

# Confirmation Of African Swine Fever Virus in Some Pigs from Ado Local Government Area of Benue State, Nigeria

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## Abstract

*Studies were undertaken to investigate and isolate African Swine Fever virus in pig in Ado local government area (LGA) of Benue state of Nigeria and this has confirmed the carrier status of the pigs in the LGA. Out of the 188 serum samples collected, 7 were obtained from the LGA and were tested using Enzyme Linked Immunosorbent Assay (ELISA). Three samples (42.86%) were positive and 4(57.14%) were negative for the test. All the serum samples (42.86%) that were positive by ELISA were also positive by polymerase chain reaction amplification (PCR). The PCR-positive pigs had higher mean WBC count, lower eosinophil and basophils count, higher mean monocytes count but the mean PCV values were not significantly different from the normal and PCR negative pigs. High mean WBC count may indicate an ongoing secondary bacterial and ASF virus infection, lower eosinophil and basophils count indicates parasitism in the pigs, higher mean monocytes count. This confirmed that pigs with demonstrable antibody should be considered as chronic carriers of the virus because it is doubtful if true recovery ever occurs. Control measures such as use of biosecurity, test and slaughter of animals that test positive for African Swine Fever (ASF) antibody with adequate compensation are suggested to eradicate the disease.*

**Keywords:** African swine fever viral antigen, Ado, Benue State.

## INTRODUCTION

African swine fever (ASF) is a notifiable, lethal hemorrhagic disease in domestic pigs (Rahimi *et al*; 2010). It is a devastating viral disease that has been threatening the pig industry worldwide (Ayoade and Adeyemi, 2003). Mortality range of 50 to 100% in various herds, was recorded in Delta State, Nigeria (Otesile, 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.(El-Hicheri, 1998).The disease is caused by African

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swine fever virus (ASFV) which is an enveloped double stranded deoxyribonucleic acid (DNA) virus belonging to genus *Asfivirus* (Sarma, 2012). Maintenance and transmission of ASFV involve cycling of virus between soft ticks of the genus *Ornithodoros* and wild pigs (warthogs, bush pigs, and giant forest boars). 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). It belongs to the family member *Asfaviridae*. It is a double stranded DNA virus that measures 200-220nm in diameter. (Ekwe and Wilkinson 2000.). 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 (Ekwe and Wilkinson, 2000, Sarma, 2012). The virus survives in chilled carcasses or in frozen meat for several weeks (Merchant and Parker, 2005). The virus can also survive 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 family is sensitive to lipid solvents and can be inactivated rapidly by 2% NaOH (Sarma, 2012). Polymerase chain reaction (PCR) is one of the most convenient, safe, and frequently used techniques for the detection and identification of ASFV (Agüero *et al.* 2003). Primer sets and probes are designed to a highly conserved region of the viral genome within the VP72 genome region (Agüero *et al.* 2003).

This work is of importance because there is need for more information on ASF antibody and antigen status in pigs in Ado LGA, Benue state of Nigeria so that control and preventive measures can be carried out to safeguard the pig population in the state. If swine production is affected by ASF in Ado LGA in Benue state, the other pork consumers and other people that come to buy from the neighbouring southern part of Nigeria are likely to suffer from meat shortage.

Methods to detect genome nucleic acids from the ASFV in infected tissues are Restriction Fragment Length Polymorphisms (RFLP), Polymerase Chain reaction (PCR) and use of DNA probes (Ayoade and Adeyemi, 2003). EURL-ASF, 2013 is a standard operation procedure for the detection of ASFV. This procedure is applied to the ASFV DNA extracted following the procedure described in the "Standard operating procedure for the extraction of ASFV DNA in any kind of porcine clinical sample such as EDTA-blood, serum and tissue homogenates and in cell culture supernatants. It is particularly useful for identifying ASFV DNA in porcine tissues that are unsuitable for virus isolation or antigen detection, because they have undergone putrefaction, or when there is good reason to believe that virus may have been inactivated before samples are received in the laboratory. Polymerase chain reaction amplification (PCR) assay using the ASF diagnostic primer sets stated above generates amplicons of 257 bp corresponding to the central portion of the p72 gene can be used to confirm the presence of ASFV DNA ( EURL-ASF, 2013).

Genomic viral DNA extraction can be carried out using viral DNA extraction kit according to manufacturer's protocol and using the primer design (PPA-1-F5'-AGTTATGGGAAACCCGAC CC-3'PPA-2-R5'-CCCTGAATCGGAGCATCCT-3').(EURL-ASF, 2013).

Whenever there is ASFV infection there is 40-50% a fall in the total leukocyte count by the fourth day of fever. There is lymphopenia and an increase in immature neutrophils. There is

hypergammaglobulinemia in chronic cases Clotting times increases from about 5 days after. From day 6 thrombocytopenia can be detected (Radostits *et al.*2007)..

## **MATERIALS AND METHODS**

### **Study Area**

The study area was Ado Local Governments Area (LGA) in Benue State of Nigeria. Ado LGA is one of the 23 LGA of Benue state. The LGA headquarter is situated in Igwumale. Ado LGA is situated in 4° 16' north and 8° east. The global location of Benue state is between Longitude 06° and 11° 3' north of the equator. (Dada *et al.*, 2010) .

The state is bounded by 6 states (Nasarawa to the north, Taraba to the east, Kogi to the west, and Enugu, Ebonyi and Cross River to the south) Including an International border with Cameroon republic to the southeast (Dada *et al.*, 2010)

### **Sample collection**

Ethical approval was sought and granted by the ethical committee for experimental purpose of College of veterinary medicine, Michael Okpara University of Agriculture, Umudike, Nigeria (MOUAU/CVM/REC/202217). 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).

Blood samples were collected from pig population from both commercially managed and traditionally managed pigs in Benue state of Nigeria. The blood was collected mainly from live animal through the jugular vein in two different tubes for each animal (one tube containing anticoagulant and vacuatainer tube without anticoagulant. All the samples collected were placed in a cooler and covered with ice pack. A total number of 188 serum samples were collected from 188 different pigs in pig population of Benue state, 7 serum samples were obtained from two villages called ukpomlokbo and ojije in the town called utonkon that is under Ado LGA Benue state of Nigeria. Other LGA where pig serum samples were obtained include Ukum, Makurdi, Gboko, Buruku, Guma, Gwer, Gwer, Tarkaa, Katsina Ala, Konshisha, Logo, Vandekya, Oturpo, Apa, Obi, Okpokwu and Ohimini

### **Antibody detection Test**

The Detection of antibody against ASF was earlier carried out by indirect Enzyme-linked immunosorbent assays (ELISA) as recommended by ID.vet Innovative Diagnostics (IDvet,310, rue Louis Pasteur-Grabels-FRANCE) who were also the supplier of the kits used for the detection of anti-African Swine fever antibodies in porcine serum and plasma samples or blood filter samples. 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

The ELISA was done in the Departments of Veterinary Pathology, Veterinary Public Health and Preventive Medicine and also National Animal Production Research Institute Ahmadu Bello University, Samaru- Zaria, Kaduna State, Samaru- Zaria is situated under Sabon gari LGA which is one of the 23 LGA under Kaduna State, is the Sabon gari LGA is situated in 11° 07' north and 7° 43' east (Dada *et al.*, 2010) situated under Sabon gari LGA which is one of

the 23 LGA under Kaduna State, is the Sabon gari LGA is situated in 11°07' north and 7°43' east (Dada *et al.*, 2010). The polymerase chain reaction (PCR) was done in the DNA lab Kaduna situated in Kaduna north LGA.

ELISA was earlier carried out on the 7(100%) serum samples from the Ado LGA in early studies (Adenaike *et al.*, 2017)

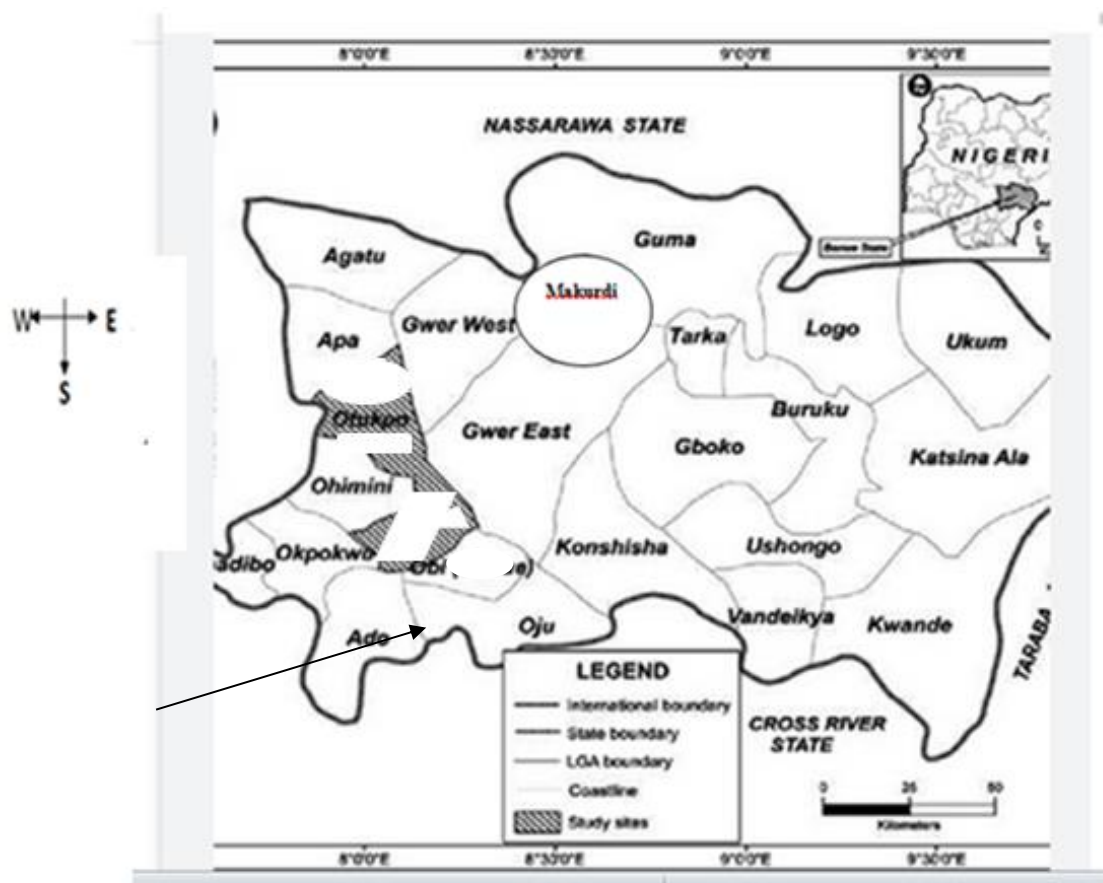


Figure 1 Map of Benue State showing Ado Local Government Areas  
Source: Dada *et al.* (2010)

Polymerase chain reaction (PCR) as recommended by European Union Reference Laboratory for ASF (EURL-ASF, 2013) was used to amplify and detect genome nucleic acids from the African Swine Fever Virus in infected sera.

Extraction of viral genomic DNA was carried out from three (3) porcine sera that earlier tested positive for indirect antibody test (EURL-ASF, 2013) using viral DNA extraction Kit according to manufacturer's protocol. Polymerase chain reaction (PCR) amplification assay was carried out using the ASF diagnostic primer set stated below, which generates amplicon of 257 bp corresponding to the central portion of the p72 gene and was used to confirm the presence of ASFV DNA.

**Table 1 African Swine Fever diagnostic primer set**

Primer	Nucleotide Sequences(5'-3')	References (Gen Bank number)	Expected Amplicon Size (bp)	References
PPA-1 -F	5'-AGTTATGGGA AACCCGACCC-3'	ASU18466	257	EURL-ASF (2013).
PPA-2 -R	5'-CCCTGAAT CGGAGCATCCT			

Key: F-Forward R-Reversed

Primers used was supplied by the laboratory called DNA LAB Kaduna (as designed by Bioneer In, USA) with the specifications as obtained from the stated reference and presented in Table 1. The dilution included 100 Picamole primer conc, 10 picamole was made by dilution with 10UI 100 picamole and 90 UI of Deionised water (DH<sub>2</sub>O), then 2µl of DNA template was added to each 0.2ml PCR tube including R<sup>+</sup>and R<sup>-</sup> controls (EURL-ASF, 2013). The reagents used are indicated in Table.2.

### Agarose gel electrophoresis

Ten microliters (10µl) of PCR products was loaded into wells of 1.5-2.0% agarose gel containing ethidium bromide of final concentration of 5µg/ml. A molecular size marker, 100bp DNA ladder was ran on both sides with PCR products. Electrophoresis was carried out in Tris Acetate EDTA buffer containing 10u of ethidium bromide at 150-200 V for 30-40minute. The mixture was poured in the gel duct kast containing the combs. It was allowed to solidify and were viewed on a U/V trans-illuminator and photographs were taken using a gel documenting machine. Amplicons were assessed and estimated from the 100 base sphere molecular sizes of the DNA ladder against their migration distance. Amplified products were kept at 4+3<sup>0</sup>C until used for the electrophoresis (max. 18 hours).

**Table 2: Reagents for Master Mix Preparation**

Pipetting Step	MasterMix Reagents	1x volume (reaction 25µl)	Final Concentration
1	H <sub>2</sub> O	17.375µl	
2	PCR buffer 10x	2.5 µ	1x
3	Cl <sub>2</sub> Mg 25mM	2 µl	2mM
4	dNTPs 10mM	2 µl	0.2mM
5	Primer-1 20µM	0.25µl	0.2µM
6	Primer-2 20µM	0.25µl	0.2µM
7	Taq Gold 5U/ µl	0.125 µl	0.025U/µl
Master Mix volume		23µl	

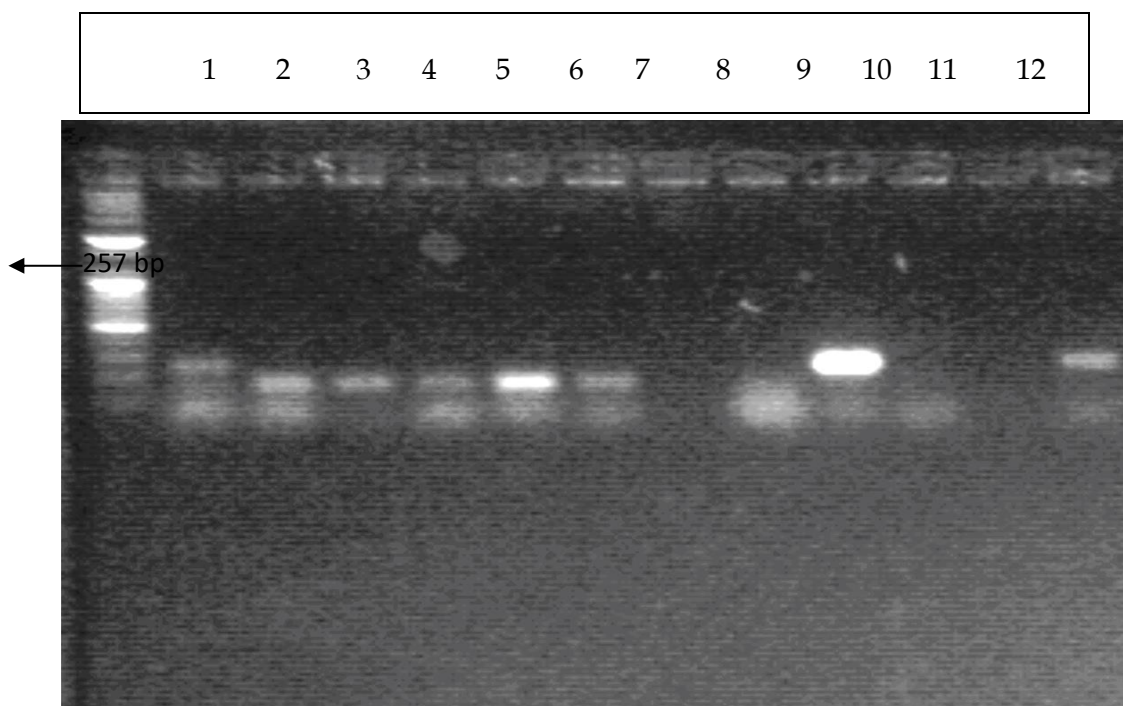
## RESULT

All the 3(42.86%) serum samples from Ado LGA that were positive by ELISA were positive by PCR.

**Table 3 : Information about the animals from Ado Local Government area and the ELISA and PCR result :**

S/N	Sex	Weight in KG	Breed Trait	S/P%	ASF antibodies or ELISA	ASF Antigen or PCR
1	F	47	Lw/cr	29.32	-	-
2	M	28	Lw/cr	100.05	+	+
3	M	33	Lw/cr	96.627	+	+
4	F	50	Lw/cr	101.92	+	+
5	F	53	Lw/cr	24.49	-	-
6	F	48	Lw/cr	15.98	-	-
7	F	30	Lw/cr	15.31	-	-

**Lw/cr: Large white cross    M: Male    S/p%: Titre Level of the Indirect antibody**  
**F: Female    ASF: African swine fever    PCR: Polymerase chain reaction**



**Plate I: Transcriptase Polymerase chain reaction (PCR) on agarose electrophoresis of 12 Sero-positive sera by I-ELISA For African Swine Fever. Lanes 3, 4 and 5 were from Ado LGA, Benue State**  
 Key: All these were sero-positive indicated by bands of 257 basespair Lane M 100 bp DNA ladder and lanes 1-12 pcramplicons

**Table 4: Mean blood parameters of pigs positive and negative to PCR African Swine Fever Virus in Pigs in Ado Local Government Area in Benue State, Nigeria**

PCR	PCV	RBC	WBC	Lym	Neu	Eso	Bas	Mon
Normal	32-50	5.0-80	11.0-22.0	4.5 - 13.0	0 - 0.8	0.05 - 2.0	0 - 0.4	0.25 -
-	31	9.767	4.667	8.967	55	28.3333		
+	32	10.633	5.667	6.533	64.667	31.667		3

Considering the differential of total WBC of pigs examined in Ado LGA, it was observed that the PCR-positive pigs had higher mean WBC count than the normal and also mean PCR negative pigs. This may indicate an ongoing progressive response to viral infection by the bone marrow. This finding is similar to the report of Karalyan *et al* (2012) who reported viral neutrophilia in an experimental infection of pigs with the ASFV Dixon *et al* (2000) cited by Karalyan *et al* (2012) also reported neutropenia and lymphopenia in the later stage of the disease. It may indicate that this pig was at its acute stage of the disease when it was sampled. The mean value of the eosinophil and basophils in the PCR-positive pigs were lower than normal values and also lower than the value of PCR-negative pigs. This indicated ASFV infection. Considering the mean of the monocytes count the value for PCR-positive pigs was much higher than normal and also higher than mean values of PCR-negative pigs. In viral infection there is increase in monocytes as described by Mandal, (2014). Monocytes and macrophages and dendritic cell progeny serve three main functions in the immune system which are phagocytosis, antigenic presentation, and cytokine production (Mandal, 2014). In Benue State, the mean PCV values of the PCR-positive pigs and PCR-negative pigs were not significantly different from the normal value even though there was higher detection rate of antibodies to ASFV infection. Both groups had than lower normal PCV. This may indicate the stage of the disease at which the blood samples were collected. Since the values were at the lower normal it may indicate an ongoing initial hemorrhagic syndrome as reported by Radostits *et al* (2007) and Zimmaerman *et al* (2012).

The mean WBC values for both PCR-positive and PCR-negative pigs were lower than normal and this may indicate an initial stage of the disease in most of the PCR-positive pigs (Karalyan *et al.*, 2012)

Considering the mean value of the monocytes, and neutrophils for PCR -positive pigs even though the differences were significant but the values were higher than that of the PCR negative pigs as well as the normal values indicating lymphocytosis, neutrophilia and monocytosis all of which are indicators of ASF virus.

The mean lymphocytes and basophils levels for the PCR-positive pig of Benue State were lower than the mean for PCR-negative pigs in the various states. This may indicate an ongoing infection of secondary bacterial organisms. This observation is similar to the report of Radostits *et al.* (2007).

Considering eosinophils level/count the mean value for the sero-positive pigs were higher than that of the s PCR negative pigs and normal values. Eosinophilia is associated with parasitism or stress. This may indicate parasitism in the pigs. Considering basophils the mean values were within normal ranges for both the sero-positive and PCR negative pigs. The observed lymphocytopenia is a feature of viral infection (Territo, 2018).

According to Radostits *et al* (2007) there is a fall in the total leukocyte count to about 40-50% of normal by the fourth day of fever. This finding is similar to this result because there was decrease in leucocyte count in the blood sample sera. However there is likely agreement with the statement by Radostits *et al* (2007) that there is lymphopenia and an increase in immature neutrophils this is because then average lymphocyte count of the positive blood sample was lower than that of the sample that tested negative while the average neutrophil count for positive count was higher than that of the samples that were negative. The monocytes counts

were higher in positive blood samples than the negative ones by PCR .This finding is different from the report of Radostits *et al* (2007), that there will be destruction of monocytes/macrophages.

The results obtained showed that from the total number of 7 blood serum samples of pigs that were collected 3 (42.86%) were positive for ELISA and while 4(57.14 %) were negative. All the 3 (42.86%) that were positive for antibodies (ELISA) were also positive for antigen (PCR). Those that were negative for antibodies (ELISA) were also negative for antigen (PCR). This has confirm the statement by Radostits *et, al*; 2007, that pigs with demonstrable antibody should be considered as chronic carriers of the virus as it is doubtful that true recovery ever occurs .This means that 42.86% of pigs in Ado LGA of Benue were chronic carriers of ASF virus.

This has serious implications especially when the disease has been confirmed by Rahimi *et, al*; 2010 (ASF) to be a notifiable, highly contagious, lethal hemorrhagic disease in domestic pigs. Ayode and Adeyemi, 2003 described ASF as a devastating viral disease currently threatening the pig industry worldwide. The present discover in Ado LGA of Benue state is a threat to the pig population in the area and could impact on economy of the pig owners and on animal protein consumption.

#### CONCLUSION AND RECOMMENDATIONS

This studies has shown that ASF antibodies and antigens are present in pig in Ado LGA of Benue state of Nigeria and this has confirmed the carrier status of the pigs in the LGA and since it could affect the food security of the low income earners in both rural and semi-urban population of Ado LGA since they depend largely on pig farming;

It is recommended that control and preventive measures to eradicate ASF in the LGA and any other place should be put in order. Biosecurity measures such as foot deep must be put in place, restriction of movement of personnel from other pig farms, visitors and butchers into the farm and farm equipment such as shovels and wears must not be borrowed or lent out. All garbage or food left overs to be giving to pigs must be cooked. All feed ingredients must be approved by the veterinary authority. Prohibition of movement of pigs from one part or the country to another that is free of the disease unless the animals are confirmed by veterinarians to be free from diseases.

Test and slaughter of animals that test positive for ASF antibody test with compensation The test should be regularly carried out on old breeder pigs and animals staying for very long time so as to eliminate carrier. Pens should be disinfected with strong caustic soda 4 months before animals are reintroduced. Ticks must be controlled and eradicated and other biosecurity measures such as quarantine, restriction of movements of animals and human beings into the farm must be practiced in order to prevent transmission of ASFV.

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