



Characterization of biosecurity practices and viral infections on pig farms in Hong Kong

Belete Haile^{a,b,*}, Congnuan Liu^a, Maura Carrai^e, Yun Young Go^{a,d}, Chi Kwan Yip^b,
Lip Tet NG^b, Yui Gordon Au Yeung^b, Kwong Ying Luk^b, Karyn A. Havas^f, Renata Ivanek^g,
Dirk U. Pfeiffer^{a,b,c}, Anne Conan^{b,h,i}, Omid Nekouei^{a,b,*}

^a Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong SAR

^b Centre for Applied One Health Research and Policy Advice, City University of Hong Kong, Hong Kong SAR

^c Department of Pathobiology and Population Sciences, Royal Veterinary College, London, United Kingdom

^d College of Veterinary Medicine, Konkuk University, Seoul, Korea

^e Tropical Futures Institute, James Cook University, Singapore, Singapore

^f Department of Public and Ecosystem Health, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

^g Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

^h ASTRE (AnimalS, health, Territories, Risks, Ecosystems), CIRAD, Harare, Zimbabwe

ⁱ ASTRE, University of Montpellier, CIRAD, INRAE, Montpellier, France

ARTICLE INFO

Keywords:

Biosecurity
Viral pathogens
Prevalence
Swine
Hong Kong

ABSTRACT

The objectives of this study were to characterize the biosecurity practices implemented on pig farms in Hong Kong and determine the between-farm prevalence of economically important viral pathogens. All active pig farms in Hong Kong ($n = 40$) were invited to participate in an interview-based survey using a Biocheck-UGent™ questionnaire to evaluate their biosecurity practices. Pen-level oral fluid samples were collected by cotton ropes to detect six target viral pathogens using RT-PCR: porcine reproductive and respiratory virus (PRRSV), porcine circovirus type-2 (PCV-2), swine influenza virus (SIV), porcine delta-coronavirus (PDCoV), porcine epidemic diarrhea virus (PEDV), and transmissible gastroenteritis virus (TGEV). Eighteen farms (45%) accepted our invitation and participated in this study. Biosecurity practices were found to be inadequate in many areas, with an average overall score of 50.1 ± 9.4 (mean \pm SD). The study farms scored higher for external biosecurity (56.4 ± 8.6) than internal biosecurity practices (43.9 ± 12.1). Among external biosecurity subcategories, breeding pig and semen purchase scored highest (93.2), while visitors and farmworkers scored lowest (23.5). In internal biosecurity, the disease management subcategory received the highest score (66.7). Only two external biosecurity subcategories, breeding pig and semen purchase (93.2), and farm location (70) exceeded the global average scores. Key deficiencies were identified in biosecurity protocols for visitors and workers, hygiene standards for feed, water, and equipment supplies, and measures to prevent disease transmission between compartments (farrowing, nursery, and finishing units). Over 90% of participating farms implemented vaccination programs for PRRSV, PCV-2, porcine parvovirus (PPV), pseudorabies virus (PRV), and classical swine fever virus (CSFV) while no farms vaccinated against SIV, and vaccination for swine coronaviruses was sporadic. All target viruses except TGEV were detected at the farm level. The between-farm prevalences among the 18 study farms were PRRSV-2 (94.4%), PRRSV-1 (38.9%), PCV-2 (83.3%), SIV (55.6%), PDCoV (16.7%), and PEDV (5.6%). We provided comprehensive baseline information on the biosecurity practices of pig farms for the first time in Hong Kong. We identified critical areas of biosecurity for improvement and offered tailored recommendations to help the producers implement more effective prevention and control strategies for infectious diseases within and between farms.

* Corresponding authors at: Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong SAR.

E-mail addresses: bhnega2-c@my.cityu.edu.hk (B. Haile), cnliu7-c@my.cityu.edu.hk (C. Liu), maura.carrai@jcu.edu.au (M. Carrai), yunyo@konkuk.ac.kr (Y.Y. Go), chiyip@cityu.edu.hk (C.K. Yip), liptetng@cityu.edu.hk (L.T. NG), gaueyung@cityu.edu.hk (Y.G. Au Yeung), kwongluk@cityu.edu.hk (K.Y. Luk), kah47@cornell.edu (K.A. Havas), ri25@cornell.edu (R. Ivanek), dirk.pfeiffer@cityu.edu.hk (D.U. Pfeiffer), anne.conan@cirad.fr (A. Conan), omid.nekouei@cityu.edu.hk (O. Nekouei).

<https://doi.org/10.1016/j.pvetmed.2025.106569>

Received 1 December 2024; Received in revised form 7 May 2025; Accepted 11 May 2025

Available online 14 May 2025

0167-5877/© 2025 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Pig farming plays an important role in the economy and food security in most Southeast and East Asian countries where pork is the predominant source of animal protein (Woonwong et al., 2020; Wang and Li, 2024). However, the pig industry faces significant challenges due to the high prevalence of infectious diseases, especially those caused by viruses (Haile et al., 2025; Qiu et al., 2020). Over the past few decades, several infectious diseases, including African swine fever (ASF), porcine reproductive and respiratory syndrome (PRRS), foot-and-mouth disease (FMD), porcine circovirus type 2 (PCV-2), and classical swine fever (CSF) have led to substantial production losses and trade disruptions, adversely affecting food security across the region (Kedkovid et al., 2020; Lee et al., 2020; Ma et al., 2021). Swine influenza virus (SIV) further compounds these challenges, posing a threat to both the pig production industry and public health (Choi et al., 2013). Its extensive genetic diversity, delineated by country-specific clades and frequent cross-border transmissions, complicates disease management efforts in the region (Saito et al., 2022). Moreover, the emergence and re-emergence of swine coronaviruses, including porcine epidemic diarrhoea virus (PEDV), swine acute diarrhoea syndrome coronavirus (SADS-CoV), porcine delta coronavirus (PDCoV), and transmissible gastroenteritis virus (TGEV) have exacerbated the situation, causing severe gastrointestinal diseases and high mortality rates in neonatal piglets (Mai et al., 2020; Thakor et al., 2022).

Pig production in Asia, particularly in Southeast Asia, is predominantly smallholder-based, often with inadequate biosecurity practices and extensive animal movements and trade of animal products, which facilitates the introduction and rapid spread of pathogens; therefore, infectious disease prevention and control remain a significant challenge (Woonwong et al., 2020; Ito et al., 2023). Biosecurity is a strategic and integrated approach for analyzing and managing the risks posed to human, animal, and plant health and associated environmental risks (Food and Agriculture Organization of the United Nations., 2023). It includes all sets of preventive measures aimed at reducing the risk of pathogen introduction (bio-exclusion) into the farm and limiting the spread of pathogens within farm premises (bio-containment) (Dewulf and Immerseel, 2019). Improved biosecurity practices can reduce the incidence of diseases, enhance farm productivity, decrease reliance on antibiotics, and contribute to the overall sustainability of livestock enterprises (Postma et al., 2016; Alarcón et al., 2021; Dhaka et al., 2023).

In Hong Kong, local pig farms are family-owned enterprises, mainly located in the New Territories (Chan, 2020). These farms primarily operate under a farrow-to-finish production system (Rosanowski et al., 2023) and focus on supplying fresh pork to meet the domestic demand. On average, they provide approximately 310 pigs daily for slaughter, accounting for 12% of live pigs consumed in Hong Kong (Agriculture Fisheries and Conservation Department., 2024a). Recent studies have highlighted the occurrences of several diseases, including PRRS, PCV-2 infection, and ASF, which pose substantial economic and animal welfare challenges for pig farms in Hong Kong (Flay et al., 2022; Go et al., 2023). There have been no reported cases of CSF in Hong Kong since 2005 (World Animal Health Information System., 2023).

The Agriculture, Fisheries, and Conservation Department (AFCD) of the Government of the Hong Kong Special Administrative Region is actively promoting the implementation and strict adherence to biosecurity measures for local pig farms to effectively mitigate the risk of disease outbreaks, such as African Swine Fever (ASF) (Agriculture Fisheries and Conservation Department., 2024c). However, the status of biosecurity practices and health and disease conditions on these farms is mostly unknown. This hinders the formulation of targeted action plans to address specific shortcomings, highlighting the urgent need for research to enhance biosecurity and health management practices, thereby improving the productivity and sustainability of the pig sector. The objectives of this study were to: 1) characterize the biosecurity practices implemented on pig farms in Hong Kong and 2) determine the

between-farm prevalences of six viral pathogens, including PRRSV, PCV-2, SIV, PEDV, PDCoV, and TGEV.

2. Material and methods

2.1. Study design

Our target population included all registered active pig farms in Hong Kong (n = 40). A cross-sectional study was conducted, and the data collection consisted of two parts: 1) a questionnaire-based interview asking about biosecurity practices; all 40 active pig farms in Hong Kong were invited to participate; 2) the collection of oral fluid samples from the farms agreeing to participate in our study to assess the between-farm prevalence of a panel of the six targeted viruses (PRRSV, SIV, PCV-2, PEDV, PDCoV, and TGEV). For enrollment, pig producers were identified using the list of farms obtained from the official records maintained by the Agriculture, Fisheries, and Conservation Department (AFCD) of the Government of the Hong Kong Special Administration Region. Producers were initially approached during their monthly association meeting organized by the Hong Kong SAR Livestock Industry Association (HKLIA) in November 2022. At this meeting, research team members provided producers with a detailed explanation of the study objectives, commitments to participation, and expected outcomes and benefits. After this introductory meeting, each producer was individually contacted by phone to obtain their informed consent verbally to participate in our study. The study protocol was evaluated and approved by the Research Ethics Sub-Committee of City University of Hong Kong (Reference Number: A-0805).

2.2. Biosecurity questionnaire

To assess biosecurity practices, the standardized Biocheck-UGent™ questionnaire protocol developed by Ghent University was used in our face-to-face interviews (Laanen et al., 2010). The Biocheck-UGent™ questionnaire is publicly accessible at www.biocheck.ugent.be in multiple languages, including Chinese. Its scoring system for pig farms consists of 109 questions, divided into six subcategories for external biosecurity and six subcategories for internal biosecurity. The scoring system and weight factors applied to each question and subcategory of the questionnaire are presented by Laanen et al. (2013). Briefly, each question was assigned a score between 0 (measure not implemented at all) and 1 (measure fully implemented). This score was then multiplied by a weight factor reflecting the importance of the biosecurity measure. The weighted scores for the relevant subcategories were summed to calculate the external or internal biosecurity score, resulting in scores ranging from 0 (complete absence of biosecurity) to 100 (assumed perfect biosecurity). The overall biosecurity score was the mean of the internal and external biosecurity scores (Laanen et al., 2013; Gelaude et al., 2014). Minor adjustments were made to the response options for two questions in the original Biocheck-UGent™ questionnaire (highlighted in Supplementary Material 1). This modification was necessary because the interviewer would have encountered difficulties interpreting and applying the original response options within the context of pig farming in Hong Kong.

To assess the farm-level vaccine coverage, our team designed 17 vaccination-related questions and added them to the interviews (Supplementary Material 2). The veterinary assistant conducting the interviews received training before the study to ensure a thorough understanding of each question. All interviews were conducted face-to-face on the farms, each lasting approximately 45–60 min.

2.3. Pen selection for oral fluid collection

To assess the presence of target viral pathogens, oral fluid samples were collected from two categories of pigs on each farm: weaners (piglets aged 28 days to 8 weeks) and growers (pigs aged 14–18 weeks).

Sampling was conducted on farms whose owners consented to participate in our biosecurity interviews. Individual pens were used as the sampling unit for collecting oral fluids, and a total of 60 pens were selected, comprising 39 weaner pens (two or three pens per farm) and 21 grower pens (one or two pens per farm).

The number of pens required to detect at least one positive case of PRRSV or PCV-2 was calculated using Epitools (<https://epitools.ausvet.com.au/ppfreedom>), following the method described by Christensen and Gardner (2000). The estimated peak prevalence of PRRSV at 8 weeks of age was estimated at 32.1% (20.8–45.0%), while the peak prevalence of PCV-2 at 16 weeks of age was estimated at 41% (30.0–55.0%) on Hong Kong pig farms in a previous study (Flay et al., 2022). For sample size calculation, the design prevalence for PRRSV and PCV-2 was set at 20% and 30%, respectively. The pooled sensitivity of RT-PCR for PRRSV and PCV-2 were assumed to be 98% (88–100%) and 100% (96–100%), respectively, based on a previous report by Hena-Diaz et al. (2020). To ensure robust estimates, the lower bounds of the sensitivity intervals (88% for PRRSV and 96% for PCV-2) were used in our calculations, with test specificity assumed to be 100% for both pathogens. Upon arrival at each study farm, active weaner and grower pens, as well as barns, were enumerated, and the average number of pigs per pen was estimated to be 29 for weaners and 30 for growers. Using these parameters, the required sample size was calculated to achieve a 95% confidence level (desired cluster sensitivity), ensuring that at least one positive pen if the disease was present at or above the level of design prevalence.

Once the required number of pens per farm was determined, sampling adhered to the following conditions. When the number of barns with weaner or grower pens exceeded the number of pens required for sampling, one pen per barn was randomly selected (i.e., a simple random sampling by assigning numbers to each pen). Conversely, when the number of barns was fewer than the required number of pens, a proportional stratified random sampling was applied, where the number of pens sampled per barn was proportional to the total pens available, and pens were randomly selected within each barn. Detailed information regarding the number of available and sampled pens, along with the estimated average number of pigs per pen for each farm, is provided in Table S1 in the Supplementary materials.

2.4. Oral fluid sample collection

Pen-level oral fluid samples were collected from weaner and grower pigs following a standardized procedure described by Prickett et al. (2008b). Briefly, within each selected pen, two cotton ropes were positioned at the shoulder height of the pigs within each selected pen. The ropes were securely fastened to the vertical bars on the side of the pen in a clean area and left in place for 30 min. Afterward, the wet portion of each rope was placed into a designated plastic bag, and oral fluids were mechanically extracted by wringing the wet parts. Ropes from the same pen were pooled in a single plastic bag, and the extraction product was transferred into 50 mL centrifuge tubes and transported in an ice box to the diagnostic laboratory at City University of Hong Kong for nucleic acid extraction and RT-PCR testing.

2.5. RNA/DNA extraction and RT-qPCR

Oral fluid samples were pre-treated by pre-centrifugation at 15,000 $\times g$ for 15 min at 4 °C, following the method adopted Gibert et al. (2017). Subsequently, viral RNA/DNA extraction was performed using the MagMAX™ Pathogen RNA/DNA Kit (Applied Biosystems™), within 24 h following the manufacturer's instructions.

Detection of PRRSV from the extracted nucleic acid was done using VetMAX™ PRRSV EU & NA 2.0 commercial Kit (Applied Biosystems, CA, USA) according to the manufacturer's instructions on Quant Studio 7 Pro Real-Time PCR. The test was done in 20 μ l of reaction volume containing 12 μ l of 3-Mix PRRS EU/NA 2.0 and 8 μ l of the extracted

nucleic acid and run at 50 °C for 5 min and 95 °C for 10 min followed by 40 cycles of 95 °C for 15 seconds, and 60 °C for 1 min. Positive (4a – EPC PRRS EU/NA 2.0, from the kit) and negative controls were included in each run. Each reaction was duplicated and considered positive if the cycle threshold (Ct) value was obtained below 40.

Porcine circovirus type 2 (PCV2) was detected using VetMAX™ Porcine PCV2 Quant Kit (Applied Biosystems, CA, USA). The real-time PCR reaction volume was 25 μ l containing 20 μ l 3 - Mix QPCV2 and 5 μ l sample. A positive (PC QPCV2 from the kit) and negative (nuclease-free water) control were used in each reaction. The reaction was run on Quant Studio 7 Pro Real-Time PCR 50 °C for 2 min, 95 °C for 10 min, followed by 95 °C for 15 s, 60 °C for 1 min for 45 cycles, and each test was conducted in duplicate reactions. A cycle threshold (Ct) value below 37, between 37 and 40, and above 40 was considered as PCV2 detected, suspected, and undetected, respectively.

To detect the swine influenza virus, MAX™-Gold SIV detection kit (Life Technologies, TX, USA) was used. Briefly, the reaction was done in 25 μ l, consisting of 12.5 μ l of 2X Multiplex RT-PCR Buffer, 2.5 μ l Multiplex RT-PCR Enzyme Mix, 1 μ l influenza virus primer probe mix, 1 μ l Nuclease-free Water, and 8 μ l of the test sample. Positive and negative controls were included in each reaction. The reaction was run at 48 °C for 10 min, 95 °C for 10 min, followed by 95 °C for 15 s, and 60 °C for 45 s for 40 cycles. Ct values below 38, between 38 and 40, and above 40 were considered positive, suspected, and undetected, respectively.

The oral fluid samples were also tested for three swine coronaviruses, porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis coronavirus (TGEV), and porcine delta coronavirus (PDCoV), using VetMAX™ PEDV/TGEV/PDCoV Ki (Applied Biosystems, CA, USA). This multiplex PCR was run at 20 μ l of reaction volume consisting of 5 μ l TaqMan® Fast Virus 1-Step Master Mix, 1 μ l VetMAX™ PEDV/TGEV/PDCoV primer probe mix, 6 μ l of nuclease-free water, and 8 μ l of sample nucleic acid according to the manufacturer's instructions protocol. The reaction was run on Quant Studio 7 Pro RT PCR at 48 °C for 10 min, 95 °C for 10 min, followed by 45 °C for 15 s and 60 °C for 45 s. The reaction was considered valid if the PEDV, TGEV, and PDCoV Ct-values were 22.5–26.5, 24.4–28.4, and 24.0–28.0, respectively.

2.6. Data analysis

The questionnaire responses and laboratory results were entered into a Microsoft Access 2007® database. All statistical analyses were conducted using Stata v18 (Stata Corp LLC, College Station, TX, USA). Summary statistics were calculated to describe biosecurity scores, vaccination coverage, and the between-farm prevalences of target viruses across the study farms. The normality of the distributions of calculated biosecurity scores was evaluated using the Shapiro-Wilk test.

The PCR test results were reported as negative (no viral detection) or positive for each target virus based on the respective Ct-value cut points established by the manufacturers of the kits. A pen was considered positive for a virus if the pooled oral fluid sample collected from that pen returned a positive PCR result. A farm was classified as positive for any of the six target viruses if at least one of the pen-level samples from either the weaner or grower cohorts tested positive. To assess the potential associations between biosecurity practices and viral infection levels on the study farms, the average scores of biosecurity practices were compared between positive and negative farms to SIV or PRRSV-1 (as the only possible ones) using two-sample T-tests with the level of significance set at 0.05.

3. Results

3.1. Farm characteristics

Of all 40 active pig farms in Hong Kong invited, 18 producers accepted and participated in our study. Data collection from these farms was conducted between November 2022 and May 2023. All

participating farms operated under a farrow-to-finish production system, where piglets were born and raised on the farm until they reached the desired market weight (of approximately 120 kg). A summary of farm characteristics and procedure demographics is presented in Table 1. Sixteen of the 18 farms (89%) reported the presence of other animal species on their premises, with dogs on 14 and cats on 13 farms.

The producers had a median of 18 years of farming experience, ranging from 2 to 50 years. The study farms employed a median of four farmworkers to manage their herds. Herd size across the study farms varied between 153 and 2533 pigs, with a median of 841.

3.2. Biosecurity level and vaccination coverage

The distributions of internal, external, and overall biosecurity scores for the 18 study farms and their respective subcategories are presented in Table 2. The average external, internal, and overall biosecurity scores were 56.4 (SD = 8.6) and 43.9 (SD = 12.1), 50.1 (SD = 9.4), respectively. The variation in the external and internal biosecurity scores across the 18 study farms is illustrated in Fig. 1. As shown in the spider plot, most farms (16/18) had higher external biosecurity scores than their internal biosecurity scores. Among the external biosecurity subcategories, the highest score was recorded for the purchase of breeding pigs, and semen (93.2), followed by the farm location subcategory (70). In contrast, lower scores were observed for visitors and farmworkers (23.5), feed, water, and equipment supply (29.4), and vermin and bird control (49).

For internal biosecurity, higher average scores were achieved in the disease management (66.7) and cleaning and disinfection (53.2) subcategories. However, several internal biosecurity subcategories demonstrated low scores, including the nursery unit (34.9), farrowing and suckling period (37.7), and finishing unit (38.1). The frequency distribution of responses provided by the producers for each question (biosecurity measure) is fully presented in Tables S2 and S3.

The average biosecurity scores of our study farms are compared with the available global averages in Fig. 2. Overall, our farms exhibited lower scores in nearly all external and internal biosecurity practices compared to the global benchmarks (Ghent University, 2025), except for two external biosecurity subcategories: 1) the purchase of breeding pigs, piglets, and semen, and 2) farm locations, in which study farms had slightly higher average scores compared to the global values (Fig. 2a).

The vaccination practices implemented by the study farms over the past two years are summarized in Table S4. All 18 farms reported vaccinating their pigs against PCV-2. Additionally, 17 farms administered vaccines against PPV, CSFV, and PRV. Vaccination for PRRSV and FMDV was reported by 16 and 15 farms, respectively. None of the farms reported vaccinating against SIV, *Brucella suis*, or PDCoV.

3.3. Viral pathogens

Oral fluid samples were collected from 60 pens, comprising 39 weaner pens and 21 grower pens. However, the ropes used for sample

Table 1
General characteristics of the 18 study pig farms in Hong Kong.

Variable	Min	1 st quartile	Median	3 rd quartile	Max
Number of sows	40	105	165	247.5	400
Number of boars	2	6	7.5	10	16
Number of weaners	40	78	210	400	1000
Number of finishers	50	105	335	675	1200
Herd size ^a	153	494	841	1171	2533
Years of farming experience	2	9	18	32	50
Number of farmworkers	1	3	4	6	11

^a Herd size was defined as the total number of pigs present on the farm during our data collection.

Table 2
External, internal, and overall biosecurity scores of the 18 study pig farms in Hong Kong by each of the 12 subcategories.

Biosecurity subcategories	Mean	SD	Median	Min	Max
External	56.4	8.6	53	43.5	76
Purchase of breeding pigs, piglets, and semen	93.2	6.2	93.8	83.3	100
Transport of animals, removal of carcasses, and Manure	57.4	13.5	53.3	34.8	87
Feed, water, and equipment supply	29.4	18.4	30	0	66.7
Visitors and farmworkers	23.5	25.5	17.6	0	70.6
Vermin and bird control	49	20.9	50	0	72.7
Location of the farm	70	16.1	65	40	100
Internal	43.9	12.1	44	24	65
Disease management	66.7	26.6	70	20	100
Farrowing and suckling period	37.7	18.1	39.3	0	71.4
Nursery unit	34.9	15.5	35.7	14.3	57.1
Finishing unit	38.1	18.7	42.9	0	71.4
Measures between compartments and use of equipment	39.5	16.4	37.5	10.7	71.4
Cleaning and disinfection	53.2	18.5	60	27.5	85
Overall	50.1	9.4	51.6	34	67

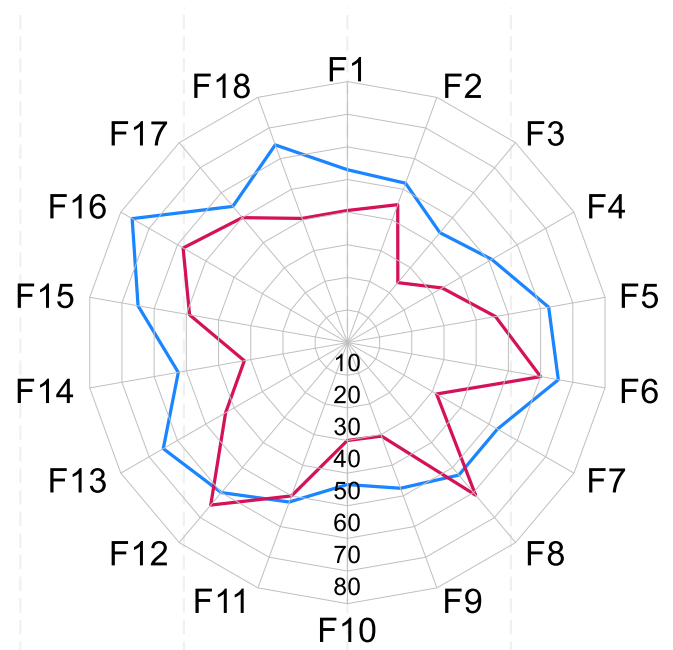


Fig. 1. Spider plot of external (blue line) and internal (red line) biosecurity scores across the 18 study pig farms in Hong Kong (labeled as F1-F18).

collection were inadequately saturated with fluids in two of the weaner pens due to the reluctance of piglets to chew. As a result, 58 samples were tested for the detection of six target viruses using RT-qPCR. The farm- and pen-level frequency distribution of the detection of these viruses and observed co-infection patterns are summarized in Table 3. Porcine reproductive and respiratory syndrome virus type 2 (PRRSV-2) was the most prevalent, detected in 94.4% of farms, followed by PCV-2, found in 83.3% of the farms. The swine influenza virus (SIV) had a between-farm prevalence of 55.6%, while PRRSV-1 was observed in 38.9% of the farms. PEDV and PDCoV were detected only on two and one farms, respectively, and TGEV was not detected at all. Co-infection with PRRSV-2 and PCV-2 was the most prevalent, detected in 77.8% of the study farms. Furthermore, triple co-infections involving PRRSV-2, PCV-2, and SIV were notably common, occurring in 50% of the study farms (Table 3).

Table 4 presents a comparative analysis of internal, external, and overall biosecurity scores between the farms testing positive and

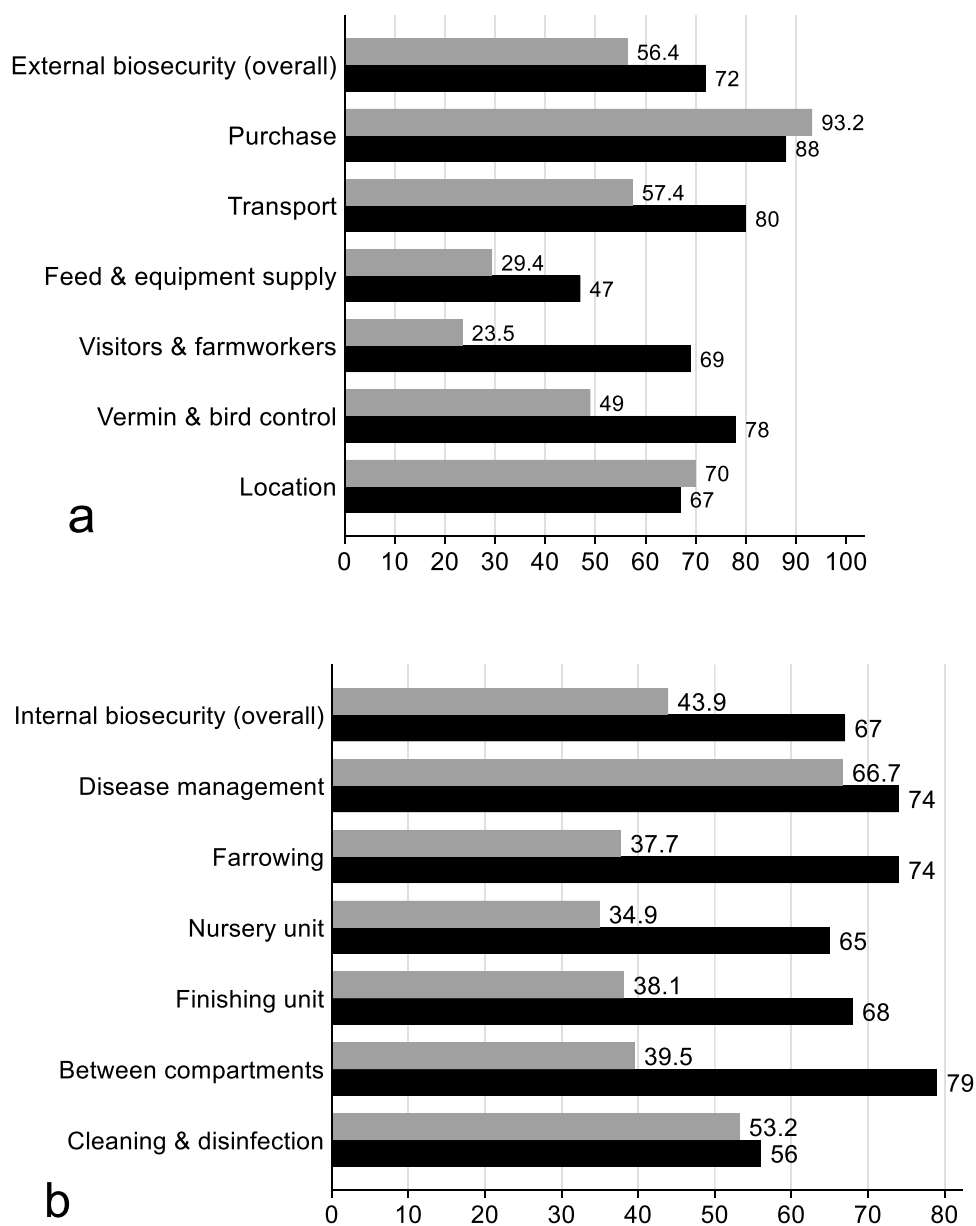


Fig. 2. Comparison of the average biosecurity scores of 18 study pig farms in Hong Kong (grey bars) with the global average scores (black bars) under external (Panel a) and internal (Panel b) biosecurity subcategories.

negative for PRRSV-1 and SIV. There were no statistically significant associations between any of the average biosecurity scores (internal, external, or overall) and the detection of PRRSV-1 or SIV on the study farms (All P-values > 0.05; Table 4).

4. Discussion

This is the first study to describe the prevailing biosecurity practices on most pig farms in Hong Kong. Our findings revealed that biosecurity practices on the study farms were generally inadequate to effectively mitigate the risk of disease introduction and spread, highlighting substantial room for improvement. Better biosecurity practices have been associated with improved production parameters, including lower mortality rates and higher average daily gain (Rodrigues da Costa et al., 2019). Higher biosecurity levels are also linked to reduced disease incidence and antimicrobial use (Postma et al., 2016).

In our study, the average external biosecurity score (56.4) was higher than the internal biosecurity score (43.9). This observation aligns with a

recent study of the Serbian pig farms by Kureljusic et al. (2024) that reported a higher external biosecurity score (75.6) compared to internal biosecurity (54.9) using the same scoring system. In contrast, Cuc et al. (2020) reported similar scores for external (53.7) and internal biosecurity (55.5) in the Vietnamese pig herds. Compared to the global average scores, our farms exhibited lower scores for all internal and most external biosecurity practices, highlighting an urgent need for improving biosecurity practices to reduce the risk of disease introduction and spread within pig herds.

Within the external biosecurity subcategory, our study revealed high scores for the purchase of breeding pigs and semen (93.2) and farm locations (70) compared to global benchmarks. The high score for the purchase of breeding pigs and semen could be attributed to the stringent local health regulations governing the importation of breeding pig stock (Agriculture Fisheries and Conservation Department., 2024b). Furthermore, producers demonstrated a high degree of diligence in implementing biosecurity practices, such as quarantine newly introduced breeding pigs, avoiding the purchase of piglets and semen from external

Table 3

Frequency distribution of farms and pens infected with six target viral pathogens tested by RT-qPCR on oral fluid samples collected from the 18 study pig farms in Hong Kong.

Infection category	Target Virus	Pen-level infection (n = 58)		
		Weaners (n = 37) No. (%)	Growers (n = 21) No. (%)	Between-farm prevalence (n = 18) No. (%)
Single infection	PRRSV-1	9 (24.3)	5 (23.8)	7 (38.9)
	PRRSV-2	32 (86.5)	12 (57.1)	17 (94.4)
	PCV-2	29 (78.4)	18 (85.7)	15 (83.3)
	SIV	17 (45.9)	7 (33.3)	10 (55.6)
	PEDV	2 (5.4)	0 (0.0)	1 (5.6)
	PDCoV	3 (8.1)	3 (14.3)	2 (11.1)
	TGEV	0 (0)	0 (0)	0 (0)
Co-infection	PRRSV-1 + PCV-2	9 (24.3)	5 (23.8)	7 (38.9)
	PRRSV-2 + PCV-2	25 (67.6)	10 (47.6)	14 (77.8)
	PRRSV-1 + SIV	5 (13.5)	2 (9.5)	5 (27.8)
	PRRSV-2 + SIV	15 (40.5)	5 (23.8)	10 (55.6)
	PCV-2 + SIV	13 (35.1)	5 (28.6)	9 (50.0)
	PRRSV-1 + PCV-2 + SIV	5 (13.5)	2 (9.5)	5 (27.8)
	PRRSV-2 + PCV-2 + SIV	11 (29.7)	5 (23.8)	9 (50.0)

Table 4

Comparison of internal, external, and overall biosecurity score averages between RT-PCR positive and negative study farms to Porcine reproductive and respiratory syndrome virus type 1 (PRRSV-1) and swine influenza virus (SIV).

Viruses	No. of farms	Internal biosecurity		External biosecurity		Overall biosecurity	
		Mean (SE)	P-value	Mean (SE)	P-value	Mean (SE)	P-value
PRRSV-1							
Positive	7	45.6 (5.0)	0.657	56.6 (2.3)	0.922	51.1 (4.3)	0.750
Negative	11	42.8 (3.4)		56.2 (4.0)		49.5 (2.6)	
SIV							
Positive	10	45.9 (4.4)	0.423	54.0 (2.4)	0.215	49.9 (3.1)	0.937
Negative	8	41.3 (3.4)		59.3 (3.3)		50.3 (3.3)	

sources, reducing the annual frequency of breeding pig deliveries, and purchasing pigs and semen from the same suppliers and herd health/sen sanitation verified farms.

The farm location has been widely recognized as a critical determinant of biosecurity and animal health due to its significant influence on the risk of disease introduction (Filippitzi et al., 2018; Ernholm et al., 2023). The relatively high score for farm location observed in our study is likely attributable to specific characteristics of the study farms. Sixty-one percent of the farms were located in areas with low pig density (<300 pigs/km²), and none reported manure spreading from other pig farms within a 500-meter radius. Moreover, nearly all farms had fencing systems to prevent the intrusion of wild boars and other animals, yet wild boars were spotted on 67 % of the farms. As wild boars act as reservoirs of several swine infectious diseases, like ASF, their presence on or near most farms poses a significant biosecurity risk (Backhans et al., 2015).

The visitors and farmworkers subcategory received the lowest score (23.5) among all external and internal subcategories. Poor biosecurity practices, including lack of farm-specific clothing and footwear and enforced downtime periods, have been associated with an increased risk of infectious disease introduction (EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2014; Pitkin et al., 2010). In our study, only three farms required visitor registrations, six farms implemented mandatory downtime periods, and none maintained a strict separation between the clean and dirty areas of the farm hygiene lock. These findings highlighted the need for stronger visitor management protocols on pig farms in Hong Kong.

The study farms also demonstrated low biosecurity scores in feed, water, and equipment supply (29.4) and vermin and bird control (49) biosecurity subcategories. Water quality monitoring was insufficient, with only three farms conducting annual microbiological testing of their water sources. Proper water management is critical for improving productivity and reducing antibiotic use in pig farming (Edwards and

Crabb, 2021). A recent pilot study conducted in Colombia demonstrated that regular cleaning of water pipes and using organic acids significantly improved productivity and delayed exposure to *Salmonella* spp. (Roldan-Henao et al., 2023). Additionally, 77.8 % of the study farms did not require feed suppliers to adhere to specific feed hygiene standards, despite feed and its ingredients being well-documented vectors for pathogens such as *Salmonella*, PEDV, and ASFV (Stewart et al., 2020; Niederwerder, 2021). Implementing audits of feed origin is a critical strategy to prevent the introduction of these infections into the herds (Alban et al., 2024).

Numerous studies have shown the role of pets, rodents, and wild birds in transmitting pathogens to and within pig farms (Backhans et al., 2011; Truong et al., 2013). In our study, birds and pets had unrestricted access to 78 % and 38 % of farm stables, respectively. These findings highlighted the need for restricting animal access to the farms, such as installing effective barriers (grids) at the air inlets.

In the internal biosecurity category, disease management scored the highest (66.7), while the nursery unit subcategory recorded the lowest score (34.9). Despite all internal biosecurity subcategories falling below global benchmarks, the study farms showed good performance in cleaning and disinfection (53.2), closely matching the global average score of 56 (Ghent University, 2025). This achievement can be attributed to the ongoing efforts of the Hong Kong SAR government in introducing cleaning and disinfection services for transport vehicles and two local slaughterhouses following the ASF outbreak in 2019. This initiative aimed to ensure compliance with standardized protocols after unloading and before departure, thereby reducing the risk of ASF and other infectious diseases (Agriculture Fisheries and Conservation Department, 2024c). Cleaning and disinfection at designated stations are identified as one of the most critical risk reduction strategies for infectious diseases (Gao et al., 2023).

Several factors can influence the implementation of biosecurity measures, including herd size, producer experience, regulatory

framework governing agricultural practices, age and condition of the facilities, as well as economic factors, such as the proportion of income allocated to biosecurity-related expenditures (Laanen et al., 2013; Wang et al., 2023; Food and Agriculture Organization of the United Nations, 2010). In Hong Kong, most pig farms are small, family-run operations that struggle with limited space and outdated infrastructure. Producers are often hesitant to invest in biosecurity infrastructure, likely due to its high operational costs and the uncertain prospects of the industry in Hong Kong (Chan, 2020; Chan and Enticott, 2023; USDA Foreign Agricultural Service., 2023). Furthermore, some producers operate on rented land, which may further discourage investment in biosecurity infrastructure due to the uncertainties around the rental agreement durations and doubt about the return of the investment. A recent study in Germany indicated that biosecurity practices, such as shoe hygiene protocols, were less frequently implemented in main and leased pig farm sites than in separately standing farm buildings (Klein et al., 2024). Addressing these challenges requires targeted interventions, such as securing land tenure and promoting the long-term economic benefits of biosecurity, to improve compliance and enhance disease control and prevention efforts.

In modern pig production, oral fluid sampling has emerged as a cost-effective and reliable method for monitoring important pathogens, including PRRSV, PCV-2, influenza A virus (IAV), *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*), and various swine coronaviruses (Prickett et al., 2008a; Hernández-García et al., 2017; Bjstrom-Kraft et al., 2018). In our study, oral fluid PCR testing revealed high between-farm prevalences for PRRSV-2 (94%), PCV-2 (83%), SIV (56%), and PRRSV-1 (39%). These findings were consistent with recent studies in Hong Kong and Mainland China, reflecting the persistent challenge of controlling these priority diseases (Flay et al., 2022; Wang et al., 2022).

Notably, co-infections with PRRSV, PCV-2, and SIV were detected on 50% of the farms, aligning with previous findings that multiple viral infections are common in pig herds and often result in more complex and severe clinical presentations than single infections (Saade et al., 2020). Furthermore, studies from China have shown that PRRSV is the predominant virus involved in co-infections with other major pathogens, such as PCV-2 and CSFV, complicating diagnosis and practical disease management (Chen et al., 2019; Ma et al., 2021; Zhao et al., 2021).

Vaccination coverage among participating farms was high, with over 83% administering vaccines against major viral pathogens, including PCV-2, CSFV, PPV, FMD, PRRSV, and PRV. This widespread adoption underscores the strong commitment of producers to disease control and reflects good access to vaccines. Achieving high vaccination rates is crucial for establishing herd immunity and maintaining effective infectious disease control (Mancera Gracia et al., 2020). Nevertheless, the persistently high between-farm prevalence of PRRSV and PCV-2, despite extensive vaccination efforts, indicates that vaccination alone is insufficient. Combining vaccination with robust biosecurity practices is critical to effectively preventing the introduction and spread of pathogens (Lopez-Moreno et al., 2022).

Surprisingly, none of the studied farms had vaccinated against SIV in the past two years, despite its detection on 56% of the farms. This highlights a gap in SIV prevention and indicates that the current biosecurity practices on these farms are inadequate to stop SIV transmission. Implementing effective vaccination programs (Sandbulte et al., 2015) together with enhanced biosecurity practices and routine surveillance is essential to reduce SIV infection and mitigate their potential risks to public health.

For swine coronaviruses, our study identified limited between-farm prevalences, with only one farm testing positive for PEDV and two for PDCoV, while none tested positive for TGEV. Vaccination against these viruses was sporadic, indicating that producers rely primarily on biosecurity to prevent outbreaks. While the current practice has seemingly limited the prevalence of these pathogens in the studied farms, swine coronaviruses remain a significant concern due to their economic impact

on the regional pig industry, particularly in China (Kong et al., 2024). Therefore, pig producers in Hong Kong should consider implementing strict biosecurity protocols and utilizing approved commercially available vaccines to minimize the risk of associated losses.

Previous studies have shown that farms with higher biosecurity scores tend to have a lower prevalence of pathogens (Stadler et al., 2024). However, this study did not observe any significant differences between the biosecurity scores and the between-farm prevalences of PRRSV-1 and SIV. This discrepancy may be attributed to several factors, including our limited number of farms (i.e., low statistical power). Among the target pathogens analysed, only PRRSV-1 and SIV had enough positive and negative farms to enable meaningful statistical comparisons.

While our study provides comprehensive insights into biosecurity practices and the status of important viral infections on pig farms in Hong Kong, it comes with some limitations that should be considered in interpreting the results. First, the collected biosecurity data relied on the participants' responses, which might have introduced a degree of bias because they might not always disclose their actual compliance with biosecurity protocols or may inaccurately assess their practices. However, we do not expect substantial bias from this source because of the small community of local producers and the familiarity of our ambulatory team with the farms' environments and producers.

The second limitation would be the generalizability of our findings to all pig farms in Hong Kong. Participation in our study was voluntary, which may have led to the exclusion of producers facing either more or less serious biosecurity challenges, potentially introducing selection bias and affecting the accuracy of biosecurity practice estimates. However, our study covered nearly half of the active farms in Hong Kong, which appeared representative in terms of production type, herd size, and outcomes, thereby likely reducing its magnitude and the potential level of concern about this source of bias.

The third limitation could be related to the timing of our data collection, which partially coincided with an ASF outbreak in Hong Kong. During this period, some producers might have reported more stringent biosecurity practices than their typical practices, potentially leading to a slight overestimation of compliance due to increased awareness and concerns about the outbreak. In addition, our original sampling protocol was affected by the availability of weaner and grower pens and the number of barns at each farm during the data collection period. While we did our best to represent the farms well by sampling a total of 60 pens across the 18 study farms, we might have still missed the viral infection statuses on some of the negative farms (i.e., a small possibility for underestimating the between-farm prevalences).

Lastly, while we intended to conduct a census and invited all active producers in Hong Kong to participate in our study (18/40 participated), the inevitable small number of farms did not allow for deeper statistical comparisons and exploring potential associations in a robust manner (e.g., between biosecurity practices and some other viral infection levels).

5. Conclusions

This study was the first comprehensive attempt to map the implemented biosecurity practices on pig farms in Hong Kong. Biosecurity practices were generally insufficient, with a more pronounced deficiency in the internal biosecurity measures. While two external biosecurity areas, including breeding pig and semen purchase and farm location, were deemed satisfactory, all other biosecurity scores fell below the global average scores, hindering the ability of producers to prevent and control diseases effectively. The high between-farm prevalences of some important viral pathogens, including PRRSV, PCV-2, and SIV, highlighted the urgent need for enhanced biosecurity interventions. Improvements should focus on restricting pets and birds from accessing stables, strengthening biosecurity protocols for farm visitors, raising hygiene standards for feed, water, and equipment supplies, and enhancing practices in specific compartments such as

farrowing, nursery, and finishing units. Targeted interventions should be directed toward farms with the most significant biosecurity deficits; providing tailored support and regular audits can help track progress and ensure sustainable improvements. Further research should prioritize investigating viral characteristics and interactions on the farms to elucidate their mechanisms of spread and persistence despite the currently high adoption rate of most vaccines. Our established baseline farm information in this study provides valuable insights to all stakeholders involved in the local pig industry in Hong Kong, including producers, veterinarians, and government authorities, to work together on improving specified biosecurity practices and developing tailored disease management and mitigation strategies.

CRedit authorship contribution statement

Belete Haile: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Congnuan Liu:** Validation, Investigation. **Maura Carrai:** Writing – review & editing, Validation, Investigation, Conceptualization. **Yun Young Go:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Conceptualization. **Chi KwanYip:** Writing – review & editing, Investigation. **Lip Tet NG:** Writing – review & editing, Investigation. **Yui Gordon Auyeung:** Writing – review & editing, Investigation. **Ying Luk:** Writing – review & editing, Investigation. **Karyn A.Havas:** Writing – review & editing, Supervision. **Renata Ivanek:** Writing – review & editing, Supervision. **Dirk U.Pfeiffer:** Writing – review & editing, Validation, Resources, Project administration, Funding acquisition. **Anne Conan:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Omid Nekouei:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the first author used ChatGPT in order to make grammatical edits in some sections. After using this tool, the authors reviewed and edited the content as needed, and they take full responsibility for the content of the manuscript

Funding

This study was funded by the Sustainable Agricultural Development Fund (SADF) of the Hong Kong SAR Government; Agriculture, Fisheries and Conservation Department (AFCD).

Disclaimer

Any opinions, findings, conclusions, or recommendations expressed in this manuscript do not reflect the views of the Government of the Hong Kong Special Administrative Region, Agriculture, Fisheries and Conservation Department (AFCD), or the Sustainable Agricultural Development Fund Advisory Committee.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

We would like to acknowledge the Sustainable Agricultural Development Fund of the Hong Kong SAR Government, Agriculture, Fisheries and Conservation Department (SADF-AFCD) for their logistical support throughout this project. We are also grateful to the pig ambulatory team members at the Centre for Applied One Health Research and Policy Advice (OHRP), City University of Hong Kong, as well as to the local pig

farmers for their kind contribution to our study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2025.106569.

References

- Agriculture Fisheries and Conservation Department., 2024a. Agriculture in Hong Kong. The Government of the Hong Kong Special Administrative Region. (https://www.afcd.gov.hk/english/agriculture/agr_hk/agr_hk.html) Accessed on 15 July 2024.
- Agriculture Fisheries and Conservation Department., 2024b. Import of live pigs for breeding purposes: The Government of the Hong Kong Special Administrative Region. (https://www.afcd.gov.hk/english/quarantine/qua_ie/qua_ie_ipab/qua_ie_ipab_ilpbp/qua_ie_ipab_ilpbp.html) Accessed on 15 July 2024.
- Agriculture Fisheries and Conservation Department., 2024c. Local pig samples test positive for ASF virus: The Government of the Hong Kong Special Administrative Region (https://www.afcd.gov.hk/english/publications/publications_press/pr2969.html) Accessed on 15 August 2024.
- Alarcón, L.V., Allepuz, A., Mateu, E., 2021. Biosecurity in pig farms: a review. *Porc. Health Manag* 7, 5. <https://doi.org/10.1186/s40813-020-00181-z>.
- Alban, L., Enemark, H., Petersen, H.H., Nielsen, L.H., 2024. Auditing of Danish pig herds for controlled housing requirements and Trichinella. *Food Waterborne Parasitol.* 37, e00247. <https://doi.org/10.1016/j.fawpar.2024.e00247>.
- Backhans, A., Fellström, C., Lambertz, S.T., 2011. Occurrence of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in small wild rodents. *Epidemiol. Infect.* 139, 1230–1238. <https://doi.org/10.1017/S0950268810002463>.
- Backhans, A., Sjölund, M., Lindberg, A., Emanuelson, U., 2015. Biosecurity level and health management practices in 60 Swedish farrow-to-finish herds. *Acta Vet. Scand.* 57, 14. <https://doi.org/10.1186/s13028-015-0103-5>.
- Bjuström-Kraft, J., Christopher-Hennings, J., Daly, R., 2018. The use of oral fluid diagnostics in swine medicine. *J. Swine Health Prod.* 26, 262–269. <https://doi.org/10.54846/jshap/1091>.
- Chan, K.W., 2020. Politics of smell: Constructing animal waste governmentality and good farming subjectivities in colonial Hong Kong. *Environ. Plan. C Polit. Space* 38, 1055–1074. <https://doi.org/10.1177/2399654420914320>.
- Chan, K.W., Enticott, G., 2023. Good methods for good farmers? Mapping the language of good farming with “diligent farmers” in Hong Kong. *J. Rural Stud.* 100, 103005. <https://doi.org/10.1016/j.jrurstud.2023.03.013>.
- Chen, N., Huang, Y., Ye, M., Li, S., Xiao, Y., Cui, B., Zhu, J., 2019. Co-infection status of classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circoviruses (PCV2 and PCV3) in eight regions of China from 2016 to 2018. *Infect. Genet. Evol.* 68, 127–135. <https://doi.org/10.1016/j.meegid.2018.12.011>.
- Choi, Y.-K., Pascua, P.N.Q., Song, M.-S., 2013. Swine Influenza Viruses: An Asian Perspective. In: Richt, J.A., Webby, R.J. (Eds.), *Swine Influenza*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 147–172.
- Christensen, J., Gardner, I.A., 2000. Herd-level interpretation of test results for epidemiologic studies of animal diseases. *Prev. Vet. Med.* 45, 83–106. [https://doi.org/10.1016/S0167-5877\(00\)00118-5](https://doi.org/10.1016/S0167-5877(00)00118-5).
- Cuc, N.T.K., Dinh, N.C., Quyen, N.T.L., Tuan, H.M., 2020. Biosecurity level practices in pig and poultry production in Vietnam. *Adv. Anim. Vet. Sci.* 8, 1068–1074. <https://doi.org/10.17582/journal.aavs/2020/8.10.1068.1074>.
- Dewulf, J., Immerseel, Fv, 2019. General principles of biosecurity in animal production and veterinary medicine. *CABI*, pp. 63–76. <https://doi.org/10.1079/9781789245684.0063>.
- Dhaka, P., Chantziaras, I., Vijay, D., Bedi, J.S., Makovska, I., Biebau, E., Dewulf, J., 2023. Can improved farm biosecurity reduce the need for antimicrobials in food animals? A scoping review. *Antibiotics* 12, 89312. (<https://www.mdpi.com/2079-6382/12/5/893>). <https://pubmed.ncbi.nlm.nih.gov/articles/PMC10215210/pdf/antibiotics-12-00893.pdf>.
- Edwards, L., Crabb, H., 2021. Water quality and management in the Australian pig industry. *Anim. Prod. Sci.* 61, 637–644. <https://doi.org/10.1071/AN20484>.
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2014. Scientific opinion on African swine fever. *EFSA J.* 12, 3628. <https://doi.org/10.2903/j.efsa.2014.3628>.
- Ernholm, L., Ståhl, K., Neimanas, A., Widgren, S., Lewerin, S., 2023. Understanding the interface between European wild boar (*Sus scrofa*) and domestic pigs (*Sus scrofa domestica*) in Sweden through a questionnaire study. *Acta Vet. Scand.* 65. <https://doi.org/10.1186/s13028-023-00705-x>.
- Filippitzi, M.E., Brinch Kruse, A., Postma, M., Sarrazin, S., Maes, D., Alban, L., Nielsen, L. R., Dewulf, J., 2018. Review of transmission routes of 24 infectious diseases preventable by biosecurity measures and comparison of the implementation of these measures in pig herds in six European countries. *Transbound. Emerg. Dis.* 65, 381–398. <https://doi.org/10.1111/tbed.12758>.
- Flay, K.J., Yang, D.A., Choi, S.C., Ip, J., Lee, S.H., Pfeiffer, D.U., 2022. First study to describe the prevalence of porcine reproductive and respiratory syndrome virus and porcine circovirus Type 2 among the farmed pig population in the Hong Kong Special Administrative Region. *Vet. Sci.* <https://doi.org/10.3390/vetsci9020080>.
- Food and Agriculture Organization of the United Nations/World Organisation for Animal Health/World Bank., 2010. Good practices for biosecurity in the pig sector – Issues and options in developing and transition countries, (<https://www.fao.org/4/i1435e/i1435e00.htm>), Accessed on 24 August 2024.

- Food and Agriculture Organization of the United Nations., 2023, Progressive Management Pathway for Terrestrial Animal Biosecurity (FAO-PMP-TAB), (<https://openknowledge.fao.org/handle/20.500.14283/cc5771en>) Accessed on 25 August 2024.
- Gao, Y., Nielsen, L.H., Boklund, A.E., de Jong, M.C.M., Alban, L., 2023. SWOT analysis of risk factors associated with introduction of African swine fever through vehicles returning after export of pigs. *Front. Vet. Sci.* 9. <https://doi.org/10.3389/fvets.2022.1049940>.
- Gelaude, P., Schlepers, M., Verlinden, M., Laanen, M., Dewulf, J., 2014. Biocheck.UGent: a quantitative tool to measure biosecurity at broiler farms and the relationship with technical performances and antimicrobial use. *Poult. Sci.* 93, 2740–2751. <https://doi.org/10.3382/ps.2014-04002>.
- Ghent University, 2025, Biocheck.UGent. Statistics for Pigs indoor -Worldwide, (<https://biocheckgent.com/en/worldwide/>), Accessed on 15 March 2025.
- Gibert, E., Martín-Valls, G., Mateu, E., 2017. Comparison of protocols for the analysis of type 1 porcine reproductive and respiratory syndrome virus by RT-PCR using oral fluids. *J. Virol. Methods* 243, 190–195. <https://doi.org/10.1016/j.jviromet.2017.02.010>.
- Go, Y.Y., Ho, J.H.P., Tam, K.W.S., Kamali, M., Zhang, Y., Lau, C.C.Y., Li, S.H., Wilson, M. T., Guo, Z., Li, R., Gu, G., Tse, M.P.Y., Hill, F.I., Batten, C., Corla, A., Flannery, J., Conan, A., Brackman, C.J., Pfeiffer, D.U., 2023. Investigation of the first African Swine fever outbreak in a domestic pig farm in Hong Kong. *Transbound. Emerg. Dis.* 2023, 1720474. <https://doi.org/10.1155/2023/1720474>.
- Henaio-Díaz, A., Giménez-Lirola, L., Baum, D.H., Zimmerman, J., 2020. Guidelines for oral fluid-based surveillance of viral pathogens in swine. *Porc. Health Manag.* 6, 28. <https://doi.org/10.1186/s40813-020-00168-w>.
- Haile, B., Karalliu, E., Ho, J., Havas, K.A., Ivanek, R., Ip, J., Xin, C., Nekouei, O., 2025. Reproductive Failure in Smallholder Pig Farms in East and Southeast Asia: A Systematic Review. *Animals (Basel)*. 15 (9), 1226. <https://doi.org/10.3390/ani15091226>.
- Hernández-García, J.A., Robben, N., Magnée, D., Eley, T.J., Dennis, I., Kayes, S.M., Thomson, J.R., Tucker, A.W., 2017. The use of oral fluids to monitor key pathogens in porcine respiratory disease complex. *Porc. Health Manag.* 3. <https://doi.org/10.1186/s40813-017-0055-4>.
- Ito, S., Kawaguchi, N., Bosch, J., Aguilar-Vega, C., Sánchez-Vizcaíno, J.M., 2023. What can we learn from the five-year African swine fever epidemic in Asia? *Front Vet. Sci.* 10. <https://doi.org/10.3389/fvets.2023.1273417>.
- Kedkovid, R., Sirisereewan, C., Thanawongnuwech, R., 2020. Major swine viral diseases: an Asian perspective after the African swine fever introduction. *Porc. Health Manag.* 6, 20. <https://doi.org/10.1186/s40813-020-00159-x>.
- Klein, L., Gerdes, U., Blome, S., Campe, A., Große, Beilage, E., 2024. Biosecurity measures for the prevention of African swine fever on German pig farms: comparison of farmers' own appraisals and external veterinary experts' evaluations. *Porc. Health Manag.* 10, 14. <https://doi.org/10.1186/s40813-024-00365-x>.
- Kong, F., Jia, H., Xiao, Q., Fang, L., Wang, Q., 2024. Prevention and control of swine enteric coronaviruses in China: a review of vaccine development and application. *Vaccines* 12, 1112. (<https://www.mdpi.com/2076-393X/12/1/111>).
- Kureljusic, B., Maletic, J., Božidar, S., Milovanović, B., Ninković, M., Nemanja, J., Prodanov-Radulovic, J., 2024. Evaluating biosecurity on selected commercial pig farms in Serbia. *Maced. Vet. Rev.* 47. <https://doi.org/10.2478/macvetrev-2024-0025>.
- Laanen, M., Beek, J., Ribbens, S., Vangroenweghe, F., Maes, D., Dewulf, J., 2010. Biosecurity on pig herds: development of an on-line scoring system and the results of the first 99 participating herds. *Vlaams Diergeneesk. Tijdschr.* 79, 302–30679.
- Laanen, M., Persoons, D., Ribbens, S., de Jong, E., Callens, B., Strubbe, M., Maes, D., Dewulf, J., 2013. Relationship between biosecurity and production/antimicrobial treatment characteristics in pig herds. *Vet. J.* 198, 508–512. <https://doi.org/10.1016/j.tvjl.2013.08.029>.
- Lee, H.S., Bui, V.N., Nguyen, H.X., Bui, A.N., Hoang, T.D., Nguyen-Viet, H., Grace Randolph, D., Wieland, B., 2020. Seroprevalences of multi-pathogen and description of farm movement in pigs in two provinces in Vietnam. *BMC Vet. Res.* 16. <https://doi.org/10.1186/s12917-020-2236-7>.
- Lopez-Moreno, G., Schmitt, C., Spronk, T., Culhane, M., Torremorell, M., 2022. Evaluation of internal farm biosecurity measures combined with sow vaccination to prevent influenza A virus infection in groups of due-to-wean pigs. *BMC Vet. Res.* 18, 393. <https://doi.org/10.1186/s12917-022-03494-z>.
- Ma, Z., Liu, M., Liu, Z., Meng, F., Wang, H., Cao, L., Li, Y., Jiao, Q., Han, Z., Liu, S., 2021. Epidemiological investigation of porcine circovirus type 2 and its coinfection rate in Shandong province in China from 2015 to 2018. *BMC Vet. Res.* 17, 17. <https://doi.org/10.1186/s12917-020-02718-4>.
- Mai, T.N., Yamazaki, W., Bui, T.P., Nguyen, V.G., Le Huynh, T.M., Mitoma, S., Daous, H. E., Kabali, E., Norimine, J., Sekiguchi, S., 2020. A descriptive survey of porcine epidemic diarrhea in pig populations in northern Vietnam. *Trop. Anim. Health Prod.* 52, 3781–3788. <https://doi.org/10.1007/s11250-020-02416-1>.
- Mancera Gracia, J.C., Pearce, D.S., Masic, A., Balasch, M., 2020. Influenza A virus in swine: epidemiology, challenges and vaccination strategies. *Front. Vet. Sci.* 7, 647. <https://doi.org/10.3389/fvets.2020.00647>.
- Niederwerder, M.C., 2021. Risk and mitigation of African swine fever virus in feed. *Anim. Open Access J. MDPI* 11, 792. <https://doi.org/10.3390/ani11030792>.
- Pitkin, A., Otake, S., Dee, S.A., Acvm, D., 2010. A one-night downtime period prevents the spread of porcine reproductive and respiratory syndrome virus and Mycoplasma hyopneumoniae by personnel and fomites (boots and coveralls). In. (<https://www.aasv.org/shap/issues/v19n6/v19n6p345.pdf>).
- Postma, M., Backhans, A., Collineau, L., Loesken, S., Sjölund, M., Belloc, C., Emanuelson, U., Grosse Beilage, E., Stärk, K.D.C., Dewulf, J., 2016. The biosecurity status and its associations with production and management characteristics in farrow-to-finish pig herds. *Animal* 10, 478–489. <https://doi.org/10.1017/S1751731115002487>.
- Prickett, J., Kim, W., Simer, R., Yoon, K.-J., Zimmerman, J., 2008a. Oral-fluid samples for surveillance of commercial growing pigs for porcine reproductive and respiratory syndrome virus and porcine circovirus type 2 infections. *J. Swine Health Prod.* 16, 86–91. <https://doi.org/10.54846/jshap/565>.
- Prickett, J., Simer, R., Christopher-Hennings, J., Yoon, K.J., Evans, R.B., Zimmerman, J. J., 2008b. Detection of Porcine reproductive and respiratory syndrome virus infection in porcine oral fluid samples: a longitudinal study under experimental conditions. *J. Vet. Diagn. Invest.* 20, 156–163. <https://doi.org/10.1177/104063870802000203>.
- Qiu, Y., Weber-Vintzel, L., Abila, R., 2020. Pig value chain in South-East Asia and the challenges to disease management. *Bull. De l'OIE* 1–2. <https://doi.org/10.20506/bull.2020.1.3120>.
- Rodrigues da Costa, M., Gasa, J., Calderón Díaz, J.A., Postma, M., Dewulf, J., McCutcheon, G., Manzanilla, E.G., 2019. Using the Biocheck.UGent™ scoring tool in Irish farrow-to-finish pig farms: assessing biosecurity and its relation to productive performance. *Porc. Health Manag.* 5. <https://doi.org/10.1186/s40813-018-0113-6>.
- Roldan-Henaio, M., Dalsgaard, A., Cardona-Castro, N., Restrepo-Rivera, L., Veleza-Angulo, L.C., Alban, L., 2023. Pilot study of the productivity and Salmonella seroprevalence in pigs administered organic acids. *Front. Vet. Sci.* 10. <https://doi.org/10.3389/fvets.2023.1123137>.
- Rosanowski, S.M., Magouras, I., Ho, W.C., Yiu, W.C.J., Pfeiffer, D.U., Zeeh, F., 2023. The challenges of pig farming in Hong Kong: a study of farmers' perceptions and attitudes towards a pig health and production management service. *BMC Vet. Res.* 19, 30. <https://doi.org/10.1186/s12917-023-03591-7>.
- Saade, G., Deblanc, C., Bougon, J., Marois-Créhan, C., Fablet, C., Auray, G., Belloc, C., Leblanc-Maridor, M., Gagnon, C.A., Zhu, J., Gottschalk, M., Summerfield, A., Simon, G., Bertho, N., Meurens, F., 2020. Coinfections and their molecular consequences in the porcine respiratory tract. *Vet. Res.* 51. <https://doi.org/10.1186/s13567-020-00807-8>.
- Saito, T., Sakuma, S., Mine, J., Uchida, Y., Hangalapura, B.N., 2022. Genetic diversity of the hemagglutinin genes of influenza A virus in asian swine populations. *Viruses* 14, 74714. (<https://www.mdpi.com/1999-4915/14/4/7471>).
- Sandbulte, M.R., Spickler, A.R., Zaabel, P.K., Roth, J.A., 2015. Optimal use of vaccines for control of influenza A virus in swine. *Vaccines* 3, 22–733. (<https://www.mdpi.com/2076-393X/3/1/22>).
- Stadler, J., Lillie-Jaschniski, K., Zwickl, S., Zoels, S., Theuns, S., Ritzmann, M., Vereecke, N., 2024. Cross-correlation between biosecurity measures and the detection of viral and bacterial agents on German farms with respiratory disease. *Transbound. Emerg. Dis.* 2024, 6205899. <https://doi.org/10.1155/2024/6205899>.
- Stewart, S.C., Dritz, S.S., Woodworth, J.C., Paulk, C.B., Jones, C.K., 2020. A review of strategies to impact swine feed biosecurity. *Anim. Health Res. Rev.* 21, 61–68. <https://doi.org/10.1017/S146625231900015X>.
- Thakor, J.C., Dinesh, M., Manikandan, R., Bindu, S., Sahoo, M., Sahoo, D., Dhawan, M., Pandey, M.K., Tiwari, R., Emran, T.B., Dhama, K., Chaicumpa, W., 2022. Swine coronaviruses (SCoVs) and their emerging threats to swine population, inter-species transmission, exploring the susceptibility of pigs for SARS-CoV-2 and zoonotic concerns. *Vet. Q* 42, 125–147. <https://doi.org/10.1080/01652176.2022.2079756>.
- Truong, Q.L., Seo, T.W., Yoon, B.-I., Kim, H.-C., Han, J.H., Hahn, T.-W., 2013. Prevalence of swine viral and bacterial pathogens in rodents and stray cats captured around pig farms in Korea. *J. Vet. Med. Sci.* 75, 1647–1650. <https://doi.org/10.1292/jvms.12-0568>.
- USDA Foreign Agricultural Service., 2023, Hong Kong: Livestock and Products Annual: Global Agricultural Information Network (GAIN). (<https://www.atohongkong.com.hk/wps/wp-content/uploads/HK2023-0029-Livestock-and-Products-Annual.pdf>), Accessed on 15 July 2024.
- Wang, H., Chen, M., Guo, Z., Shen, Y., Chen, Y., Luo, T., Liu, Y., Li, J., Wang, F., Wan, J., 2023. The influencing factors of "Post-African Swine Fever" pig farm biosecurity: evidence from Sichuan Province, China. *Animals* 13. <https://doi.org/10.3390/ani13190503>.
- Wang, L., Li, D., 2024. Invited Review - Current status, challenges and prospects for pig production in Asia. *Anim. Biosci.* 37, 742–754. <https://doi.org/10.5713/ab.23.0303>.
- Wang, J., Zhu, X., Cai, C., Pan, X., Wang, C., 2022. Risk analysis of viral diseases in infected pig farms during the lockdown period in China, January to May 2020. *Int. J. Env. Res. Public Health* 19, 3215. <https://doi.org/10.3390/ijerph19063215>.
- Woonwong, Y., Do Tien, D., Thanawongnuwech, R., 2020. The future of the pig industry after the introduction of african swine fever into Asia. *Anim. Front.* 10, 30–37. <https://doi.org/10.1093/af/vfa037>.
- World Animal Health Information System., 2023, Six-monthly report., (<https://wahis.woah.org/#/smr-management>) Accessed on 5 August 2024.
- Zhao, D., Yang, B., Yuan, X., Shen, C., Zhang, D., Shi, X., Zhang, T., Cui, H., Yang, J., Chen, X., Hao, Y., Zheng, H., Zhang, K., Liu, X., 2021. Advanced research in porcine reproductive and respiratory syndrome virus co-infection with other pathogens in Swine. *Front Vet. Sci.* 8. <https://doi.org/10.3389/fvets.2021.699561>.