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USMANU DANFODIYO UNIVERSITY
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A five-year survey of African swine fever outbreaks in Plateau State, Nigeria

NJ Luther*, D Shamaki, TM Joannis and FO Fasina

Viral Research Department, National Veterinary Research Institute (NVRI), Vom. Nigeria

*Correspondence author: Tel.: +2348035895497; E-mail: luthernj1@yahoo.com

Abstract

Reported here is a five-year account of outbreaks of African swine fever (ASF) in Plateau state, which devastated swine production and almost threw the whole pig induatry of the state in total disarray. Although veterinary authorities from 15 local government areas (LGA) of the state kreported the lsuspicion of the ldiseas, confirmation by laboratory test was obtained only from 8 LGA's namely: Jos-North, Jos-South, Mangu, Pankshin, Langtang North, LantangSouth, Shendam and quaan-pan, representing all the three zones of the state (Northern, central and southern zones). Since its initial outbreak in 1999, the disease; has remained enzootic in the state. A control programme ,involving improved management, major surveillance operation, heightened public awareness and a market value compensation scheme for slaughtered pigs is suggested.

Key words: African Swine Fever, Antibodies, Immunoblotting, I-Elisa, Optical Density.

Introduction

African swine fever was first described in Kenya at the beginning of the nineteenth century (Montgomery, 1921). Since then the disease has been reported in a large numbers of African countries at the south of the equator (Wilkinson, 1981; Plowright, 1984). African swine fever virus (ASFV) is the causative agent of ASF. This is a unique, complex, icosahedral DNA arbvirus belonging to the genus asfivirus in the family asfaviridae (Dixon *et al.*, 2000). The virus multiplies in the cytoplasm of the infected cells. In nature, the perpetuation and transmission of this ;virus involve the cycling of virus between the agasid soft ticks Ornithodoros Moubata and wild pig populations (Warthogs, bush pigs) in sub-Saharan Africa (Plowright *et al.*, 1969a; Plowright *et al.*, 1969b). This association is unlikely ever to be eliminated; hence making it potentially very difficult to eradicate the disease in Africa and it appears that this continent will be the main reservoir of ASF for the foreseeable future (Majiyagbe *et al.*, 2004). In this type of established age-long relationship of host-parasite association, prolonged infection occurs without any manifestation of clinical signs of the disease. However, in domestic pigs, ASF is a high significant disease. It is considered to be most serious and dreaded epidemic diseases of domestic pigs which may be introduced into previously uninfected countries at any time from epidemic areas of the world. There is no treatment or effective prophylaxis against the disease. The disease occurs in several forms ranging from peracute (sudden death with few, if any, previous clinical signs) to sub-clinical or inapparent (Moulton and Coggins 1968; Mebus *et al.*, 1988).

In Africa however, ASF appears mostly in the acute form with very high mortality anf characterized by fever, anorexia, leukopemia, and haemorrhages of the internal organs and skins (especially around the ears, snout and flanks) (Mebus and Dardiri, 1980; Gomezvillamandos *et al.*, 1995).

The disease entered Nigeria around 1997 through the southwestern region most probably by infected pig/or pig products smuggled through the Nigerian boarder from neighboring Benin Republic (Anon, 1998). Amongst the earliest cases reported in Nigeria was an outbreak at the co-operative farm, Oko-okba, Agege-Lagos, between mid 1997 and early 1998 (Odemuyiwa *et al.*, 2000). Within a period the disease became widely distributed through pig producing areas of Nigeria (Majiyagbe, 1999; Luther *et al.*, 2002) and has been causing a lot of devastation to the national pig herd. At present, the current situation of ASF in Nigeria revealed that virtually all pig producing centers are affected. The disease has been confirmed through laboratory tests in 18 states of the federation, covering southwest, southeast and central states (Majiyagbe *et al.*, 2004).

In Plateau state, pig production play an important and integral role in the agricultural system and a large percentage of the human populace are engage in pig production lat both traditional and small-scale commercial levels. Here on the Plateau, despite certain negative, cultural and religious sentiments especially from the extreme parts of the \north fresh pork is finding ready acceptance especially amongst the expatriates Asians and Europeans who troop in their numbers from all nook crannies of the state to purchase pork at the Jos abattoir.

Until the sudden outbreak of ASF in 1999, pig production on the Plateau been one of the fastest growing arms of livestock industry. The disease first surfaced at Gada Biyu in Jos North LGA area between mid August and early September 1999 involving free-ranging pigs and with very high mortality. At least an estimated number of 700 pigs were lost. In Langtang North and South LGA's, the disease was noticed in April 2000 after the report of unusually high mortality and morbidity rates among pigs in Pajak, Zinni, Mabudi and Sabon Gida. Later the disease then spread from these primary foci (Jos North, Langtang North and South local government areas) to other parts of the state namely Jos South, Mangu, Pankshin, Shendam, and Quaanpan within one year. Initial investigation carried out withn these primary foci showed that the disease was restricted to free-range scavenging local domestic pigs kept by villagers rather than intensively managed institutional farms.

The estimated pig mortality in the state as a consequence of the disease is summarized in the attached annexture (Table 2). Other 7 local government areas where no laboratory confirmation has been made had reported outbreaks and are now great risk. To date, there is no officially recorded financial figure of death due to ASF across the state. However, the estimated pig mortality contained in Table 2 translates to a huge loss financially with damaging and serious socioeconomic implications.

Materials and Methods

Collection of Samples

A total samples were collected from pigs in Jos North, Jos South, Mangu, Pankishn, Langtang North and South, Shendam and Quaanpan as illustrated in table 1. These samples were derived from the various submissions made by the respective LGA's from suspected field cases and those collected by the staff NVRI Vom during field investigations. These samples comprised of lymph nodes, spleen, kidneys and sera, which were appropriately processed.

Antibody Detection

Antibody detection by indirect ELISA and immunoblotting assay were done according to standard protocols of the OIE manual of standards for diagnostic tests and vaccines (OIE 2000). Briefly, electrophoretically separated ASF viral protein resolved in 17% polyacrylamide gels (PAGE) were transferred under a constant current intensity unto nitrocellulose filters (Jhohndson *et al.*, 1982). The filters were cut into strips

then blocked using 2 non-fat dried milk in phosphate buffer saline (PBS) at PH 7.5 for 15 minutes in order to saturate the remaining free protein binding sites. After discarding the blocking solution, 0.5ml of test and control sera were added at a dilution of 1.50 in PBS/2% milk at PH 7.2 and incubated at 370C for 45 minutes with continuous agitation. Penultimate to this was washing step of at least 4 times using PBS/2% milk (PH 7.2).

If specific antibodies are present in the serum, the antigens in the strip will react with the serum antibodies resulting to the formation of an immuno-complex, which will be developed following addition of an A-peroxidase conjugate and 4-chloro-1-naphtol as substrate. This complex is visualized as bands on the nitrocellulose filters.

Antibody detection by I-ELISA was similarly done according to standard protocols of the OIE, manual of standard for diagnostic test and vaccines (OIE 2000). Reagents and control sera used were supplied by Onderstepoort Institute for Exotic Diseases, Pretoria and Republic of South Africa.

Briefly, flat bottomed Nun Maxisorp ELISA microtitre plastic plates (Nune-Immunol-Maxisorp, Valdeolmos, Madrid, Spain) were sensitized with 0.01ml/well of ASF antigen diluted to 1:1600 in carbonate/bicarbonate buffer, PH 9.6, overnight in sealed containers at +40C. After washing, 0.01ml of test control sera (ASF positive and ASF negative serum) at 1:30 lin phosphate mbuffered saline (PBS)/Tween 20 (20µ1 of serum + 600µ1 PBS/:Tween 20) PH 7.2, was added per well and plates were incubated for 1hr at 370C on incubator shaker. After washing, 100µ1 of protein-A horseradish peroxidase-conjugated rabbit anti-swine immunoglobin dilute 1:2000 in PBS Tween 200 (PH 7.2) was added per well and the plates incubated for another 1hr on incubator shaker. After the final washing, substrate (3% [hydrogen peroxide, H2O2) was added to chromogen (Orthopheylenediamine, OPD) at 4µ1 of H2O2 per ml of OPD and incubated at room temperature for 20 minutes. The reaction was stopped by the addition of 100µ1 of 1M sulphuric acid to each well of the plates using a multichannel micropipette. Each serum was tested in two wells. Positive and negative control sera included on each plates was tested in four wells. All washing stages involve at least five washes with 0.5% Tween 20 in PBS, PH 7.2. All optical densities (OD) of each plate well was measured by an ELISA plate reader, connected to an IBM computer at 46y2nm filter. A serum was considered positive if it has an OD value grearer than the cut off point and vice-versa.

Cut-off = Mean OD negative serum x 1 + Mean OD Positive Serum x 0.2.

Spleen, lymph nodes, kidney and lung tissue were tested for jASFV genomic DNA by the PCR according to standard protocol of the OIE manual of standards for diagnostic test and vaccine (OIE 2000). DNA extraction was done using guanidinium thiocyanate method as modified by Bloom *et al.*, (1990). Reagent and buffers used for both the DNA extraction and PCR were molecular grade and supplied by Ondersteppoort Institute for Exotic Diseases, Republic of South Africa.

Results

The various samples submitted from the field and those collected during field investigations involved a variety of tissue comprising lympnodes, spleen, kidney and lungs totaling 325. Also a total \of 207 sera were processed and analyzed in the appropriate test to determine the presence or absence of ASF antibodies.

Using the highly sensitive polymerase chain reaction (PCR) techniques, representative samples from all the zones of Plateau (North, central and Southern) were positive for ASF virus. Antibodies to ASFV were found in all zones. Of a total of 207 sera tested by I-ELISA, 70 were positive (Table 1). A Limited number of the I-ELISA negative samples gave positive results by immunoblotting assay.

Table 1: Materials Collected From Pigs for Laboratory Analysis for ASF

Local Government Area	Serum	Lymph nodes	Spleen	Lung	Kidney
Jos-North	60	20	10	9	8
Jos-South	45	15	12	11	3
Mangu	38	7	3	8	-
Panshin	20	3	1	1	1
Langtang-North	18	3	2	-	-
Langtang-South	11	5	3	-	-
Shendam	8	1	1	1	-
Quaan-Pan	7	-	-	-	-
Total	207	54	32	30	12

Table 2: African Swine Fever Outbreaks in Plateau State Local Government Reports (1999-2004)

L.G.A	Affected Areas	Laboratory Report	No. Affected
Jos, North	Gada Biu, Congo-Russia, Tudun Wada, Hwolshe, Nassarawa Gowm	ASFV antibody detected by I-ELISA	700 dead out of 1,200 that were exposed.
Jos, South	Bukuru, Kaduna Vom, LID, Kuru Pig farm, Zawan.	ASFV antibody and Antigen detected by I-ELISA and PCR	500 dead out of 700 on exposed farms.
Mangu	Angwan Mata, Dawo, Daika, M/Pushit	ASFV antibody detected from Daika	200 dead out of 350 on affected farms.

Pankshin	Jing, Pankshin town, Dawaki	ASFV antibody detected from Dawaki	150 dead, 500 killed
Langtang North	Pajak, Zimi, Dam site, Wase road, Pilgani	ASFV antibody detected	800 killed 200 dead
Langtang South	Mabudi	ASFV antigen detected	No. figure available
Shendam	Congo, Total Gleeson	ASFV antibodies antigen detected	350 killed No figure of kdeaths
Quaan-Pan	Kwalla, Domak	ASFV antibodies detected	No. figure available

Discussion

The results obtained from the various laboratory tests conducted on the field samples collected from suspect cases of ASF, confirmed the occurrence of ASF in Plateau state. The geographical distribution of the disease in the state is very instructive. All the three zones of the state were affected by the disease leading to severe economic losses. Affected peasant farmers especially rural women who keep pig as a reserve source of ready and regular cash for meeting family needs suffered severe economic losses. The result of this enormous economic losses has further exacerbated the problem of poverty amongst the farmers in the state,. In fact, instead of upliftment, it has further increased the level of poverty and rendered poverty alleviation effort amongst the rural populace a far dream.

Knowledge and understanding of the epidemiology of ASF is crucial to the necessary steps required for the formulation of policies strategies for control. One major problem with ASF virus is that is it resistant to inactivation and may remain viable for long period in formites, infected pig tissues, meat and processed pig products; pigs that survive ASF infections may become carriers. Also many wild suid species and feral pigs are susceptible to ASF virus but may not develop overt disease. These factors, coupled with the fact that the argasid ticks ornithodoros moubata are capable of transmitting the virus, make ASF more difficult to eradicate and control.

The only available option for ASF eradication and control is stamping out by slaughter and disposal of all infected and potentially infected pigs. This is to be accompanied by quarantine, pig movement controls, prohibitions on the sale of potentially infected pig products. Affected farms should be depopulated and the premises thoroughly disinfected with appropriate disinfectants such as 2% sodium hydroxide, detergent and phenol substitutes, sodium or calcium hypochochlorite and iodine compounds. Such disinfected premises should left fallow for a period not less than 3 month before restockings are made from ASF free stocks. As a general rule, these fallow periods would be shorter in hot climate than in cold or temperate climates.

It is essential that farmers who have had their pigs slaughtered or property destroyed as part of an ASF eradication campaign be fairly compensated with the current market value of the animals and goods. Compensation should be paid without delay. Compensation here implies not only the payment for the market value of the pigs but also under certain circumstances, replacement of stock may be offered in lieu of monetary compensation.

At present, there is no officially coordinated control programme for ASF in Nigeria. The role played by ASF in limiting pig production and its economic impact on the livestock industry in Nigeria has been widely recognized (Majiyagbe *et al.*, 2004). To date, officially recorded deaths across the country as a result of ASF are well over 0.5 million pigs and this translate to a huge loss financially with a serious and damaging socioeconomic implications (Majiyagbe *et al.*, 2004). Considering the obvious of all allowing the disease run rampant from place to place and causing serious threat to profitable pig farming, there is a need to plan and institute a control programme for this disease in the country.

References

Anonymous, FAO/TCP/NIR/1822) (1998): Control of African swine fever in Western Nigeria.

Bloom, R.,Sol, C.J, Salimans, MMM, Jansens, C.L., Wertheim-Van Dillen, P.M.E and VanDer Noordaa, J (1990): Rapid and simple method of purification of nucleic acids. *Journal of Clinical Microbiology*. **28**:495-503.

Dixon, L.K., Costa J. V: Escribano, J. M., Rock, D.L., Venuela, E and Wilkinsons P.J (2000): Family Aspaviridae. In: *Virus Taxonomy, Seventh report of the International committee on taxonomy of viruses*. Academic Press 159-165.

Gomez-villamandos, J., Hervas, J., Mendez, A., Carraso, L., Villeda, C., Wilkinson, P and Sierra, M. (1995): Experimental African swine fever: apoptosis of lymphocytes and virus replication in other cells. *Journal of General Virology*, **76**:2399-2405.

Luther N. J., Majiyagbe, K. A., Shamaki, D and Umoh, J.U. (2001): Serological investigation of slaughtered pig from Plateau and Kaduna for evidence of African swine fever virus antibodies. *Sokoto Journal of Veterinary Sciences*, **3**(2): 44-46.

Majiyagbe, K.A(1999): African swine fever: In *FAO/FGN training workshop on control and eradication of African swine fever in Nigeria*. 16-17 November, 1999. National Veterinary Research Institute, Vom.

Majiyagbe, K.A., Shamaki D., Luther N.J and Udeani T.K.C (2004): African swine fever epidemics. The Nigerian experience 1997-2004. *Vom Journal of Veterinary Science*, **1**(1): 138-153.

Mebus, C. A.(1998): African swine fever. *Advances in Virus Research*, **35**: 251-269.

Mentgomery R.E (1921): On a farm of African swine fever occurring in British east African (Kenya colony). *Journal of Comparative Pathology and Therapeutics*, **34**:159-191.

Moulton J and Coggins L (1968): Comparison of lesions in the acute and chronic African swine fever.

Odemuyiwa S.O., Issac A.A., Ammer an M.W., Adebowale T P, Alaka O O., Oyedele O I., Soyelu K O., Olaleye D O., Otesile E B and Muller, C P (2000): An outbreak of African swine fever in Nigeria: Virus isolation and molecular characterization of the VP 72 Gene of the first isolote from West Africa. *Virus Genes*, **20**(2): 139-142.

Ploright In: Parker J and Pierce M.A (1969(b)): African swine fever in ticks (*Ornithodoros moubata* Murray) collected from burrows in Tanzania. *Nature (London)*, **221**:1071-1073.

Plowright W.(1984): African swine fever. In: *Principles of Bacteriology, Virology and Immunity. Vol.4 (7th edition)* London: Edward Arnold. pp. 53-54.

Wilkison P.J (1981): African swine fever. In: *Virus Disease of Food Animals, vol 2*. Disease Monographs Edition. Gibbs, E.P.J. 767 – 786. London: Academic press.

Mebus, C.A and Dardiri, A. H.H (1980): Western hemisphere isolates of Africa swine fever virus: a symptomatic carriers and resistance to challenge inoculation. *American Journal of Veterinary Research*, **41**:186-1969.

Office of International Epizootics Manual of Standards for Diagnostic Test and Vaccines (2000): 4th Edition. 189 – 198.

Hawkes, R; Niday E and Cordion J. (1982): A dot immunobinding assay for monodonal and other antibodies.

Jhonson T.K; Yuen, L; Denell, R.W and Consigli, R (1983): Efficient transfer of proteins from acetic urea and isoelectric focusing gels to nitrocellulose membrane filters with retention of protein antigenicity. *Biochemistry*, **133**:126 – 131.