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# Surveillance and detection of African swine fever on abbatoir in different municipalities of third district of Cagayan, Philippines

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**ABSTRACT**

African Swine Fever (ASF), driven by the African Swine Fever Virus (ASFV), persistently undermines the Philippine swine industry, characterized by its significant transmissibility, resilience in the environment, and the lack of an available vaccine. This investigation carried out active monitoring in abattoirs across five municipalities in the 3rd District of Cagayan, Philippines, to determine the ASFV infection in slaughtered pigs and analyze the slaughter facilities' contribution to disease spread. Two hundred thirty nine (239) whole blood samples were systematically collected and organized into 92 groups, subsequently analyzed for ASFV utilizing real-time PCR techniques. The findings indicated a positivity rate of 29.35%, with 27 pooled samples (70 individual pigs) yielding positive results. The highest positivity rate was observed in Solana at 9.78%, with Tuao and Amulung at 6.52%, Tuguegarao at 4.35%, and Peñablanca at 2.17%. All five towns displayed confirmed ASFV, which emphasizes slaughterhouses as possible ASF sources particularly in areas with low pre-slaughter screening and poor biosecurity. These findings suggest that infected pigs may have acquired the virus on farms before being delivered to slaughterhouses. The findings support the need of more stringent biosecurity rules, improved farm-level screening, public education among backyard hog raisers, and legislative laws to stop sick pigs from finding their way into slaughter and market chains. ASF control and eradication in Cagayan depend critically on strengthening surveillance and inspection processes at slaughterhouses.

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## INTRODUCTION

Numerous farmed pigs, feral pigs, and wild boars have died from African swine fever (ASF), a highly contagious viral hemorrhagic disease that has drastically reduced the worldwide pork industry (World Organisation for Animal Health (OIE), 2015). This disease is brought on by a complex DNA virus to the Asfarviridae family. Both direct exposure with sick animals and indirect contact with fomites, tick vectors, and ill animals themselves, can all spread ASF. The infection can manifest in a number of forms, including acute, subacute, chronic, and peracute.

The virus was initially identified in Kenya, Africa in 1921 and is now spreading across Europe and Asia. With its high contagiousness, high mortality rate, and devastating economic impact on both the national and international trade of animals and animal products, the World Animal Health Organization (OIE) vigilantly keeps track of ASF as it is regarded as the most notifiable diseases of domestic pigs. At present, there is no cure or commercial vaccine for the virus, so control depends on rapid laboratory detection and strict sanitary measures (Sanchez-Vizcaino, 2006).

Surveillance is necessary for early detection and is a crucial element of eradication strategies. The clinical presentation of ASF is necessary for the early identification of the virus, as it dictates the timing of disease symptoms that alert farmers, livestock raisers, and others who visually interact with domestic pigs, including veterinarians during pre-slaughter examinations.

Research indicates that ASFv can disseminate at a relatively slow rate in experimental contexts, while exhibiting rapid transmission in the wild (Guinat *et al.*, 2016; Guinat *et al.*, 2014). To control and stop ASFv in a country, you need to know how the eco-social system works. This means that we need to know enough about the pork food systems and the population of wild boars in the area.

Slaughterhouses, or abattoirs, have a big impact on whether or not ASF is present and how easily it spreads. Before pigs are slaughtered, they must be checked for clinical signs of ASF, and if any are found, the right steps must be taken for the pig and the group of animals it belongs to. The facilities, the workflow, and the management at slaughterhouses all have a big impact on how much ASF spreads.

## MATERIAL AND METHODS

### Research design

This study employed a quantitative cross-sectional design in which pig blood samples from municipalities in the 3rd District of Cagayan were analyzed using real-time PCR (RT-PCR) to determine the presence of African swine fever virus (ASFV). The analysis focused on estimating the incidence and positivity rates of ASF among the sampled population.

This study utilized a cross-sectional surveillance methodology, analyzing pig blood samples from the municipalities of the 3rd District of Cagayan through RT-PCR to detect the presence of the ASF virus. The qualitative aspect of the study focuses on the incidence rate of newly reported ASF cases.

### Sampling technique

The study used convenience sampling. Samples were collected twice in one week per municipality during the regular operation schedule of the municipal slaughterhouses. The total pig population for slaughter was identified, ranging from 6 to 30 animals per schedule (except for the Tuguegarao City abattoir, with daily operations). Historical data such as the name of the owner, address of the farm, gender of the animal, and population were also collected. Whole blood was collected prior to slaughter using 3 ml vacuum seal collection tube with EDTA. The collected sample was cooled for transport and submission to the laboratory. A total of 239 blood samples were collected, which include 100 samples from Solana, 50 samples from Tuguegarao City, 43 samples from Tuao, 30 samples from Amulung, and lastly, 16 samples from Peñablanca.

**Locale of the study**

Whole blood samples were collected in the government managed slaughterhouses found in the 5 municipalities of the 3rd district of Cagayan Valley: Amulung, Solana, Tuao, Peñablanca, and Tuguegarao City. Collected samples were submitted regional laboratory for diagnostic test. Data gathering was done within the months of February to May of 2021.

**Research instruments**

The study utilized whole blood samples and employed RT-PCR for analysis. The routine procedure for the QIAamp Viral RNA Mini Kit (QIAGEN) extraction was used to prepare blood samples and reagents. The collected DNA/RNA was then tested for African swine fever using the Agpath ID One Step-RT PCR kit. To avoid contamination, proper lab practices and protocols were followed.

**Data gathering procedure**

Samples were processed according to the standard procedure for PCR virus isolation. Whole blood samples were pooled and extracted for RT PCR testing. A total of 92 samples were pooled depending upon the number of animals the owner had. Blood samples and reagents were prepared based on the standard procedure for extraction of Qiamp Viral RNA Mini kit. The extracted DNA/RNA were subjected to RTPCR testing for African swine fever using Agpath ID One Step-RT PCR kit. Good laboratory practices and procedures were followed to prevent contamination.

**Analysis of the data**

Data collected was analyzed using descriptive statistics such as percentages and tabulations. The result of the study per municipalities were interpreted using the positivity and incidence rate formula.

**Positivity rate**

The positivity rate was the percentage of all blood samples that were tested and were positive of ASF viral antigen. It was obtained using the formula:

Positive rate=(Number of positive pooled samples/Total number of pooled blood samples tested)×100

**Incidence rate**

Incidence was the term used to describe the number of new cases of ASF over a certain amount of time. The study employed incidence as the count of new cases per unit of population, rather than the tally of new cases within a community, as is customary among certain epidemiologists. Incidence rates are especially useful for figuring out incidence in studies with fixed populations and dynamic populations that have long follow-up times.

Incidence Rate =Number of pigs tested positive in pooled blood samples/Total number of pigs in pooled blood samples tested

**RESULTS AND DISCUSSION**

The African swine fever virus (ASFV), a large, enveloped double-stranded DNA virus belonging to the Asfivirus genus within the Asfarviridae family, was the causative agent of ASF (Dixon *et al.*, 2005).The disease was destroying the pig farming business in the Philippines. The stability of ASFV in the environment and in infected pork (Taylor, 2020), the lack of a vaccine, and inadequate biosecurity and preventive measures were the main reasons why ASF spread so quickly (Petrini *et al.*, 2019).

And since the Philippines was one of the major consumers of pork meat, many people were affected, especially the backyard and commercial hog raisers. As of now, ASF transmission was continuously affecting 11 municipalities in Cagayan Valley. The disease was spread in different premises, such as in slaughterhouses.

Swine are slaughtered and processed in the slaughterhouse facilities of each municipality before being distributed to the public market. Blood and entrails were also cleaned and sold in the public market.

Before the pigs were killed, three milliliters of whole blood were taken from each one. RADDL tested samples taken from slaughterhouses in Solana, Tuguegarao, Amulung, Tuao, and Peñablanca. We got 239 blood samples from pigs that had been killed at different slaughterhouses. There were no signs of the disease, and all of the animals looked healthy. The meat inspectors said that looking at the meat didn't show anything important. There were 100 blood samples taken from Solana, 50 from Tuguegarao, 43 from Tuao, 30 from Amulung, and 16 from Peñablanca, Cagayan. We made 92 pools of blood samples based on who owned them, and then we used RT-PCR to test each pool for viral antigen. The QIAamp mini–Viral RNA Mini Kit (QIAGEN) was used to separate nucleic acids (DNA and RNA) from the samples.

The kit worked well for ASFV DNA isolation and was appropriate for DNA extraction (King *et al.*, 2003). The Real Time-Polymerase Chain Reaction was used to test the extracted DNA. According to the results, there were confirmed cases of African Swine Fever in every municipality.

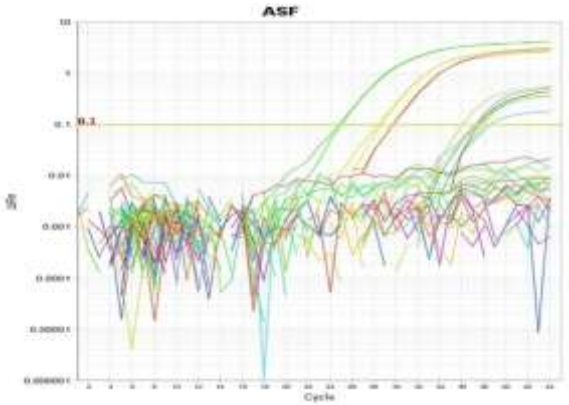


**Fig. 1.** The amplification plot of real-time PCR for african swine fever

Fig. 1 displays the analysis results and the amplification plot for the various tested blood samples. The delta Rn value is the difference between the Rn value of an experimental reaction and the Rn value of the device's baseline signal. It is shown on the y-axis. This parameter accurately quantifies the strength of the signal derived from a specific set of PCR conditions. The cycle threshold, which is shown on the x-axis, is the point at which the curve first diverges from the baseline in a way that is statistically significant. In a qualitative assay, a sample was considered positive when it exceeded the statistical noise threshold. In a quantitative PCR, the cycle number at which this happened was used to make a standard curve and find the initial template. The second point, known as an inflection point, was halfway up the curve's "second phase." This is where the slope of the curve changed from going up with each cycle to going down with each cycle. The plot shows that a number of samples made a curve that looked like a sigmoid. This means that the fluorescence curve was normal and the CT value was less than 40. The result was good. A Ct value of 0 means that the samples are not positive. If a sigmoidal plot was seen and the analysis was done twice to make sure it was right, samples with a Ct value of 35 or higher were called weak. Samples with a Ct value greater than 38 were deemed negative.

Table 1 showed the total number of blood samples that were tested positive for ASF. Results showed that all municipalities where blood samples were collected tested positive in African Swine Fever virus. A total of 92 samples were extracted and tested through RT- PCR machine with Agpath one step RT-PCR reagents. Control samples of both positive and negative were included in each nucleic acid extraction run (King *et al.*, 2003). The municipality of Solana showed high chance of ASF infection with 9 positives for ASF viral antigen out of 29 pools tested. Tuguegarao had a total of 4 positive pools out of 12 tested, Peñablanca with 2 positive pools out of 6 tested. Result from Amulung showed that 6 out of 13 pools were positive while the municipality of Tuao had 6 positive from 32 pools tested. Based on the number of positive cases, the municipalities with the most cases were Solana.

Table 2 showed the positivity rate of infected pooled samples per municipal slaughterhouses. The positivity rate was determined by dividing the number of pools that tested positive by the total

number of pooled samples tested, and the result was multiplied by 100. Solana had the highest positivity rate at 9.78%, followed by Tuao and Amulung, both at 6.52%. Tuguegarao had 4.35% of the votes, and Peñablanca had 2.17%, which was the lowest. It was thought that the high positivity rate from the samples was directly related to the high number of positive cases in the municipality. A high positivity rate of samples from slaughterhouses can indicate that slaughterhouses can be a potential source of infection, thus serving an important role in the transmission of African Swine Fever There was a high chance that pigs with African swine fever were killed and processed in registered facilities like municipal slaughterhouses because the pigs that were allowed into the facilities were not properly screened. The low indemnification cost was another factor, especially for the pigs that were ready to be slaughtered. This made owners or hog raisers choose to sell the pigs for slaughter. This practice has been previously noted to result in significant outbreak scenarios (Dixon *et al.,* 2020).

**Table 1.** Number of negative and positive pooled blood samples per municipalities

| Municipality | Positive | Negative | Total number of pooled blood samples |
|---|---|---|---|
| Solana, Cagayan | 9 | 20 | 29 |
| Tuguegarao City | 4 | 8 | 12 |
| Tuao, Cagayan | 6 | 26 | 32 |
| Amulung, Cagayan | 6 | 7 | 13 |
| Peñablanca | 2 | 4 | 6 |
| Total | 27 | 65 | 92 |

**Table 2.** Positivity rate of ASF based on pooled samples per municipalities

| Municipality | Positive | Samples | Positivity rate |
|---|---|---|---|
| Solana, Cagayan | 9 | 29 | 9.78 |
| Tuguegarao City, Cagayan | 4 | 12 | 4.35 |
| Tuao, Cagayan | 6 | 32 | 6.52 |
| Amulung, Cagayan | 6 | 13 | 6.52 |
| Peñablanca, Cagayan | 2 | 6 | 2.17 |
| Total | 27 | 92 | 29.35 |

Fig. 2 showed the order of which municipalities have the highest positivity rate. Arranged from highest to lowest were Solana, followed by Tuao, Amulung, Tuguegarao and lastly Peñablanca. Based on the study, Solana and Tuao have the most cases of African swine fever since there were many backyard hog raisers in these municipalities. Backyard hog raisers maintained an average of 5 heads which they can easily sell, or slaughter based on needs and biosecurity practices were hardly ever followed. Peñablanca and Amulung have low positivity rate since there were few hog raisers in these municipalities. While Tuguegarao slaughterhouse with high scale operation based on the number of slaughtered hogs daily, showed low positivity rate mainly due to the biosecurity measures and information campaign implemented by the local government.

On average, the positivity rate in the 3rd District of Cagayan was 29.35%, representing 27 positive pooled blood samples out of a total of 92 pooled samples tested. The overall positivity rate was 29.35%, indicating the proportion of pooled samples that tested positive for ASFV, while the incidence rate was 29.29%, representing the proportion of infected individual pigs among the total tested population. This finding reflects only the samples collected from slaughterhouses in the 3rd District of Cagayan. No clinical signs of African swine fever (ASF) were observed in the pigs delivered for slaughter; therefore, it is suggested that the virus may have been acquired prior to their arrival at the slaughterhouses.

Further, we can safely hypothesize that pigs acquired the disease from the farm (Ekue *et al.,* 1989). Based on the study of Thomas *et al.* (2016), particularly crucial to the management of ASF is the existence of infected pigs at slaughterhouses and, consequently, the spread of potentially infectious meat to butcheries throughout the research area. A significant risk factor for outbreaks that seem to be separate from the

sylvatic cycle is the transportation of diseased pigs to an abattoir and the transportation of infected pork products, according to earlier research.

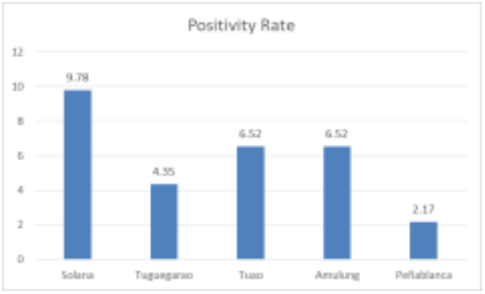


**Fig. 2.** The graphical sketch of the positivity rate

These studies proved the spread of ASF and because there was no effective vaccine or treatment, many pigs were buried or killed to avoid further transmission of the virus. Several surveillance efforts were being conducted from national and local government as well as the academe to determine the spread of the disease in the province as well as identify possible sources of infection to be a basis for the province's eradication program. Still, the government disease eradication efforts should benefit from well-informed hog raisers and slaughterhouse's personnel on the role of the slaughterhouses and aggregation points such as the public markets in the spread and transmission of ASF. Also, a more vigilant monitoring particularly in the slaughterhouses for potentially infected pigs should be conducted such as testing of source farms or the pigs before slaughter as well as strengthening of meat condemnation procedure in the slaughterhouses.

**Table 3.** The incidence rate of ASF per municipal slaughterhouses of 3rd district Cagayan valley

| Number of pigs in positive pooled samples | Total number of pigs in pooled samples tested | Incidence rate (%) |
|---|---|---|
| Solana – 9 pools = 29 | 100 | 12.13 |
| Tuguegarao – 4 pools = 16 | 50 | 6.69 |
| Tuao – 6 pools = 9 | 43 | 3.77 |
| Amulung – 6 pools = 13 | 30 | 5.44 |
| Peñablanca – 2 pools = 03 | 16 | 1.26 |
| Total = 70 | 239 | 29.29 |

The incidence rate of ASF in the 3rd District of Cagayan was 29.29%. A total of 27 pools tested positive mentioned in Table 3. Blood samples were pooled based on the owner of the pig where individual blood samples were collected with the assumption that since they came from one owner or farm, one positive pig will render all pigs from one source positive. The 27 positive pools in the study consisted of 70 individual blood samples. On the other hand, a total of 239 blood collected from individual pigs. In an interview conducted, Cagayan Provincial Veterinary Office Chief Dr. Noli Buen in 2021, ASF was present in 38 barangays of 11 municipalities affecting 1,019 hog raisers in the province. Dr. Buen mentioned that a total of 3,692 domestic pigs from the infected barangays have been culled to prevent the spread of the disease.

## CONCLUSION

The presence of African Swine Fever (ASF) in pigs meant for slaughter across all five municipalities was confirmed by this investigation's successful surveillance at slaughterhouses and aggregation points in the 3rd District of Cagayan. 239 separate blood samples were combined into 92 samples, which were then subjected to RT-PCR processing. According to the findings, 70 pigs had positive ASF viral antigen tests, resulting in a 29.35% positivity and incidence rate. The findings provide conclusive evidence of ASF infection in local slaughtered pigs.

The discovery of ASF in pigs at slaughterhouses raises the possibility of virus transmission at crucial control points, such as meat distribution networks and public marketplaces. This demonstrates how urgently improved oversight and legal procedures at slaughterhouses are needed. Slaughterhouses may serve as hubs for the spread of ASF if nothing is done.

## RECOMMENDATION(S)

### Department of agriculture

Community-level information on active and subclinical infections will be obtained through mass screening of pigs raised in backyards throughout the five municipalities. The FAO and WOAH surveillance

frameworks advise this approach, which emphasizes syndromic reporting and active sampling as ways to identify ASF early. In smallholder settings, a common containment strategy supported by FAO guidelines is the strategic culling or quarantining of exposed or infected pigs, coordinated with local veterinary authorities.

**Municipal agriculture office**

The Philippines' "Bantay ASF sa Barangay" campaign and FAO/OIE guidelines for awareness, education, and behavior modification are echoed by focused outreach programs for backyard farmers that teach them to identify ASF symptoms, follow biosecurity guidelines, and conduct disease-prevention measures.

Farms are evaluated and certified according to biosecurity profiles, which encourages adherence and promotes safer production methods that lower the risk of disease entry.

**National meat inspection service**

Continuity-of-business permitting guidance, which requires pre-movement isolation and testing, particularly when entering control zones, is followed by local government units that enact policies that forbid the movement of untested or infected pigs into slaughterhouses and public markets.

The National Meat Inspection Service follows EU and FAO guidelines that prioritize post-slaughter inspections to identify questionable cases and stop further spread by implementing better condemnation standards and appropriately disposing of infectious carcasses.

**Public information agency**

ASF's effects on livelihoods, food security, and the local economy should be communicated to consumers and stakeholders through public advisories, seminars, and media engagement. To increase awareness and encourage community compliance, communication tactics such as the national ASF campaign in the Philippines encourage the use of consistent messaging through LGU channels.

In order to effectively combat ASF in the 3rd District of Cagayan, these measures work together to form a comprehensive strategy that is based on surveillance, readiness, stakeholder engagement, regulation, and communication. This strategy builds on local frameworks and international best practices.

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