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Original research

Seroprevalence of African swine fever in pigs in Lere and Zaria Local Government Areas of Kaduna State Nigeria

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ABSTRACT

African swine fever (ASF) is the most important disease limiting small holder pig production in Nigeria, causing mortality as high as 100% resulting in total loss of investment. African swine fever is of public health concern because it is a recurring disease in many countries including Nigeria. This study was undertaken to investigate the presence of antibody (IgG) against African Swine Fever Virus (ASFV) among pig population in Lere and Zaria local government areas (LGAs) of Kaduna State of Nigeria. A total of 45 blood samples were collected from different suspected pigs from Lere and Zaria local government areas (LGAs). Eighteen serum samples were obtained from eighteen different suspected pigs from the pig population in Lere LGA while twenty seven serum samples were also obtained from twenty seven different suspected pigs at Zaria LGA. The samples were tested by conventiolan indirect Enzyme-linked immunosorbent assays (ELISA). The results showed 2 samples representing 11.11% of the total samples collected from Lere LGA were positive for ASF antibody. On the contrary, none of the 27 samples obtained from Zaria LGA was positive. Therefore, the overall seroprevalence was 4.4%. Pigs with demonstrable antibody should be considered as carriers of the virus because it is uncertain if true recovery ever occurred in them. Control measures such as use of biosecurity, routine test and slaughter of animals that tested positive for ASF antibody were recommended. Further studies are also suggested to involve larger sample size and better diagnostic technique such as polymerase chain reaction, having observed that ELISA –detected ASFV antibodies do not transfer into clinical ASF.

Keywords: African swine fever, antibody, serum, Lere, Zaria , Kaduna State.

INTRODUCTION

African swine fever (ASF) is a devastating viral disease currently threatening the pig industry worldwide (Ayoade & Adeyemi, 2003). It is a notifiable, lethal hemorrhagic disease of domestic pigs (Rahimi *et al;* 2010). Mortality range of 50 to 100% in various herds was recorded in Delta State, Nigeria (Otesile *et al;* 2005). A total of 125,000 pigs died of ASF from September, 1997 to October, 1998 in Lagos, Ogun, Kaduna, Benue, Enugu, Akwa Ibom, Rivers, Plateau and Delta states in Nigeria (Hicheri, 1998). The disease is caused by *African swine fever virus* (ASFV) which is an enveloped double stranded deoxyribonucleic acid (DNA) virus measuring 200 – 220nm in diameter (Sarma, 2012). Different strains of *ASFV* vary in their ability to cause disease, but there is one serotype of the virus detectable by blood antibody test (EU, 2010). It a member of the family *Asfaviridae* and is a double stranded DNA virus that measures 200-220nm in diameter (Ekwe & Wilkinson, 2000).

Maintenance and transmission of *ASFV* involve cycling of virus between soft ticks of the genus *Ornitodorus* and wild pigs (warthogs, bush pigs, and giant forest boars). The virus survives in chilled carcasses or in frozen meat for several weeks (Merchant & Parker, 2005). In uncooked products, such as dried sausage and ham, the virus can persist for 3 to 6 months. The virus can be viable in garbage containing meat scraps that have not been heated to 65° C for 1 hr. The dried virus is not destroyed by exposure to 40°C in 15 days. It is preserved in 0.5% phenol in 50% per cent glycerin mixture at room temperature for 536 days. The virus is quite stable since it remains viable for 11 days at room

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temperature, 15 weeks in carcasses, 5 months in processed hams and 6 months in bone marrow (Merchant & Parker, 2005).

The family is sensitive to lipid solvents and can be inactivated rapidly by 2% Sodium Hydroxide NaOH (Sarma, 2012). The virus can also be acquired through ingestion of contaminated feed (Rahimi *et, al;* 2010). Different strains of *ASFV* vary in their ability to cause disease, but there is one serotype of the virus detectable by blood antibody test (EU, 2010). The DNA of the virus is circular and has 170-190 Kbp, nucleoprotein core surrounded by an icosahedral sheet and possesses an outer envelope. The viral genome codes for about 34 structural protein and some nonstructural proteins (Sarma, 2012; Ekwe & Wilkinson, 2000).

The virus is transmissible directly from one domestic pig to another or from pig products to domestic pigs, without the involvement of intermediate host such as sylvatic hosts or arthropod vectors. Many studies have reported a direct transmission between infected bush pigs and domestic pigs, and between pigs-to-pigs in domestic cycle through contact. The most common route of transmission is through mouth or the upper respiratory system provoking proliferation of lymphocytes and reticular cells (Ayoade & Adeyemi, 2003). Infection through the respiratory tract leads to replication of the virus in pharyngeal tonsils and lymph nodes that drain the nasal mucosa, eventually culminating into viraemia. Consequently, all secretions and excretions (urine and faeces) contain large amounts of infectious virus (Murphy *et, al.,* 1999; Radostits *et al.,* 2007) through which infection spread rapidly to other areas and animals.

Kaduna State is in North Western part of Nigeria and an acknowledge point of swine production in Northern Nigeria compared to other Northern States due to religious sentiment against pork consumption. Presently, there is dearth of information on ASF antibody status in pig production in Kaduna State. Therefore, this study aimed to determine the seroprevalence of ASF in pigs in two local areas of Kaduna States towards developing a control strategy to ensure a healthy pig population.

MATERIALS AND METHODS

STUDY AREA

The study areas for this study were Lere and Zaria Local Government Areas (LGAs) in Kaduna State. Kaduna State are situated between Longitude 06° and 11° 3'N of the equator. The State occupies an area of approximately 48,732.2 square kilometers. Blood samples were collected from pig population from both commercially managed and traditionally managed pigs within Lere and Zaria LGAs (Figures I and II).

ACTIVITY BEFORE THE COLLECTION OF SAMPLES FOR ANALYSIS

The following were taken from live animals or before animals are slaughtered: The breed or breed trait and sex of each animal were recorded; the weights of the animals were taken mostly with the use of weighing band which gave more than 97% accuracy of the animal weights. The body of each animal was carefully examined with magnifying glass or hand lens for any lesion on the skin and to see if there were any ectoparasites (eg Mites or tick).

SAMPLE COLLECTION

Ethical approval was sought and granted by the ethical

Figure 1 Map of Nigeria showing Kaduna State used for the study (Dada *et al* , 2010)

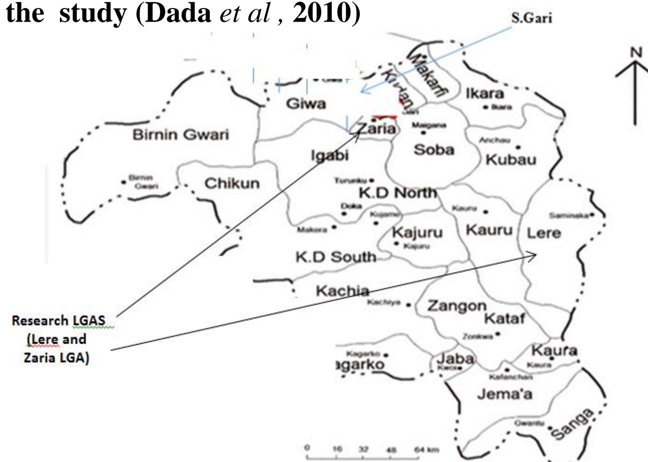

Figure II. Map of Kaduna State and its Local Government Areas area showing study areas, Lere and Zaria Local Government Areas (Dada *et al* , 2010) .

committee for experimental purpose of College of veterinary medicine, Michael Okpara University of Agriculture, Umudike, Nigeria (MOUAU/CVM/REC/202217). A total of 45 blood samples were collected from different suspected pigs from Lere and Zaria LGAs of Kaduna state of Nigeria. Eighteen blood samples (5 to 10 mls) from eighteen different suspected pigs from Lere LGA and twenty seven blood serum samples from twenty seven different suspected pigs from Zaria LGAs. The blood was collected from live animals through the jugular vein in two different tubes for each animal, one tube containing anticoagulant and the other vacutainer tube without anticoagulant. All the samples collected were placed in a cooler and covered with ice pack until further processing.

ANTIBODY DETECTION TEST

The detection of antibody against ASF was carried out by indirect Enzyme-linked immunosorbent assays (ELISA) as recommended by the manufacturer (IDvet,310, rue Louis Pasteur-Grabels-FRANCE).

ELISA was carried out in a 96-well plate as described in previous study (Asambe, Sackey, & Tekdek, 2018). Briefly, 100 µl of dilution Buffer 14 to each well, which was followed by addition of 10 µl of the negative control buffer (supplied with the kit) to wells A1, B1 and A2, and B2. Another volume of 10 µl of the positive control buffer was added to the wells C1, D1 and C2, D2. Then after, 10 µl of test sample was added the remaining wells in duplicate with adjacent wells. The plate was later incubated for 45 min at 21°C (± 5°C). The wells were emptied and washed 3 times with approximately 300 µl of washing solution. Drying of the wells was avoided during washing. The conjugate (10×) was reconstituted into 1× using the diluting buffer. A volume of 100 µl the latter was added to all the wells and the plate was incubated at 21°C (±5°C) for 3 mins. The wells were rinsed again and then added, 100 µl of the Substrate Solution for incubation at 21°C (±5°C) for 3 mins in the dark. Lastly, the reaction was stopped by adding 100 µl of the Stop Solution to each well. Antigen antibody reaction took place in the positive wells to give a brownish colouration due to horseradish peroxidase. The Optical density (OD) of all samples was simultaneously measured at 450 nm wavelength using an ELISA microplate reader (UNIEQUP ELISA Reader, Germany, A3 2009 Model).

RESULT

The details of all the 45 pigs used for this study and the results of seroprevalence of ASF in the Lere and Zaria LGAs of Kaduna States are presented in Tables I. There were 12 males and 6 females pigs for the Lere LGA while, the number of males and females at Zaria LGA were 15 and 12 respectively. The breeds of pigs involved were large white, large white crossbreeds and cross between local breeds and other undefined breeds as well as local breeds.

Only two pigs that represented 11.1% of the total samples in Lere LGA tested positive while the remaining 16 that represented 88.9% were negative. On the contrary, there was no positive results in the pigs located at the Zaria LGA, all the 27 pigs (100%) were negative. The overall percentage positive was 4.4%.

DISCUSSION

This study was conducted to determine sero-prevalence ASF in Lere and Zaria LGAs of Kaduna States and showed a total prevalence of 4.4% which is far less than 37% sero-prevalence reported by Patrick et al. (2020) in apparently healthy pigs in South-Kivu Province of the Democratic Republic of Congo.

ASF is endemic in Southwest Nigeria and the results of the present study is far lower than 28% reported by Awosanya *et al.* (2015) with higher prevalence during the dry season compared to rainy season. On the contrary, a total seroprevalence of 2.8% was reported in Benue which is further delineated into two categories, prevalences of 7 (1.7%) and 6 (13.6%) were observed in piggeries and in Makurdi slaughter slab, respectively (Asambe *et al.,* 2018). It seems there is less prevalence at piggery pen than slaughter slabs. According to Umaru *et al.* (2014), thirty six samples (out of 289) representing 12.9% were positive for ASF in Taraba State.

According to Radostits *et al.* (2007), pigs with demonstrable antibody should be considered as carriers of the virus since it is uncertain that true recovery ever occurs or the strain is unable to cause clinical disease in the herd. However, that number remains a carrier of ASF in the herd. By implication, the 2 pigs showing positive for ASFV antibody are carriers in Lere LGA of Kaduna State. On the contrary, there was no positive result obtained from the 27 samples taken from Zaria LGA. Nevertheless, it is reasonable to exercise caution in declaring Zaria LGA free of ASF considering the number of samples tested. Ideally, the minimum number of sample in the present study ought to should have considered the present ASF prevalence in the study area such that is enough to show significance in the sample size.

It was noted that sero- positive boar was 65 Kg while sero-positive female weighed 75 Kg. This may likely be due to the fact the male might have been used as breeder by many pig farmers in that area and this gave chance to carer of the virus by the boar which may later spread the disease to other pigs. Similarly, the only positive sow that was 75 Kg by weight must have been retained for a long period of time possibly due to her higher litter number per farrowing and

Table 1: Showing the information about the animals from Lere Local Government (1-18 samples) and Zaria (19-45) LGA areas and the S/P% as calculated from ELISA result

S/N	G/N	Sex	Wg in Kg	Breed	S/P % Titre Level	Antibody	LGA
1	1	M	65	Lw/cr	100.88	+	Lere LGA
2	2	M	50	Lwcrss	11.63	-	
3	3	M	55	Lw/cr	07.15	-	
4	4	F	55	Lw/cr	11.46	-	
5	5	M	55	Lw/cr	09.41	-	
6	6	F	60	Lw/cr	33.72	-	
7	7	M	65	Lw/cr	11.43	-	
8	8	M	55	Lw/cr	09.00	-	
9	9	M	55	Lw/cr	07.79	-	
10	10	F	50	Lw/cr	08.90	-	
11	11	M	60	Lw/cr	08.36	-	
12	12	F	75	Lw/cr	93.76	+	
13	13	M	55	Lw/cr	07.49	-	
14	14	F	50	Lw/cr	08.97	-	
15	15	F	55	Lw/cr	08.60	-	
16	16	M	50	Lw/cr	38.57	-	
17	17	M	60	Lw/cr	11.33	-	
18	18	M	45	Lw/cr	08.33	-	
19	1	F	65	Lw/Cross	10.20	-	Zaria LGA
20	2	F	100	Lw/Cross	09.91	-	
21	3	M	50	Lw/Cross	14.69	-	
22	4	M	50	Lw/Cross	11.58	-	
23	5	M	30	Lw/Cross	08.76	-	
24	6	F	45	Lw/Cross	13.42	-	
25	7	M	20	Lw/Cross	17.86	-	
26	8	F	20	Lw/Cross	10.89	-	
27	9	M	20	Lw/Cross	10.54	-	
28	10	F	20	Lw/Cross	12.21	-	
29	11	M	20	Lw/Cross	13.60	-	
30	12	M	20	Lw/Cross	11.87	-	
31	13	F	20	Lw/Cross	12.73	-	
32	14	M	20	Lw/Cross	11.87	-	
33	15	M	20	Lw/Cross	16.91	-	
34	16	F	30	Lw/Cross	12.74	-	
35	17	M	25	Lw/Cross	06.54	-	
36	18	F	15	Lw/Cross	16.11	-	
37	19	T	15	Lw/Cross	34.33	-	
38	20	M	15	Lw/Cross	25.14	-	
39	21	M	15	Lw/Cross	17.17	-	
40	22	M	15	Lw/Cross	13.12	-	
41	23	M	45	Lw/Cross	12.10	-	
42	24	F	45	Lw/Cross	13.88	-	
43	25	F	35	Lw/Cross	06.50	-	
44	26	M	35	Lw/Cross	14.51	-	
45	27	F	30	Lw/Cross	16.32	-	

Keys: Lw: Large white breeds Lw/cr: Large white crossbreeds Cros: Cross Between local beeds and other undefined breeds. LC: Local breeds M: Male F: Female

good mothering ability. This coupled with the fact that adult sows are traditionally not easily sold out or slaughtered by farmers so as to give room for fast proliferation.

Probing further, some associated factors that may be responsible for the 11.1% ASFV seropositive obtained for Lere LGA and nil obtained for Zaria LGA were considered. The piggery farms where samples were obtained in Zaria were not more than 2 years old and had no neighbouring pens within their vicinity. In sharp contrast to Zaria LGA, the pigs at Lere LGA had been reared for long time by many families and many were on free range system of management. The free range system in pig production is a major factor responsible for increased risk of ASF (Payne *et al.,* 2021).

ASF has been included among notifiable animal disease according to the World Animal Health Organization (WOAH). ASF was first discovered in Kenya, Africa, in 1921. Before 1957, ASF outbreaks occurred only in Africa and subsequently spread to Europe and the Americas. The disease seems to be recurring almost on annual basis in Nigeria and ASF has assumed a new enzootic status where most infected farms are at subclinical level (Awosanya *et al.,* 2015).

So many variants of the ASFV genotypes have been reported in Sub-Sahara Africa and these include genotypes I, II, III, IV, V, VI and VII as well as many others. However, there are few studies on genomic characterisation of ASF in Nigeria. Adedeji et al. (2021) were the first to report ASFV genotype II in Nigeria and West Africa. At the same times, there is difference in the virulence among the strains. Some strains are mild and may not be able to cause clinical ASF. Interpretation of this result requires a caveat, since presence of ASFV antibody does not necessarily translates into true recovery and clinical ASF especially if the strains is of lower virulence (Atuhaire *et al.,* 2013). However, the pigs with ASF antibodies are carrier for potential spread and reinfection of ASF in the future. Sometimes, mutation of one ASF strain to another had been reported (Awosanya *et al.,* 2021).

CONCLUSION AND RECOMMENDATION

This study has shown that ASF antibodies were present in pig in Lere LGA of Kaduna State of Nigeria and this has confirmed the carrier status of the pigs in the local government area. The study has also shown that ASF antibodies were absent in pig in Zaria LGA of the same State. African swine fever has been described by Rahimi *et, al;* (2010) as a notifiable, highly lethal haemorrhagic population and could impact on economy of the pig owners and on animal protein consumption.

One of the major control measures is to ensure strict adherence to biosecurity guidelines (Fasina et al., 2020). Efforts should be made to contain infection where it has occurred (bio-containment) and then, bio-exclusion to prevent further spread of infection in to piggery pens free of infection (Mutua & Dione, 2021). Control of personnel from other pig farms, visitors, butchers and provision of foot deep are essential to curtail infection. Other measures include quarantine, compulsory slaughter of infected and contact animals and other animals at risk with adequate compensation to the owners and disinfection of contaminated pig pens with strong solution of caustic soda. Such pens have to be made to rest for about four months before new animals are reintroduced since the site can remain infective for over 3 months. Lastly, further studies are also suggested to involve larger sample size and better diagnostic technique such as polymerase chain reaction, having observed that ELISA – detected ASFV antibodies do not transfer into clinical ASF and such animals remains carrier that sustain the recurrence and re-emergence of ASF after its apparent eradication.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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