MAKERERE UNIVERSITY

ASSESSMENT OF RESIDUAL AFRICAN SWINE FEVER VIRUS DNA PRESENCE ON PIG FARM SURFACES FOLLOWING OUTBREAKS IN BUSUKUMA AND NAKAWA DIVISIONS

BY

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AUGUST 2026

DECLARATION

I, AHURIRA MARVIN declare that this report is my original compilation and that none of its section(s) is/are plagiarized. I further declare that this report has never been submitted to any university for the award of any degree.

Signature .....Date 21/08/2026.....

APPROVAL

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DEDICATION

To my dear parents, Mr. Robert Mwebesa and Mrs. Jennifer Bongyereire, for your belief in my education; to my brothers Solomon, Armstrong, Joshua, and Kevin; to my lecturers; and to everyone who supported me through this journey - this work is dedicated to you.

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LIST OF ACRONYMS

Acronym	Full Meanings
ASF	African swine fever
ASFV	African swine fever virus
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries
NCDLVs	Nucleocytoplasmic large DNA viruses
UV	Ultraviolet
PBS	Phosphate-buffered saline
OIE	World Organization for Animal Health
TAE	Tris-acetate-EDTA
GDS	Gel documentation system

ABSTRACT

African swine fever (ASF), caused by the African swine fever virus (ASFV), remains a major constraint to pig production in Uganda. ASFV DNA can remain detectable on farm surfaces after infected pigs are removed. However, field-based information on the persistence of the viral DNA in endemic settings following real outbreaks is limited. This study assessed the presence of residual ASFV DNA on farm surfaces and fomites following routine post-outbreak cleaning practices. A cross-sectional study design was employed, covering five purposively selected pig farms in Busukuma and Nakawa Divisions in Wakiso and Kampala districts, respectively. Structured questionnaires were administered to capture farm characteristics and post-outbreak cleaning practices. One swab sample was collected from each of six targeted surfaces and fomites per farm (pen floors, pen walls, feeders, water troughs, cleaning tools, and footwear), giving 30 samples in total. DNA was extracted from all the samples and screened using conventional polymerase chain reaction (PCR) targeting the p72 gene. All 30 samples tested negative for ASFV DNA, despite variation in herd size, cleaning practices, and time since the last sick pig was removed. These findings suggest that ASFV DNA on farm surfaces is unlikely to be detected at post-outbreak intervals of three to nine weeks in comparable settings. The study recommends a fallow period of four to nine weeks before restocking, and calls for longitudinal studies to separate the effects of cleaning from natural environmental decay.

Keywords: **African swine fever virus (ASFV); environmental persistence; polymerase chain reaction (PCR); post-outbreak cleaning; Uganda**

INTRODUCTION

1.1. Background

African swine fever (ASF) is a highly contagious and frequently fatal viral disease affecting domestic pigs and wild suids, caused by African swine fever virus (ASFV). ASFV is a large, enveloped, double-stranded DNA virus classified within the family Asfarviridae (Dixon et al., 2020; Guinat et al., 2016). In naïve pig populations, the disease presents with severe hemorrhagic lesions and mortality rates can approach 100%. Globally, ASF is one of the most devastating transboundary animal diseases, resulting in huge economic losses due to pig mortality, disruption of pork trade, and substantial costs associated with outbreak control, surveillance, and biosecurity enforcement (Sánchez-Cordón et al., 2018).

ASFV originated from sub-Saharan Africa, and the region remains endemic for the disease. Virus maintenance is supported by complex transmission cycles involving domestic pigs, wild suids, and soft ticks of the genus Ornithodoros (Dixon et al., 2020; Jori et al., 2013). However, in many endemic settings such as Uganda, ASF epidemiology is dominated by the domestic transmission cycle, where pig-to-pig transmission and indirect spread via contaminated fomites play a central role (Chenais, 2017; Chenais et al., 2018). ASFV is transmitted to domestic pigs through various routes, such as direct contact with infected animals, ingestion of contaminated pork or pork products, and, in some settings, bites from infected soft ticks. Indirect transmission via contaminated farm surfaces, equipment, feeders, water troughs, and handling tools is important in low-biosecurity systems (Chenais et al., 2017; Guinat et al., 2016).

ASFV is notably stable on surfaces and fomites. The virus can persist in organic material and on contaminated farm surfaces for extended periods, even after infected pigs are removed. ASFV can survive for prolonged periods in blood, tissues, feces, and urine, all of which are abundantly shed by infected pigs and can contaminate the farm environment during outbreaks (de Carvalho Ferreira et al., 2012; Sánchez-Cordón et al., 2018). Importantly, the persistence of ASFV DNA does not necessarily indicate the presence of viable virus; however, it reflects prior contamination and provides insight into the effectiveness of cleaning and disinfection procedures following an outbreak. Such contaminated environments may act as sources of re-exposure if decontamination is inadequate. Detection of ASFV in environmental samples using polymerase chain reaction

(PCR) provides a sensitive indicator of residual contamination and ongoing biosecurity risk (Agüero et al., 2003; Kosowska et al., 2021).

Despite the recognized importance of surface contamination in sustaining ASF outbreaks, there is limited field-based information from endemic African settings on the distribution of residual ASFV DNA on typical pig farm surfaces and fomites after outbreaks end (Chenais et al., 2017; Mutua & Dione, 2021). Most available evidence is derived from experimental studies conducted under controlled laboratory conditions or from outbreaks in non-endemic regions such as Europe and Asia, which may not fully reflect conditions in Ugandan pig rearing systems characterized (Kwon et al., 2024).

This study therefore assesses residual ASFV DNA presence on targeted farm surfaces and fomites following routine management after an outbreak.

1.2. Problem Statement

African swine fever is endemic in Uganda and causes recurrent outbreaks with severe impacts on pig production and livelihoods. Despite the implementation of quarantines, depopulation, and movement restrictions, outbreaks frequently recur throughout the year within the same localities (Chenais, 2017; Wampande et al., 2025). Much of the available evidence on ASFV environmental persistence originates from laboratory-based studies or outbreaks in non-endemic regions, which may not accurately reflect actual conditions on Ugandan farms (Carlson et al., 2020; de Carvalho Ferreira et al., 2012). In addition, field-based information from endemic African settings on the presence of residual ASFV DNA in pig farm environments following routine post-outbreak cleaning practices is limited (Chenais et al., 2017; Mutua & Dione, 2021). This absence of context-specific data constrains the development of evidence-based recommendations for safe restocking of farms after ASF outbreaks (Chenais, 2017).

1.3. Objectives of the study

1.3.1. General objective

To determine the presence of residual African swine fever virus (ASFV) DNA on selected farm surfaces and fomites following routine management after an outbreak.

1.3.2. Specific objectives

a) To describe the characteristics of the selected pig farms and their post-outbreak cleaning practices.
b) To determine the presence of residual ASFV DNA on targeted farm surfaces and fomites.

1.4. Research Question and Hypothesis

a) What are the characteristics of the selected pig farms and their post-outbreak cleaning practices?
b) *H_0 (null hypothesis):* There is no residual ASFV DNA on the targeted farm surfaces and fomites after routine post-outbreak cleaning practices.
H_1 (alternative hypothesis): There is residual ASFV DNA on the targeted farm surfaces and fomites after routine post-outbreak cleaning practices.

1.5. Justification of the study

African swine fever remains endemic in Uganda, with outbreaks reported in over 66% of districts between 2010 and 2023. Despite efforts by the Department of Animal Health (MAAIF), recurrent outbreaks continue to occur within the same localities. These outbreaks severely affect Uganda's pig sector. They destroy farmers' livelihoods due to disruption of pork production and trade, thus maintaining poverty cycles. No Ugandan farm-level studies have assessed residual ASFV DNA contamination on common pig farm surfaces following the end of the outbreaks. Existing global data are derived mainly from laboratory or non-endemic settings and do not reflect conditions in Ugandan pig production systems. This has made it difficult for the Animal Disease Control Division to develop evidence-based post-outbreak interventions for use by affected farmers.

This study provides locally relevant evidence by assessing residual ASFV DNA contamination on targeted farm surfaces following routine cleaning practices after ASF outbreaks. The findings of the study can inform decision-making by the Animal Disease Control Division regarding post-outbreak farm management guidelines, enabling the development of practical recommendations that can be disseminated to farmers through veterinary officers.

1.6. Conceptual Framework

The conceptual framework of this study (Figure 1) is based on the premise that the detection of residual ASFV DNA after routine cleaning practices is influenced by the characteristics of the farm and the specific cleaning practices employed by farmers.

Farm characteristics such as the type of production system and pen floor type, together with post-outbreak cleaning practices, form the contextual factors. The outcome of interest is the presence of residual ASFV DNA on six targeted surfaces and fomites: pen floors, pen walls, feeders, water troughs, cleaning tools, and footwear used by farm workers.

This framework assumes that residual ASFV DNA may persist variably across the different surfaces, and that documenting both farm context and contamination patterns can provide useful insights into environmental contamination of pig farms.

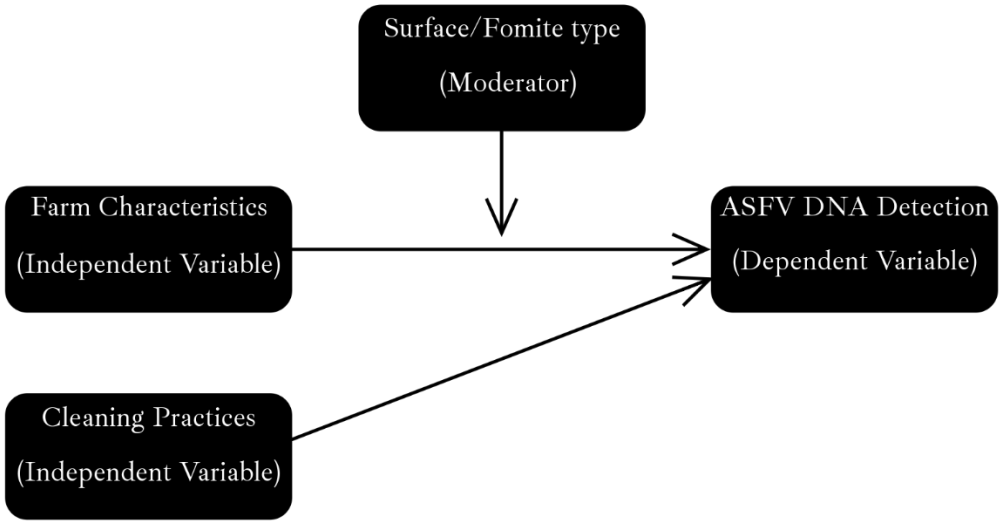


Figure 1: Conceptual framework of the study

LITERATURE REVIEW

2.1. The Pig Production Sector in Uganda

Pig keeping is one of the fastest-growing livestock sub-sectors in Uganda and provides a vital source of income, especially for women and youth. The national pig population has risen from roughly 0.19 million in the early 1980s to 3.2 million in 2008 and reached 7.1 million by 2021, kept by about 2.2 million households. The central and western regions have the highest pig densities (Dione et al., 2022; Ouma et al., 2014; Uganda Bureau of Statistics, 2024). Ugandans also eat more pork per person than their East African counterparts, with a per capita consumption rate estimated at 3.4 kg/person/year (Rao et al., 2025).

Almost all production is small-scale and low-input. Production systems range from free-range or tethered pigs in rural areas to semi-intensive and fully intensive units in peri-urban zones (Dione et al., 2022; Ouma et al., 2014). Farmers keep pigs mainly for income generation, but also value the manure and the quick turnover that short reproductive cycles allow. The feed used is usually locally available crop residues, kitchen waste, and forages (Dione et al., 2022).

Despite its importance, the sector still struggles with disease pressure especially ASF, poor market linkages, limited access to quality inputs, inadequate housing, and seasonal feed availability (Aliro et al., 2022; Ouma et al., 2014).

2.2. Production and Economic Impact of African Swine Fever in Uganda

ASF is by far the biggest biological and economic constraint on pig production in Uganda. Outbreaks regularly kill almost entire herds (Chenais, 2017; Wampande et al., 2025). Direct losses arise from high mortality, emergency culling, and disruption of breeding and herd-rebuilding cycles. Indirect impacts include trade bans, quarantine restrictions, panic selling, and falling prices. Smallholder farmers, who rarely have savings or insurance, can face months of lost income, debt, and food insecurity (Aliro et al., 2022; Chenais, 2017).

Studies in northern Uganda have shown that ASF outbreaks are negatively associated with economic outputs, including gross margins from pig enterprises and overall pig herd value. Bigger herds lose more in absolute terms and the effects often last several production cycles (Chenais, 2017). Traders and butchers further down the value chain are also affected, thereby perpetuating poverty cycles in communities that depend on pig rearing (Chenais, 2017; Ouma et al., 2014).

2.3. Structure and Biology of the African Swine Fever Virus

Understanding why ASF is so hard to control requires looking at the virus itself. ASFV is a large, enveloped, double-stranded DNA virus and the sole member of the Asfarviridae family, genus Asfivirus (Alonso et al., 2018; Gaudreault et al., 2020). Its genome is 170 to 194 kilobase pairs long and codes for up to 50 structural proteins. The particles are roughly 200 nm across, and have a complex multilayered structure that includes inner and outer capsids along with lipoprotein membranes (Andrés et al., 2020; Carrascosa et al., 1984; Dixon et al., 2019). This architecture places ASFV among nucleocytoplasmic large DNA viruses (NCLDVs), a monophyletic group capable of infecting diverse eukaryotic hosts (Iyer et al., 2006).

In domestic pigs, the virus causes a lethal hemorrhagic fever with mortality rates approaching 100% in acute cases. Clinical outcomes range from peracute to chronic, depending on the strain, infection dose, route, and host factors (Boklund et al., 2018; Salguero, 2020; Sánchez-Vizcaíno et al., 2015). After initial replication in tonsils or pharyngeal mucosa, the virus spreads to lymph nodes and tissues (Greig, 1972). All pig breeds and age groups are fully susceptible and, despite decades of work, no commercial vaccine is yet available because of the virus's complex proteome and immune-evasion mechanisms (Gaudreault et al., 2020).

2.4. Transmission of African Swine Fever and Environmental Contamination

ASFV spreads both directly and indirectly. Direct contact with infected pigs or their secretions facilitates rapid spread, with blood serving as a primary vehicle (Eblé et al., 2019; Olesen et al., 2024). Indirect transmission routes include contaminated fomites, vehicles, personnel, feed products, and environmental surfaces, as well as movement of infected animals or pork products (Gaudreault et al., 2020; More et al., 2018). Wild boars are a major reservoir in Europe and Asia, while soft ticks enable sylvatic cycles in parts of Africa (Jori et al., 2023; More et al., 2018).

Environmental contamination is central to the epidemiology of ASF. Infected pigs shed huge amounts of virus in blood, saliva, feces, urine, and tissues (Dixon et al., 2020; Guinat et al., 2016). These media easily contaminate housing surfaces, equipment, manure, soil, feed, and water (Chenais et al., 2017; Costard et al., 2013). Shedding often starts before clinical signs appear, allowing contamination to spread across multiple farm locations by the time the disease is noticed (Chenais et al., 2019; Guinat et al., 2016). This creates contamination zones in pig housing and surrounding areas (Carlson et al., 2020; Chenais et al., 2017).

Movement of people, animals and farm tools also shapes contamination distribution (Chenais et al., 2019; Costard et al., 2013). Farm workers and visitors can mechanically transfer the virus on footwear, clothing, tools, or vehicles, spreading it from infected areas to unaffected ones (Chenais et al., 2017; Costard et al., 2013; Guinat et al., 2016). Improperly disposed carcasses create localized contamination hotpots, releasing substantial amounts of virus into surrounding soil and organic material (Carlson et al., 2020; Chenais et al., 2019).

2.5. Persistence of ASFV DNA in the Environment

ASFV is remarkably hardy outside the host, largely because of its robust particle structure and stable genome (Gaudreault et al., 2020; Mazur-Panasiuk et al., 2019; Zheng et al., 2023). The infectious virus inactivates rapidly under field conditions, usually within days to weeks, whereas viral DNA can remain detectable for weeks to months (Davies et al., 2017; Zheng et al., 2023).

Experimental evaluations of genome stability show that ASFV DNA tolerates many stresses but degrades under conditions favoring hydrolysis or enzymatic activity, such as higher temperatures, desiccation, UV exposure, and microbial presence (Zheng et al., 2023). Organic matter provides protection to the viral DNA, but microbial degradation and environmental fluctuations speed-up decay over time (Prodelalova et al., 2022). In feces and urine, infectious virus half-lives are short, due to quick inactivation by bacterial enzymes and pH shifts, while DNA half-lives extend to 8-9 days in feces across temperatures and 19-33 days in urine (Davies et al., 2017; Nuanualsuwan et al., 2022). Detection windows in oral fluid are usually limited to 14-35 days, depending on temperature (Davies et al., 2017).

On solid surfaces, DNA persistence depends on porosity and temperature. Non-porous materials retain DNA longer at ambient conditions than porous ones, although organic contaminants reduce stability (Kwon et al., 2025; Nuanualsuwan et al., 2022).

2.6. Cleaning, Disinfection, Fallow Periods, and Restocking of Farms

Given ASFV's environmental persistence, thorough cleaning and disinfection are essential. Effective cleaning requires mechanical removal of organic matter followed by application of appropriate disinfectants. This involves removal of visible dirt, manure, feed residues, and other organic material from surfaces, equipment, and housing (Alarcón et al., 2021; De Lorenzi et al., 2020). Disinfectants lose their effectiveness when organic load is high (Alarcón et al., 2021; Mutua & Dione, 2021).

Once surfaces look clean, approved virucides such as alkalis, chlorine compounds, aldehydes, peroxides, or phenolic derivatives are applied at sufficient concentrations and contact times (De Lorenzi et al., 2020; Mutua & Dione, 2021). Affordable options such as lime or household bleach can work but require proper dilution and application to inactivate the virus (Aliro et al., 2022; Mutua & Dione, 2021).

Fallow periods of 1-3 months are commonly recommended to allow degradation of the virus in soil, manure, and surfaces, with longer periods advised in humid or organic-rich environments (Aliro et al., 2022; Mutua & Dione, 2021). Monitoring of the farms during fallow periods by environmental sampling can confirm reduced contamination before restocking (Alarcón et al., 2021). Restocking carries a risk of disease reintroduction if residual ASFV remains or external sources are contaminated, so sourcing pigs from ASF-free areas, quarantine of new pigs, and phased introduction are emphasized (Aliro et al., 2022; Mutua & Dione, 2021). In practice, money and logistics often limit how carefully these steps are followed (Aliro et al., 2022).

MATERIALS AND METHODS

3.1. Study design

This study employed a cross-sectional design. Surface swabbing was conducted during a single sampling event on each farm.

3.2. Study area

The study was conducted in Busukuma and Nakawa Divisions (Figure 2), located in Wakiso and Kampala Districts respectively. These areas lie within the central region of Uganda, which has high pig populations and has consistently recorded high numbers of ASF outbreaks (Wampande et al., 2025).

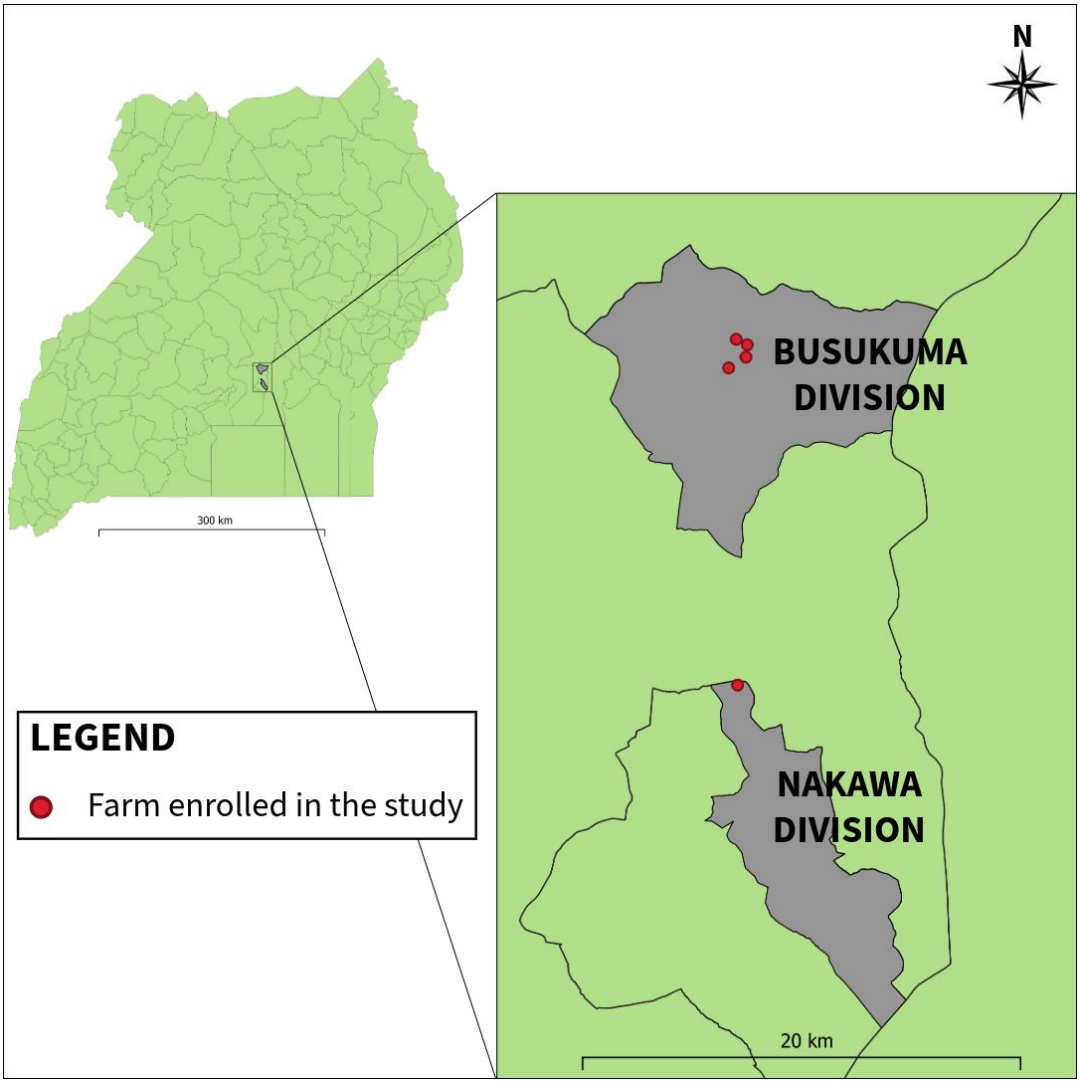


Figure 2: Map showing the study area

3.3. Sampling strategy

Purposive sampling was used to select the farms to be assessed in the study. A total of five farms was purposively selected based on the following inclusion criteria:

- Located in Busukuma Division or Nakawa Division.
- Had experienced an ASF outbreak within the last 3 months.
- Willingness of the farm owner to participate in the study.

Efforts were made to include farms that represented variation in production systems and pen floor types.

3.4. Study population

The study population comprised surfaces and fomites on the selected pig farms. The targeted surfaces were; pen floors, pen walls, feeders, water troughs, cleaning equipment, and footwear used by farm workers.

Live animals and humans were not sampled.

3.5. Ethical considerations

An introduction letter from Makerere University (College of Veterinary Medicine, Animal Resources and Biosecurity) (Appendix III) was used to request permission from authorities and farm owners prior to data collection. Informed consent (Appendix I) was obtained from all participating farm owners.

3.6. Sample size

On each selected farm, one sample was collected from each of the six targeted surfaces/fomites. This resulted in 6 samples per farm and a total of 30 samples across the five farms. Sampling points within each surface were purposively chosen to target likely contamination hotspots.

3.7. Sample collection and preservation

A questionnaire (Appendix I) was administered to each farm owner to capture relevant farm characteristics such as pen floor type, outbreak date, and cleaning practices.

Surface samples were collected aseptically using sterile swab sticks (GongDong, Zhejiang GongDong Medical Technology Co., Ltd.), pre-moistened with sterile phosphate-buffered saline (PBS). Each sampling point was swabbed using firm vertical and horizontal strokes. After

swabbing, each swab was placed back into its respective container, and transferred to a specimen bag. Samples were transported on ice packs and stored at -20°C within 4 hours of collection.

3.8. Laboratory analyses

3.8.1. DNA extraction

DNA was extracted using the Quick-DNA™ Miniprep Kit (Zymo Research Corp., USA) according to the manufacturer's instructions, with minor modifications as described below.

Samples were processed in the laboratory in batches. For each swab, 1 ml of sterile PBS was added to the container and agitated to release cellular material and debris into suspension. From this suspension, 1 ml was transferred into a correspondingly labeled 1.5 ml microtube, followed by 500 µl of lysis buffer, and the mixture was agitated and incubated at room temperature for 10 minutes. From each microtube, 500 µl of the lysate was loaded onto a labeled spin column fitted with a collection tube and centrifuged at 10,000 × g for 1 minute. The flow-through was discarded, and this loading step was repeated twice more with fresh 500 µl aliquots from the same microtube, each time centrifuging under the same conditions, so that the full lysate volume passed through the column. The collection tubes were then emptied and 500 µl of pre-wash buffer (AW1) was added to each spin column, followed by centrifugation (10,000 × g, 1 minute). This was followed by a second wash with 500 µl of wash buffer (AW2), centrifuged under the same conditions. After discarding the collection tubes, each spin column was transferred to a clean, labeled microtube, and 50 µl of elution buffer was added directly to the column matrix. Columns were incubated at room temperature for 5 minutes to allow the buffer to rehydrate and release bound DNA, then centrifuged for 30 seconds at 10,000 × g to elute.

The spin columns were discarded, and the eluted DNA was stored at -20°C in the microtubes until PCR analysis.

3.8.2. Conventional PCR

Conventional PCR targeted the conserved p72 gene using the OIE-recommended diagnostic primers DiagP1 (5'-ATGGATACCGAGGGAATAGC-3') and DiagP2 (5'-CTTACCGATGAAAATGATAC-3'), which amplify a 257 bp fragment (Agüero et al., 2003). Each reaction was carried out in a 12.5 µl volume comprising 6.25 µl of 2× Reaction Mix

(Invitrogen, P/N 55177), 0.4 µl of SuperScript III RT/Platinum Taq mix, 0.25 µl of each primer, 2.85 µl of PCR water, and 2.5 µl of extracted DNA template.

A master mix containing all reagents except the DNA template was prepared once per batch of samples, and 10 µl was aliquoted into individual 0.2 ml PCR tubes. To each tube, 2.5 µl of DNA extract was then added from the corresponding extraction tube. Every PCR tube was clearly labelled with its sample ID before use. Thermal cycling conditions consisted of an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of 94°C for 15 seconds, 50°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 5 minutes. Each batch of samples included a positive control (ASFV DNA template) and a negative control (PCR water without template) to verify assay functionality and detect contamination.

3.8.3. Gel Electrophoresis and Visualization

PCR products were resolved by electrophoresis on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide in 1× TAE buffer. The gel was run at 121 V for 20 minutes and visualized under UV light using a gel documentation system (Enduro GDS). A DNA molecular weight ladder was included on each gel, and samples producing a clear band of approximately 257 bp were considered positive for ASFV DNA.

RESULTS

4.1. Farm characteristics

Five pig farms were enrolled in the study on 22nd and 23rd June 2026. Four were located in Busukuma Division, Nansana Municipality, Wakiso District, and one was in Nakawa Division, Kampala District. A structured questionnaire (Appendix I) was administered to each farm owner after obtaining consent. Farm 01 had the largest herd (300 pigs), while the remaining farms had considerably smaller herds, ranging from 5 to 30 pigs (Figure 3). The time interval between removal of the last sick pig and sample collection ranged from 23 to 64 days, with a median of 38 days (Figure 4). Table 1 gives the full details on the farm characteristics.

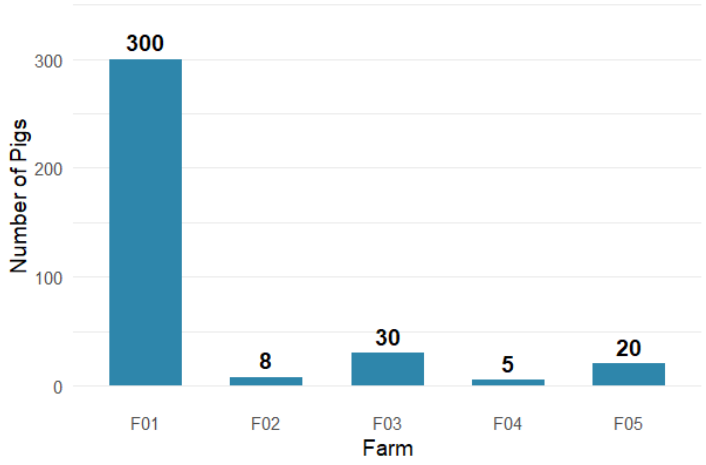


Figure 3: Herd size per farm

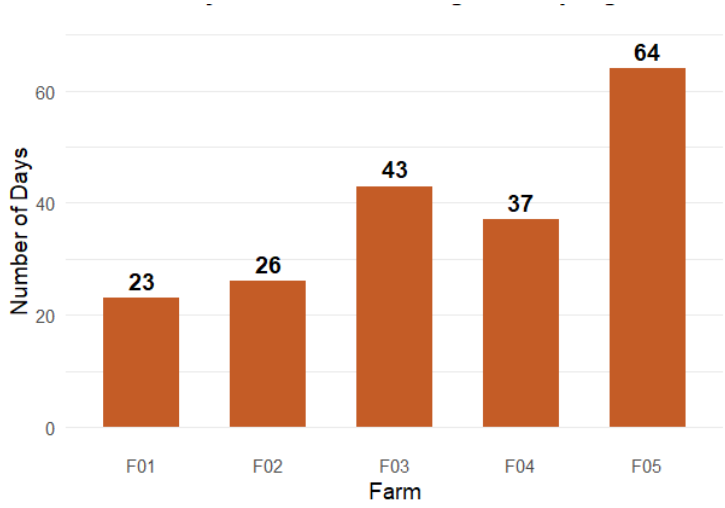


Figure 4: Days from last sick pig to sampling

Table 1: Characteristics of the selected pig farms and post-outbreak cleaning practices

Farm	F01	F02	F03	F04	F05
Location	Buso	Buso	Buso	Buso	Walufumbe
System	Intensive	Semi-intensive	Semi-intensive	Semi-intensive	Semi-intensive
Wall material	Concrete / Brick	Wood	Concrete / Brick	Concrete / Brick	Concrete / Brick
Floor Material	Concrete	Concrete	Concrete	Earth	Concrete
Herd Size	300	8	30	5	20
Outbreak Date	15/05/2026	16/05/2026	28/04/2026	02/05/2026	12/04/2026
Date Last Sick Pig Removed	30/05/2026	27/05/2026	10/05/2026	16/05/2026	20/04/2026
Cleaned?	Yes	No	Yes	Yes	Yes
Date Cleaning done	06/06/2026	-	12/05/2026	17/05/2026	22/04/2026
Disinfectant	Norocleanse	None	Water only	JIK	Biosafe, JIK
Days to Sample	23	26	43	37	64

4.2. Post-outbreak cleaning practices

Four of the five farms reported carrying out some form of post-outbreak cleaning. The number of targeted surfaces cleaned varied across farms. Floors, feeders and water troughs were the most commonly cleaned surfaces (reported by four farms), while walls were not cleaned on all the five farms (Figure 5). Details of the surfaces and fomites cleaned on each farm are provided in Appendix II (Table 2).

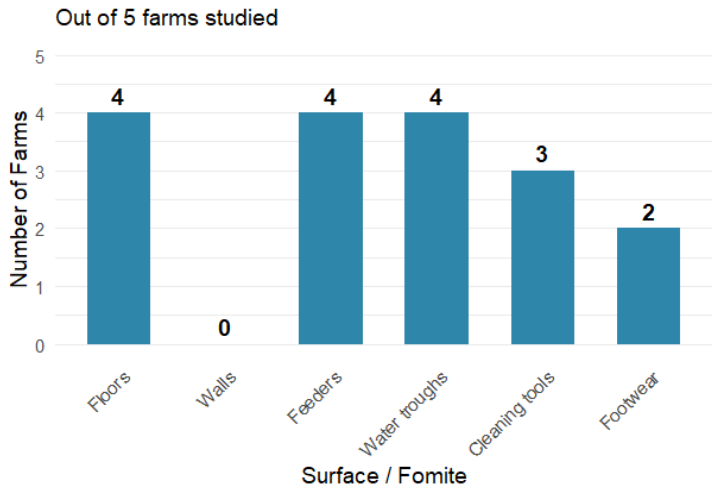


Figure 5: Number of farms that cleaned each surface

4.3. Detection of residual ASFV DNA

A total of 30 environmental samples were collected and processed, comprising 6 samples from each of the 5 enrolled farms. DNA extraction was successful for all 30 samples.

None of the 30 samples produced the expected 257 bp amplicon (Figure 6). All samples were therefore classified as negative for residual ASFV DNA. The overall detection rate was 0%. These findings led to a failure to reject the null hypothesis (H_0), which stated that there is no residual ASFV DNA on the targeted farm surfaces and fomites after routine post-outbreak cleaning practices.

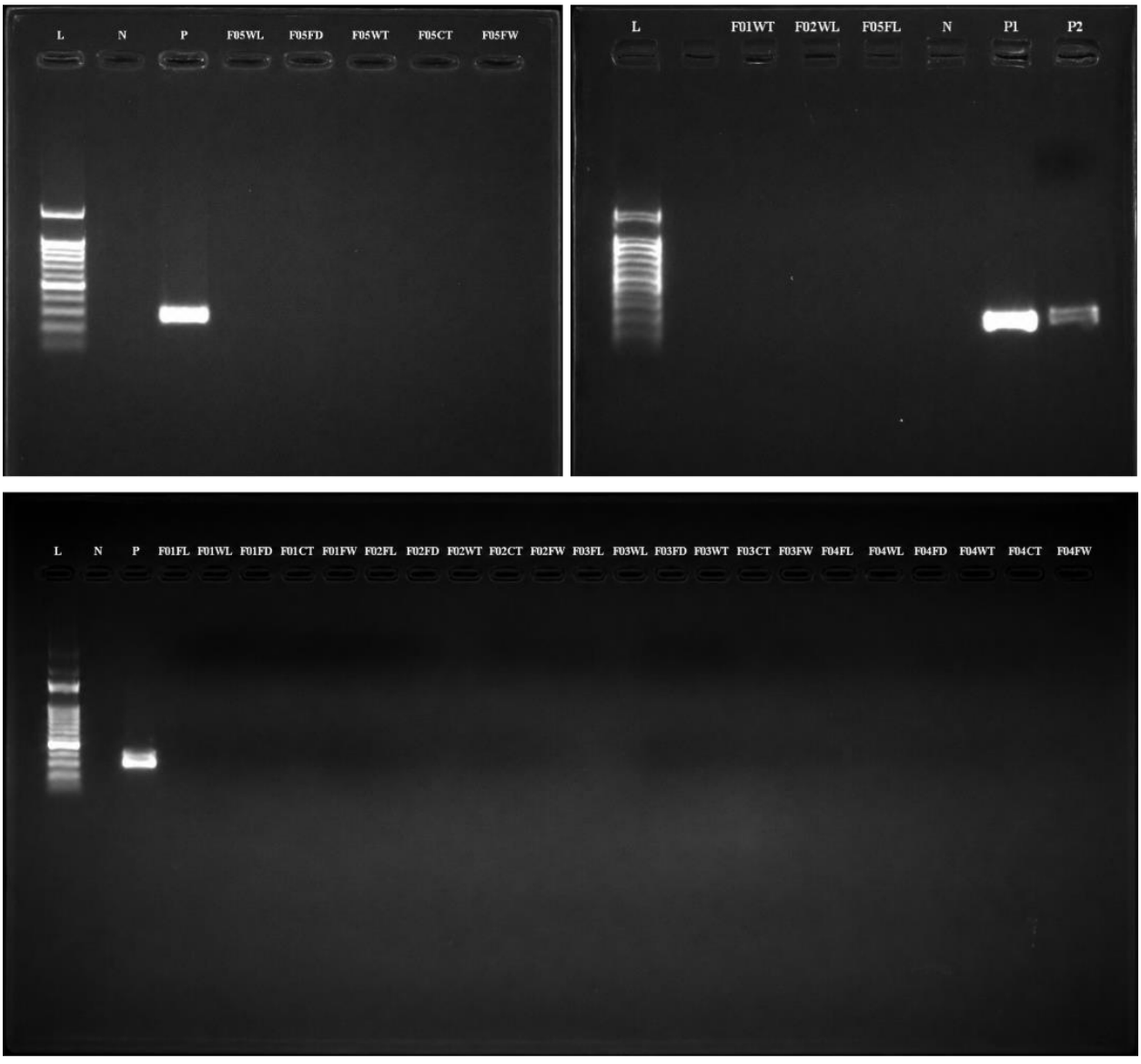


Figure 6: 1.5% agarose gel electrophoresis showing PCR amplification of target DNA from the environmental samples collected from five pig farms.

L = 100 bp DNA ladder; N = negative control; P, P1, and P2 = positive controls. Sample lanes are identified using the format FxxYY, where F01-F05 denote Farms 1-5 and the suffix indicates the sampled surface: FL = floor, WL = wall, FD = feeder, WT = water trough, CT = cleaning tools, and FW = footwear. The presence of a PCR amplicon at the expected product size indicates detection of the target DNA in the corresponding environmental sample, whereas the absence of a visible band indicates a negative result. The negative control showed no amplification, confirming the absence of contamination, while the positive controls produced the expected amplification products, validating the PCR assay.

DISCUSSION

The study was aimed at assessing the presence of residual ASFV DNA on surfaces and fomites on pig farms in Busukuma and Nakawa Divisions following outbreaks. PCR analysis of the samples showed complete absence of ASFV DNA on pen floors, walls, feeders, water troughs, cleaning tools, and footwear from all the five farms. This was so despite the variation in herd size (Figure 3), time since the last sick pig was removed (Figure 4), and post-outbreak cleaning practices (Figure 5). These results led to a failure to reject the null hypothesis (H_0) that no residual ASFV DNA is present on farm surfaces and fomites following routine post-outbreak cleaning practices, and provide no statistical evidence in support of the alternative hypothesis (H_1).

This outcome is consistent with experimental data on environmental stability of ASFV DNA. Laboratory studies have shown that while infectious ASFV is unstable outside the host, its DNA persists for longer periods. However, the viral DNA eventually undergoes degradation through hydrolysis, microbial activity, and exposure to high temperatures and UV radiation (Zheng et al., 2023). For example, in organic matrices, DNA half-lives are reported as 8-9 days with detection windows limited to 14-35 days, depending on temperature (Davies et al., 2017). Since sampling was done 23-64 days after removal of the last sick pig, and considering the warm, humid conditions characteristic of Uganda, the absence of detectable ASFV DNA is not shocking. Therefore, the results from this study provide field-based evidence that persistence models derived from laboratory studies translate reasonably well to real farm environments in an endemic setting.

However, these findings ought to be treated with caution since sampling was conducted at a single time-point several weeks after the outbreaks had run their courses. Similarly, much time had passed since the cleaning events for the farms that were cleaned. It is therefore not possible to tell whether the observed absence of detectable DNA resulted from the reported cleaning practices, or from natural environmental decay processes. Mutua & Dione (2021) observed that cleaning in Ugandan smallholder pig production systems is often incomplete, suggesting that farms that performed post-outbreak cleaning may not have removed all organic matter. This contributes to the difficulty of attributing the negative results to the reported cleaning. To more conclusively establish the process responsible for the zero detection, a study incorporating serial sampling before and shortly after the removal of the last sick pig both for farms that perform cleaning and those that do not would be required. Without such longitudinal data, these results cannot be

interpreted as evidence that routine cleaning was effective, nor can they be used to infer that cleaning following an outbreak is unnecessary.

Despite this limitation, the outcomes of this study have important practical implications for ASF outbreak management in Uganda. They provide locally generated evidence suggesting that ASFV DNA on farm surfaces and fomites is unlikely to be detected at post-outbreak intervals of roughly three to nine weeks under the environmental conditions that were present in the study area. This information can therefore be used by veterinary officers and the Animal Disease Control Division when advising farmers on appropriate fallow periods before restocking, provided that other biosecurity measures are maintained.

Several other limitations nonetheless affect the interpretation and generalizability of the findings. The purposive selection of only five farms, and the collection of only one swab per surface/fomite type limit the study's capacity to capture spatial variation in contamination. This has however been shown to be heterogeneous during ASF outbreaks (Chenais et al., 2017). As a result, this study does not conclusively show the absence of contamination. The study relies on self-reported cleaning practices, without verification of the procedures that were used, such as disinfectant concentrations and contact times, also creates uncertainty. Given the nature of this data, the results could be biased. These limitations suggest that these findings should be taken as preliminary rather than definitive evidence of environmental clearance.

These findings give a number of directions for future research. Longitudinal studies incorporating serial sampling at early post-outbreak time points would allow clearer differentiation between the effects of cleaning interventions and natural environmental decay on viral DNA persistence. The application of quantitative PCR, and where possible virus isolation, would provide more detailed information on both the quantity and infectivity of any residual virus. In addition, bigger studies that incorporate independently verified cleaning protocols and include follow-up of restocked farms would strengthen the evidence base for refining guidelines on post-outbreak management in Uganda's pig sector.

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

This study has demonstrated that residual ASFV DNA was not detected on the targeted farm surfaces and fomites after three to nine weeks following outbreaks in Busukuma and Nakawa Divisions. While these findings are consistent with laboratory data on ASFV DNA persistence and provide valuable field-based evidence from an endemic setting, the single sampling event limits the extent to which the contribution of cleaning to the observed results can be determined. Nevertheless, the study contributes useful information on the environmental persistence of ASFV DNA under Ugandan conditions and underscores the need for longitudinal field studies to better understand post-outbreak contamination dynamics and to support the development of practical recommendations for pig farmers.

6.2. Recommendations

Based on the results of this study, the following recommendations are proposed:

For pig farmers and veterinary officers

An adequate fallow period of at least four to nine weeks should be observed before restocking pig farms following ASF outbreaks, together with other biosecurity measures.

For the Animal Disease Control Division and policymakers

- National guidelines on post-outbreak farm management should include clear recommendations on appropriate fallow periods before restocking.
- Further efforts should be directed towards generating field evidence on the effectiveness of cleaning and disinfection practices under Ugandan smallholder conditions.

This study was limited by its small sample size, single sampling event, and reliance on self-reported data from the farmers.

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APPENDICES

APPENDIX I: Questionnaire

INFORMED CONSENT

I am Ahurira Marvin, a final-year student at Makerere University (CoVAB), conducting research on *Assessment of Residual African Swine Fever Virus DNA Presence on Pig Farm Surfaces Following Outbreaks in Busukuma and Nakawa Divisions*. This involves answering a short questionnaire and collecting surface swab samples.

Participation is voluntary, you may withdraw at any time, and your information will be kept confidential and used only for academic purposes. There are no risks to you or your farm.

Contact: +256 763 041359 | marvinahurira2022@gmail.com

☐ **I have understood the above and agree to participate.**

Farmer's Name: _____

Date: ____ /____ /_____ (DD/MM/YYYY) Contact: _____

FARM NAME: _____

LOCATION: Latitude: _____ Longitude: _____

Village: _____ Parish: _____

Sub-county: _____

County: _____

District: _____

1. What is the production system used on the farm?

☐ Free-range / Tethering ☐ Semi-intensive ☐ Intensive

2. What is the farm's pen wall material?

☐ Concrete / Brick ☐ Metal / Wire

☐ Wood ☐ Mixed

3. What is the farm's main pen floor material?

☐ Concrete ☐ Wood ☐ Earth ☐ Mixed

4. How many pigs were on the farm during the outbreak? _____
5. When did the ASF outbreak begin? ____ /____ /_____ (DD/MM/YYYY)
6. When was the last sick pig removed (or when did it die)? ____ /____ /_____ (DD/MM/YYYY)
7. Was the farm cleaned after the outbreak?

☐ Yes ☐ No

8. If yes, when was the cleaning done? ____ /____ /_____ (DD/MM/YYYY)
9. What disinfectant was used for cleaning? _____
10. Which surfaces were cleaned? (tick all that apply)

☐ Pen floors ☐ Feeders ☐ Footwear

☐ Pen walls ☐ Water troughs ☐ Cleaning tools

APPENDIX II: Results

Table 2: Surfaces cleaned on each farm (✓ = Cleaned, ✗ = Not cleaned)

Farm	Floors	Walls	Feeders	Water troughs	Cleaning tools	Footwear
F01	✓	✗	✓	✓	✓	✓
F02	✗	✗	✗	✗	✗	✗
F03	✓	✗	✓	✓	✗	✗
F04	✓	✗	✓	✓	✓	✗
F05	✓	✗	✓	✓	✓	✓

APPENDIX III: Introduction letter

MAKERERE  **UNIVERSITY**

COLLEGE OF VETERINARY MEDICINE, ANIMAL RESOURCES AND BIOSECURITY

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SCHOOL OF VETERINARY MEDICINE AND ANIMAL RESOURCES (SVAR)

Department of Livestock and Industrial Resources

Date: 18th /06/2026

TO:

WHOM IT MAY CONCERN

Dear Sir/Madam,

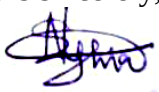
RE: INTRODUCTION OF AHURIRA MARVIN (Reg. No.:23/U/0129)

This is to introduce you to Ahurira Marvin, a final-year student of the Bachelor of Animal Production Technology and Management (BAPTM), in the Department of Livestock and Industrial Resources (LIR), School of Veterinary Medicine and Animal Resources (SVAR), College of Veterinary Medicine and Animal Resources and Biosecurity (COVAB).

As part of the requirements to complete this degree program, a student must undertake research and submit a research dissertation upon completion. Marvin proposed a research study titled ***"Assessment of Residual African Swine Fever Virus DNA Presence on Pig Farm Surfaces Following Outbreaks in Busukuma and Nakawa Divisions".*** *The research will involve* collection of samples and administering questionnaires in June 2026. All ethical and biosafety protocols, farm confidentiality, and voluntary participation will be adhered to. The data collected shall be used strictly for academic purposes.

Your support for this research by allowing Marvin to collect data will be greatly appreciated. If you need any further information, do not hesitate to contact me at the details below.

Yours sincerely,



Dr. Sylvia Nalubwama, PhD.
BAP program coordinator
Tel:0782552706; Email: drnalubwama@gmail.com