



Detection of African swine fever virus (ASFV) antibodies in pigs in Taraba state north east Nigeria

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Abstract

A serological study was conducted between 2011 and 2013 to detect the presence of antibodies to African swine fever virus (ASFV) in Pigs in Taraba State, north eastern Nigeria. Sixty (60) pig farms were studied. Questionnaires (N=885) consisting of sections related to the farmer and the animal were distributed and blood samples taken to analyze for the African swine fever virus antibodies. All the 60 questionnaires were answered by the farmers, while 885 were retrieved for the individual pig studied. Of the 60 famers, only 13 (21.7%) were aware of the disease while 47 (78.3%) were ignorant ($P<0.05$). Out of the 885 pigs examined, none (0%) was infected nor showed any clinical signs of the infection. However, 117 (13.2%) out of the 885 sera samples analyzed for the three year period were positive for ASFV antibodies. The results obtained in 2013 (22.5%) was significantly higher ($P<0.05$) those in 2011 (12.9%) and 2012 (4.0%). Although male pigs recorded higher seroprevalence (13.7%) than the females (12.9%), this was no statistically significant ($P>0.05$). The highest seroprevalence (16.3%) was recorded in the Northern zone in 2011, followed by 10.0% and 5.0% in Southern and Central zones respectively. In the year 2012, no ASFV antibody was detected from the 100 and 99 pigs examined from Central and Northern zones respectively. However, the Central zone recorded a seroprevalence of 12.0%. In 2013, the sero-prevalence in Southern (29.0%) and Central zones (24.0%) is significantly higher ($P<0.05$) than in the Northern zone. The sero-prevalence obtained may pose a great risk in the study area, it is therefore recommended that adequate veterinary services, proper sanitary measures and monitoring of pig movement be instituted to avert an outbreaks.

Keywords: African swine fever virus, Antibodies, ELISA, Pigs, Taraba State, North eastern Nigeria

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Introduction

Pigs belong to the genus *Sus*, and family *Suidae*, and include the domestic pig and the common Eurasian wild boar (*Sus scrofa*). Other genera include the Babirusa and the Warthog. Domesticated pigs are raised commercially for meat (pork, hams, gammon or bacon), accounting for 38% of worldwide meat production. In Nigeria, pig production is practiced mainly by extensive system (Pathiraja *et al.*, 1986; Rekwot *et al.*, 2003) but depends largely on climatic factors, social and religious beliefs (Ogunniyi & Omoteso, 2011).

Pigs can live in virtually any productive habitat that can provide enough water to sustain its large size and also availability of forage. Pigs are prone to many disease causing agents such as parasites,

viruses, bacteria and protozoa. One of the devastating pig diseases of economic importance worldwide is African swine fever (ASF) (Penrith, 2009; Costard *et al.*, 2013).

African swine fever (ASF) is a highly contagious viral disease of domestic pigs caused by a large double stranded DNA virus, African swine fever virus (ASFV) which predominantly replicates in the cytoplasm (Costard *et al.*, 2013) and transmitted by *Ornithodoros* ticks. The virus is present in excretions and secretions of the infected pigs and remains in blood and other tissues for long period of time (Costard *et al.*, 2013). ASFV is resistant to different modes of inactivation, can exist in the

environment for several days and is stable at pH 4-10.

The disease is endemic in Africa and poses a serious threat to pig production as a result of economic losses (Kperebeyi & Amotsuka, 2010; Saka *et al.*, 2010), and also loss of nutrients in the form of protein and fats (Okoth *et al.*, 2013). The virus infects the pigs through either sylvatic or domestic cycle (Okoth *et al.*, 2013). The sylvatic cycle occurs in warthogs (*Phacochoerus aethiopicus*) with asymptomatic infection and in the *Ornithodoros* ticks (Costard *et al.*, 2013). The domestic cycle involves infection of the pigs by ticks (*Ornithodoros moubata*) that have been fed on the infected warthogs. Direct transmission between bush pigs and domestic pigs, and also in-between domestic pigs through contact has been reported (Penrith *et al.*, 2004; Costard *et al.*, 2013). ASF in domestic pig population is associated with high morbidity and mortality rates leading to economic loss; however, the trend decreased in endemic areas resulting from acquired immunity due to prolonged exposure or low infectivity (Fasina *et al.*, 2010). Recovered pigs serve as reservoirs and, hence, play important role in the spread of the virus to susceptible pig populations (Leitão *et al.*, 2001; Sánchez-Vizcaíno & Arias, 2012).

ASF outbreaks have been reported in pig farms across Nigeria (Odemuyiwa *et al.*, 2000; Olugasa, 2007; Kperebeyi & Amotsuka, 2010; Owoludun *et al.*, 2010; Saka *et al.*, 2010; Fasina *et al.*, 2012). Similarly, Olugasa (2007) in his study reported a seroprevalence of 50% (Ondo), 52.5% (Oyo), 59.8% (Lagos) and 60.7% (Ogun) in south west Nigeria while Saka *et al.* (2010) reported 70% in Osun and 88% with 79.2% mortality in Lagos, Nigeria respectively. This study is aimed at providing information on the seroprevalence of ASFV antibodies in apparently healthy pigs in Taraba state, Nigeria.

Materials and methods

Study area

The study was conducted in Taraba state. Taraba state is located in the north-eastern geopolitical zone of Nigeria, bordered on the west by Gombe and Plateau states while by Adamawa state to the North-east and it also shares its south western boundary with Benue state. An international boundary exists on the Eastern parts of the state that separates Taraba state from the Republic of Cameroun. It has an estimated land area of about 59,365.2sq.km and lies between latitudes 6^o25"N and 9^o30"N and between longitudes 9^o30"E and 11^o45"E (GSN, 1994). The state lies within the tropical zone and has a vegetation of low forest in the southern part and grassland in the northern

parts. Farming, fishing, mining and pastoralism are the predominant occupations of the people. The study covered the three Senatorial Zones, namely; Central Zone (Bali and Gassol L.G.As), Northern Zone (Ardo-kola, Jalingo and Zing L.G.A.s) and Southern Zone (Donga, Takum, Ussa and Wukari LGAs).

Structured questionnaires

Structured questionnaires (N=885) consisting of sections related to the famers and pigs were distributed for individual response prior to sample collection. Therefore, 60 questionnaires were answered by the 60 famers while 885 were retrieved for the 885 pigs studied. The questionnaire involved the awareness or ignorance of the infection, age, sex and occurrence of infection among others.

Samples collection

The sampling sites were selected based on the availability of the pigs in the areas and the sampling techniques was done by convenient sampling techniques based also on the availability of the pigs and willingness of the farmer to allow for the study to be carried out in his or her farms. Therefore, all the pigs found within the accepted farms were sampled. Five (5) ml of blood samples were collected by veni-puncture via the ear vein using syringe and needle. All the samples collected were placed in sterile blood sample bottles and allowed to stand for 30 minutes before centrifuging. The sera were separated, placed in ice packs and transported to the Viral Research Unit, National Veterinary Research Institute (NVRI), Vom, Plateau State for the detection of the ASF antibodies by ELISA procedure.

Laboratory procedures

The assay was performed using the ELISA kit (INGEZIM PPR COMPAC 11.PPA.K3) according to the manufacturer's instructions. Fifty µl of the supplied diluent were added to each well. Then 50 µl of positive control sera were added to two wells (A1 and B1) followed by the addition of 50 µl of negative control sera (A2 and B2). 50 µl of the sera samples were added to the remaining wells. The plates were sealed and incubated at 37°C for 1 hour. The wells were then emptied into a container with NaOH solution and washed. 100 µl of the specific conjugate were added, sealed and incubated at 37°C for 30 minutes. The wells were then washed and 100 µl of substrate added to each well and the plate kept at room temperature for 15 minutes. Finally, 100 µl of stop solution was added and the optical density (OD) of each well was read at 450nm.

Interpretation of the results

- i. Cut off calculation
 Positive cut off = negative control (NC)-[(NC-Positive control (PC)) × 0.5]
 Negative cut off = negative control (NC)-[(NC- Positive control (PC)) × 0.4]
 Where negative control (NC) = OD of negative control sera
 Positive control (PC) = OD of positive control sera

$$X\% = \frac{NC-Sample\ OD}{NC-PC} \times 100$$
- ii. Interpretation of results
- Serum samples with OD lower than positive cut off were considered positive to ASFV antibodies.
 - Serum samples with OD higher than negative cut off were considered negative to ASFV antibodies.
 - Serum samples with OD values between both cuts off were considered as doubtful.

Statistical analysis

Data obtained from the study were analyzed using statistical package for social science (SPSS) Version 13 and presented in the form of tables. Prevalence was calculated by dividing the number positive by the total number examined and multiplied by 100. Chi-square at 5% level of confidence was used to determine the differences in occurrence between

the different variables. A P value of <0.05 was considered significant in all comparisons.

Results

Sixty pig farms were studied and a questionnaire was given for each of the pig examined. Thus, 885 questionnaires were distributed in all and same numbers of blood samples were taken and analyzed for the ASFV antibodies. Of the 60 famer respondents, only 13 (21.7%) were aware of the disease while 47 (78.3%) were ignorant (Table 1).

Out of the 885 sera samples analyzed for the three year period, 117 (13.2%) were positive for ASFV antibodies. The results obtained in 2013 (22.5%) was significantly higher ($P < 0.05$) than in 2012 (4.0%) and 2011 (12.9%) respectively (Table 3). Although male pigs had the higher sero-prevalence (13.7%) than the females (12.9%), there was no significant difference ($P > 0.05$) in the sero-prevalence between the two genders (Table 2). According to the zones/locations, the highest sero-prevalence (16.3%) was recorded in the Northern zone in 2011, followed by 10.0% and 5.0% in Southern and Central zones respectively (Table 3). In the year 2012, no ASFV antibody was detected from the 100 and 99 pigs examined from Central and Northern zones; however, the Central zone recorded a sero-prevalence of 12.0% out of the 100 pigs examined. In 2013, the sero-prevalence in Southern (29.0%) and Central zones (24.0%) is significantly higher ($P < 0.05$) than in the Northern zone (Table 3).

Table 1: Questionnaire's results on the awareness and occurrence of ASF in Taraba State

Zone	No. of farms	Aware	Ignorant	No. of animals examined	Infection	Not infected
Central	18	3 (16.7)	15 (83.3)	280	0	280 (100)
Northern	25	6 (24.0)	19 (96.0)	365	0	365 (100)
Southern	20	4 (20.0)	16 (80.0)	240	0	240 (100)
Total	60	13 (21.7)	47 (78.3)	885	0	885 (100)

Numbers in brackets indicate percentages

Table 2: Sero-prevalence of African Swine Fever Virus (ASFV) in Taraba State, North-eastern Nigeria

Year	All Animals		Male		Female	
	No. examined	No. (%) positive	No. examined	No. (%) positive	No. examined	No. (%) positive
2011	280	36 (12.9) ^a	115	15 (13.0) ^a	165	21 (12.5) ^a
2012	299	12 (4.0) ^b	121	3 (2.5) ^b	178	9 (5.1) ^b
2013	306	69 (22.5) ^c	122	31 (25.4) ^c	184	38 (20.7) ^c
Total	885	117 (13.2)	358	49 (13.7) ^d	527	68 (12.9) ^d

Values in the same column with different superscripts differ significantly ($P < 0.05$)

Table 3: Sero-prevalence of African Swine Fever Virus (ASFV) according to the Zones/Locations in Taraba State, North-eastern Nigeria

Zone/Location	2011		2012		2013	
	No. examined	No. (%) positive	No. examined	No. (%) positive	No. examined	No. (%) positive
Central						
Bali	40	2 (5.0)	60	0 (0.0)	51	15 (29.4)
Gassol	40	2 (5.0)	40	0 (0.0)	49	9 (18.4)
Sub Total	80	4 (5.0)^a	100	0 (0.0)^a	100	24 (24.0)^a
Northern						
Ardo-Kola	39	2 (5.1)	30	0 (0.0)	21	6 (28.6)
Jalingo	90	15 (16.7)	54	0 (0.0)	48	9 (18.8)
Zing	31	9 (29.0)	15	0 (0.0)	106	16 (15.1)
Sub Total	160	26 (16.3)^b	99	0 (0.0)^a	106	16 (15.1)^b
Southern						
Donga	-	-	11	2 (18.2)	-	-
Takum	20	0 (0.0)	20	0 (0.0)	30	12 (40.0)
Ussa	20	2 (10.0)	-	-	29	11 (37.9)
Wukari	-	-	69	10 (14.5)	41	6 (14.6)
Sub Total	40	4 (10.0)^c	100	12 (12.0)^b	100	29 (29.0)^c
Total	280	36 (12.9)^d	299	12 (4.0)^d	306	69 (22.5)^d

Values in the same column with different superscripts and bolden differ significantly ($P < 0.05$)

Discussion

In this study, 885 samples obtained from apparently healthy pigs were examined for African swine fever antibodies using sera from 60 different farms across the state between 2011 and 2013. The results showed that greater percentage (83.3%) of farmers had no knowledge about the disease nor recorded any case of the disease in their farms. Those that had knowledge about it also did not record any case or witness any clinical sign of it in their farms. Also, a sero-prevalence of 13.2% (117/885) was observed from the 885 pigs examined with no statistical difference ($P < 0.05$) in the sero-prevalence between males (13.7%) and females (12.9%). The presence of the ASFV antibodies in apparently healthy pigs is a suggestion that the disease might be endemic in this area but there is absence or lack of proper reporting system. Also, outbreak and clinical disease might occur but confused or mistaken for other diseases. Factors that contributed to the sero-prevalence were largely management related such as poor hygiene and feeding methods. The results obtained from this study are lower when compared to the 33.3% reported by Luther *et al.* (2008) and 50.75% by Owolodun *et al.* (2007) in Plateau state and North central Nigeria. These studies both used tissue samples consisting of visceral lymph nodes, liver, spleen and kidneys and analyzed them using polymerase chain reaction (PCR) rather than the sera samples used in the present study. Outbreak of ASF was confirmed and reported in Kumo, Gombe State, Nigeria (Mailafiya and Iliya, 2009). This area is in close border to

Taraba State and ASF being a transboundary disease may be transferred to Taraba State. ASF is known to spread from one place to the other in Nigeria through pigs and pig products movements over considerable distances due to human factor which were considered the most relevant means involved in the ASF spread. The result can also be compared with those reported by Olugasa (2007) and Saka *et al.* (2010) who obtained an overall prevalence rate of 65.2% and 88% in commercial pig herds in south west Nigeria and in Lagos State, Nigeria respectively. The differences could be due to absence of risk factors such as large population of pigs and wild species coupled with the religious beliefs prohibiting the rearing of pigs in some areas of the state.

As observed, there was a decrease in the sero-prevalence between 2011 and 2012 and a subtle increase in 2013. The explanation is that these pigs are not kept for long period of times in the farms, but sold when they reached certain sizes and also when farmer needs money. At a latter time the farmers purchased more pigs to re-stock the farm. So, the increase in 2013 may probably be as a result of the purchase of pigs from neighboring states which are known to be ASF endemic. The results also showed that the ASFV antibodies were detected throughout the three year period and in all the three zones with the exception of 2012 in which there was no occurrence in the Central and Northern zones respectively. This could be attributed to absence of biosecurity measures and proper environmental and management practices

against ASF due largely to ignorance of the disease. The occurrence in all the period of the study and in all the three geographical zones confirmed the occurrence of ASF in Taraba State and may suggest that the infection could be endemic in the area, but factors necessary for outbreak were absent or not ignited. Also, ASFV varies in virulence from the highly pathogenic isolates that can cause 100% mortality to low virulence isolates (Costard *et al.* 2013; Okoth *et al.*, 2013). Hence, the presence of antibodies of ASFV in pigs examined from this study may suggest infection with low virulence strains.

ASF have been reported in pig farms in Nigeria and considered endemic (Odemuyiwa *et al.*, 2000; Kperegbeyi & Amotsuka, 2010; Owoludun *et al.*, 2010; (Olugasa, 2007; Saka *et al.*, 2010; Fasina *et al.*, 2012). Higher mortality was recorded during its introduction, which decreased later, but with

higher sero-prevalence observed frequently in apparently health pigs.

From the results obtained, it was concluded that African swine fever exists in apparently healthy pigs in Taraba State with a sero-prevalence of 13.2%. This is of concern and needs urgent attention to forestall possible spread and outbreaks. This study therefore recommends adequate veterinary services and proper sanitary measures to be adopted by all farms to avoid spread and occurrence of outbreaks.

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