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Molecular surveillance of African swine fever virus in raw pork and blood samples from wet markets and abattoirs in Tuguegarao City, Cagayan

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ABSTRACT

African Swine Fever (ASF) is a highly lethal transboundary disease that endangers swine production and food security. This study conducted active molecular surveillance to detect ASF virus (ASFV) in raw pork and whole-blood samples collected from local government–sanctioned wet markets in Tuguegarao City. From August to October 2025, a total of 180 specimens (30 meat and 30 whole-blood samples per month) were collected, pooled by stall owner and market, and transported cold for laboratory processing. Nucleic acids were extracted and screened using real-time PCR assays targeting the *VP72* (B646L) gene, with appropriate extraction, weak and strong positive, and no-template controls to ensure run validity. Results showed that all specimens produced undetermined cycle threshold (*Ct*) values and lacked characteristic amplification curves, yielding 0/180 PCR positives. Quality control amplification of positive controls and flat negative controls confirmed assay performance. Exact Clopper–Pearson binomial analysis generated a two-sided 95% confidence interval of 0.00 to 0.02029 for true prevalence, placing an upper bound of 2.03%. These findings indicate ASFV was not detected from any of the samples analyzed, while acknowledging potential limitations such as pooling, sample size, viral loads below the assay limit of detection, or nucleic acid degradation. The study recommends sustained surveillance with increased sample numbers and duration, biosecurity assessments at market and farm levels, and data linkage with live-swine surveillance to strengthen early detection and protect local pork value chains.

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INTRODUCTION

African swine fever (ASF) is a highly contagious and often fatal viral disease of domestic pigs and wild boar, with mortality in naïve populations that may approach 100%, making it a major threat to pork production, trade, and food security worldwide (Costard *et al.*, 2009). The disease is caused by African swine fever virus (ASFV), a large double-stranded DNA virus and the only known member of the family Asfarviridae, whose complex biology contributes to its persistence, adaptability, and cross-border spread (Costard *et al.*, 2009). Since its re-emergence outside Africa, the global spread of ASF has been driven largely by the movement of live pigs and pork products, as well as weaknesses in biosecurity systems (Costard *et al.*, 2009; Mulumba-Mfumu *et al.*, 2019; Li *et al.*, 2022). ASFV is also not known to infect humans, so prevention and surveillance remain focused on animal health and production systems within a One Health framework (Hsu *et al.*, 2024; Costard *et al.*, 2009).

The transmission of ASFV is shaped by several interconnected ecological and production-based cycles. In Africa, the disease may circulate through a sylvatic cycle involving wild suids and Ornithodoros ticks, a domestic tick–pig cycle, and a domestic cycle driven by pig-to-pig transmission and contaminated pork products, while in Eurasian settings a wild boar–habitat cycle is also important (Costard *et al.*, 2009). In Eastern, Central, and Southern Africa, these cycles may coexist, creating a highly complex epidemiological landscape that is further complicated by variable surveillance capacity, genotype circulation, and control practices across countries (Mulumba-Mfumu *et al.*, 2019). This complexity explains why ASF remains difficult to eradicate and why region-specific biosecurity and surveillance strategies are necessary. In Southeast Asia, meanwhile, ASF spread has been associated with multiple introductions and continuing transmission events, and some studies have even shown that public search activity may reflect outbreak dynamics, suggesting that non-traditional surveillance tools may complement conventional epidemiology (Hsu *et al.*, 2024).

In Asia, ASF has been strongly associated with genotype II and the expansion of a Georgia-origin lineage that later diversified across China, India, and Southeast Asia (Zhang *et al.*, 2023; Xin *et al.*, 2023; Mahajan *et al.*, 2022; Mthombeni *et al.*, 2023; Chernyshev *et al.*, 2024). The first confirmed detection in continental Asia occurred in China in August 2018, with outbreaks rapidly reported in multiple provinces shortly afterward and official responses involving culling and emergency control measures (Li and Tian, 2018). By 2019, the disease had spread to several neighboring countries and regions in East and Southeast Asia, including Mongolia, Vietnam, Cambodia, Hong Kong, North Korea, Laos, the Philippines, Myanmar, Indonesia, Timor-Leste, and South Korea (Ito *et al.*, 2023). The rapid regional spread has been linked to live pig movement, swill feeding, contaminated vehicles, and wild boar involvement, demonstrating the role of both human-mediated and ecological pathways in ASF transmission (Cheng and Ward, 2022; Ito *et al.*, 2022). These patterns underscore the need for sustained genomic and field surveillance to detect new introductions, sublineages, and possible transmission bridges across countries (Zhang *et al.*, 2023; Chernyshev *et al.*, 2024).

In the Philippines, ASF was first confirmed in August 2019 after laboratory testing of samples from sick pigs in Rizal, and subsequent outbreaks were documented in nearby areas of Rizal and Bulacan, prompting mass culling and emergency response actions by the Department of Agriculture (ter Beek, 2019; Department of Agriculture, 2019). The disease later spread across the country and affected all 17 administrative regions, with Hsu *et al.* (2023) reporting 19,697 ASF farm outbreaks from August 2019 to July 2022. Their analysis showed a seasonal pattern, with outbreaks peaking from August to October and declining around April to May, and identified Central Luzon, Regions I and II as major clustering areas while Western and Central Visayas

remained ASF-free during the study period (Hsu *et al.*, 2023). The spread in the Philippines has been associated with contaminated feed, swill feeding, movement of workers and veterinary personnel between farms, the role of middlemen in pig trade, and possible wild boar exposure in certain regions (Hsu *et al.*, 2023). Because ASF can present initially with non-specific signs such as fever and hemorrhagic lymph nodes, early recognition of clinical and pathological indicators is essential, and Filipino swine producers have been shown to recognize many of the disease's clinical signs, which is valuable for early intervention and outbreak containment (Hsu *et al.*, 2023).

Diagnostic capacity and surveillance infrastructure are central to ASF control because no vaccine or specific treatment is currently available. According to the WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, ASF diagnosis may involve viral detection through virus isolation, antigen detection, and PCR or real-time PCR, while serological testing may use ELISA, IFAT, IPT, and IBT to detect antibodies that usually appear within 7 to 10 days after infection (WOAH, 2024). In the Philippines, diagnostic approaches currently include real-time PCR, isothermal assays, lateral flow assays, antigen ELISA, antibody ELISA, and lateral flow devices, with national confirmation handled by the Animal Disease Diagnosis and Reference Laboratory of the Bureau of Animal Industry (OIE, 2021). Regional laboratories were also established to provide provisional diagnosis and once competent in real-time PCR, to release results through the National ASF Task Force, while additional DA and private laboratories have been accredited to expand testing capacity (OIE, 2021). At the same time, local innovations such as the ASFV Nanogold Biosensor and the MTek GenAmplify ASF PCR Detection Kit reflect national efforts to make testing faster, cheaper, and more accessible (Del Rosario, 2022). Beyond diagnostics, ASF risk is also sustained by environmental persistence and contaminated animal products, as ASFV can survive in carcasses and pork products, including bone marrow from buried carcasses and raw or processed pork, which highlights the importance of monitoring pork as a potential reservoir of infection (Arzumanyan *et al.*, 2021; Fernandez-Colorado *et al.*, 2024; Serdeña *et al.*, 2024). Blood likewise remains a key diagnostic matrix during acute infection, with studies showing detectable ASFV DNA during viremia, although long-term systemic persistence in recovered pigs remains more uncertain and context-dependent (Zsak *et al.*, 2005; Nieto-Pelegrín *et al.*, 2015; Patrick, 2019; Gaudreault *et al.*, 2020; Pornthummawat *et al.*, 2021).

Government intervention and local capacity building are therefore essential components of ASF mitigation in the Philippines. After the first confirmed cases, the national government declared a state of calamity through Presidential Proclamation No. 1143 and strengthened coordination with hog raisers, local government units (LGUs), and the private sector to implement contingency plans, surveillance systems, and biosecurity measures in both affected and ASF-free areas. Biosecurity remains a core principle of disease prevention because it aims to reduce the introduction of pathogens from outside the farm and their spread within the farm, yet implementation is often weak in smallholder systems that lack resources and technical support (Alarcon *et al.*, 2021; Cooper *et al.*, 2022).

Studies in the Philippines show that many farmers are aware of ASF risks but still need stronger education on vaccination, footbaths, swill-feed control, reporting, and proper carcass disposal, while some communities prefer to bury infected animals rather than report them to authorities (Bernardes and Peña Jr., 2020; Uy and Uy, 2023). These findings suggest that empowering LGUs through training, closer coordination with municipal agriculturists and veterinarians, and stronger surveillance and disease monitoring are critical to improving local preparedness (Uy and Uy, 2023; Bernardes and Peña Jr., 2020). This need is intensified by the shortage of veterinary personnel

in many areas and by the heavy economic dependence of backyard raisers on swine production, making targeted support for smallholders especially important to the recovery and stability of the swine value chain (Jorca, 2021). This study aims to determine the presence of African swine fever virus (ASFV) in raw pork and whole-blood samples collected from selected wet markets and abattoirs in Tuguegarao City through molecular surveillance using real-time polymerase chain reaction (qPCR), with specific detection of the *VP72* (B646L) gene. It further aims to establish the positivity rate of ASFV in the collected meat and blood samples during the study period, thereby providing baseline data that may contribute to the monitoring of ASFV contamination in pork products and the assessment of potential risks associated with the circulation of the virus in local meat supply chains.

MATERIALS AND METHODS

Research design

This study utilized a descriptive design. This research design is best suited for studies which describe a situation or phenomenon under study. In this study, the researcher aims to describe the positivity rate of African Swin Fever from pork sold in the wet markets and abattoir in Tuguegarao City, Cagayan.

Identification of study area and sampling sites

The study was conducted in Local Government sanctioning wet markets and abattoir in the city of Tuguegarao. Collected meat and blood samples were subjected to diagnostic molecular testing through Real-Time Polymerase Chain Reaction test.

Sample collection

Meat samples were collected from wet markets while blood samples were collected from abattoir. A total of 30 bloods and 30 meat samples were collected every month from August to October. This was coordinated with the Local Government Unit of Tuguegarao City and the management of wet markets. A 100-gram meat samples per owner were collected in the early morning and were put in sterile zip locks and stored in ice box prior to transport to the laboratory. Moreover, blood samples collected from abattoir are stored in EDTA tubes and were also stored in ice box prior to sample preparation in the laboratory.

Nucleic acid extraction

Collected meat samples were pooled depending on the number of samples per stall owner. Samples were also grouped per wet market. A 0.5–1 gram portion of each collected sample was mixed with 1× phosphate-buffered saline (PBS) and vortexed until homogenized. The samples were then centrifuged at 14,000 rpm for 2 minutes. A 140 µL portion of the supernatant was then transferred to a 1.5 mL microtube. Meat samples were subjected to nucleic acid extraction following the protocols utilizing the QIAamp Viral RNA Kit.

Controls for RT-PCR assay

A total of five (5) controls, two (2) negative and three (3) positive controls were utilized in the assay. No Template Control (NTC) was used as a negative control to assess cross-contamination in the assay while Extraction Negative (E-) was used as negative control to assess cross contamination in the extraction process. On the other hand, Extraction Positive (E+) was used as positive control for the assessment of the extraction process while both Weak Positive (C+) and Strong Positive (C++) were utilized to assess the sensitivity of the assay and the performance of the assay components respectively. Assay validity was based on the results of the controls where Negative controls must not have any characteristic of amplification curves, and No *Ct* values are detected. Moreover, positive controls must show characteristics of amplification curves and *Ct* values must fall within acceptable range of 30 – 33.

Nucleic acid amplification assay

Extracted nucleic acid from meat and blood samples were subjected to UV-Vis Spectrophotometer using Nanodrop™ 2000c for the measurement of nucleic acid concentration prior to polymerase chain reaction. Protocols for the Real-time Polymerase Chain Reaction test were based on the standard procedures of the Agpath -ID One Step RT PCR.

The *VP72* gene which codes for the major capsid proteins of ASF was targeted using the following primers and probe: ASF *Fwd* 5'CTG CTC ATG GTA TCA ATC TTA TCG A-3', ASF *Rev* 5'-GAT ACC ACA AGA TCR GCC GT-3', and ASF *Prb* FAM-CCA CGG GAG GAA TAC CAA CCC AGT G-TAMRA.

Furthermore, amplification of the target gene was performed utilizing the following thermal cycling parameters in the RT-PCR Assay: RT-Reactivation: 45^{0}C for 10 minutes and RT Deactivation: 95^{0}C for another 10 minutes; Denaturation (95^{0}C) and Annealing (60^{0}C) for 15 and 45 seconds respectively for 45 cycle rounds.

Evaluation of PCR results

Cycle threshold (*Ct*) values and the shape of the amplification curve were utilized to assess the results. Positive results were interpreted as results showing amplification curves with logarithmic, linear, and plateau phase where *Ct* values range less than 40 after 45 cycles. No *Ct* value or undetected and showing no characteristics of amplification curve similar to the positive control were interpreted as negative. Furthermore, *Ct* values greater than 40 but less than 45 are warranted for retest.

Statistical analysis

Data collected was analyzed using descriptive statistics such as percentages and tabulations. The result of the study will also be interpreted using the positivity rate formula. Positivity rate will be determined as follows:

Positive Rate= {(Number of positive pooled samples)/ (Total number of samples tested)}×100

RESULTS AND DISCUSSION

Thirty meat and thirty whole blood samples were collected every month from August to October. Samples were processed and analyzed using Quantitative Polymerase Chain Reaction Assay (FAST 7500) targeting the *VP72* gene of the African Swine Fever Virus. Table 1 summarizes the results by sample type (meat vs. whole blood) for each month of collection and additionally reports the mean and range of cycle threshold (Ct) values observed among specimens.

Summary of results shown in Table 1 indicates zero qPCR-positive samples for all months (August-October) characterized by undetermined cycle threshold values. Furthermore, Fig. 1 provides the amplification curve of the assay showcasing no amplification of the sample nucleic acid beyond the threshold of 0.1.

Fig. 1 highlights the result of the nucleic acid amplification wherein none among the samples were able to reach a threshold of 0.1 which is indicative that no nucleic acid has been amplified after 35 cycles. Given these findings, the lack of threshold crossing among the sample curves indicates that all specimens tested negative for ASFV genetic material under the assay conditions used. This suggests either (1) the absence of *VP72* sequences in the collected meat and whole-blood samples or (2) viral concentrations below the detection limit of the qPCR assay. In either scenario, the amplification profiles in Fig. 1 collectively demonstrate that no measurable nucleic acid amplification occurred during the 35-cycle reaction, reinforcing the conclusion that all analyzed samples were negative for ASFV by qPCR during the August–October sampling period.

Similar findings have been reported in surveillance studies where ASFV was not detected in sampled populations despite continued regional risk, highlighting the value of active surveillance in confirming disease absence or very low prevalence under field conditions (Hsu *et al.*, 2023; Fernandez-Colorado *et al.*, 2024).

Table 2 summarizes the total positivity rate of the samples per sample tested where 0.00% positivity rate was recorded across the three-month sampling period. However, it must be noted that observing zero positives does not prove the pathogen is absolutely absent but would only mean no detections in the tested samples.

Furthermore, a Clopper-Pearson Confidence Interval at 95% places at 0.00000 ≤ p ≤ 0.02029 for the underlying infection prevalence given that 0 positives were observed in the sample set which means that, based on these data, we are 95% confident the true prevalence in the sampled population lies between 0% and about 2.03%. Consequently, while no infections were detected, the sample size and randomness of sampling allow for the possibility of very low-level circulation up to roughly 2% that this study could have missed. Nonetheless, because the positive control amplified and the no-template control stayed below the threshold, the runs are technically valid which supports treating these table entries as true negatives under the assay conditions used.

Nevertheless, surveillance studies emphasize that negative molecular findings should be interpreted cautiously because low viral loads, limited sample size, and heterogeneous pathogen distribution may reduce the probability of detection, particularly in low-prevalence settings (WOAH, 2024; Cheng and Ward, 2022).

Table 1. Summary of results and mean *Ct* values showing zero number of samples tested positive for ASFV throughout the collection period

Month	Meat		Whole blood	
	Number of positive samples	Mean *Ct* values	Number of positive samples	Mean *Ct* values
August	0	Undetermined	0	Undetermined
September	0	Undetermined	0	Undetermined
October	0	Undetermined	0	Undetermined

*Undetermined *Ct* values indicate no detected viral nucleic acid.

Table 2. Summary of positivity rate per sample type

Month	Meat		Whole blood	
	Number of positive samples	Positivity rate	Number of positive samples	Positivity rate
August	0	0.00 %	0	0.00 %
September	0	0.00 %	0	0.00 %
October	0	0.00 %	0	0.00 %

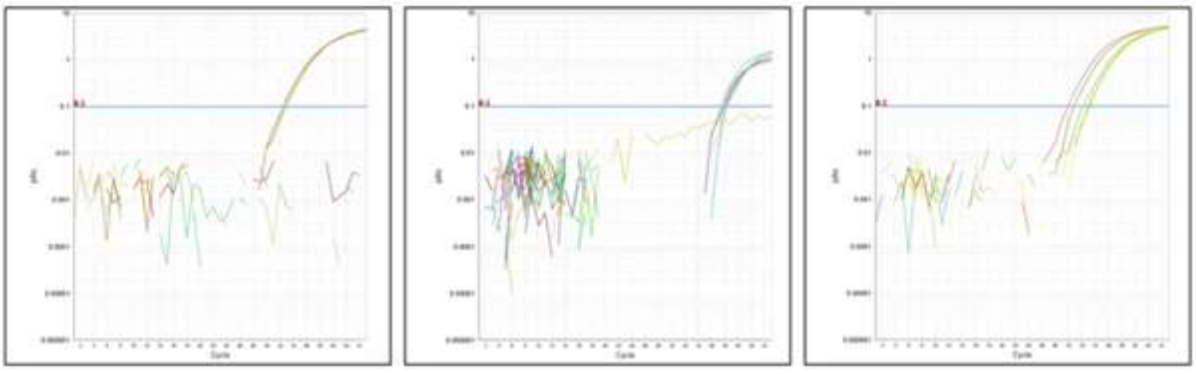

Fig. 1. (L to R) Amplification plots of samples analyzed for August, September, and October

The qPCR surveillance of 180 specimens (30 meat + 30 whole blood each month, Aug - Oct) returned zero detections for ASFV *VP72* and the exact Clopper–Pearson 95% confidence interval (0.00–0.02029) implies that, with 95% confidence, the true prevalence in the sampled population is no greater than 2.03%. Practically, this combination of negative test results and a relatively small upper-bound on prevalence is reassuring for local producers, traders, and consumers as it reduces the likelihood of a widespread, undetected outbreak in the sampled markets and slaughterhouse during the study period and therefore supports continued market activity without emergency movement bans or mass culling,

provided follow-up surveillance and routine biosecurity remain in place.

This observation is consistent with reports showing that sustained surveillance, rapid diagnostics, and early response systems are essential for maintaining confidence in swine production systems while minimizing unnecessary disruptions to trade and market activities (Costard *et al.*, 2009; Ito *et al.*, 2023).

However, it is to be noted that this data do not demonstrate absolute freedom from ASFV. Low-level or geographically focal infection below the statistical detection limit could still exist and would require more intensive, targeted sampling (e.g., at high-risk farms, slaughterhouses or sentinel herds) to exclude with higher confidence. Continued vigilance is therefore guaranteed to protect market stability and consumer confidence.

Similar recommendations have been advanced in Asian ASF control programs, where routine surveillance and risk-based monitoring have been identified as critical tools for early detection and prevention of disease re-emergence (Ito *et al.*, 2022; Hsu *et al.*, 2024).

Furthermore, the positivity rate of 0.00% and the absence of positive results from the samples obtained from the local markets and slaughterhouses can be attributed to several factors beyond the laboratory.

Based on the recent ASF Bulletins released by the Bureau of Animal Industry, there have been no cases of ASF in the city in the past months. This is attributable to the efforts of the Local Government Unit of Tuguegarao City. If the LGU had applied movement controls, market hygiene, surveillance, and other standard prevention measures, those actions can prevent introduction or early spread of ASF, yielding zero detection.

Previous studies have demonstrated that strict implementation of biosecurity protocols, movement control measures, and farm-level disease monitoring significantly reduce the likelihood of ASF introduction and transmission within susceptible pig populations (Alarcón *et al.*, 2021; Cooper *et al.*, 2022).

Recommended measures by international agencies (biosecurity, movement restrictions, swill feeding prohibitions, market inspections) are known to reduce risk.

Moreover, the results of this study are parallel to the current trend of active cases in the region and in the whole Philippines where lower number of cases is detected. ASF Bulletin No. 2025-20 records only 3 active cases in the region with only 1 active case in the province of Cagayan. This lower number of cases of ASF can be traced from the efforts of the national and local stakeholders in combatting the spread of disease.

The declining number of reported outbreaks is also consistent with observations from other regions where coordinated surveillance, rapid laboratory confirmation, and targeted containment measures contributed to reductions in ASF spread over time (Mulumba-Mfumu *et al.*, 2019; Hsu *et al.*, 2023).

Routine sampling in markets and farms, combined with more intensive, risk-based sampling in high-risk settings such as backyard operations, free-range systems, and premises with recent pig introductions substantially strengthens overall surveillance capacity. Implementing this multi-tiered approach increases the probability of detecting any circulating African swine fever virus at an early stage and, in turn, narrows the upper confidence bound on estimated prevalence. This leads to more precise assessments of disease status and enhances the reliability of declaring an area free or nearly free of infection.

Such surveillance frameworks are widely recommended by international animal health authorities because they improve sensitivity for detecting low-level infection while supporting

evidence-based disease freedom assessments (WOAH, 2024; OIE, 2021).

Furthermore, implementing and strictly enforcing movement permits, veterinary checkpoints, and science-based zoning systems such as restricted, controlled, and protection areas for live pigs and pig-derived products play a critical role in limiting the spread of disease should it be introduced.

These regulatory measures help ensure that only animals and products verified as safe can enter or move within designated zones, thereby reducing the likelihood of undetected viral transmission and safeguarding the broader swine industry.

Similar zoning and compartmentalization approaches have been successfully applied in several ASF-affected countries to minimize disease spread while allowing safe movement of animals and animal products from low-risk areas (Costard *et al.*, 2009; Ito *et al.*, 2023).

In totality, this study reflects effective biosafety and biosecurity measures implemented at the local government level with big implications for the local pork industry of the city and the local and provincial economy as a whole.

CONCLUSION

The following conclusions are drawn based on the results of the analyses of meat and blood samples collected through August to October 2025. There were no ASFV nucleic acid detected based on the target *VP72* gene in all samples across all collection months. Quantitatively, there is a 0.00% positivity rate of ASFV in meat and blood samples collected in selected local markets and slaughterhouses within the city. Furthermore, prevalence of the ASFV by Clopper-Pearson Interval reveals a very low upper-bound at 2.03%, suggesting a very low prevalence of ASFV in the sampled population.

In totality, the following are recommended to address the gaps in this study, widen its scope, and strengthen the statistical significance of the low-to-zero prevalence of ASFV in the local wet markets and slaughterhouse of Tuguegarao City. First is to increase sample size with higher number of samples collected and the length of sampling collection to determine the long-term prevalence of ASFV in the local wet markets and slaughterhouses of the city.

There is also a need to conduct biosecurity analysis of sampling sites, including local practices and biosafety implementations in the locality. Lastly, it is recommended to correlate data on pork products with the positivity case results of live swine in the city.

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