



Molecular detection of African swine fever virus in apparently healthy domestic pigs in Nasarawa state, Nigeria

SA Ayas¹, CJ Bot¹, AR Jambol² & PD Luka^{2*}

^{1.} Federal College of Animal Health and Production Technology, Vom, Plateau state, Nigeria

^{2.} Biotechnology Division, National Veterinary Research Institute, Vom, Nigeria

*Correspondence: Tel.: +2348073919739; E-mail: pamluka08@gmail.com

Abstract

A cross-sectional survey was carried out to investigate the presence of African swine fever virus (ASFV) in domestic pigs in Nasarawa state. This state is surrounded by other states that had earlier reported ASF outbreaks in domestic pigs. Pig blood was collected and screened for ASFV DNA by using polymerase chain reaction (PCR) technique. Apparent healthy pigs from Doma, Awe, Obi, Keana and Lafia local government areas of Nasarawa state were screened. A total of 1.9% (2/103) of the samples were found to be positive from Keana and Lafia. Of the 103 farmers surveyed, 58.3 % were females and 41.7% males. Management of pigs was predominantly semi-intensive (87.4%) with most of the pens built with mud bricks (51.5%), 31% built with concrete and 18.4% with wooden materials. Most of the farmers have formal education while 14.6% do not have any form of education. However, 23.3% of farms had a history of tick infestation around pens which is a predisposing factor for ASF. In conclusion, Nasarawa state is not free of ASFV. Pigs in the state are owned by smallholder farmers (within an average range of 1-20 pigs) with a sizeable number without education. More females are involved in production compared to males should be supported as a strategy for poverty alleviation.

Keywords: Apparently healthy, ASFV, Nasarawa state, PCR, Production characteristics

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Introduction

African swine fever (ASF) is a highly contagious and fatal viral disease of both wild and domestic pigs. ASF virus belongs to the family Asfarviridae and genus Asfivirus (Dixon *et al.*, 1999). ASFV is the only DNA virus reported to be transmitted by arthropods. It is highly contagious and transmission is by direct contact between infected and susceptible pigs or by contact with infectious secretions/excretion resulting in up to 100% morbidity in exposed pigs herd with mortality varying from 0 and 100% depending on the strain, host, dose and the route of entry of the virus (Atuhaire *et al.*, 2013). The virus can survive for 15 weeks in putrefied blood, 70 days in blood on wooden boards, 11 days in faeces at room temperature, 3 hours at 50°C, 18 months in pig blood at room temperature, 150 days in boned meat at 39°C and 140 days in salted dried hams (Vallee *et al.*, 2001).

ASF is a viral disease with severe consequences, and its expanding and continuous spread has been considered as a global threat to the pig industry worldwide (Costard *et al.*, 2009). When introduced into a new territory, the high potential to spread, absence of vaccines or treatment makes it difficult to control. However, for strategic prevention and eradication, effective control policy needs to be put in place to protect pig production industry for the economic benefits.

Pig production is a long-standing practice in most sub-Saharan Africa (FAO, 2000). Rapid urban growth and government agricultural policies in the 1980s had supported a four-fold rise in pig population in Nigeria before the advent of African swine fever (ASF) in 1997 (El-Hicheri, 1998). As a cheap source of proteins for human consumption, with low economic input in production, it became an increasing activity undertaken by women and youths in and around cities and towns where the

ASFV has remained a threat to the development of the pig industry in Nigeria and globally. The disease was first described in 1921 in Kenya and remained restricted to Africa until 1957, when it entered Portugal and other parts of Europe but was eventually eradicated through test and slaughter policy (Costard *et al.*, 2009). Its destructive potential was well appreciated in 1957 when it occurred outside Africa (FAO, 2000). Several risk factors and bio-security measures have been identified as persistence factors to ASF in Nigeria but majorly, across the sub-Saharan Africa, the soft tick (*Ornithodoros spp*) and warthogs have been responsible for the disease occurrence in domestic pigs.

Transmission through warthog and soft ticks has not been reported in West Africa, although ASF virus genome has been detected from a warthog and bush pig in Nigeria (Luther *et al.*, 2007; Owolodun *et al.*, 2010). From the first report of ASF in 1997 in Nigeria (Odemuyiwa *et al.*, 2000) the disease has spread to 18 of the 36 states of Nigeria (Owolodun *et al.*, 2010) including Plateau, Kaduna and Benue states

which borders Nasarawa state. The aims of this study were firstly to describe the pig production characteristics and secondly, to actively investigate the presence of ASF in southern senatorial district of Nasarawa state using polymerase chain reaction technique.

Materials and methods

Study area and design

The study was carried out in the southern senatorial zone of Nasarawa state. The state is bordered in the north by Kaduna state, to the west by the Federal Capital Territory, Abuja, to the south by Kogi and Benue states and to the east by Taraba and Plateau states. Politically, the state is divided into three national Senatorial Districts (North, West and South). Nasarawa state consists of thirteen (13) local government areas (LGA) (Figure 1). The state is characterized by tropical sub-humid climate with two distinct seasons, the wet season (May-October) and dry season (November – April). Annual rainfall ranges from 1100mm to 2000mm. The temperature is generally high during the day, particularly during the months of March and April. The mean monthly temperature in the state ranges between 20°C and 34°C. The vegetation falls within the southern guinea savanna zone.



Figure 1: Map of Nigeria showing Nasarawa state and the tree senatorial districts

A total of 103 open-ended questionnaires were administered to collect demographic and production data from individual pig farmers where pigs/farms were sampled. Purpose of the study was discussed and informed consent obtained from farmers. Twenty (20) questionnaires were administered and retrieved from each of the five local government areas within the senatorial district. The data generated was subjected to analysis of variance (ANOVA) for statistical significance at $p < 0.05$ using GraphPad Prism 5 (La Jolla, CA 92037 USA).

Sample collection

The simple size ($n = 103$) was determined at a 95% confidence level using an expected prevalence of 9% (Fasina *et al.*, 2010), an absolute precision of 6.6 % and an estimated within-class coefficient $P = 0.8$ with an inflation coefficient of 25.

A total of 103 blood samples were collected via the ear and femoral vein: twenty (20) samples each obtained from Awe, Doma, Keana, Obi and 23 from Lafia LGAs, respectively. Five (5) ml of blood per farm was collected into ethylenediaminetetraacetic acid (EDTA) and transported on ice to the Biotechnology Division of the National Veterinary Research Institute, Vom,

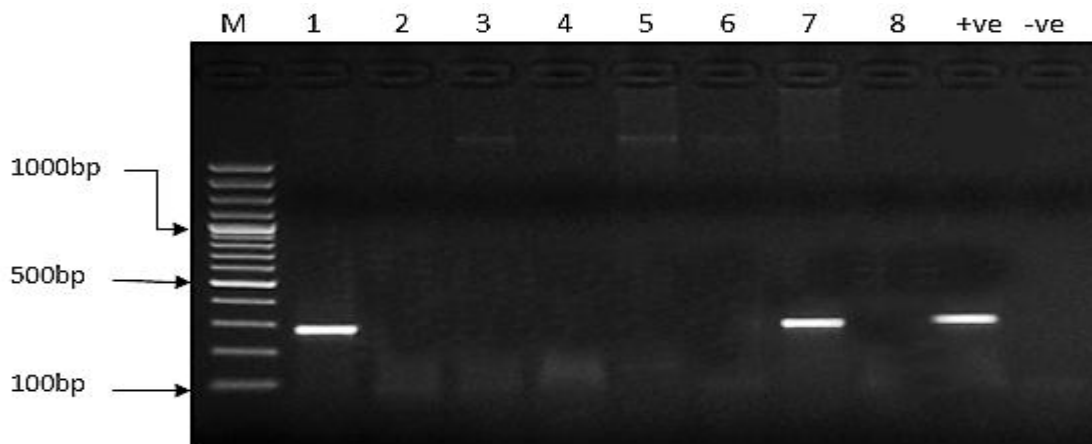


Figure 2: PCR amplicon of ASFV showing the band size of 278 bp. Lane M: molecular marker 100 bp, lanes 1 -8: samples, +ve: positive control and -ve: negative control

Nigeria and stored at -20°C until tested.

DNA detection by PCR

DNA was extracted from blood samples collected using QIAGEN[®] DNA Mini prep kit according to the manufacturer's instructions. The quality of DNA was checked on an Eppendorf BioPhotometer[®] plus (Eppendorf AG, Hamburg Germany) at A260/A280 and checked on 1.5% agarose gel electrophoresis. polymerase chain reaction (PCR) was performed according to the Manual of Diagnostic Tests and Vaccines (OIE, 2008). Lyophilized freeze-dried E70 virus from the reference laboratory for ASF (CISA-INIA, Madrid Spain) was used as PCR positive control. ASF-specific primers (oligonucleotide primers) targeting the major capsid protein (VP72 gene) amplifying a 278-bp fragment within the conserved region was employed: ASF1F: 5'-ATG GAT ACC GAG GGA ATA GC-3' and ASF2R: 5'-CTT ACC GAT GAA AAT GAT AC-3' (OIE, 2008). The PCR mix had the following constituents: 1x PCR buffer (50 mM KCl, 10 mM Tris-HCl), 2 mM MgCl_2 , 0.4 μM concentration of primers, 0.2 mM dinucleotide triphosphates (dNTPs) and 2.5U Taq polymerase in a total volume of 25 μl . Cycling conditions were 94°C 15s, 30 cycles of 94°C 15s, 62°C 15s, 72°C and a final extension at 72°C for 5mins. The PCR products were visualized by electrophoresis on a 2 % agarose gel.

Results

PCR detection of ASFV

Analysis of the 103 field samples with ASF1F/ASF2R yielded an amplicon of the expected size of 278 bp (Figure 1) in 2 (1.9%) samples from Keana (lane 1) and Lafia (lane 7) LGAs (Figure 1). The positivity of 1.9% by PCR confirmed the presence of ASFV in Nasarawa state.

Production characteristics

In summary, the results of this study revealed that 42% of pigs within the study area were owned by

men against 58% by women (Table 1). However, this was statistically not significant ($p < 0.05$).

Taking into account the rural and urban settings surveyed, the management systems practiced by farmers was primarily semi intensive (87.4%), with a few who were into intensive (8.7%) and extensive (3.9%) management methods, respectively. The result obtained was highly statistically significant ($p = 0.0001$) (Table 1).

Majority of the farmers surveyed had attained at some form of education (Table 1). Tertiary, secondary and primary education was 41.7, 26.2 and 17.5 percent, respectively. However, 14.6% of the farmers do not have any form of education. Educational level of the farmers was statistically significant ($p=0.042$).

A high rate of respondents provided some form of housing for their pigs. Three forms of housing were identified: concrete (30.1%), mud bricks (51.5%) and wooden pens (18.4%). The low cost housing eventually predisposes the pigs to diseases. Statistical analysis reveal significance of $p=0.0053$ (Table 1).

Similarly, 23.3% of farmers reported to have seen ticks on their farms or pens while 76.7% said they have never seen ticks (Table 1). This data was not statistically significant.

Discussion

African swine fever has been recognized as one of the priority diseases of pigs by FAO and OIE, jeopardizing the socio-economy of farmers globally. In this study, ASFV was detected in 2 (1.9%) of the total samples collected from Nasarawa state. Until now, no molecular detection has been carried out in the state. The result obtained revealed that apparently healthy domestic pigs indicate the presence of ASFV DNA. Movement of pigs from some north central states southwards and the pressure of disease burden from neighbouring infected states may have been responsible for the spread. However, the confirmation of ASFV may

Table 1: Characteristics of pig ownership, marital status, household size, management method, educational level, pig housing type and report of ticks in the study areas where “n” denotes the number of households visited

Variable	Doma (n=20)		Awe (n=20)		Obi (n=20)		Keana (n=20)		Lafia (n=23)		p-value	Total = 103 Overall %
	Num	%	Num	%	Num	%	Num	%	Num	%		
Ownership of pigs												
Male	10	50	10	50	6	30	6	30	11	47.8	0.1817	43
Female	10	50	10	50	14	70	14	70	12	52.2		60
Marital status												
Married	17	85	18	90	16	80	17	85	17	73.9	0.0008***	85
Single	3	15	2	10	4	20	3	15	6	26.1		18
Household size												
1 to 5	2	10	11	55	0	0	7	35	15	65.2	0.8436	35
6 to 10	8	40	3	15	12	60	4	20	5	21.7		32
11 >	10	50	6	30	8	40	9	45	3	13.1		36
Management method												
Intensive	2	10	0	0	0	0	2	20	5	21.7	0.0001***	9
Semi intensive	18	90	18	80	20	100	18	80	16	69.6		90
Extensive	0	0	2	20	0	0	0	0	2	8.7		4
Educational level												
None	3	15	3	15	7	35	2	10	0	0	0.04170*	15
Primary	1	5	4	20	5	25	4	20	4	17.4		18
Secondary	3	15	6	30	4	20	7	35	7	30.4		27
Tertiary	13	65	7	35	4	20	7	35	12	52.2		43
Pig housing type												
Concrete	5	25	6	30	6	30	8	40	6	30	0.0053**	31
Mould bricks	13	65	11	55	10	50	6	30	13	56.5		53
Wooden	2	10	3	15	4	20	6	30	4	17.4		19
Report on ticks												
Yes	2	10	1	5	5	25	0	0	16	75	0.1192	24
No	18	90	19	95	15	75	20	100	7	25		79

Num – number

% - percentage

p-value refers to the level of the difference between the proportions from the 5 local government areas

suggest a number of scenarios i.e. (i) infected pigs never get reported by farmers, (ii) the disease reporting system is not efficient (Tsofo, 2010), (iii) perhaps the positive pigs were brought in from infected locations or (iv) the pigs were carriers from previously infected herd (Costard *et al.*, 2015).

The result obtained also revealed that 41.7% of the ownership of pigs within the study area were by males against 58.3% by females (Table 1). This is in

agreement with the FAO report (FAO, 2000) that more women were into pig production compared to men. However, the finding was not statistically significant. Our findings also disagree with Nwanta *et al.* (2011) who reported that more men (66%) were into pig production compared to women (23%) in Southern Nigeria. Similarly, our findings also observed a very high proportion of farmers who are married (84%) and involved in swine production

compared to singles (16%). This is also in agreement with Igwe *et al.* (2013) in Abia state, south east Nigeria who observe 67% of farmers who are married compared to 23 % of farmers being single and involved in pig production.

Although the survey was carried out in both rural and urban settings, the management methods practiced by farmers were: semi intensive (87.4%), intensive (8.7%) and extensive (3.9%) methods, respectively. The result obtained observed that more farmers keep their pigs in an enclosure especially during the rains and open them to roam when the rains are over and crops have been harvested. Our results were similar to those of Ironkwe & Amefule (2008) in Rivers state, south-south Nigeria who reported a very few (10%) respondent who practice intensive method. Management systems are closely related to feeding and source of feeds. However, pigs under intensive system perform better than extensive system (Rekwot *et al.*, 2003). Additionally, our study revealed that household members are within the age that can be involved in production activities (Table 1).

Majority of the farmers surveyed had attained at least some form of education (Table 1). However, some farmers do not have any form of education. Educational level of farmers was considered relevant to understanding extension services made available to them. This finding is in agreement with Adesehinwa *et al.* (2010) in Oyo state, southwest Nigeria who reported high level of education among pig farmers. Farmer's level of education can benefit

the farmer, extension officers and veterinarians who give professional advice in terms of extension messages especially in the application of new technologies. Above all, education has been shown to improve productivity and make literate farmers to adopt new technologies faster and easier (Mishra *et al.*, 2009)

In addition, majority of respondents provide some form of housing for their pigs. The low cost housing eventually predisposes the pigs to diseases. From the three categories, the most durable is cement and zinc roof and enables the maintenance of hygiene. Some farmers (23.3%) reported seeing ticks on their farms or pens while 76.7% said they have never seen ticks. Ticks are vectors of several diseases and can serve as long-time carriers in the transmission and persistence of diseases such as African swine fever (Penrith *et al.*, 2004).

In conclusion, ASFV is circulating in domestic pigs in the southern senatorial district of Nasarawa state. Pig production in the state is basically a preoccupation of women with majority of farmers having formal education. Most pig farms are small scaled with a majority of farmers practicing semi intensive management method. However, pig housing was observed to be very poor with low hygiene which may predispose pigs to diseases. We recommend farmers awareness in this regard, enlightenment on the maintenance of good hygiene where it is not possible to upgrade the quality of housing.

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