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African Swine Fever (Asf) Control and Repopulation Strategies in Tropical Condition: Effectiveness of Cleaning and Disinfection Protocols

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Research Article

Keywords: African swine fever, cleaning and disinfection, biosecurity, repopulation

Posted Date: March 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9001682/v1

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Version of Record: A version of this preprint was published at Tropical Animal Health and Production on September 22nd, 2026. See the published version at https://doi.org/10.1007/s11250-026-05360-8.

Abstract

This study aimed to evaluate the effectiveness of existing cleaning and disinfection (C&D) protocols in inactivating African swine fever virus (ASFV) on affected pig farms in Malaysia. This work also identifies farm-level biosecurity weaknesses that contribute to ASF risk. Farms with a history of ASF infection were selected on the basis of specific biosecurity and C&D criteria. A structured questionnaire was distributed to the farmers to collect information on farm background and risk factors related to C&D and water sources. Environmental swabs collected post-C&D were tested via real-time polymerase chain reaction (qPCR). Data obtained from the questionnaire survey and environmental qPCR testing were analyzed via SPSS statistical software. Chi-square tests and one-way analysis of variance (ANOVA) were applied to determine significant differences in ASFV detection outcomes across farms and to identify associations between specific biosecurity practices and the presence of ASFV. These findings provide practical guidance for farmers, highlighting how effective C&D practices can enhance biosecurity and support repopulation efforts following ASF outbreaks.

1. Introduction

African swine fever virus (ASFV) was first reported in Kenya in 1921 and initially emerged in sub-Saharan African nations (Li et al. 2022). By 1960, ASFV genotype I had been introduced into Europe from western Africa, and in 2007, ASFV genotype II had spread from eastern Africa and subsequently disseminated widely throughout Europe. In 2018, ASFV genotype II reached China via Russia (Li et al. 2022). Following its introduction into China, ASF rapidly spread to neighboring regions, including Mongolia, Hong Kong, North Korea, South Korea, and Southeast Asian countries such as Vietnam, Cambodia, Laos, the Philippines, Myanmar, Timor-Leste, Indonesia, Papua New Guinea and India (*ASF Situation in Asia & Pacific Update* 2025; Kim et al. 2021; Blome et al. 2020). In February 2021, Malaysia reported confirmed ASF cases in backyard pigs across several districts in Sabah (C.K. 2021).

ASFV is a large, double-stranded DNA virus with an envelope ranging from 170–194 kbp in genome size and measuring approximately 172–191 nm in diameter (Nuanualsuwan et al. 2022; Blome et al 2020). According to the World Organization for Animal Health (WOAH), ASF is a highly contagious viral disease affecting both domestic and wild pigs, with mortality rates reaching 100% (Liu et al. 2021). ASFV can be transmitted directly via the fecal–oral route, such as via exposure to infectious blood, excretions, and secretions from infected pigs, and can consume contaminated feed as well as mechanical transmission, e.g., contaminated fomites, ticks, lice, and flies (C.K. 2021; Guinat et al. 2016).

The incubation period for ASF typically ranges from 3–5 days, with most infected pigs dying within 7–13 days post-infection (Guinat et al. 2016). Clinical signs and pathological lesions vary, depending on the virulence of the virus isolate, infection dose, route of transmission and host susceptibility (Salguero 2020). ASFV isolates are categorized as highly virulent, moderately virulent or low virulent, with clinical presentations ranging from peracute to chronic forms (Salguero 2020). The clinical and histopathological features of ASF are similar to those of classical swine fever (CSF), salmonellosis and highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) (Kim et al. 2021).

Currently, there is no effective treatment or commercial vaccine for ASF due to the limited understanding of the protective immune response in pigs (Wang et al. 2024). This lack of therapeutic options has made ASF extremely difficult to control. The primary strategies for controlling ASF include stamping out infected herds, implementing rigorous C&D protocols and ensuring strict biosecurity to prevent the spread or reinfection of ASF. Physical isolation of the herd can be achieved by maintaining adequate distances between farms, fully fencing the herd, and installing a closed entrance to the farm area, which also reduces the risk of transmission from wild boars (Kim et al. 2021; Madec et al. 2025). Proper carcass disposal remains a key component of biosecurity (Kim et al. 2021).

Since 2021, ASF outbreaks have affected multiple pig farms in Malaysia. In the absence of vaccination, effective C&D remains critical for virus control and safe repopulation. However, swine farms across the country employ a wide variety of unstandardized C&D practices that have not been scientifically validated under local tropical conditions. The hot and humid climate, combined with the presence of organic matter, may reduce disinfectant efficacy and consequently increase the risk of ASF. To date, data on the effects of these protocols in eliminating ASFV are limited. This research investigated the possibility of the C&D method enhancing repopulation planning and reducing the risk of recurrence.

C&D is an important part of biosecurity. A properly executed C&D protocol can effectively remove pathogens and prevent disease recurrence. There are 7 steps in a complete C&D protocol: (I) dry cleaning, (II) wet cleaning, (III) rinsing, (IV) initial drying, (V) disinfection, (VI) final drying and (VII) testing the efficiency of the procedure (Štukelj et al. 2021). Dry cleaning refers to the removal of gross contamination, organic material, and debris, whereas wet cleaning involves water and soap/detergent (De Lorenzi et al. 2020; Štukelj et al. 2021). Disinfection refers to the use of chemicals or heat to destroy or eliminate pathogens (Štukelj et al. 2021). The cleaning process is generally conducted from cleaner to dirtier areas to avoid cross-contamination.

WOAH recommends the following disinfectants for ASFV inactivation (Guberti et al. 2019):

Class of Disinfectants	**Examples**
Aldehydes	Glutaraldehyde, Formaldehyde
Chlorine compounds	Sodium hypochlorite, Calcium hypochlorite
Iodine compounds	Iodophor, Potassium tetraglicine triiodide
Alkalis	Calcium hydroxide, Sodium hydroxide (Caustic soda)
Oxidizing acids	Peracetic acid, Peroxygen compounds
Quaternary Ammonium compounds (QACs)	Benzalkonium chloride, Didecyl dimethyl ammonium chloride

Although studies have been conducted in countries such as Italy, China, and the United Kingdom to evaluate the efficacy of commercial disinfectants against ASFV, there is a lack of equivalent studies under tropical farm conditions that use Malaysia as a tropical example. Therefore, this study assessed the efficacy of various commercial disinfectants commonly used in tropical areas under field conditions and tested their effectiveness. Therefore, it aims to identify effective C&D strategies and recommendations to improve biosecurity and support repopulation.

2. Materials and methods

This study focused on pig farms in Malaysia that had previously experienced confirmed ASF outbreaks. Farms were selected on the basis of specific criteria related to key C&D practices, including water sources, the use of foaming agents, and the types of disinfectants applied.

A structured questionnaire survey was conducted among farm managers or farm owners to assess current biosecurity practices. Responses were obtained from 37 pig farms across the country, covering five major biosecurity domains, namely, (I) personnel entry, (II) farm structure, (III) farm and pig house cleaning and disinfection, (IV) carcass disposal management and (V) water source management.

Following the questionnaire survey, environmental swab sampling was conducted on six selected pig farms located in Peninsular Malaysia. The cleaning and disinfection procedures were carried out according to each farm's routine

practices, supplemented with recommended C&D steps where applicable. Environmental swab samples were collected from pig pens, feeding areas, walls, floors, vehicles, and other relevant environmental locations.

All the samples were tested for the presence of the ASF virus via real-time polymerase chain reaction (qPCR). A cycle threshold (Ct) value of 38 was used as the cutoff point, whereby samples with Ct values below 38 were classified as ASFV positive, whereas samples with Ct values of 38 or above were classified as ASFV negative.

All the data collected from the environmental swabs and questionnaire survey were analyzed via SPSS statistical software (IBM SPSS Statistics 31.0.1.0). Questionnaire responses and environmental swab results were analyzed independently and interpreted complementarily because of differences in sample size and geographic coverage.

The environmental swab results were analyzed in two ways. First, the ASFV detection status (positive or negative) was analyzed via chi-square tests to determine whether the frequency of ASFV-positive environmental samples differed among farms. Second, one-way analysis of variance (ANOVA) was used to assess differences in mean Ct values among farms with ASFV-positive environmental samples only (Ct < 38).

Post hoc multiple comparison tests were not performed following ANOVA because at least one farm group contained fewer than two ASFV-positive samples. Therefore, differences in Ct values among farms were interpreted via the overall ANOVA results in conjunction with descriptive statistics, including mean Ct values and confidence intervals.

For the questionnaire survey, chi-square tests of independence were performed via crosstabulation. Crosstab analysis was used to assess the associations between farms and individual biosecurity criteria within each domain. A p value of less than 0.05 was considered statistically significant.

3. Results

3.1 Environmental Swab Analysis

3.1.1 Environmental swab sampling following C&D practices

Environmental swab sampling was conducted across six farms following routine cleaning and disinfection (C&D) procedures. Environmental samples were collected from pig pens, feeding areas, walls, and floors following cleaning and disinfection procedures routinely applied by each farm. ASFV detection was determined via real-time PCR, with a Ct cutoff value of 38, where samples with Ct values below this threshold were classified as ASFV positive.

The cleaning and disinfection practices implemented by each farm, along with the corresponding ASFV environmental swab outcomes and statistical results, are summarized in Table 1 (refer to Fig. 1 for the 7 steps of C&D). Differences were observed among farms in terms of disinfectant type, inclusion of drying steps, and water treatment methods.

Chi-square analysis (Table 2) revealed a significant association between farm and ASFV detection status ($p < 0.05$), indicating that the proportion of ASFV-positive environmental swabs differed significantly among farms. Farm A recorded no ASFV-positive swabs, with all sampled locations testing negative, whereas ASFV-positive swabs were detected in Farms B, C, D, E, and F. Multiple positive samples were observed in Farms B, D, E, and F across several environmental sites, including pig housing areas, vehicles, and waste-related locations. Farm C recorded only one ASFV-positive sample, which was detected at the sewage pond (waste-related location). The distribution of ASFV-positive and ASFV-negative samples across farms is summarized in Fig. 2.

Table 1
C&D steps for each farm

C&D Steps	Farm A	Farm B	Farm C	Farm D	Farm E	Farm F
Water Source	Pond	Pond	Underground water	Pond	Pond	Underground water
Dry Cleaning	Yes	Yes	Yes	Yes	Yes	Yes
Wet Cleaning	Yes, using foaming agent	Yes, with water	Yes, using foaming agent	Yes, with water	Yes, with water	Yes, with water
Rinsing & drying	Yes	Rinsing without drying	Yes	Yes	No	Rinsing without drying
Disinfection	Environment: Caustic soda 4% Water: Chlorine	Environment: Iodophor 1:200 Water: No treatment, using recycle water	Environment: Quaternary ammonium compounds & Glutaraldehyde 1:200 + Heat Water: Calcium hydroxide 30ppm + UV light	Environment: Caustic soda 4% Water: Chlorine	Environment: Quaternary ammonium compounds & Glutaraldehyde 1:200 Water: NA	Environment: Caustic soda Water: H_2O_2 1:15000

Table 2
Association between farm and ASFV detection status

Chi-Square Tests			
	Value	**df**	**Asymptotic Significance (2-sided)**
Pearson Chi-Square	67.825[a]	5	< .001
Likelihood Ratio	87.294	5	< .001
Linear-by-Linear Association	28.675	1	< .001
N of Valid Cases	98		
a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 5.39.			

3.1.2 Comparison of Ct values among ASFV-positive samples

To further evaluate the degree of environmental contamination, Ct values from ASFV-positive samples (Ct < 38) were analyzed via one-way analysis of variance (ANOVA). Only positive samples were included in this analysis.

One-way ANOVA revealed significant differences in the mean Ct values among the farms ($p < 0.05$), as shown in Table 3. Farm A was excluded from the Ct value analysis, as no ASFV-positive environmental samples were identified. Descriptive analysis (Table 4) indicated that some farms had lower mean Ct values, suggesting higher residual environmental contamination, whereas others recorded higher mean Ct values, indicating lower viral loads following cleaning and disinfection. Post hoc multiple comparison tests were not conducted because there were insufficient ASFV-positive samples in at least one farm group.

Table 3
Ct values are significantly different between positive farms

ANOVA					
Ct value					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	71.139	4	17.785	5.755	< .001
Within Groups	139.076	45	3.091		
Total	210.215	49			

Table 4
Descriptive analysis of the average Ct value of each ASFV-positive farm

Descriptives								
Ct value								
					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
B	13	33.1054	1.65945	.46025	32.1026	34.1082	29.75	35.60
C	1	37.3200					37.32	37.32
D	11	35.5582	1.33759	.40330	34.6596	36.4568	33.87	37.66
E	9	36.0467	2.91270	.97090	33.8078	38.2856	28.43	37.90
F	16	35.6119	1.16243	.29061	34.9925	36.2313	33.58	37.91
Total	50	35.0608	2.07126	.29292	34.4722	25.6494	28.43	37.91

3.2 Questionnaire Survey on Biosecurity Practices

A total of 37 pig farms, represented by farm managers or owners, completed the survey. Chi-square tests of independence conducted via crosstabulation revealed significant differences in specific biosecurity criteria across multiple domains ($p < 0.05$).

No significant differences were detected among farms for any criteria under the carcass disposal management domain, suggesting relatively consistent carcass handling practices. For the farm structure domain, regular cleaning of weeds and sewage pits within the farm area was significantly different among farms ($p < 0.05$). This suggests that variations in environmental hygiene practices may influence pest presence and disease persistence.

Within farm and pig house C&D, two criteria significantly differed among farms ($p < 0.05$): the use of caustic soda for disinfecting pig houses and the cleaning and soaking of removable facilities such as slats, feed troughs, drinkers, and nipples in disinfectants. These findings indicate heterogeneity in the intensity and effectiveness of C&D protocols implemented by different farms.

Under the personnel entry domain, the presence and use of showers and changing rooms significantly differed among farms ($p < 0.05$), reflecting variability in personnel biosecurity control measures. Within the water source domain, the

use of underground water significantly differed among farms ($p < 0.05$), indicating that differences in water sourcing practices may affect biosecurity risk.

Table 5 highlights that significant differences were observed across various domains, particularly farm structure, C&D, personnel entry and water source management, whereas carcass disposal practices were not significantly different. These findings highlight substantial variation in the effectiveness of farm-level cleaning and disinfection practices, warranting further interpretation in relation to specific biosecurity measures.

Table 5
Summary of significant biosecurity criteria identified by chi-square crosstab analysis (questionnaire survey, n = 37 farms)

1.	Domain	Biosecurity Criterion	Chi-square Significance	Interpretation
	Carcass Disposal Management	**All criteria assessed**	**Not significant (p > 0.05)**	**Carcass disposal practices were relatively consistent across farms, suggesting basic compliance following ASF outbreaks.**
2.	Farm Structure	Regular cleaning of weeds and sewage pits within the farm area	Significant (p = 0.04)	Indicates variation in environmental hygiene and pest habitat control, which may influence ASFV persistence and mechanical transmission (Mutua & Dione 2021).
3.	Farm and Pig House Cleaning and Disinfection	(i) Use of caustic soda for pig house disinfection	Significant (p = 0.034)	Reflects differences in disinfectant selection and stringency of cleaning protocols, potentially affecting ASFV inactivation (Tian et al. 2020).
		(ii) Removable facilities (slats, feed troughs, drinkers, nipples) cleaned and soaked in disinfectants	Significant (p = 0.025)	Suggests variability in thoroughness of cleaning practices; removal and separate disinfection improve disinfectant efficacy by reducing organic load. ASFV in feces able to survive for 60 to 100 days while liquid manure can survive 84 days at 17°C (Štukelj et al. 2021).
4.	Personnel Entry and Movement Control	Presence and use of showers and changing rooms	Significant (p = 0.047)	Highlights differences in personnel biosecurity; inadequate entry controls may increase risk of ASFV introduction via human movement (Mutua & Dione 2021).
5.	Water Source Management	Use of underground water	Significant (p = 0.015)	Indicates variation in water sourcing practices; untreated or poorly protected water sources may pose indirect biosecurity risks. Surface water will have the risk of contamination due to wild boar carcass might be detected nearby (Cukor et al. 2020; Bergmann et al. 2021).

4. Discussions

The questionnaire survey and environmental swab analyses addressed two complementary but distinct objectives. The questionnaire survey provided an overview of biosecurity practices currently implemented across pig farms in Malaysia, whereas the environmental swab analysis offered direct evidence of the effectiveness of cleaning and disinfection procedures in reducing environmental ASFV contamination.

ASFV DNA detection by real-time PCR does not necessarily indicate the presence of infectious or viable viruses. Viral genomic material may persist on environmental surfaces even after effective virus inactivation through cleaning and

disinfection. Consequently, the ASFV-positive qPCR results in this study were interpreted as evidence of residual contamination rather than confirmation of active environmental infectivity.

An environmental swab study revealed clear differences in environmental ASFV contamination among farms, which were closely associated with variations in cleaning and disinfection practices. The combined use of ASFV detection frequency and Ct value analysis provided a comprehensive assessment of environmental biosecurity performance.

Farm A presented the highest level of environmental hygiene, as evidenced by the complete absence of ASFV-positive environmental swabs. The inclusion of a drying step and effective water management likely enhanced virus inactivation and reduced the potential for environmental persistence of ASFV.

Farm C generally resulted in effective cleaning and disinfection outcomes, with only a single ASFV-positive swab detected at the sewage pond. The high Ct value associated with this sample suggests a low viral load, indicating that the detected viral material may represent residual contamination rather than active environmental persistence. However, environmental reservoirs must be rigorously decontaminated, as these sites can retain viable ASFV for extended periods. Experimental studies have shown that urine is the most stable medium for ASFV survival because it has a longer viral half-life than feces and oral fluids do (Davies et al. 2015).

In addition, inadequate cleaning and disinfection of waste-handling areas may allow residual ASFV genetic material to persist in the farm environment and increase the risk of mechanical dissemination. Waste management sites may attract or be accessed by mechanical vectors such as flies, rodents, and scavenging reptiles, which have been implicated in the indirect spread of ASFV between contaminated environments and susceptible pig populations. Farm C's use of combined disinfectant strategies, including the use of quaternary ammonium compounds with glutaraldehyde and heat treatment, along with water treatment with calcium hydroxide and ultraviolet (UV) light, likely contributed to the overall reduction in environmental contamination. However, the detection of ASFV at waste management sites highlights the importance of targeted disinfection in high-risk areas, as inadequate decontamination may facilitate virus persistence and potential spread via mechanical vectors such as flies, rodents, and other scavenging animals, even in farms with otherwise effective C&D protocols, underscoring the importance of reinforcing targeted biosecurity measures at these high-risk interfaces to minimize the risk of ASFV persistence, environmental spread, and potential farm-level recurrence.

In contrast, Farms B, D, E, and F presented higher levels of environmental ASFV contamination, as reflected by multiple ASFV-positive swabs and lower Ct values. These farms commonly lack one or more critical C&D components, such as adequate drying or consistent water treatment. In particular, reliance on water-only wet cleaning, the absence of drying steps, and the use of untreated or recycled water may have reduced the overall effectiveness of virus inactivation, allowing ASFV to persist in the environment despite disinfection efforts. These farms need to emphasize water disinfection, as the water sources used on these farms have shown potential for ASFV contamination. They can consider the application of calcium hydroxide or UV light, as both are effective in water treatment (Qiu et al. 2025; Štukelj et al. 2021). UV light is cost effective, easy to install and easy to maintain. It inactivates microorganisms via several mechanisms, such as the formation of cyclobutane pyrimidine dimers in nucleic acids, which ultimately inhibits transcription and replication (Štukelj et al. 2021). According to a previous study (Rutala & Weber 2024), rinsing without drying prior to disinfection is inappropriate, as residual water can dilute the disinfectant, resulting in a weaker solution than that required for successful microbial inactivation.

There are only two farms, Farm A and Farm C, that use foaming agents during wet cleaning. These farms had the lowest or no ASFVs detected. The use of foam to carry disinfectants provides distinct benefits over conventional liquid methods (Štukelj et al. 2021). Mechanically, the foam matrix allows for prolonged contact time between the chemical

agent and the surface, which is critical for inactivating viruses such as ASFV (Chlibek et al. 2006). Foam also offers uniform application across even, ragged, or vertical surfaces, and the embedded surfactant ensures deeper penetration and reduces the negative impact of residual surface contaminants (Chlibek et al. 2006). Furthermore, foam application is economically superior because of its high efficiency in minimizing water and chemical consumption, improving both safety and efficacy (Chlibek et al. 2006).

Moreover, 50% of the selected farms reported using caustic soda (sodium hydroxide) as an environmental disinfectant. Caustic soda is recognized as an effective disinfectant because of its strong hygroscopic properties, which inhibit microorganism growth by decreasing the humidity of the environment (Tian et al. 2020). However, it is highly corrosive and poses significant occupational health risks if improperly handled, including severe ocular and respiratory irritation as well as skin damage such as dermatitis, hair loss and tissue necrosis (Ahmadi 2019). Chlorine is another good disinfectant for water disinfection, as it produces hydrochloric acid and hypochloric acid when dissolved in water (Tian et al. 2020; Jackman et al. 2025). The application of flames to the surfaces of installations, equipment, walls, and floors is also recommended (Tian et al. 2020). Importantly, the effectiveness of all disinfectants is highly dependent on the correct preparation, appropriate concentration, and proper application to ensure adequate microbial inactivation.

Overall, these findings indicate that stringent, multistep cleaning and disinfection protocols, particularly those incorporating effective disinfectants, sufficient drying time, and appropriate water treatment, are strongly associated with reduced environmental ASFV contamination. Farms implementing comprehensive C&D measures achieved superior outcomes and may be better positioned to support ASF prevention and safe repopulation.

The questionnaire findings demonstrated that although certain biosecurity practices, such as carcass disposal, appeared to be relatively standardized, substantial variability existed in farm structural hygiene, cleaning and disinfection protocols, personnel entry controls, and water source management. These inconsistencies may contribute to differences in the farm-level risk of ASF persistence and reinfection.

5. Conclusions

This study demonstrated that effective C&D practices play a critical role in reducing environmental ASFV contamination and strengthening farm biosecurity. Farms that implemented comprehensive, multistep C&D protocols —particularly those incorporating nonsurface water sources, adequate drying periods, appropriate disinfectant selection and dosage, and strict personnel biosecurity measures—showed superior outcomes and may be better positioned for safe repopulation following ASF outbreaks.

The results further suggest that the use of underground water is preferable for minimizing contamination risk, whereas surface water, if utilized, should be adequately covered and subjected to periodic disinfection. An effective C&D sequence involves dry cleaning to remove organic matter, followed by wet cleaning with foaming agents, thorough rinsing, sufficient drying, and subsequent disinfection at appropriate concentrations. The combined use of questionnaire-based assessment and environmental surveillance provides practical guidance supported by field data, contributing to improved farm-level biosecurity and long-term ASF prevention strategies in tropical countries.

Declarations

6. Acknowledgment

I would like to express my deepest gratitude to Assoc. Prof. Dr. Ooi Peck Toung and Dr. Michelle Fong Wai Cheng for their invaluable guidance and support throughout this study. I am also sincerely thankful to my family for being my

unwavering pillar of strength.

Funding

The authors received research support from Boehringer Ingelheim (Malaysia) Sdn. Bhd. and Agritech Enterprise Sdn. Bhd.

Competing Interests

The authors declare that they have no relevant financial or nonfinancial competing interests.

Author Contributions

All the authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Shi Ling Soh. The first draft of the manuscript was written by Shi Ling Soh, and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are not publicly available owing to company-related confidence constraints but are available from the corresponding author upon reasonable request.

Ethics Approval

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was granted by the Ethics Committee of University Putra Malaysia (JKEUPM-2025-423).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

The authors confirm that informed consent for publication was obtained from all participants for the data presented in Tables 1 and 5 and Fig. 2.

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Figures

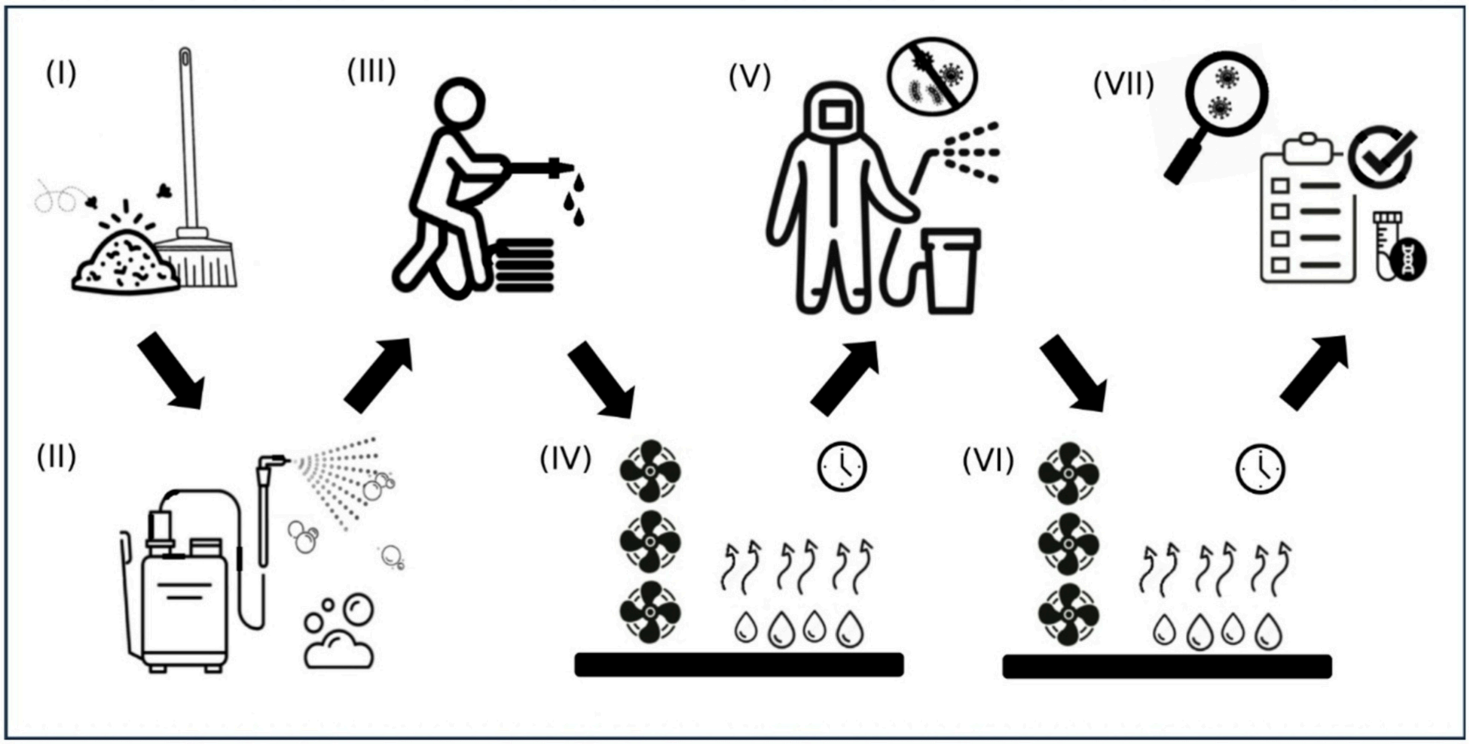


Figure 1

The comprehensive 7-step C&D protocol involves sequential processes critical for eliminating ASFV. The procedure begins with dry cleaning to remove gross organic matter, followed by wet cleaning using a foaming agent/surfactant for deep cleaning and extended contact time. The surfaces are then thoroughly rinsed. The crucial initial drying step ensures that the disinfectant remains at its required concentration. The fifth step is disinfection, where the approved chemical agent is applied at the correct dosage. This step is succeeded by final drying, which secures the treatment. The process concludes with efficacy testing via methods such as qPCR or the adenosine triphosphate (ATP) test

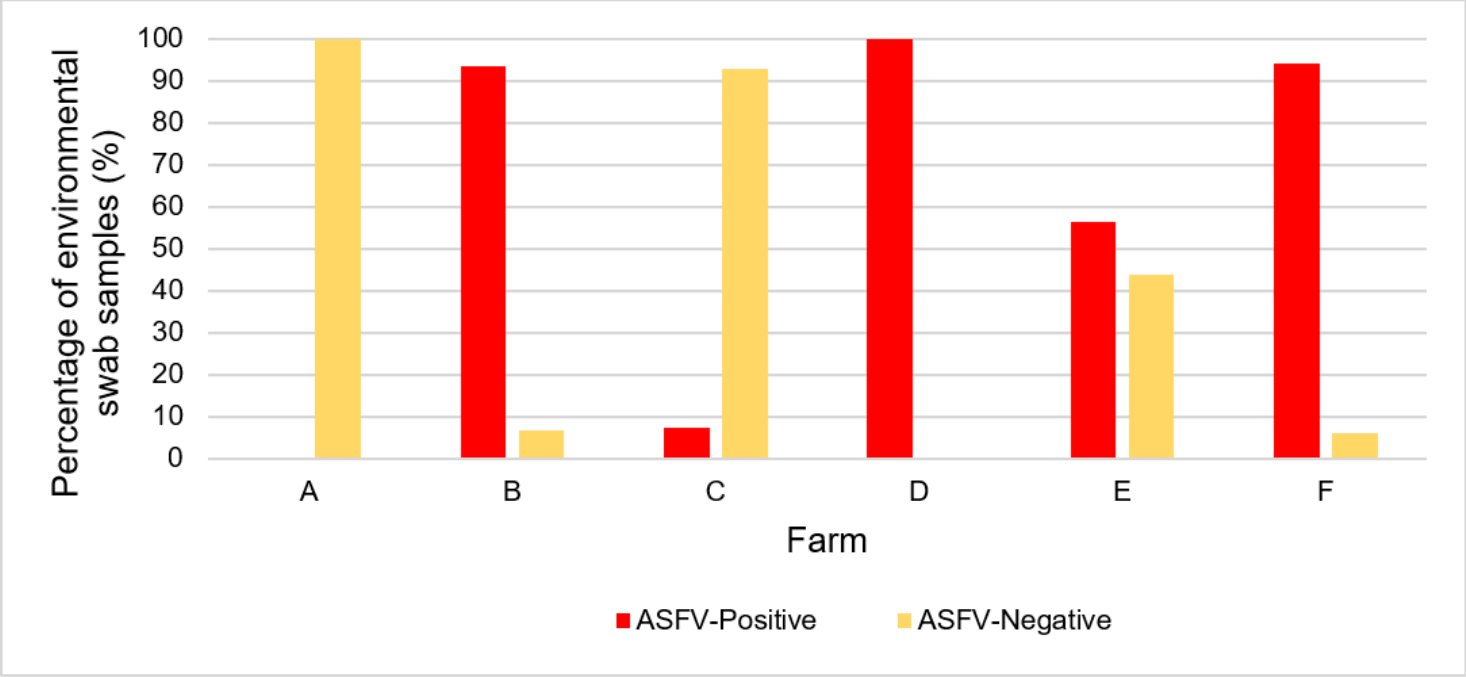


Figure 2

Distribution of ASFV-positive and ASFV-negative environmental swab samples collected from six pig farms following routine cleaning and disinfection procedures