a role in the spread and maintenance of the disease (96). Indeed, a study that spatially quantified the predicted risk of ASFV infection in wild boars across Asia identified Southeast Asia as concentrating the highest risk areas (72).

5. ASF subregional update in South Asia

5.1. ASF epidemic status

South Asia is a relatively new region for the emergence of ASF, with the disease confirmed in three countries to date (India, Bhutan, and Nepal). Compared with other Asian regions with outbreaks spreading across the entire region, the spatial distribution of ASF is centered in the northeast area, which appears to be gradually spreading westward (Figure 7). The first ASF infection was confirmed in India in January 2020. Abnormal swine mortality was reported in Assam and Arunachal Pradesh in early January 2020 and later diagnosed as ASF positive (147). The virus strains isolated were 100% identical in nucleotide sequence to ASFV in Asia and Europe, including China, South Korea, Vietnam, Georgia, and Hungary (148). The following year, Bhutan became infected in May, and Nepal confirmed its first case of ASF in March 2022. The precise decrease in pig numbers due to ASF is unknown, but approximately 54,000 pigs died by July 2021 in India (149). Throughout the region, 130 notifications have been reported to FAOEMPRES-i to date, all from domestic pigs except one case from wild boar. In 2022, 63 notifications, the highest number to date, were recorded, owing to the large number of outbreaks observed in Nepal, along with ongoing outbreaks in India. As of the end of June 2023, outbreaks continue to be reported from various areas.

5.2. Pig industry and wild boar distribution

5.2.1. Pig industry

Approximately 9 million pigs are raised in India, 45% of which are in the northeastern states (150). The northeastern region has the largest pig population, followed by eastern, southern, central, northern, and some western regions of India (147). About 90% of pigs are raised by resource-poor smallholder farmers (149), and pig farming is of great importance for the livelihoods of the rural poor, especially in these states (148). Swill feeding is common, with pigs roaming freely for food in both rural and urban areas. Among small farmers, traders usually travel between villages to collect pigs and bring them to livestock markets and slaughterhouses (151). Commercial pig farms with large-scale pig production in India are scarce and are mostly found in peri-urban areas (152).

The demand for the pork industry in Nepal has increased significantly in recent years, with the number of pigs increasing from 1.1 million in 2011 to 1.6 million in 2021 (153). Although there are some modern pig farms, the majority of these are dominated by small-scale farmers (154). In common with pig production in India, most pigs are raised by scavenging activities utilizing food waste (155). Many of these pigs are slaughtered on their farms due to the lack of slaughterhouses (156).

5.2.2. Wild boar distribution

Information on wild boar populations and distribution throughout South Asia is not available. However, the Indian crested boar (*Sus scrofa cristatus*) is found in most protected wildlife areas and is widely distributed in India, Sri Lanka, Nepal, Thailand, and Myanmar (157). The northeastern states of India, especially those with forest cover exceeding 65% and large wild boar populations, are considered a major threat to the spread of ASF infection (147, 149, 158).

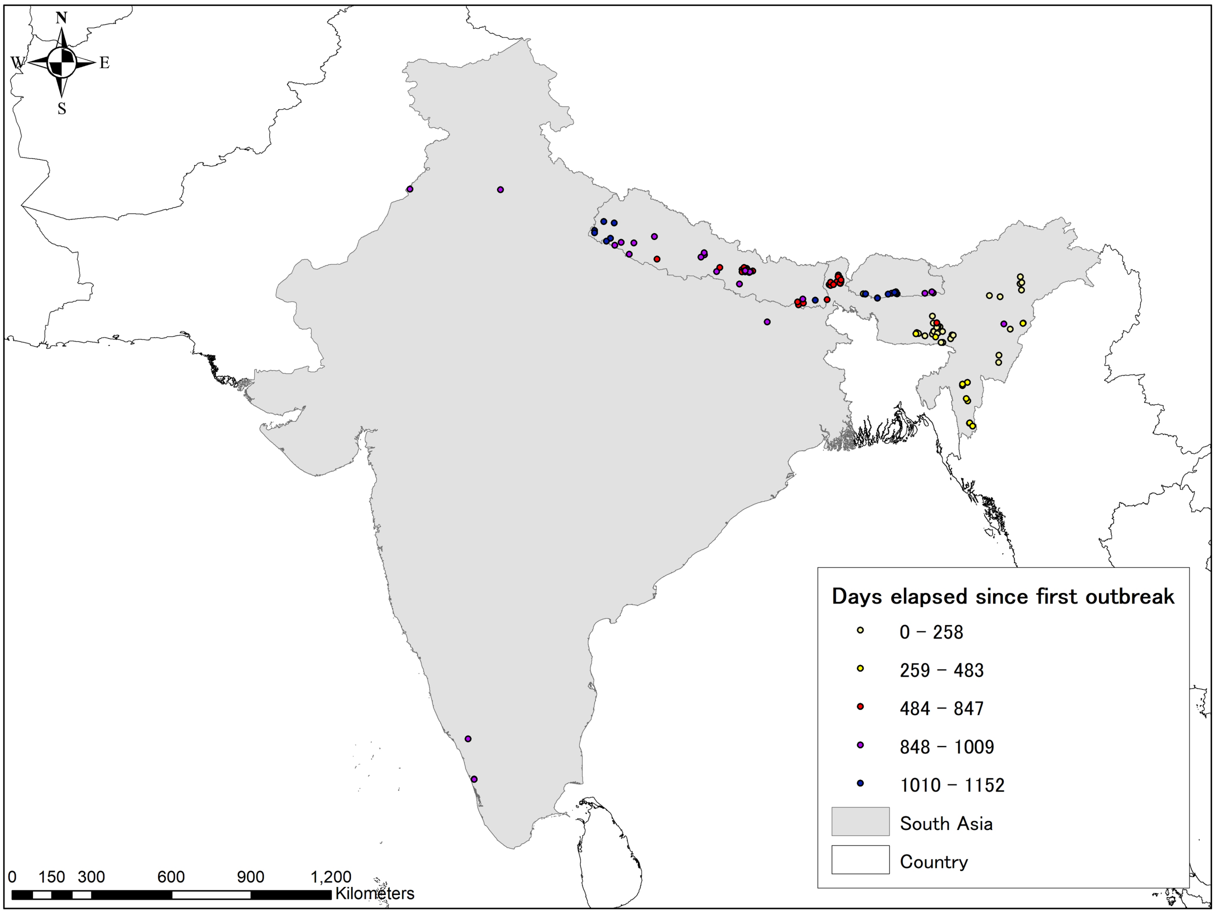


FIGURE 7
ASF evolution in South Asia as of June 30, 2023, based on the EMPRES-i database. Lighter colors indicate earlier stages, darker colors indicate more recent occurrences in this region. The map was depicted using ArcGIS 10.8.1 (ESRI, Redlands, CA, United States).

5.3. Risk factors and control measures

Many rural farmers lack general knowledge about infectious diseases and often fail to report infections. Subsequently, the risk of disease spread is high when animals from uncertain sources are purchased. In most rural and remote areas, pigs are slaughtered on home grounds or in open meat markets in the absence of organized abattoirs, and the run-off derived from these slaughterhouses is directly accessible to animals. Free-range pig production, the movement of virus-contaminated pigs, and lack of basic biosecurity measures are major risk factors in India, as in other Asian countries (147). In Nepal, the first ASF outbreaks in various swine production areas in the Kathmandu Valley were suspected to be caused by swill feeding (159).

Many of the Northeastern states of India share borders with Tibet, China, Myanmar, and Bangladesh, and there are no restrictions on the movement of people or goods, thus posing a continuous risk of ASF introduction into the country (150, 160). This is shared with other countries, and Nepal also suggests a risk of pigs entering illegally across the border. Additionally, there is a continuous transmission risk of ASF to wild boars via forest routes adjacent to India-Nepal National Parks and Reserves (154).

No official cases of ASF in wild boars have yet been reported in India, nonetheless, a wild boar carcass found in a northeastern state was positive for ASF (161). It is more likely that the disease originated from infected domestic pigs rather than spreading among wild boars. As confirmed in other countries, disposal of infected carcasses in rivers during the early stages of the epidemic may have caused further spread of the disease (147). ASF outbreaks have been reported around the Brahmaputra River, a tributary of which flows through national parks and wildlife sanctuaries in northeastern India. Most of the densely distributed domestic pigs in this area are backyard farms with inadequate biosecurity measures and are a major threat to the wild boars that abound in this area (147). In addition, several states in the northeastern region are prone to flooding, raising concerns about the spread of ASF associated with animal movements (149). It is hypothesized that the early ASF outbreaks in India involved wild boars (direct transmission among wild boars, indirect transmission via their habitat, and contact between wild boars and domestic pigs) and the subsequent domestic transmission cycle involved disease transmission among domestic pigs via contaminated pig products/fomites (150).

There is limited research on soft ticks, particularly *Ornithodoros* species, in the region; their geographic distribution is yet to be defined. Moreover, there is no official evidence of the involvement of *Ornithodoros* species in the current ASF outbreak in northeastern India. However, studies associated with soft tick distribution modeling are considered very important for disease prevention (147).

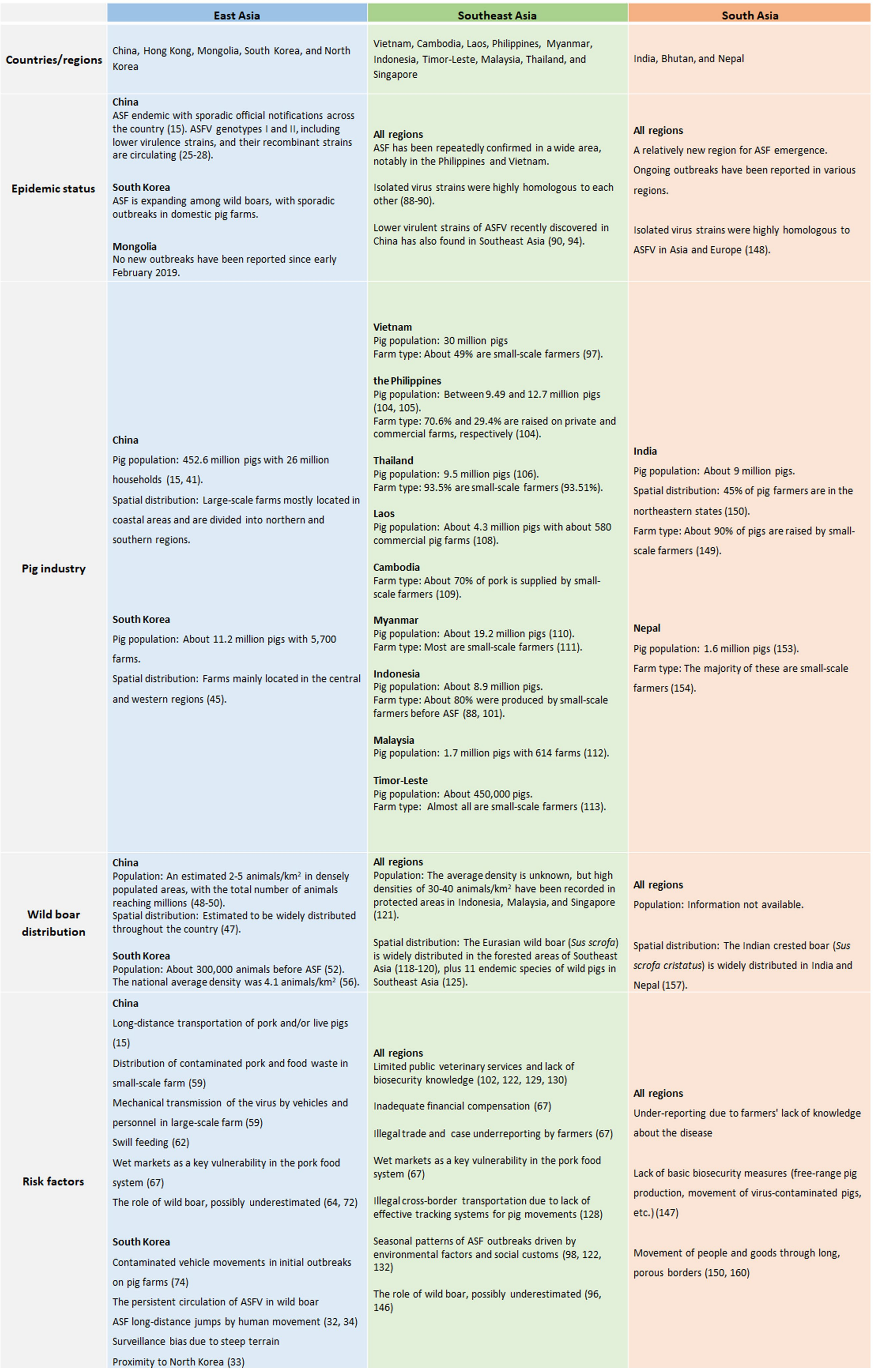
6. Discussion

The epidemiological status and related information for each of the regions described above are briefly summarized in Figure 8. The ASF epidemic situation in Asia has become more complex and disease control more challenging. Apart from Mongolia, where all ASF events have been resolved, the disease is still widely distributed throughout affected Asian countries. As the world's top pork-producing countries include China and other Asian countries such as Vietnam, South Korea, Japan, and the Philippines, more serious consequences for the entire global swine industry can be anticipated. Small-scale farmers with low biosecurity levels have traditionally played an important role in pork production in most Asian countries, implying that ASF management will be challenging. Note that this is not a problem exclusive to Asia, it is observed worldwide.

The information available regarding wild boars is limited, mostly sourced from South Korea, where wild boars play a major role in the expansion of ASF. However, this does not imply that the ASF risk of wild boar should be neglected in the other countries. Wild boars are abundant throughout Asia, and cases have been officially reported in half of the 18 ASF-affected countries/regions. In many countries, limited resources are allocated to wildlife surveillance (162), and, therefore, the potential underestimation of the wildlife epidemiological situation should also be fully considered (121, 123). Risk factors and their priorities differ among these countries, as this review has shown. ASF management strategies should aim to accommodate differences in swine husbandry, wild boar distribution, priority risk, culture, and social values across regions.

In this review, we have summarized the ASF outbreak situation in Asia based on officially reported information. However, the number of notifications does not always accurately reflect the epidemic status (162). For example, if a disease is endemic in a country's territory, the WOAH standard allows these diseases to be reported in a six-monthly report (163). Also, each country has its own epidemiological unit for disease reporting, thus caution is required when interpreting simply by comparing report numbers. As a result, the number of disease notifications differs between WAHIS, EMPRES-i, and the country's own databases, as queried by some literature (6, 7, 80, 98). Such discrepancies should always be considered, along with underreporting at the point of data collection. As small and medium-sized farms (<500 head) account for 99% of the swine industry in China, a complete and accurate picture of the number of slaughters and deaths on these farms is challenging to obtain (164). As mentioned above, backyard farms and small farms are the norm in affected Asian countries, hence this concern is likely to be common to most countries. One of the feared possibilities is that the disease becomes endemic, with periodic outbreaks affecting the food system (47). In some countries, the number of outbreaks has already subsided, with only sporadic reports from various locations; however, it has not been determined whether this is due to the data gap or reflects the actual situation. This review is based on publicly accessible information and published literature, which biases the amount of information by region. Paradoxically, this underscores the need for further research.

Before 2018, ASF was mainly distributed in Africa and Europe. The current epidemic status and the significance of the swine industry indicate that Asia and Europe are most likely to be the main players in the ASF epidemic for a time to come. The two regions are closely linked historically and geographically and have much in common. As the ASF expansion in Europe has influenced the emergence of ASF in Asia, the Asian ASF epidemic is surely a new concern for Europe, as well as the rest of the world. ASF control remains a top priority for the WOAH and FAO, hence initiatives are underway within the Global Framework for the Progressive Control of Transboundary Animal



	East Asia	Southeast Asia	South Asia
Countries/regions	China, Hong Kong, Mongolia, South Korea, and North Korea	Vietnam, Cambodia, Laos, Philippines, Myanmar, Indonesia, Timor-Leste, Malaysia, Thailand, and Singapore	India, Bhutan, and Nepal
Epidemic status	**China** ASF endemic with sporadic official notifications across the country (15). ASFV genotypes I and II, including lower virulence strains, and their recombinant strains are circulating (25-28). **South Korea** ASF is expanding among wild boars, with sporadic outbreaks in domestic pig farms. **Mongolia** No new outbreaks have been reported since early February 2019.	**All regions** ASF has been repeatedly confirmed in a wide area, notably in the Philippines and Vietnam. Isolated virus strains were highly homologous to each other (88-90). Lower virulent strains of ASFV recently discovered in China has also found in Southeast Asia (90, 94).	**All regions** A relatively new region for ASF emergence. Ongoing outbreaks have been reported in various regions. Isolated virus strains were highly homologous to ASFV in Asia and Europe (148).
Pig industry	**China** Pig population: 452.6 million pigs with 26 million households (15, 41). Spatial distribution: Large-scale farms mostly located in coastal areas and are divided into northern and southern regions. **South Korea** Pig population: About 11.2 million pigs with 5,700 farms. Spatial distribution: Farms mainly located in the central and western regions (45).	**Vietnam** Pig population: 30 million pigs Farm type: About 49% are small-scale farmers (97). **the Philippines** Pig population: Between 9.49 and 12.7 million pigs (104, 105). Farm type: 70.6% and 29.4% are raised on private and commercial farms, respectively (104). **Thailand** Pig population: 9.5 million pigs (106). Farm type: 93.5% are small-scale farmers (93.51%). **Laos** Pig population: About 4.3 million pigs with about 580 commercial pig farms (108). **Cambodia** Farm type: About 70% of pork is supplied by small-scale farmers (109). **Myanmar** Pig population: About 19.2 million pigs (110). Farm type: Most are small-scale farmers (111). **Indonesia** Pig population: About 8.9 million pigs. Farm type: About 80% were produced by small-scale farmers before ASF (88, 101). **Malaysia** Pig population: 1.7 million pigs with 614 farms (112). **Timor-Leste** Pig population: About 450,000 pigs. Farm type: Almost all are small-scale farmers (113).	**India** Pig population: About 9 million pigs. Spatial distribution: 45% of pig farmers are in the northeastern states (150). Farm type: About 90% of pigs are raised by small-scale farmers (149). **Nepal** Pig population: 1.6 million pigs (153). Farm type: The majority of these are small-scale farmers (154).
Wild boar distribution	**China** Population: An estimated 2-5 animals/km² in densely populated areas, with the total number of animals reaching millions (48-50). Spatial distribution: Estimated to be widely distributed throughout the country (47). **South Korea** Population: About 300,000 animals before ASF (52). The national average density was 4.1 animals/km² (56).	**All regions** Population: The average density is unknown, but high densities of 30-40 animals/km² have been recorded in protected areas in Indonesia, Malaysia, and Singapore (121). Spatial distribution: The Eurasian wild boar (*Sus scrofa*) is widely distributed in the forested areas of Southeast Asia (118-120), plus 11 endemic species of wild pigs in Southeast Asia (125).	**All regions** Population: Information not available. Spatial distribution: The Indian crested boar (*Sus scrofa cristatus*) is widely distributed in India and Nepal (157).
Risk factors	**China** Long-distance transportation of pork and/or live pigs (15) Distribution of contaminated pork and food waste in small-scale farm (59) Mechanical transmission of the virus by vehicles and personnel in large-scale farm (59) Swill feeding (62) Wet markets as a key vulnerability in the pork food system (67) The role of wild boar, possibly underestimated (64, 72) **South Korea** Contaminated vehicle movements in initial outbreaks on pig farms (74) The persistent circulation of ASFV in wild boar ASF long-distance jumps by human movement (32, 34) Surveillance bias due to steep terrain Proximity to North Korea (33)	**All regions** Limited public veterinary services and lack of biosecurity knowledge (102, 122, 129, 130) Inadequate financial compensation (67) Illegal trade and case underreporting by farmers (67) Wet markets as a key vulnerability in the pork food system (67) Illegal cross-border transportation due to lack of effective tracking systems for pig movements (128) Seasonal patterns of ASF outbreaks driven by environmental factors and social customs (98, 122, 132) The role of wild boar, possibly underestimated (96, 146)	**All regions** Under-reporting due to farmers' lack of knowledge about the disease Lack of basic biosecurity measures (free-range pig production, movement of virus-contaminated pigs, etc.) (147) Movement of people and goods through long, porous borders (150, 160)

FIGURE 8
Brief summary of the epidemiological situation and relevant information for each region presented in this study.

Diseases (GF-TADs) to implement risk-based control strategies on a regional scale. This includes technical assistance to Asian countries for ASF diagnosis and epidemiological interpretation of the situation (165, 166). Much has been learned in Europe over the past 16 years, yet not enough to contain the disease. In Asia, where this disease is spreading at an unprecedented rate, the importance of cooperation and collaboration between countries is emphasized, along with greater efforts for disease control (167). There is much to be learned from this experience to prevent another disaster.

Author contributions

SI: Conceptualization, Writing – original draft, Writing – review & editing. NK: Conceptualization, Writing – review & editing. JB: Writing – review & editing. CA-V: Writing – review & editing. JS-V: Supervision, Validation, Writing – review & editing.

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Conflict of interest

Publisher's note

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Retrospective investigation of the 2019 African swine fever epidemic within smallholder pig farms in Oudomxay province, Lao PDR

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The 2019 African swine fever (ASF) outbreak in the Lao People's Democratic Republic (Lao PDR or Laos) represented a major epidemiologic event where a transitioning lower-middle income nation (LMIC) experienced a viral epidemic in a naïve pig population. The diversity of pig management styles creates challenges for local and regional policymakers when formulating recommendations to control an ASF outbreak. The aim of this study were to investigate the management of pigs in villages of Oudomxay province that were affected by ASF in 2019, as a case study in a smallholder pig-rasing system in northern Laos. The frequencies of well known risk factors were measured in the affected villages and the timelines and household level stock losses due to the outbreak were investigated. These findings were compared to data available from a similar outbreak in the southern province of Savannakhet. Disease control implications of these findings are discussed. Mean losses were 3.0–23.3 pigs per household, with a mean lost herd value of USD 349, 95% CI (294–415). These pig losses reflect those estimated in Savannakhet (6.7 pigs per household). However, the financial loss estimated per household was higher, USD 349 versus USD 215, possibly due to higher pig values and a higher input/output management approach in Oudomxay. The investigation revealed the presence of numerous ASF risk factors, such as swill-feeding and free-ranging. In addition, poor biosecurity practices – such as inappropriate garbage disposal and slaughtering – that could contaminate the environment were present. ASF cases occurred across all villages between June and December 2019, with outbreak periods ranging from 22–103 days. These values are consistent with the outbreak in Savannakhet; however, notable differences in management styles were observed. These findings demonstrate the need for more disease control resources from the village to the Governmental level. Villages need support in enacting context appropriate biosecurity measures, whilst the ongoing surveillance and investigation of ASF require investment in logistical and veterinary resources at the Governmental level.

KEYWORDS

African swine fever, animal health economics, Lao PDR, pig production, smallholder, village, outbreak investigation

1. Introduction

The 2019 African swine fever (ASF) outbreak in the Lao People's Democratic Republic (Lao PDR or Laos) remains a unique epidemiologic event where a transitioning lower-middle income nation (LMIC) experienced a viral epidemic in a naïve pig population. The first published data on the village-level impacts of this outbreak were collected in ASF-affected villages in the Southern province of Savannakhet (1).

Laos is a nation known for its diversity. While broadly classified as Lao-Tai, Hmong-Mein, Tibeto-Burman and Mon-Khmer, the Lao government recognises an additional forty-nine minority ethnic groups making up the Lao people, each with unique cultural practices, languages, and agricultural management styles (2, 3). There are distinct differences in the farming styles of those living in the Mekong floodplains and those in the mountainous regions that dominate the northern segment of the country (4). The diversity of pig management styles creates challenges for local and regional policymakers when formulating recommendations to control an ASF outbreak. For example, very few households reported the practice of swill-feeding their pigs in the Savannakhet ASF outbreak (1). Given this context, actions to prevent ASF might be adjusted to be more context appropriate. For example, the expenditure of minimal resources to prevent swill-feeding might be of lower priority when large amounts of foreign trader activity occur simultaneously (1).

In 2019, more than 150 confirmed ASF outbreaks occurred across Laos in just over six months (5). This followed the arrival of ASF in China from the Caucasus in 2018, with its subsequent spread to Vietnam, Cambodia in 2019 and more recently to Thailand (6). Due to the scale of the outbreak, an in-depth retrospective outbreak investigation to examine possible causative agents, the presence of known ASF risk factors, and impacts upon livelihoods was not possible. The risk factors for an outbreak are likely to vary between regions, requiring an investigation of ASF epidemic risk factors in a representative "northern" region. Based on the information generated, appropriate biosecurity recommendations can be made to prevent future ASF outbreaks by recognising the diversity of Laos' pig farming communities.

The aim of this study was to investigate the management of pigs in villages of Oudomxay province (northern Laos) that were affected by ASF in 2019. The region was recommended by local animal health stakeholders as a good representation of the outbreak in northern Laos, in tandem with the work peformed in southern Laos. The frequencies of well known risk factors and other challenges to biosecurity in the management styles of the affected villages were assessed, and the timelines and household level stock losses due to the outbreak were investigated. In addition, these findings were compared to ASF data available from Savannakhet province (southern Laos). The disease control implications of these findings are discussed.

2. Materials and methods

2.1. Prior outbreak data

The Lao animal health services are governed by the Department of Livestock and Fisheries (DLF) of the Ministry of Agriculture, Forestry and Fisheries. At the village level, the Village Veterinary Worker (VVW) is a lay-person who has received a small amount of training from governmental and non-governmental organisations in basic veterinary care and diagnostics, they are the first person most villagers will consult upon illness in their animals (7). Upon the outbreak of ASF in the smallholder villages of Thapangtong district, the local VVW reported the unusual clinical signs and mortalities to their local District Agriculture and Fishery Office (DAFO), which in turn reported to the Provincial Agriculture and Fishery Office (PAFO) of the DLF (1). The initial outbreak investigations were performed by the DAFO/PAFO teams, who sent diagnostic samples and an outbreak report to the National Animal Health Laboratory (NAHL) and DLF, respectively. Upon confirmation, these already resource-poor teams returned to perform prevention and control activities such as culling, disinfection, movement controls and public awareness campaigns (1).

The villages included in this investigation were a census of all village-level outbreaks in Oudomxay province, where multiple households were affected ($n = $ seven villages, official reporting data in Table 1). Oudomxay province was chosen purposively on the recommendation of the DLF both due to the high number of ASF cases and the availability of veterinary resources to perform questionnaires. All confirmed case villages tested positive Taqman rt-PCR for ASF on whole blood samples of clinically affected pigs at the Lao National Animal Health Laboratory (NAHL) (8). The locations of the villages where latitude and longitudinal data were available are shown in Figure 1. The location data was obtained from the 2011 Lao Agricultural Census and the DIVA-GIS Gazetteer (9).

The data collected by the Oudomxay PAFO at the time of the initial outbreak investigation in 2019 are presented in Table 1, based on a central spreadsheet (Microsoft Excel) kept by the Oudomxay PAFO team. Upon their arrival at an ASF suspected village, local investigators sampled a small number of pigs showing ASF-like clinical signs. However, the number of pigs and the households sampled were not recorded. The team recorded the total number of pig-owning households in the village, the number of pigs at risk, the number of households affected (household morbidity) and the number of pigs that had already died (mortality).

After an initial investigation, the PAFO team sent samples to the NAHL for testing and a report to the DLF, which are recorded in Table 2. The DLF Found Date was obtained from the PAFO records, the NAHL report date and diagnosis date were collated from the NAHL's Pathogen Asset Control System, a diagnostic sample inventory database.

TABLE 1 Morbidity and mortality data captured by the Oudomxay PAFO staff in the 2019 ASF outbreak.

Village	Affected households (total households)	Affected pigs (total pigs at risk)	Morbidity (household)	Mortality (pigs)	Deaths/ affected HH[1]
Doneant	15 (101)	55 (101)	15%	54%	3.7
Homsouk	9 (93)	509 (725)	10%	70%	56.6
Houythong	19 (50)	144 (162)	38%	89%	7.6
Huanamkham	49 (80)	236 (273)	61%	86%	4.8
Huaycharng	12 (68)	223 (316)	18%	71%	18.6
Huaylerm	58 (101)	167 (169)	57%	99%	2.9
Pangthong	12 (68)	32 (181)	18%	18%	2.7
Total	174 (561)	1,366 (1927)	31%	71%	7.9

[1] HH – household.

2.2. Sampling methodology

The study period was defined as 1 June 2019 to 1 January 2020. A case village was a village with PCR confirmation of ASF in porcine blood samples during the study period based on the NAHL diagnosis date. A case household was defined as any household where pigs displayed signs of ASF, as defined in Sánchez-Vizcaíno et al. (10), and a case animal displayed clinical signs of ASF. Ethics approval for the questionnaires was obtained from the University of Sydney Human Ethics Committee under approval number (2019/725).

Where villages had less than twenty-five households affected by ASF, the investigators aimed to perform as close to a household-level census as possible. In the case of a village with more than twenty-five ASF-affected households, the same methods were used as those in Matsumoto et al. (1). The maximum number of interviews was determined for simplicity of instruction to the field teams and ability to complete surveys within the allocated days. The Village Chief or VVW drew up a list of all available ASF-affected households. A random number generator (Microsoft Excel) was used to select a list of twenty-five households. In both scenarios, some households were not available on the days of the questionnaire and replacements could not be found, and they were not included in the investigation. Furthermore, some ASF-affected households fell outside of the study period or clinical signs consistent with ASF were not observed. Of the 161 participant households, 108 met the case definition for ASF-affected in this study, and only their data is presented in the outbreak investigation section of this paper. However, the data from all 161 households are still presented for informative purposes relating to the biosecurity and management of pigs in the affected villages.

2.3. Questionnaire design and administration

The questionnaire comprised twenty-eight open and closed questions on the households' herd size, structure, price value, management styles (encompassing feeding, housing, and health practices), trading history prior to the outbreak and disease history during the outbreak period. Questions were designed based upon literature review of risk factors for ASF, with a focus on gaining relevant epidemiologic data for an outbreak investigation. A detailed explanation of the questionnaire design process, which included a round of pilot testing prior to the final questionnaires performed in Savannakhet province in 2019, and a copy of the survey can be found in Matsumoto et al. (1, 11).

The data presented here is at the household level; however, the epidemic curves present the total number of pig mortalities per day since there was notable variation in the herd sizes between households and therefore the number of daily mortalities.

Travel restrictions within Laos substantially delayed the delivery of the questionnaires during the SARS-CoV 2019 pandemic; as such, all questionnaires were completed in December 2020. The questionnaire delivery otherwise mirrored that of Matsumoto et al. (1). The questionnaires were performed in the Lao language by DLF officers from PAFO and DAFO. After the questionnaire, each participant was given an educational t-shirt about ASF in pigs to recognise the donation of their time.

2.4. Data handling and analysis

All questionnaires were translated from Lao to English and entered into Microsoft Excel by a team of Lao veterinarians working at NAHL. The data were then exported into RStudio for cleaning, descriptive analyses, financial analysis and outbreak investigation (12).

Financial losses were calculated using the questionnaire data, by summing the farmer reported value of the pigs lost during the outbreak. For example if they reported owning one sow worth 800,000 Lao kip (LAK) and two piglets worth 100,000 LAK each, the lost herd value was 1,000,000 LAK. The value was then converted into 2019 USD for reporting (8814.07 LAK to 1 USD). This was then reported as the lost herd value.

3. Results

3.1. Pig management and biosecurity practices prior to the outbreak

3.1.1. Overall herd demography

Across the seven villages, there was considerable variation in the herd sizes of households. For this reason, the average herd demography

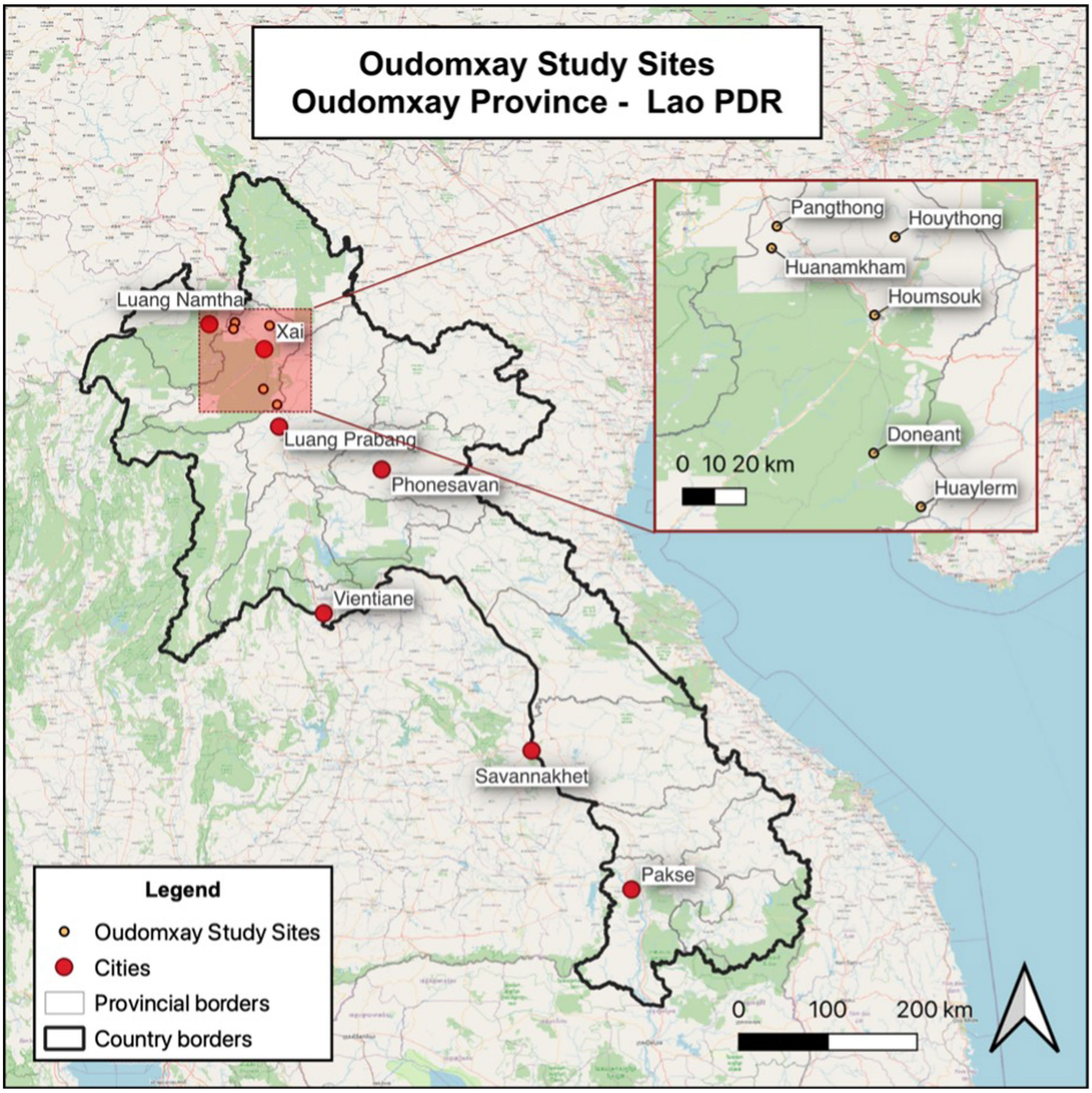

FIGURE 1
Map of Oudomxay province showing 2019 ASF outbreak locations; Huaycharng coordinates not available.

TABLE 2 Reporting dates from the DLF and NAHL for villages affected by the 2019 ASF outbreak in Oudomxay, Laos.

Village	DLF found date	NAHL report date	NAHL diagnosis
Doneant	8/08/2019	4/08/2019	6/08/2019
Homsouk	19/07/2019	18/07/2019	19/07/2019
Houythong	1/08/2019	1/08/2019	1/08/2019
Huanamkham	16/08/2019	29/08/2019	30/08/2019
Huaycharng	20/06/2019	–	–
Huaylerm	29/07/2019	28/07/2019	28/07/2019
Pangthong	2/08/2019	3/08/2019	3/08/2019

is presented here by herd size from large (more than nine pigs) to very small (less than three pigs) (Table 3). The sizes demonstrated here were chosen based on the previous data in Savannakhet where households tended to own a sow with one to six of her piglets (1). Larger and medium herds tended to include more fattening pigs and have more piglets per sow, while smaller herds had equivalent numbers or more sows than piglets. Very few households owned a boar.

3.1.2. Housing styles

The questionnaire participants typically kept their pigs in household-based pens (72.7%). Another 14.9% kept their pigs in some form of free-ranging, including free-ranging during the day and penning them at night. Other housing methods included multi-household pens, tethering the pigs in the garden, or keeping the pigs

TABLE 3 Pig herd demography of ASF-affected villages in Oudomxay, Laos.

Herd size (total no. pigs)	HH[1]	Average herd size (pigs)	Piglets	Fatteners	Sows	Boar
Large (>9)	33	16.4	12.1	1.5	1.9	0.9
Medium (7–9)	38	7.9	4.5	1.1	1.6	0.7
Small (3–6)	53	4.3	1.7	1.0	1.3	0.4
Very small (<3)	37	1.4	0.2	0.6	0.7	0.0

[1]HH – households.

TABLE 4 Housing styles in ASF-affected villages of Oudomxay, Laos.

Housing type	*N*	%	Average weekly cost (USD)
Penned – communal pen	20	12.4	11.55
Free-range	24	14.9	11.77
Other*	31	19.3	25.77
Penned – own pigs	117	72.7	11.34
No answer	1	0.6	0

*For respondants that did not identify their pig housing style as penned or free-range.

in the crawl space under the house (Table 4). It was common for farmers to provide multiple housing methods from the list of responses (i.e., free-range AND tethered) for their pigs, likely reflecting seasonal changes in housing styles reported by Okello et al. (13).

3.1.3. Feeding and water sources

The participants reported feeding commercial brand diets (19.9%), local vegetable products (15.5%) or a mixture of the commercial and vegetable diets (14.3%). To determine the frequency of pig exposure to potentially infective meat, smallholders were asked "Do you feed swill to your pigs?" with the follow up question "If yes do you cook it beforehand?," as well as "Do you feed household scraps to your pigs." Swill feeding (1.9%) and household scrap feeding (37.3%) were both reported. An additional ten households (6.2%) did not respond to the direct question of swill feeding their pigs, but then reported cooking the swill that they fed to their pigs, and another three reported that they did not cook the swill or were not sure (1.9%).

Commercial diets such as that of Charoen Pokphand Foods and Thai and Vietnamese brands were reported as common pig feeds, often combined rather than brand exclusive. Local vegetable products mentioned included rice and rice bran, maize, cassava, pumpkins, and banana tree flowers/stems. The most common primary water source was the local river (22.8%) or a communal well in the village (21.0%). It was unclear in the data if the pigs could freely access these water sources or if the water was transported to the pigs' enclosures.

3.1.4. Local slaughtering practices

Across the study population, many households did not routinely slaughter their own pigs (40.4%). Of the households that reported home slaughtering of their stock for food or ceremonial purposes, the majority (39.8%) slaughtered one to three pigs annually in an area outside the home (Table 5). Lao regulations state that all slaughter should occur at an official slaughterpoint, and home slaughter can only occur for ceremonial reasons and under the supervision of the VC and VVW (14).

Qualitative information on the utilisation of home-killed pork demonstrated that most tissues (blood, skin, viscera, bones and offal) were kept for food. A small number of questionnaire respondents reported leaving blood or viscera (1.6% and 0.8%, respectively) on the ground. The practice of feeding pork bones to the dogs in the village was relatively common (31.6%).

3.1.5. Trading activities

Four smallholder pig trading activities were recorded during the study period in Doneant, Huaycharng and Huaylerm villages. However, smallholder participants also reported numerous trading activities outside of the study period, ranging November 2018 – November 2020 (thirty purchases, two sales and thirteen who reported a date of trading without providing further information). Only three were reported from local traders; the rest were either transactions between neighbours or no answer was given. The trader purchases all occurred in Homsouk village in 2020. The average number of pigs bought in a transaction was 4.8, and the average number sold was 3.5. Prior to trading, pig owners are required to complete a number of health, vaccination and ownership transfer certificates – however further questioning on this topic was outside the scope of the survey (14).

3.1.6. Pig contact structures

Some of the surveyed smallholders in Oudomxay reported their pigs had contact with their neighbours' pigs and with wild boar daily (4% and 8%, respectively). However an additional 92% provided no answers for the question on their neighbours' pigs, and 93% did not answer the question on wild boar.

3.1.7. Disease management

VVWs attended 5.0% of households during the study period. None of the VVW visits occurred on the same days as one another. Smallholder farmers reported a wide range of common therapeutic agents for pigs, such as penicillin–streptomycin and oxytetracycline. Five farms (3.1%) performed routine piglet prophylactic care, such as iron and vitamin injections. Households spent an annual average of USD 10.59 on medications and USD 3.02 on vaccines for their pigs.

In the event of a disease outbreak, the questionnaire participants were asked to describe how they disposed of the carcasses of disease-affected animals. Most participants buried their dead pigs at depths ranging from less than one metre to greater than two metres, with the most common depth being one to one-and-a-half metres (91.8%). Other disposal methods included burning the carcasses (2.0%) or retaining the carcass for consumption (0.7%).

3.2. 2019 African swine fever outbreak

3.2.1. DLF outbreak data

The Oudomxay PAFO of the DLF provided the outbreak data they collected during the ASF epidemic, including data on the numbers of affected pigs and households (Tables 1, 2). The DLF reported finding the ASF-affected villages 20 June 2019–16 August 2019, whilst the NAHL received the reports of these cases 18 July 2019–29 August 2019; in some cases, the dates of case detection by the DLF were after receipt of an official report at the NAHL (*n* = three of six villages with NAHL report dates). Huaycharng village had no dates recorded and no explanation was provided. There is no centralised database to corroborate these dates.

The DLF provided population data for the study. In the study villages, 10%–61% of all households and 18%–99% of all pigs were clinically affected when the DLF commenced control measures (Tables 1, 2).

3.2.2. Questionnaire household outbreak data

The number of questionnaire participants affected by ASF and their herd sizes are shown in Table 6. Of the 161 participants, 108 fit the study case definition for households and pigs, and their data are presented here. The mean losses of pigs per household ranged from three to twenty-three mortalities, and the mean value of the lost herds across all households was USD 349, 95% CI [294, 415]. In Homsouk, Huaycharng and Pangthong, more survey participants fit the study's case definition for an ASF-affected household than were recorded in the original DLF data.

TABLE 5 Home slaughtering locations of ASF-affected villages in Oudomxay, Laos.

Butchering location	*N*	%
Home – inside area of the home	56	34.8
Home – outside area of the home	78	48.5
Village – dedicated area	1	0.6
No answer	26	16.2

The first reported mortalities consistent with ASF clinical signs occurred in July 2019 in Panthong village (Table 7). The epidemic curve (Figure 2) shows that peaks in mortalities occurred between July and October of 2019, with sporadic mortalities continuing through December 2019. The villages where the first reported mortality occurred on 1 August may not represent an accurate date, as the first date of the week or month was recorded if the participants could not remember an exact date (Table 7).

3.2.3. Clinical presentation

The average owner-reported interval between the onset of clinical signs and mortality was 3.6 days (IQR five days, Figure 3). Fifty-three households reported the clinical interval to be two days or less, suggestive of a peracute outbreak, whilst sixty-eight households reported clinical periods of three days or more, suggestive of more acute syndromes.

The questionnaire participants were asked to describe their affected pigs' early and late clinical signs. The most common early clinical signs reported were weakness (29.9%), sudden death (32.0%) and anorexia (25.9%). The most common late clinical signs were death (40.7%) and reddened body or reddened spots on the body (15.3% and 6.8%, respectively). Other late clinical signs observed included conjunctivitis, fever, salivation, tremors, and reddened papillae.

4. Discussion

4.1. Outbreak investigation

The outbreak periods where mortalities were occurring based on the questionnaire were 22–103 days, likely reflecting the variations in both number of households affected per village (nine to fifty-eight households in the DLF data) and the number of affected pigs across the villages (32–509 pigs in the DLF data). In addition to population size, differences in management styles, such as the prevalence of free-ranging or swill-feeding, likely influenced the speed of spread between households and, therefore, the variation in outbreak lengths. The outbreak periods in the smaller Oudomxay villages were similar to those of Savannakhet, with inter-quartile ranges of 5.5–35 days (1).

TABLE 6 Retrospective outbreak investigation questionnaire mortality data from households affected by the 2019 ASF outbreak in Oudomxay, Laos.

Village	No. participant HH[a]	Mortalities (no. pigs)	Mean losses/HH[a] (SD)	DLF reported cases (*n*, %)
Doneant	14	42	3 (2.4)	15 (93%)
Homsouk	10	233	23.3 (16.3)	9 (111%)[b]
Houythong	12	62	5.2 (2.9)	19 (63%)
Huanamkham	25	153	6.1 (2.8)	49 (51%)
Huaycharng	17	125	7.4 (4)	12 (142%)[b]
Huaylerm	14	60	4.3 (2.6)	58 (24%)
Pangthong	16	121	7.6 (3.8)	12 (133%)[b]
Overall	108	796	7.4 (7.7)	174 (62%)

[a] HH – household.

[b] Villages where more households fit the study case definition than reported in the DLF data.

NAHL reports and diagnosis dates, DLF dates and questionnaire response timelines varied across the villages. The DLF reported the outbreaks between 20 June and 16 August 2019, whilst the first mortalities with consistent clinical signs occurred between 10 July 2019 and 22 August 2019. A centralised disease reporting database and future work investigating the period of whole-village infectivity will aid in clarifying the dates and periods of outbreaks under investigation, and future work should aim to compare the risk factors between villages within the study.

The number of pig mortalities per household was similar in the Lao DLF and the questionnaire data, with discrepancies likely caused by recall bias and random error related to the specific participating households in larger villages. Mean losses were 3.0–23.3 pigs per household, with a mean lost herd value of USD 349, 95% CI [294, 415]. These pig losses are consistent with those of Savannakhet (6.7 pigs per household). However, the financial loss estimated in Savannakhet (USD 215 per household) is notably different (1). The variation in management styles between the two locations may be a plausible explanation for the differences in herd loss value. A full gross margin analysis would aid comparison of financial losses caused by the outbreaks at both locations, as feed inputs and health inputs were also higher in Oudomxay households than in Savannakhet households before the outbreak.

In managing the ASF outbreak, the Lao DLF performed culling and disinfection activities at the whole village level upon PCR confirmation of ASF. The NAHL lacked the resources to perform concurrent testing for similar clinical syndromes. The case definition for ASF in the individual pig was based on clinical signs rather than molecular diagnostics in this study. ASF clinical signs are notoriously non-specific, particularly in peracute and acute cases (10). It is possible that other similar diseases, such as Classical Swine Fever or Highly-Pathogenic Porcine Respiratory and Reproductive Syndrome Virus, were present and causing concurrent mortalities at the same time as the ASF outbreak. The study period was limited to June–December 2019 to reduce overestimating mortalities caused by similar diseases, to align with the first reported incursion of ASF into Laos in June 2019 (5). Ongoing active surveillance activities and abattoir-based surveillance would aid in better understanding the background disease load on smallholder pig farmers impacted by an ASF outbreak.

An additional and unexpected source of error in this study was the SARS-CoV-19 pandemic. The pandemic forced deployment of the questionnaires to be delayed by over 12 months. This long delay created difficulties for the farmers in remembering exact case numbers, clinical signs, and dates of events. Some of this recall may also be due to education levels or access to accurate data recording tools within smallholder villages. A centralised reporting system combined with lifetime traceability on animals would allow for more accurate timelines and calculations of stock losses in future outbreaks.

TABLE 7 Retrospective timeline data from households affected by the 2019 ASF outbreak in Oudomxay, Laos.

Village	Case dates			Outbreak period (days, IQR)
	First	Median	Last	
Doneant	22/09/2019	7/11/2019	21/12/2019	90 (48)
Homsouk	1/08/2019[1]	1/08/2019[1]	2/09/2019	32 (23)
Houythong	1/08/2019[1]	1/08/2019[1]	1/08/2019[1]	–
Huanamkham	1/08/2019[1]	20/08/2019	23/08/2019	22 (9)
Huaycharng	1/08/2019[1]	1/08/2019[1]	23/09/2019	53
Huaylerm	22/08/2019	2/09/2019	3/12/2019	103 (27)
Pangthong	10/07/2019	30/07/2019	4/08/2019	25 (5)
Overall	10/07/2019	1/08/2019	21/12/2019	164 (27)

[1] Household could not remember dates of cases.

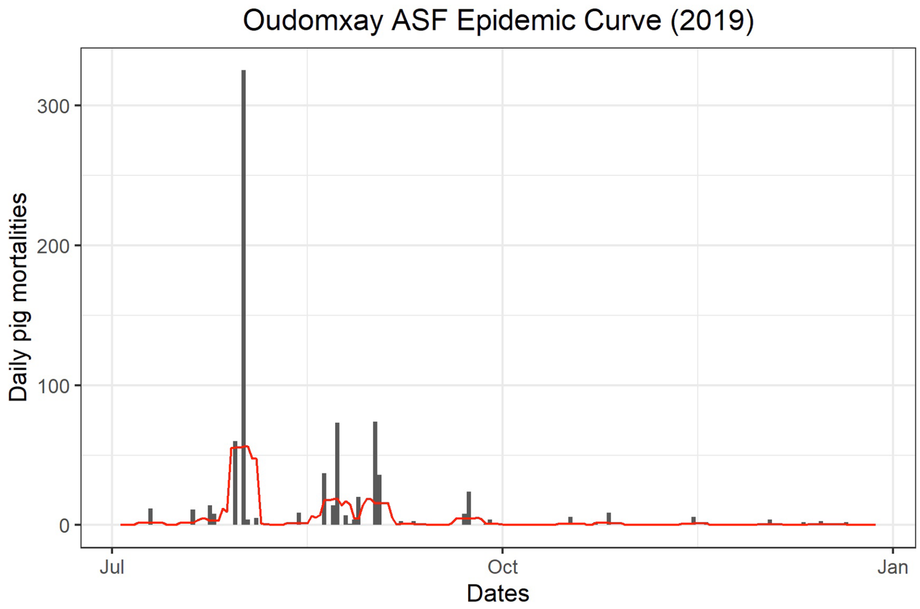

FIGURE 2
Epidemic curve of owner-reported pig mortalities during the 2019 ASF outbreak in Oudomxay, Laos.

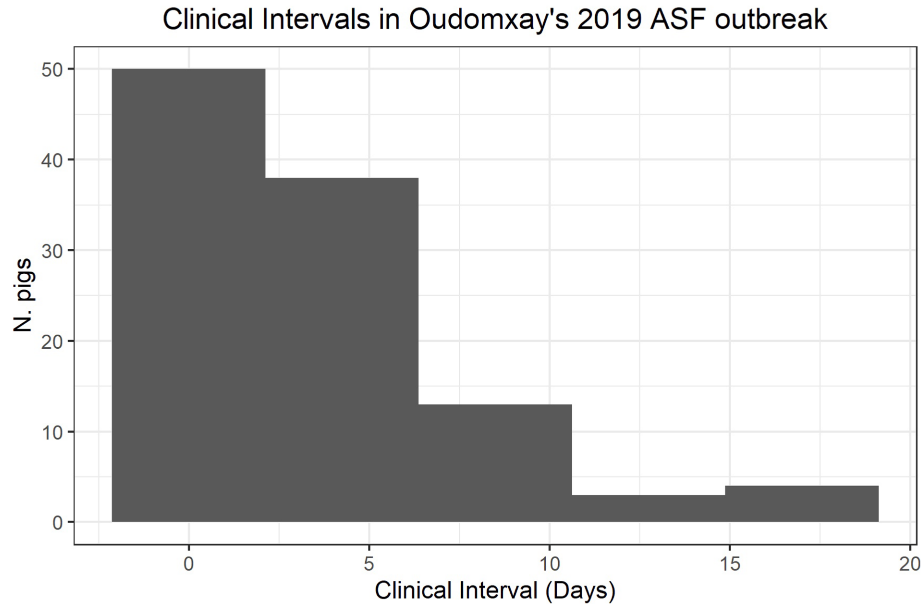

FIGURE 3
Intervals from the appearance of clinical signs to mortalities in pigs affected by the 2019 ASF outbreak in Oudomxay, Laos.

Additionally, training and resources for VVWs to record mortality or morbidity events in their communities would allow for cross-referencing by the local veterinary authorities in future studies. Further household training could include animal health management records, with disease events, mortalities and medication/vaccination administrations recorded in a simple wall calendar or similar. This could further be strengthened with production records to include reproductive outcomes such as farrowing dates and trading records for historical reference.

4.2. Investigation of management and risks to biosecurity

This study aimed to investigate management that could increase disease transmission risk through direct and indirect contacts, such as free-ranging or trader activities. Whilst only 1.9% of farmers reported feeding swill to their pigs, 37.3% reported feeding household scraps. An additional 6.2% reported cooking the swill, but did not report feeding swill. The confusion around these responses suggest that questions around feeding practices need to be clearly defined, given feeding household scraps *is* swill feeding. Slaughter products left out or improperly disinfected create a source of ASF environmental contamination. All tissues and secretions from the ASF-affected pig are infectious (15–17). The blood of a viraemic pig is extremely contagious, and ASFV contained in infected faeces can survive in the environment for up to 3.7–8.5 days, depending on the ambient temperature (15–17). Environmental contamination is of particular concern in smallholder settings where farmers may attempt to salvage pork meat products from their slaughtered or suddenly deceased animals. Many farmers (31.6%) reported giving pork bones to their dogs after slaughter, allowing parts of an infectious carcass to be spread well beyond the initial slaughter site and across the village. In scenarios where free-ranging occurs, the behaviour of domestic free-range pigs likely mirrors that of wild hogs, where they remain in family groups but come into contact at common resources (18, 19).

The use of common water sources noted in this study must be investigated further as a potential cause of spread. This complex interplay warrants close observation to understand all possible risk factors within the smallholder village. Supporting local veterinary workers in their biosecurity and consulting skills would provide a regionally relevant pathway to understanding the biosecurity challenges of smallholder pig farming.

The pig-raising styles in Oudomxay was notably different from Savannakhet prior to the 2019 ASF outbreak. Oudomxay households tended to keep more pigs in higher-density settings, with more money invested in feeding, medicating, and housing their pigs. Herd sizes were larger and of higher value than the previously surveyed ASF-affected households in Savannakhet. The increased value per pig is likely due to the higher value feed and medical inputs utilised amongst the Oudomxay participants, such as commercial brand diets and routine piglet care. Adding more nutrient-dense commercial feeds and vegetable crops to the diet of a village pig can improve both the growth rate of the piglet and the reproductive performance of a sow (20). In addition, routine piglet health care, such as iron injections and vitamin supplementation, is well reported to improve piglet survivability (21). In the large herds, the piglet-to-sow ratio was approximately six piglets per sow (1). Whilst not comparable to benchmarks for commercial operations, this is dramatically higher than the three piglets per sow reported in Savannakhet. Both estimates are comparable to similar studies in non-outbreak conditions. In a study of small farms (less than 30 sows) in the northern provinces of Xayaburi and Phongsaly, households reported an average of 7–7.2 piglets born alive and 4.3–6.2 piglets weaned per sow (22). The variation in value and herd sizes may be due to management styles or a more general shift in Lao farming styles reflecting the broader pattern of economic growth in the region. Ongoing assessment of both management and value chains can help pork stakeholders better understand the changing needs of their industry as Lao progresses beyond the status of a least-developed country. The reported low number of boar ownership would also warrant further investigation, as renting or borrowing boars, or practicing artificial insemination could also represent an ASF transmission risk factor, despite its lack of regulation in Lao PDR (14).

The medical protocols described in the questionnaire are of interest beyond investigating an ASF outbreak. Participant households reported using common veterinary antibiotics such as oxytetracycline and penicillin–streptomycin, with the majority reporting their use for weakness or fever as a two-to-three day course. Antibiotics are not recommended for treating ASF because it is a viral disease. Future studies into antimicrobial use amongst smallholders would be of value, as one smallholder reported using weekly oxytetracycline and vitamin injections on their fattening pigs. Prophylactic antimicrobial usage can effectively reduce the load of common respiratory pathogens in pigs and grow larger marketable animals. Oral antibiotics are common in commercial operations (23, 24). However, the routine use of antimicrobials on-farm significantly increases the risk of antimicrobial resistance (AMR) (25). Smallholder farming systems lack the resources to manage the emergence of AMR; in Timor-Leste, a study of smallholders found that only 12.7% understood what antibiotics were, and even fewer knew their mode of action (26). The utilisation of antimicrobials amongst Lao smallholders must be further studied to protect the future of smallholder pig health and welfare.

Trader activity during an outbreak provides numerous possibilities for anthropogenic ASF spread between villages (27). The emergency sale of sick pigs is a well-documented behaviour among smallholder farmers (28, 29). Most recorded trading activities occurred between neighbours within close geographical proximity, with some trader activities occurring after the study period. The lack of detail on the identities of trading partners and often the quantities of animals sold may reflect cultural attitudes towards the Government and reporting of income. Furthermore, a lack of resources to accurately track or record trading data may have contributed to this problem. In Xayaburi province, a social network analysis demonstrated that most trader-smallholder interactions occurred in a discrete network, with a small number of traders servicing a specific region (30). A social network analysis of smallholder and trader activity in the region would allow investigators to understand the dynamics of trading interactions as a potential route for disease transmission. This behaviour may vary between ethnic groups and geographical regions.

The presence of VVW in smallholder villages allows nations with under-resourced veterinary services to provide baseline animal health and welfare services but simultaneously increase the risk of disease transmission if not managed correctly (31). The Lao DLF report provides training to the local VVWs when resources become available (7). In ASF outbreaks, there have been occurrences where veterinarians and para-veterinarians inadvertently become mechanical fomite vectors as they travelled between ASF-affected sites without suitably disinfecting their tools and equipment (32). Recent work in neighbouring Cambodia demonstrated gaps in para-veterinary service biosecurity (33). Savannakhet VVWs reported washing their syringes and needles in soap and water rather than complete disinfection or using new needles and syringes between animals, which would not allow for sufficient disinfection of ASFV (1). Eight households recorded a VVW visit during the outbreak period, which may have allowed for ASF transmission between herds. Ongoing training and support for LMIC paraveterinary and veterinary services will support smallholder animal health and welfare whilst reducing iatrogenic infection risk.

The purposive nature of performing the study in Oudomxay province introduces a source of selection bias which must be accounted for when extrapolating the findings to larger ASF modelling projects and decision-making. The selection bias may therefore over- or under-represent the management and outbreak data findings. The regions selected in this study and the work of Matsumoto et al. (1) were nominated by the Lao DLF as representative regions for northern and southern Laos due to their accessibility and the availability of on-ground veterinary resources. For this reason, both studies may represent a best-case scenario for pig-rearing and outbreak management. Despite this potential bias, many of the descriptive results align with previous work on pig-rearing in Lao smallholder villages in other provinces like Savannakhet, Luang Prabang, Phongsaly and Xayaburi (1, 3, 22, 34). Ongoing research into the impact of the ASF outbreak on smallholder farming across various Lao contexts is necessary.

This study identified numerous transmission pathways by which ASF could spread within – and between villages in an outbreak. Housing, slaughter, wild-boar contacts, feeding, and watering protocols observed in this study allow for effective contact between infectious and susceptible pigs, whilst trading and para-veterinary activities could hasten the spread of the disease between whole villages. The presence of wild boar in Laos has been confirmed *in camera* trap studies between 2013 and 2017, however the distribution, ecology and nature of interactions with domestic pigs are poorly understood, and this study therefore presents a rare piece of information suggesting that contact occasionally occurs between these groups (35, 36). Regarding the frequency of risk factor events, it appears that between-village activities occur less frequently and may be a more resource-efficient method of controlling disease spread. Within the village, further research is necessary to understand the best methods for reducing household-to-household spread. An initial pilot performed in Timor-Leste found that combining public awareness campaigns with simple, community-driven biosecurity strategies such as fencing and reduced free-ranging and cleaning measures appeared to reduce the incidence of ASF (37). Potential focus areas for Lao smallholders may include these approaches and adapted methods for household garbage disposal and feeding practices to optimise biosecurity.

Smallholder farming at its best is a regenerative system in the cyclical nature of inputs and outputs, with minimal waste and highly efficient utilisation of all resources. Using pig manure as a fertiliser for crops is beneficial and a potential source of contamination in an outbreak. Almost all farmers reported the consumption of all pig products, including offal, an important practice for a region where almost a third of all children are stunted in their growth due to low protein (38). Traditional outbreak questionnaire studies such as this may fail to capture the nuances of such a system. In non-outbreak scenarios, a more complex understanding of Lao smallholder agriculture may be developed using methods such as system dynamics and spatial group model building, as has been piloted in Timor-Leste (39). Future studies into developing a smallholder biosecurity assessment tool that is sensitive to this style of farming and cultural practices would allow local animal health staff and outreach organisations to teach smallholder pig farmers good biosecurity practices whilst efficiently maintaining their outputs.

The investigation of the 2019 ASF outbreaks in Oudomxay province showed that practices recognised as risk factors for ASF were present among the 7 villages, such as swill-feeding and free-ranging. In addition, poor biosecurity practices, such as inappropriate garbage disposal and slaughtering that would contaminate the environment, were present. These findings demonstrate the need for increased resources from the village to the Governmental level. Villages need support in enacting context-appropriate biosecurity measures, whilst the ongoing surveillance and investigation of ASF require investment into logistical and veterinary resources at the Governmental level. The findings of this research provides outlines for future work in supporting smallholder farming in rural areas both within and beyond the South East Asian context.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by University of Sydney Human Ethics Committee under approval number (2019/725). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

NM: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. JS-L: Project administration, Writing – review & editing. TH: Formal analysis, Methodology, Writing – review & editing. JY: Conceptualization, Supervision, Writing – review & editing. MW: Supervision, Writing – review & editing. BD: Writing – review & editing. WT: Project administration, Writing – review & editing. SK: Project administration, Writing – review & editing. J-AT: Supervision, Writing – review & editing. RB: Supervision, Writing – review & editing. SB: Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Estimating the time of infection for African swine fever in pig farms in Korea

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African swine fever (ASF) is a highly contagious and lethal disease with characteristics of hemorrhagic fever. ASF outbreaks in pig farms significantly damage the entire pork industry. Understanding the transmission dynamics of ASF is crucial to effectively respond. Notably, it is important to know when the infection started on the outbreak farm. This study aimed at establishing a procedure for estimating the time of infection on pig farms affected by the ASF outbreak in Korea. The protocol for sampling to detect ASF virus infection, the estimation of the time interval between infection and detection, and the estimation of the infection stage parameters for the simulation model were described. After infection, fattening sheds (9.8 days in median) had the longest detection time compared with pregnant (8.6 days) or farrowing sheds (8.0 days). The intervals were 8.8 days for farrow-to-finisher farms, 7.0 days for farrow-to-weaning farms, and 9.5 days for fattening farms. The findings of this study provide valuable insights into ASF outbreaks in pig farms thus, improving the disease control ability.

KEYWORDS

African swine fever, pig farm, time of infection, simulation, Korea

1 Introduction

African swine fever (ASF) is a highly contagious and lethal disease affecting *Suidae* (domestic pigs and wild boars) and is characterized by hemorrhagic fever. It is caused by the ASF virus belonging to the *Asfarviridae* family (1). ASF is a disease listed by the World Organization for Animal Health, and its outbreaks in pig farms significantly damage the pork industry (2).

Since the 2018 ASF outbreak in China, it has spread to several Asian countries. In May and September 2019, an outbreak was reported in the Democratic People's Republic of Korea (North Korea) and the Republic of Korea (South Korea, hereafter Korea), respectively. The first outbreak in Korea was reported in Paju, Gyeonggi-do Province, located approximately seven kilometers south of the border with North Korea (3–5). Fourteen outbreaks were confirmed in 2019, while only two outbreaks occurred in 2020. The number of outbreaks increased to five in 2021, followed by seven in 2022. As of July 2023, nine ASF outbreaks have been confirmed, bringing the total number of outbreaks to 37 since the index case in September 2019 (6). All outbreak farms were located in the northern part of the country (see ASF outbreak map at https://mafra.go.kr/FMD-AI2/map/ASF/ASF_map.jsp).

Upon ASF confirmation, all pigs on the outbreak farms were promptly culled and an outbreak investigation was conducted. Following the protocol of controlling ASF, vehicles, people, and goods entering and exiting the farm 21 days before the outbreak must be investigated, along with the usual livestock management and animal disease control on the farm (7). The outbreak investigation was conducted to identify the infection source, the introduction pathway of the virus to farms, and the farms at high risk in epidemiological relation to the current outbreak. The high-risk period, when the virus was most likely introduced into the outbreak farm and spread to other farms, is particularly a critical period requiring intensive investigation. To calculate this period, it is necessary to determine the time when the first infection occurred in animals on the outbreak farm. To estimate the likely time for an infection event, the evidence must be provided, and a basis for scientifically explaining the evidence is required (8). Moreover, the basis must be applied equally to all events. The criteria for estimating the infection time have already been established for the foot-and-mouth disease (9) and highly pathogenic avian influenza (10). No such study has yet been published on ASF. This study aimed at establishing a procedure for estimating the time of virus infection on pig farms affected by the ASF outbreak in Korea.

2 Materials and methods

2.1 Sample collection to detect African swine fever on pig farms

Detection of ASF outbreak farms in Korea is divided into two routes: reporting animals suspected of the disease and surveillance. Professionals in the livestock industry receive education repeatedly to promptly notify if any of the following applies: (1) death in sows and an increased number of stunted fattening pigs; (2) high fever over 40°C; (3) unexplained abortion or stillbirth; (4) daily mortality for all age groups higher than the average for the past 10 days (11). Sample collection following a report of suspected animals must include all dead and ill animals. Samples were blood from live animals and tissue from dead animals. Samples should also be collected from seemingly normal animals nearby (12). On the other hand, when conducting surveillance, blood sample is collected from 10 heads per farm at least once yearly from pig farms nationwide. In annual surveillance, samples are first collected from pigs in high-risk sheds and pens. The 10 heads comprise five sows and five fattening pigs. Additionally, when pigs are shipped out from farms located in intensive management areas, i.e., where the ASF virus was detected in wild boars, a test was also performed with blood samples. For shipping out fattening pigs, samples were collected from 10 heads. And all sows to be shipped out were tested (13).

Once a positive animal to ASF antigen test was identified, samples were additionally collected from the animals around it that looked normal. Blood samples were obtained from all sheds on the farm, 10 animals per shed, not only from those with positive animals (14). Samples were also obtained from additional dead animals found during the outbreak investigation, with oral and nasal swab sampling. Samples were tested using polymerase chain reaction (PCR) for antigen detection and enzyme linked immunosorbent assays for antibody detection. Details on the detection methods were described in the papers published (15, 16).

The proportion of antigen-positive animals, defined as prevalence, was calculated for each shed in which an antigen-positive animal was identified. The prevalence was calculated by including the pigs for which the presence of antigen was verified using PCR by the National reference Laboratory (Foreign Animal Disease Division of the Animal and Plant Quarantine Agency).

2.2 Estimating the time of infection

The time of the first infection of a herd in an ASF outbreak is estimated by considering factors, including mortality and antigen and antibody detection. Mortality is the first priority criterion and can be applied upon confirmation of the first ASF antigen-positive animal. When ASF infection was confirmed in a deceased animal, 1,000 random numbers following a Poisson distribution with the lambda parameter set as the time from infection to death were generated using the programing language R.[1] The infection date for each animal with a confirmed infection was estimated by subtracting the number of days that corresponded to the quartile of the random numbers generated from the animal's date of death.

For the second priority criterion, a program to simulate within-herd transmission was run to determine the date that predicts the number of antigen-positive animals, cumulative mortality, and the number of antibody-positive animals on the sampling day. By subtracting the number of predicted days from the sampling day, the time of the first infection in the shed was determined. If an antigen-positive animal was detected in more than one shed, the infection time was estimated per shed.

After estimating the infection time via mortality and simulation, the values were combined using random forest model written in R. However, the simulation program was run when there were at least three antigen-positive animals in a shed. For outbreak farms with antibody-positive animals, it was assumed that more than 10 days had passed since the infection onset, and the simulation results were compared for confirmation. However, the number of antibody-positive animals was not used to calculate the number of days after infection. Epidemiological factors, including people and vehicles entering and exiting the farm and events, were also considered in the final decision on the estimated infection time. Estimating the infection time on ASF outbreak farms was conducted for 34 of 37 confirmed ASF outbreak farms in Korea between 2019 and 2023. Two backyard farms and one farm with small number of native Korean pigs were excluded from the estimation.

2.3 Simulation program

The spread of the ASF virus within an infected pig shed was simulated using a mathematical modeling program with SLIR compartments: susceptible (S), latently infected (L), infectious (I), and removed (R). In this model, "removed" refers to the deceased or survived by developing antibodies. The simulation program was constructed using the programing language R. During the nn-day simulation period, the numbers of animals in the S, L, I, and R conditions and the number of newly infected animals were calculated for day i + 1. They were calculated based on the number of animals in

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[1] https://www.r-project/org

each condition on the previous day (i), and the calculation was executed daily.

Latent period, number of days between infection and death, the number of days needed for antibody formation, and the percentage of dead or antibody-forming animals were entered as a constant in the model. Notably, a coefficient was needed to initiate simulation on the spread of infectious diseases. The rate at which new infections occur through contact between infectious and susceptible animals in a pig herd, was defined as "within-herd transmission coefficient." The within-herd transmission coefficient was set to a unique value based on the herd type (0.9 for pregnant, 0.8 for farrowing, and 1.0 for fattening). The simulation was iterated, and the results were compared with real data (outbreak investigation and the animal study) to select the most appropriate values. Data from the outbreak investigation were records of daily mortality in the infected sheds. Regarding the animal study data, the time when the virus was detected in unvaccinated animals housed in the same pen as the inoculated animals was referenced (16). Estimation of other input values needed to run the program is discussed in Sections 2.4 and 2.5.

The simulation was executed with the number of animals to be simulated, assuming that the infection was started with one infectious animal then spread in the herd. The simulation program was iterated 1,000 times per execution.

2.4 Pathogenicity of the African swine fever virus

The parameter values for the infection stage of the ASF virus in pigs were estimated based on the results of the infection challenge experiments conducted using virus samples from ASF outbreak farms in Korea. The viruses in the pathogenicity experiments were 2019 Paju isolates (Korea/Pig/Paju1/2019), 2020 Hwacheon isolates (Korea/Pig/Hwacheon1/2020), 2021 Yeongwol isolates (Korea/Pig/Yeongwol/2021), 2021 Inje isolates (Korea/Pig/Inje1/2021), 2022 Hongcheon isolates (Korea/Pig/Hongcheon/2022), and 2023 January Pocheon isolates (Korea/Pig/Pocheon1/2023). The six isolates (i.e., the viruses isolated in the six outbreak farms) were injected intramuscularly into eight-week-old landrace pigs. As shown in Table 1, the study had six experimental groups with 22 animals ($n = 3–5$ per group). The experimental animals inoculated intramuscularly died within 10 days, and all animals in contact with them died within 18 days. Accordingly, the viruses were identified as highly virulent ASFVs that cause an acute clinical course and belonged to the p72 genotype II and CD2v serogroup 8 (15, 16).

The experiments recorded the time (in days) until the onset of viremia (the presence of the virus in the bloodstream), detection of the virus in the oral or nasal cavity, and the onset of high fever with a body temperature of 40°C or over. The onset date of virus detection in the oral or nasal cavity was reported as "one to 2 days after the onset of viremia." Therefore, for each animal, the onset date of virus detection in the oral or nasal cavity was estimated by adding a randomly assigned value of "one or 2 days" to the viremia onset date with R.

TABLE 1 ASF outbreak status and experiments with infection challenge of ASF virus isolated from the outbreak farms.

Year	Number of ASF outbreaks confirmed in pig farms	Number of experiments with ASF virus from outbreak farms (Number of animals in each experiment)
2019	14	1 (3)
2020	2	1 (3)
2021	5	2 (3, 3)
2022	9	1 (5)
2023	7 (as of July)	1 (5)
Total	37	6 (22)

2.5 The infection stage parameters

The parameters for the infection stage required to run the simulation model were defined, and their values were calculated using the animal study results.

The ASF infection stages required to run the simulation model were defined as follows: (1) The latent period was defined as the interval from the day of inoculation of the virus into the experimental animals to the day the virus was first detected in the nasal or oral cavity; (2) The time from infection to death was defined as the interval between infection and death; (3) The duration of infectiousness was estimated from the beginning of virus detection in the nasal or oral cavity to death; (4) The incubation period was defined as the interval from the day of infection to the day when high fever with a body temperature of 40°C or more was measured (Figure 1).

Individual experimental measurements in each infection stage were combined as pooled means and 95% confidence intervals (95% CI). For this meta-analysis, "metaphor" package with mixed effect option was used in R. Both the characteristics of the six individual experiments (fixed effect) and overall variability (random effect) were considered through mixed effect model. The values of the infection stage were also expressed as probability density function. A goodness-of-fit test was conducted using "fitdistrplus package" in R to measure the difference between experimental measurements and several continuous probability distributions (i.e., Gamma, Logistic, Normal, Lognormal, and Weibull), the distribution with the smallest values of the Akaike information criterion and Bayesian information criterion was selected.

3 Results

3.1 Pig farms with outbreak of African swine fever

The 37 pig farms where ASF outbreaks were confirmed between 2019 and 2023 comprised 26 (70.3%) farrow-to-finisher farms, four (10.8%) farrow-to-weaning farms, and four (10.8%) fattening farms. The remaining three (8.1%) farms were one farm with Korean native black pigs and two backyard farms. Of the 34 commercial pig farms, the infected sheds where pigs tested positive for ASF virus antigens (referred to as antigen-positive animals) were found in 19 (55.9%) pregnant sheds, six (17.6%) farrowing sheds, and nine (26.5%) fattening (finisher) sheds. Outbreaks in sows (pregnant or farrowing

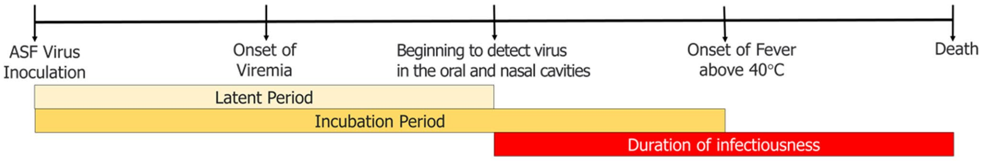

FIGURE 1
Definition of parameters for the ASF infection stage in pigs.

TABLE 2 Status of ASF-infected sheds on pig farms confirmed between 2019 and 2023 in Korea.

Infected Shed	Number of outbreak farms	Number of pigs in infected shed (A)*	Antigen-positive pigs		Number of deaths in relation to ASF
			Number (B)*	Prevalence (B/A, %)*	
Pregnant	19	107 (91, 223)	6 (4, 11)	3.9 (2.2, 6.9)	2 (0, 3)
Farrow	6	126 (78, 262)	8 (5, 10)	2.3 (2.0, 11.4)	3 (1, 8)
Fattening (Finisher)	9	332 (227, 665)	10 (3, 11)	1.2 (0.8, 5.0)	4 (0, 6)
Total	34[1]	180 (101, 351)	6 (4, 11)	2.8 (1.5, 6.7)	2 (0, 5)

[1] Exclude two backyard farms and one farm with native Korean black pigs.
*Quartile statistics: median (1st quartile, 3rd quartile).

pigs) accounted for 82.4% (28 farms) of the total. The number of pigs in the infected sheds, antigen-positive animals, and deaths increased in the pregnant, farrow, and fattening sheds order. Conversely, based on the number of pigs per shed, the highest prevalence was observed in pregnant sheds. Table 2 shows the data on the infected sheds in ASF outbreak farms in Korea. Antibodies were detected in one farm with positive cases in farrowing sheds and three farms with positive cases in fattening sheds. No cases of antibody detection were reported in pregnant sheds. The two farms with positive cases in the fattening sheds were excluded from antibody tests.

3.2 Values for the infection stage of African swine fever

Table 3 shows the values for the infection stages of ASF. For the latent period, the range was 2–5 days, with a pooled mean (95% CI) of 4.3 (3.7–4.8) days. For the incubation period, a mean of 4.3 (3.4–5.2) days was estimated, and the range was 3–7 days. For the infection time to death, the range was 4–10 days, and the mean was 9.0 (8.9–9.1) days. Consequently, the duration of infectiousness ranged from 1 to 6 days, with a mean of 4.4 (3.6–5.2) days.

3.3 Simulation of within-herd transmission

The simulation program was executed using the first quartile (Q1), median, and third quartile (Q3) values of the number of pigs in infected sheds in Table 2 and the coefficient of transmission based on shed type. The simulation output revealed that the spread of ASF virus infection was relatively faster in small herds compared to large herds. Although the absolute number of infected individuals differed based on herd size and shed type, the trends of increase, peak, and decrease at each stage of infection were similar. The period with the highest daily number of newly infected animals was 16–18 days for Q1, 17–19 days for the median, and 19–22 days for Q3 values of the number of pigs. The highest prevalence was below 40% in sows (38.3–39.0% for pregnant sheds and 35.7–36.6% for farrowing sheds) and above 40% (40.7–41.4%) for fattening sheds. The highest prevalence reached at 23–24 days for Q1, 24–26 days for the median, and 26–28 days for Q3 values of the number of pigs in the shed.

The ASF-confirmed deceased pigs were regarded as being infected 7–11 days before death, which was the interquartile range of the random values generated (with lambda = 9), the median value of time to death (Table 3).

Following the infection in the animals and spread within the pig herds, it was expected to reach the prevalence of detection (shown in Table 2) at 8–11 days in pregnant sheds (3.9%), 5–11 days in farrowing sheds (2.3%), and 6–10 days in fattening sheds (1.2%). Supposing the ASF outbreaks were not recognized and no response measures were implemented, then, the entire herd was expected to die within 64 days (for the median number of pigs) in pregnant sheds, 70 days in farrowing sheds, and 77 days in fattening sheds from the time of infection (Figure 2).

3.4 Time of infection on the African swine fever outbreak farm

The time interval from infection to detection was estimated to be a median of 9.0 (Q1–Q3, 7.8–10.5) days in ASF outbreak farms in Korea. The median (Q1–Q3) intervals were 8.6 (7.8–10.5) days in pregnant sheds, 8.0 (7.9–8.6) days in farrowing sheds, and 9.8 (9.0–12.8) days in fattening (finisher) sheds. In real outbreak, the fattening sheds had the longest detection time after infection. When considering the livestock type on the outbreak farms, the median (Q1–Q3) intervals were 8.8 (7.8–12.8) days for farrow-to-finisher farms, 7.0 (3.9–10.1) days for farrow-to-weaning farms, and 9.5 (7.9–11.3) days for fattening farms (Table 4).

TABLE 3 **Infection stage duration parameters estimated based on the experiment of ASFV infection challenge.**

Infection stage parameter	Values*	Pooled mean (95% CI)	Probability distribution
Latent period	4 (4, 5)	4.3 (3.7–4.8)	Lognormal (meanlog = 1.5, sdlog = 0.2)
Incubation period	5 (4, 5)	4.3 (3.4–5.2)	Normal (mean = 4.7, sd = 1.1)
Time to death	9 (8, 9)	9.0 (8.9–9.1)	Lognormal (meanlog = 2.1, sdlog = 0.2)
Duration of infectiousness	5 (5, 6)	4.4 (3.6–5.2)	Weibull (shape = 3.7, scale = 4.5)

*Estimated based on experimental observation, Quartile statistics: median (1st quartile, 3rd quartile).

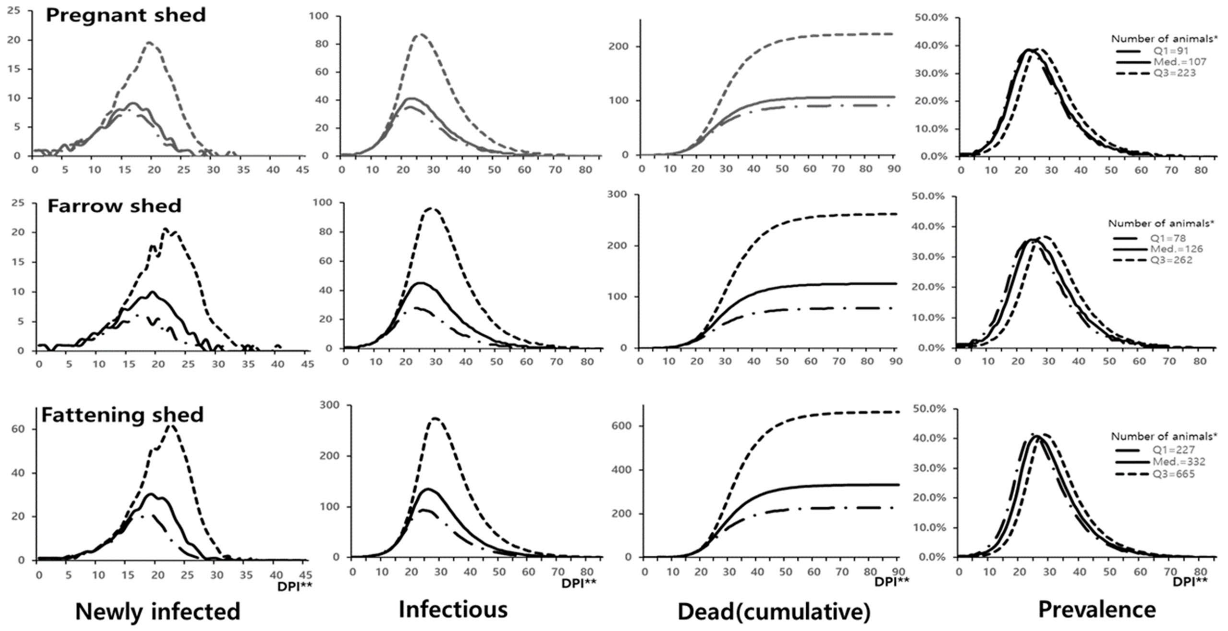

FIGURE 2
Within-herd transmission of ASF infection according to the type of infected shed. *Number of animals Q1: 1st quartile, Med.: Median, Q3: 3rd quartile. **DPI: Days post introduction (start of infection).

4 Discussion

An understanding of within-herd transmission dynamics is important for an effective response to an infectious animal disease and requires knowledge of when the infection started on the farm. Simulation models can be particularly helpful in such cases (17, 18). If the herd size is small and the number of cases is low, then, the infection time can be estimated based on the number of days since the onset of the oldest appearing clinical symptoms or lesions, and the incubation period. However, if many animals such as pigs or poultry are housed together, and many have become ill or died simultaneously, simulation can be used to predict the number of newly infected, infectious, and dead animals on daily basis. The values of the input parameters affect the prediction accuracy (19–21).

TABLE 4 **Time from infection to detection in the ASF outbreak pig farms in Korea between 2019 and 2023.**

Infected pigs		Number of outbreak farms	Time (days) from infection to detection*
Shed	Pregnant	19	8.6 (7.8, 10.5)
	Farrow	6	8.0 (7.8, 8.6)
	Fattening (Finisher)	9	9.0 (9.8, 12.8)
Farm	Farrow-to-finisher	26	8.8 (7.8–12.8)
	Farrow-to-weaning	4	7.0 (3.9–10.1)
	Fattening	4	9.5 (7.9–11.3)
Total		34[1]	9.0 (7.8, 10.5)

[1] Exclude two backyard farms and one farm with native Korean black pigs.
*Quartile statistics: median (1st quartile, 3rd quartile).

Based on the infection challenge using the ASF virus isolated from farms in Korea between 2019 and 2021, it was found that all viruses belonged to the strain causing an acute form of illness (15). The ASF virus from outbreaks in 2022 and 2023 was also determined to be in the acute form. Viremia was detected 2–5 days after inoculation, followed by detection in the nasal or oral cavity within 1–2 days, and death occurred 4–9 days later (15). Similar experiments conducted in other countries showed that viremia was detected 2–5 days after infection challenge, followed by detection in oral, nasal, or rectal swabs within 1–2 days. The incubation period until clinical symptoms, such as high fever, appeared at 3–5 days and death occurred at 6–10 days after inoculation (22–25). The results of our study, which calculated a 95% CI, were consistent with that of studies with infection challenges in Korea and other countries. The estimated time until virus detection in the oral or nasal cavity ranged from 3.7 to 4.8 days, the incubation period ranged from 3.4 to 5.2 days, and mortality ranged from 8.9 to 9.1 days (Table 3).

Viremia was detected 10–13 days after inoculation in pigs that were in contact with virus-inoculated pigs (22, 24). Pigs in contact with the inoculum developed clinical symptoms after 9 days (25) and 6 to 7 days after inoculation (26). The duration of infectiousness was 3.6–5.2 days in our study compared with 2.9 days from the nasal cavity and 3.2 days from the oral cavity with the virus from Georgia 2007 (22). The infectious period ranged from 2 to 9 days in Europe (27).

Studies on within-herd transmission between pigs have mainly focused on direct contact (28, 29). However, indirect transmission through viruses in the environment can still occur. For instance, an experiment conducted in Poland showed that healthy pigs that entered a pen emptied for 1 day after being occupied by ASF-infected pigs, exhibited severe clinical symptoms within a week (30). In pig farms in Korea, pigs have contact with each other within the same pen, but there is more indirect contact with pigs in other pens through human behavior, including tool usage. The reproductive number (R_0), representing transmission between pigs, has been reported to be 2.8 (95% CI 1.3–4.8) within pens and 1.4 (0.6–2.4) between pens (31). The R_0 for within-herd transmission varies from 1.6 to 24.2 in different studies, based on the breeding type and measurement method (32). In our study, both direct and indirect transmission routes were considered when determining the within-herd transmission coefficient. The smallest coefficient was assigned to the farrowing shed, where the farrowing sow stays in an individual stall with her suckling piglets, with a value of 0.8. Based on the Enforcement Rule of the Livestock Industry Act, In Korea, pregnant sheds are required to be in the form of grouping pens by 2029 (33). As of 2023, the transition of the pregnant pig shed from stall to grouping pen has commenced. Consequently, the transmission coefficient for the pregnant shed was set to 0.9. The within-herd transmission coefficient was the highest for fattening pigs (1.0), where pigs could come into close contact with pen mates.

Based on this study, it is estimated that ASF can be detected after 8.0 (95% CI 7.8–8.6) days from infection in farrowing sheds, 8.6 (7.8–10.5) days for pregnant sheds, and 9.0 (9.8–12.8) days for finishers at farrowing sheds (Table 4). Various factors, such as the coefficient used, the number of animals, and the work patterns in the pig farms may have influenced the simulation results. In Korean ASF outbreak farms, the estimated infection-to-detection time (7.8–10.5 days) was found to be shorter than the period suggested by a Danish study (13–19 days) (18) and an experiment using a moderately virulent virus (more than 20 days) (28). The relatively rapid detection in Korea may be attributed to the intensive breeding system and careful identification of ill and dead pigs. Detecting and reporting animals showing abnormalities, such as sudden death, is crucial for early ASF detection and containment. A study that reconstructed the spread patterns within a large-scale pig farm in Latvia based on an ASF investigation suggested that the first infected animal died within a week after infection, but went unnoticed (34). In Korea, sudden death is the most frequently observed symptom reported by farmers in ASF outbreak farms (35). The Korean government has established criteria for reporting suspected ASF cases, which include sudden death in sows and daily mortality higher than the average for the last 10 days in all age groups (11). Prompt reporting of a deceased animal observed on a pig farm effectively enhances the efficiency of ASF response.

Besides ASF-related deaths, this study calculated the prevalence of antigen-positive populations compared with the total number of pigs in the infected sheds. Although laboratory tests were not conducted on all pigs in the affected sheds, specimens were obtained from all ill and deceased pigs and their cohabitants, ensuring that the most visibly affected animals were included in the tests. It took 9.0 (range 7.8–10.5) d for 2.8% (95% CI 1.5–6.7%) of the pigs in the sheds to test positive for the ASF antigen (Tables 2, 4).

5 Conclusion

In this study, a systemic procedure for estimating the time of introduction of ASF virus into a pig farm upon the confirmation of an ASF outbreak on the farm was established. The procedure was also applied to estimate the time of infection and the time interval from infection to detection (i.e., the period during which there is a risk of unknowingly releasing the virus from the outbreak farm). The findings provide valuable insights into ASF outbreaks in pig farms, particularly those with intensive management systems, such as those in Korea. This study will help facilitate early ASF detection and implementation of preventive measures, thus improving the ability to control and manage the disease.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

HY: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. YS: Formal analysis, Software, Writing – review & editing. K-SK: Data curation, Formal analysis, Software, Writing – review & editing. IL: Formal analysis, Writing – review & editing. Y-HK: Resources. EL: Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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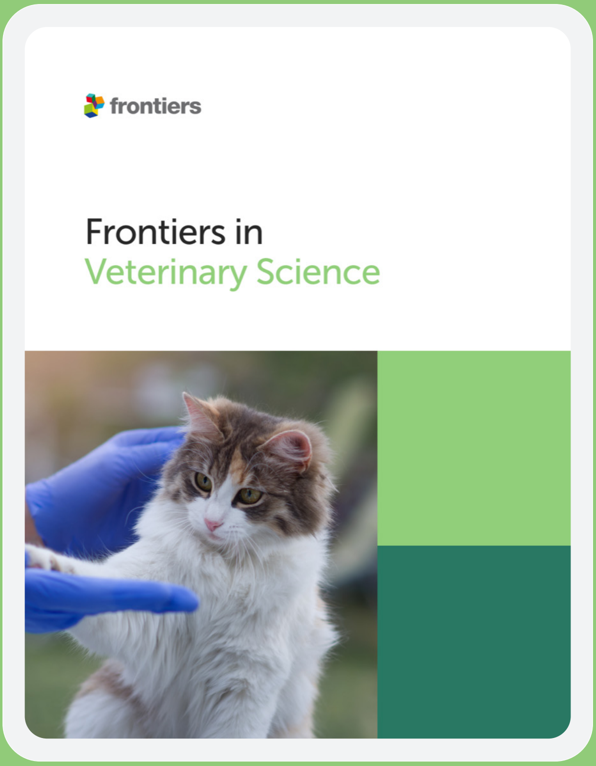

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