

RESEARCH PAPER

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Surveillance and detection of the occurrence of African swine fever in abattoirs in the different municipalities of the second district of Cagayan, Philippines

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ABSTRACT

African swine fever (ASF) is a highly contagious hemorrhagic viral disease of domestic and wild pigs, which is responsible for serious economic and production losses. It is caused by a large DNA virus of the Asfarviridae family. The study was conducted to determine positive cases of African swine fever on pigs from different slaughterhouses in municipalities of second district in Cagayan. A total of 362 blood samples were collected in 6 municipal slaughterhouses. The samples were analyzed in the Regional Animal Disease Diagnostic Laboratory (RADDL). The viral DNA of ASF was extracted from the blood samples using the QIAamp Viral RNA Mini Kit (QIAGEN) and was identified through the RT-PCR (ASFV p72 gene-based real-time PCR assay). Two municipalities involve in the study resulted positive of African Swine Fever with a total of 60 out of 362 pigs. The municipality with the highest positive case and incidence rate is Sto. Nino with 41 out of 56 pigs are infected (incidence rate is 11.32%) followed by Piat with 19 pigs out of 96 pigs (incidence rate is 5.25%) and the rest no incidence of African Swine-Fever. The result obtained from the study indicates that out of 230 pooled samples (362 pigs), 35 (60 pigs) are showing 15.22% positivity rate and 16.57% incidence.

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INTRODUCTION

African swine fever (ASF) is a devastating haemorrhagic fever of pigs with mortality rates approaching 100 per cent. It causes major economic losses, threatens food security and limits pig production in affected countries. ASFV is a large DNA virus that replicates in the cytoplasm and is the only member of the Asfarviridae family. The virus encodes 150–165 proteins, which have ‘essential’ functions in virus replication, as well as ‘non-essential’ roles in host interactions, including evasion of host defences; for example, many proteins inhibit the early innate responses, including type I interferon and cell death pathways (Dixon *et al.*, 2019).

African swine fever (ASF) was first identified in East Africa in the early 1900s as a disease causing high mortality in domestic pigs (*Sus scrofa domesticus*). It was quickly established that warthogs (*Phacochoerus africanus*) could be a source of infection (Montgomery, 1921) and that this host, along with a species of soft ticks (*Ornithodoros* spp.) which live in warthog burrows, could be persistently infected with ASF virus (ASFV) without showing signs of disease (Plowright *et al.*, 1994).

ASF has a severe socio-economic impact, both in areas where it is newly introduced and where it is endemic. The high impact is most apparent in countries with a significant commercial pig industry. In Africa, ASF has potentially devastating effects on the commercial and subsistence pig production sectors, but the greatest losses are usually inflicted on the poorer pig producers who are less likely to implement effective prevention and control strategies (Edelsten and Chinombo, 1995) or basic biosecurity. The farmers also often lack financial resources to restart production in the absence of compensation schemes. In countries such as Cote d'Ivoire and Madagascar, the introduction of ASF resulted in the loss of between 30 and 50 per cent of the pig population (El Hicheri *et al.*, 1998; Roger *et al.*, 2001).

Although ASF was first described almost a century ago, controlling the disease has proven to be a

challenge, in particular because no vaccine is available. Following introduction to ASFV-free countries, the only control measures available are strict quarantine and biosecurity, animal movement restrictions and slaughtering affected/exposed animals.

MATERIALS AND METHODS

Location of the study

The research study is conducted in the different slaughter house located in different municipality of the second district of Cagayan namely: Abulug, Allacapan, Ballesteros, Calayan, Claveria, Lasam, Pamplona, Piat, Rizal, Sanchez-Mira, Santa Praxedes and Santo Niño (Fig. 1). This study was carried out within the month of January to March of 2021.

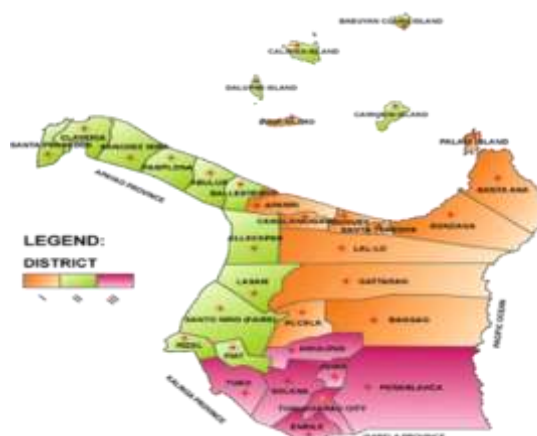


Fig. 1. Showing the three districts of Cagayan

Sample collection

Samples will be collected during regular operation schedule of the municipal slaughterhouses. The total pig population for slaughterhouse will be identified ranging from 6-12 animals per schedule for the period of 3 months (conducted 2-3 times a week). From 6-30 animals per schedule. Historical data: name of the owner, address of the farm, gender of the animal and population will be collected. Whole blood was collected prior to slaughter using 3ml EDTA. The collected sample is cooled for transport and submission to the laboratory. Whole blood will be collected prior to slaughter (Fig. 2 & 3).



Fig. 2. Collection of blood samples



Fig. 3. Blood samples collected



Fig. 4. Pooling of blood samples



Fig. 5. Extraction of blood samples



Fig. 6. Biosafety cabinet and real time polymerase chain reaction machine

Laboratory diagnosis

Samples were processed according to the standard procedure for PCR virus isolation. Whole blood samples were pooled and extracted for RT PCR testing. Using micropipette, blood samples were pooled (500 μ l) depending upon the number of animals the owner had. Standard procedure of Qiamp Viral RNA Mini kit was used for extraction. Reagents were prepared prior extraction process. The extracted DNA/RNA undergone RT-PCR testing after extraction. African swine fever rt pcr protocol is followed, the kit used is Agpath ID One Step-RT PCR. Before proceeding to the procedure, good laboratory practices is followed for the procedure to be successful and to prevent contamination of the virus (Figs. 4-6).

Research design

This study utilized a descriptive cross-sectional research approach to ascertain the prevalence of African Swine Fever Virus (ASFV) in pigs slaughtered in designated municipal slaughterhouses.

The design centered on laboratory detection via real-time reverse transcription polymerase chain reaction (RT-PCR), alongside the acquisition of pertinent animal and farm-level data. During the usual operating hours of the slaughterhouses, data and biological samples were gathered throughout a set three-month period.

The cross-sectional method made it possible to check the ASFV status of pigs that were brought in for slaughter at a certain period, which gave useful data for disease monitoring and public health response.

Sampling technique

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Research instruments

The main tool that the researchers used to collect data for this study was a standardized Data Collection Form/Laboratory Surveillance Checklist that they made. The equipment was made to systematically collect descriptive data, sample information, and lab findings needed to figure out how many pigs slaughtered in certain municipal slaughterhouses have African Swine Fever (ASF) and how many were positive for it.

The tool made sure that data was recorded the same way at all study sites, which made it easier to do proper statistical analysis.

Data gathering procedure

This study's data collection included both primary laboratory data and secondary descriptive data gathered throughout the blood sample collecting process at municipal slaughterhouses.

During the planned slaughterhouse procedures, animals that were eligible were found, and full blood samples were taken before the pigs were killed, following established biosafety and animal handling rules. To keep the owners of the animals private while still being able to trace each blood sample, each one was given a unique identifying code.

A structured data recording form was used to encode all of the data that was collected. Using the assigned identifying codes, the findings of the RT-PCR analysis were compared to the information on the animals and farms.

Data collection was done all the time during the three-month study so that results could be grouped by municipality. Before data encoding and statistical analysis, completed data forms were checked for completeness and correctness.

Analysis of the data

Data collected will be analysed using descriptive statistics such as percentages and tabulation form. The result of the study per municipalities will also be interpreted using the incidence 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:

$$PR = (\text{Number of positive pooled samples} / \text{Total number of pooled blood samples tested}) \times 100$$

Incidence rate

Incidence refers to the occurrence of new cases of disease or injury in a population over a specified period of time. Although some epidemiologists use incidence to mean the number of new cases in a community, others use incidence to mean the number of new cases per unit of population.

$$IR = \text{Number of new cases of disease during specified time interval} / \text{Population at start of time interval}$$

Incidence rates are particularly advantageous when trying to measure incidence in studies with dynamic populations and in studies with fixed populations with relatively long follow-up time.

RESULTS AND DISCUSSION

ASF was caused by African swine fever virus (ASFV); a large, enveloped double-stranded DNA virus belonging to the Asfivirus genus of the Asfarviridae family (Dixon *et al.*, 2005). African swine fever attacked the swine industry of the Philippines. The fastidious transmission of ASF was due to the lack of a vaccine, (Petrini, 2019) the stability of ASFV in the environment and in infected pork (Taylor, 2020) and less biosecurity and preventive measures. 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 Province. 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. Three millilitre whole blood were collected from each pig prior to slaughter.

Samples collected from slaughterhouses in Piat, Sto. Nino, Lasam, Abulug, Ballesteros and Sanchez Mira were submitted to RADDL for testing. A total of 362 blood samples were collected from butchered pigs in different slaughterhouses. No signs indicative of the disease was observed, and all animals appeared to be asymptomatic. Visual examination by the meat inspectors reported no significant findings. There were 96 blood samples collected from Piat, 56 blood samples from Sto. Nino, 50 blood samples from Lasam, 60 blood samples from Abulug, 50 blood samples from Ballesteros and 50 blood samples from Sanchez Mira Cagayan. The blood samples were pooled based on the owner resulting to 240 pools processed and tested for viral antigen through RT-PCR. Nucleic acids (DNA and RNA) were isolated from the samples using the QIAamp mini-Viral RNA Mini Kit (QIAGEN). The kit was suitable for the extraction of DNA and has been used successfully for ASFV DNA isolation (King *et al.*, 2003). The extracted DNA was tested using Real Time-Polymerase Chain Reaction. The result indicates that 2 of the municipalities had positive cases of African Swine Fever.

Fig. 7 shows the result of the analysis and the amplification plot of the different blood samples tested. The y axis represents the delta Rn value, the Rn value of an experimental reaction minus the Rn value of the baseline signal generated by the instrument. This parameter reliably calculates the magnitude of the specific signal generated from a given set of PCR conditions.

While the x axis represents the cycle threshold this was basically the point where the curve first clearly rises off baseline to a statistically significant degree. The crossing of this statistical noise threshold was the basis for calling a sample positive in a qualitative assay, and

the cycle number at which it occurs was the basis for generation of a standard curve and quantitation of starting template in a quantitative PCR.

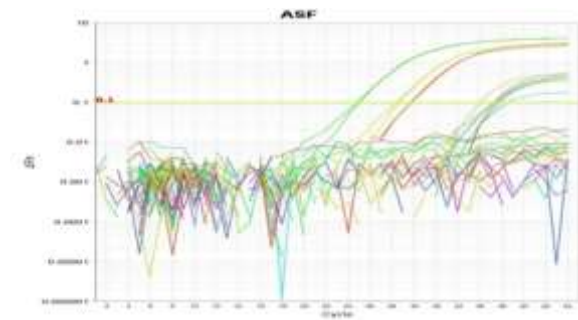


Fig. 7. The amplification plot of real-time PCR for detection of African swine fever in the blood samples tested

The second point was a point midway up the apparent “second phase” of the curve, where there was an imperceptible change from the curve increasing in slope each cycle, to decreasing in slope each cycle, referred to as an inflection point. As we can see in the plot, several samples forming sigmoid shape curve were present which implies a typical fluorescence curve with a Ct value of less than 40, the reading of this result was Positive. No Ct value implies negative samples. Samples giving a Ct value ≥ 35 were considered weak if a sigmoidal plot was observed and the analysis was repeated twice for confirmation. Samples showing a Ct value >38 were considered negative.

Table 1 shows the total number of blood samples that were tested positive for ASF. A total of 230 pooled 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). Results showed that 2 of the municipalities, where blood samples were collected, tested positive for the African Swine Fever virus. The municipality of Sto. Nino showed a high chance of ASF infection with 22 positives for ASF viral antigen out of 34 pools tested. Municipality Piat had a second high chance of ASF infection with a total of 1 positives out of 54 pools tested. And, the rest of the

municipalities had no ASF-positive infection, which includes: Lasam, Abullug, and Sanchez Mira with. Based on the number of positive cases, the municipality with the most case were Sto. Nino.

Table 2 shows the positivity rate of infected pooled samples of the municipal slaughterhouses. Positivity rate was derived by dividing the number of pools that tested positive and the total number of pooled samples tested multiply by 100. The municipality with the highest positivity rate at 9.57% were in Sto. Nino followed by Piat were 5.65% and the rest of the Municipality were 0% detected positivity. High percentage of positivity rate from the collected samples was assumed directly proportional to the

high number of positive cases in the municipality. High positivity rate of samples from slaughterhouses can indicate that slaughterhouses can be a potential source of infection thus serves an important role in the transmission of the disease African Swine Fever. The likelihood of African swine fever positive pigs slaughtered and processed in a registered facilities such as the municipal slaughterhouses were high due to lack of proper screening of pigs accepted in the facilities. Another factor was the low indemnification cost particularly for the slaughter-ready pigs which forced owner or hog raisers to opt selling the pigs for slaughter. This practice was previously observed to lead to large outbreak scenarios (Dixon *et al.*, 2020).

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

Municipality	Positive	Negative	Total # of pooled blood and spleen samples
	Whole blood	Whole blood	Whole blood
Piat	13	41	54
Sto. Nino	22	12	34
Lasam	0	29	29
Abullug	0	34	34
Ballesteros	0	29	29
Sanchez Mira	0	50	50
Total	35	195	230

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

Municipality	Positive	Samples	Positivity rate per municipality	Positivity rate (%)
Piat	13	54	24.07%	5.65%
Sto. Nino	22	34	64.70%	9.57%
Lasam	0	29	0%	0%
Abullug	0	34	0%	0%
Ballesteros	0	29	0%	0%
Sanchez Mira	0	50	0%	0%
Total	35	230		15.22%

Fig. 8. Show which municipalities have the highest positivity rate of African Swine Fever (ASF). In which, Municipality of Sto. Niño and Piat showed the positivity rate of ASF, while the other Municipality, Lasam, Abullug, Ballesteros, and Sanchez Mira where showed zero (0) positivity rate of ASF.

Based on the study, Piat and Sto. Niño, have the most case of ASF 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. Lasam, Abullug, Ballesteros, and Sanchez Mira showed zero (0) cases of ASF since there were few hog raisers in these municipalities and mainly due to the biosecurity measures and information campaign implemented by the local government.

On average, the positivity rate of second district Cagayan was 15.22% which includes 35 positive pooled blood samples out of the total 230 pooled blood samples

tested. This rate only reflects the samples collected from the slaughterhouses in District 2 of Cagayan. No clinical signs of African Swine Fever were seen in the pigs delivered and slaughtered, therefore, it is safe to hypothesize that the virus was acquired before the pigs were delivered to the slaughterhouse. Further, we can safely hypothesize that pigs acquired the disease from the farm.



Fig. 8. The graphical sketch of the positivity rate of African swine-fever

Based on the study of Thomas *et al.* (2016), the presence of infected pigs at abattoirs and in turn the dispersal of potentially infective meat to butcheries across the study site is of particular importance to the

control of ASF. Previous studies identified the movement of infected pigs to an abattoir and the movement of infected pork products as being a major risk factor for outbreaks, which appear to be distinct from the sylvatic cycle. 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 personnel on the role of the slaughterhouses and aggregation points such as the public markets in the spread and transmission of ASF. Also, 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 second district Cagayan Province

Municipality	Number of pigs in positive pooled samples	Total number of pigs in pooled samples tested	Incidence rate per municipality	Incidence rate (%)
Sto. Nino	22 pools = 41pigs	56	73.21%	11.32%
Piat	13 pools = 19 pigs	96	19.79%	5.25%
Lasam	0 pools = 0 pig	50	0%	0%
Abulug	0 pools = 0 pig	60	0%	0%
Ballesteros	0 pools = 0 pig	50	0%	0%
Sanchez Mira	0 pools = 0 pig	50	0%	0%
Total	35 pools = 60 pigs	362		16.57%

Overall, the incidence rate of ASF in the 2nd District of Cagayan was 16.57%. A total of 35 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 35 positive pools in the study consisted of 60 individual blood samples. On the other hand, a total of 362 blood collected from individual pigs. In an interview conducted, Cagayan Provincial Veterinary Office Chief Dr. Noli Buen (2021), ASF was present 38 barangay 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

Using real-time PCR detection, this study verified the existence of the African swine fever virus (ASFV) in municipal slaughterhouses located in the 2nd District of Cagayan Province. 35 pools, or 60 pigs, of the 230 pooled blood samples (362 individual pigs) that were tested tested positive, resulting in an overall positivity

rate of 15.22% and an incidence rate of 16.57%. Of the six municipalities that were part of the study, Sto. Lasam, Abulug, Ballesteros, and Sanchez Mira did not exhibit any cases during the sampling period, while Niño had the highest positivity and incidence rates, followed by Piat.

The fact that every sampled pig looked clinically healthy before being killed highlights the silent spread of ASFV and the drawbacks of depending only on visual inspection and standard meat inspection practices. Pigs found to be ASFV-positive at slaughterhouses indicate that the infection most likely started at the farm level and that slaughterhouses could be important hubs for the spread of the disease, especially in cases where biosecurity, traceability, and pre-slaughter screening are insufficient.

The results emphasize the critical need for regular molecular testing of high-risk animals, tighter biosecurity regulations at the farm and slaughterhouse levels, and improved surveillance systems. An effective ASF prevention and control strategy must include improved indemnity policies, more stringent movement control of pigs and pork products, and improved information campaigns aimed at backyard hog raisers. To prevent further spread and support the province's ASF eradication efforts, ongoing cooperation between local government entities, veterinary authorities, and stakeholders is essential.

RECOMMENDATION(S)

Based on the findings of this study confirming the presence of African swine fever virus (ASFV) in selected municipal slaughterhouses in THE District 2 towns of Cagayan Province, the following recommendations are proposed:

Strengthen pre-slaughter screening and surveillance: Mandatory pre-slaughter testing using real-time PCR should be implemented, particularly for pigs originating from high-risk municipalities. Routine molecular surveillance in slaughterhouses must be institutionalized to detect asymptomatic carriers and prevent entry of infected animals into the food chain.

Enhance farm-level biosecurity measures: Local government units (LGUs) and veterinary offices should intensify biosecurity campaigns, especially among backyard hog raisers. Standardized protocols on disinfection, controlled farm access, proper waste disposal, and regulated animal movement must be strictly enforced.

Improve traceability and movement control: A strengthened animal movement permit system and traceability mechanism should be adopted to track pigs from farm to slaughterhouse. Strict quarantine checkpoints and monitoring of inter-municipal transport of pigs and pork products are essential to minimize disease spread.

Capacity building for slaughterhouse personnel: Continuous training of meat inspectors and slaughterhouse workers on ASF recognition, biosafety, and biosecurity practices should be conducted. Emphasis must be placed on early detection, proper handling of suspect animals, and strict meat condemnation procedures.

Review and enhance indemnification policies: Compensation schemes for affected hog raisers should be reviewed to ensure fair and timely indemnification. Adequate financial support may discourage the sale of potentially infected pigs for slaughter and reduce underreporting of suspected cases.

Sustain public awareness and information campaigns: Community-based education programs should be strengthened to increase awareness about ASF transmission, prevention strategies, and the risks associated with improper disposal and movement of pork products.

Further research and expanded surveillance: Future studies should include farm-level sampling, environmental testing, and longitudinal monitoring to better understand transmission dynamics. Expansion of surveillance to other districts of the province is recommended to provide a comprehensive epidemiological profile.

Collectively, these measures will contribute to a more robust ASF prevention and control framework and support ongoing eradication efforts within the province.

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