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ANTIBODY DETECTION IN SUSPECTED CARRIERS OF AFRICAN SWINE FEVER VIRUS IN ADO LOCAL GOVERNMENT AREA OF BENUE STATE, NIGERIA

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ABSTRACT

Studies were undertaken to investigate the presence of antibodies against African Swine Fever Virus (ASFV) in pig populations of Ado local government area (LGA) of Benue State. Out of the 188 pigs examined 7 sera samples were obtained and assessed by indirect Enzyme-linked immunosorbent assays (ELISA) .Three samples (42.86%) were positive and 4 (57.14%) negative for antibodies against ASFV. Since 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 ASF antibody with adequate compensation are suggested to eradicate the disease.

Keywords: African swine fever, serum, antibody, Ado LGA, Benue State.

INTRODUCTION

African swine fever (ASF) is a notifiable lethal hemorrhagic disease in domestic pigs (Rahimi *et al.,* 2010).The disease isa devastating viral disease currently 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 *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 belonging to the genus, *Asfivirus* (Sarma, 2012). Maintenance and transmission of ASFV involve cycling of virus between soft ticks of the genus *Ornitodoros* 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 is a member of the family *Asfaviridae*, 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 icosahedra 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). 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^{o}C for 1 hr. The family is sensitive to lipid solvents and can be inactivated rapidly by 2% NaOH (Sarma, 2012).

Ado is one of the Local Government Areas (LGA) in Benue State that is also known for swine production in Nigeria (RIMS, 1992). The LGA is one of the main source of pigs to the southern part of Nigeria. This means that if swine production is affected by ASF in the LGA, it will affect the people in the community and also some neighboring states mostly in the southern part of

Nigeria. It is therefore important to assess the ASF status of pigs in the area so that control and preventive measures can be carried out in order to save the pig population in the area.

This work is of importance because there is scarcity of information on ASF antibody status in pigs in Benue state of Nigeria. If swine production is affected by ASF 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. It is therefore important to assess the ASF status of pigs in the area so that control and preventive measures can be instituted safeguarded the pig population in Benue State.

MATERIALS AND METHODS

Study Area

This study was conducted in Ado Local Governments Area (LGA) in Benue State, Nigeria. Ado LGA is one of the 23 LGA of Benue state which is between longitude 06^{o} and 11^{o} 3'north of the equator (Dada *et al*., 2010). Benue 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) with an international border with Cameroon republic to the southeast (Dada *et al*., 2010).

Sample collection

Out of the 188 pigs examined, 7 sera samples were obtained and assessed by indirect enzyme-linked immunosorbent assays (ELISA). 10ml of blood was collected from pigs through the jugular vein into two tubes (one tube containing anticoagulant and the other without anticoagulant). All the samples collected were placed in an ice pack and transported to the laboratories located in Ahmadu Bello University, Zaria, Kaduna State. The laboratories are those of Departments of Veterinary Pathology, Veterinary Public Health and Preventive Medicine and also National Animal Production Research Institute. The following were taken from live animals: breed, sex and weight using a weighing band. The body of each animal was carefully examined for skin lesions or ectoparasites with the aid of a magnifying glass.

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) 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.

Description and Principles of Indirect ELISA for the detection of anti-African swine fever antibodies in porcine serum and plasma samples or blood filter samples

Even-numbered microwells were coated with p32, p62 and p72 ASFV recombinant proteins, odd-numbered wells are uncoated. Serum samples to be tested and controls were added to even and odd- numbered wells. Anti-ASFV antibodies with the aid of micropipette, if present will form an antigen-antibody complex. After washing, an anti-multi- species horseradish peroxidase (HRP) conjugate is added to the wells. It fixes to the antibodies, forming an antigen-antibody-conjugate-HRP in the presence of antibodies, blue solutions which become yellow after addition of the stop solution. In the absence of antibodies, no coloration appears. The microplates were red at 450 nm. ELISA recorder and computer will help in recording, and result sheets for recording all the result shown by the ELISA recorder and computer.

Wash solution preparation

Wash concentrate (20X) was brought to room temperature and mixed thoroughly to ensure that the wash concentrate (20X) is completely solubilized. The Solution 1X was prepared by diluting the wash concentrate (20X) to 1/20 in distilled water.

Procedure for testing of serum samples

All reagents were allowed to come to room temperature (21^{o} C $\pm$ 5^{o}C) before use. All reagents were homogenized by inversion or vortex. Each sample was deposited twice (adjacently in even and odd-numbered wells).

Serum samples

	No Ag 1	**Ag 2**	**No Ag 3**	**Ag 4**	**No Ag 5**	**Ag 6**	**No Ag 7**	**Ag 8**	**No Ag 9**	**Ag 10**	**No Ag 11**	**Ag 12**
A	NC	NC	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
B	NC	NC	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
C	PC	PC	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
D	PC	PC	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
E	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
F	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
G	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
H	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44

Figure 1. Showing plate map. Samples above are deposited in duplicate in adjacent even and odd- numbered wells. No Ag=Uncoated well Ag=Coated well, NC=Negative control, PC = Positive control, S= sample

The following were added as instructed by the manufacturer of the kits.
100 ul of Dilution Buffer 14 to each well.10 ul of the negative control to wells A1, B1 and A2, and B2, 10 ul of the positive control to wells C1, D1 and C2, D2.10ul of each sample to be tested to the remaining wells. Each sample was deposited twice (adjacently in even and odd-numbered wells). It was later incubated for 45 min at 21^{o}C ($\pm$ 5^{o}C). The wells were emptied and washed 3 times with approximately 300 ul of washed solution. Drying of the wells was avoided during washing. Conjugate 1Xwas prepared by diluting the concentrated conjugate 10X to 1/10 in Dilution Buffer 100 ul of the conjugate 1X was added to each well It was incubated for 3 min at 21^{o} C ($\pm$ 5^{o}C).The wells were emptied and washed 3 times with approximately 300 ul of washed solution. Drying of the wells was avoided during washing.
100 ul of the Substrate Solution was added to each well. It was incubated for 15 min $\pm$ 2min at 21^{o} C ($\pm$ 5^{o}C) in the dark.
100 ul of the Stop Solution was added to each well in order to stop the reaction.
The O.D was red and recorded at 450 nm.

Validation

O.D result was calculated as follows

O.D net = O.D even well-O.D odd well

Any OD value that is negative, the sample was given O.D net as value of Zero

Interpretation

well- O.D result was calculated as follows

O.D net = O.Deven- O.D odd well

S/P percentage (S/p%) was calculated

$$S/P\% = \frac{\text{Net OD sample}}{\text{net OD PC}} \quad X100$$

Samples with a S/p%

With less than or equal to 50% are considered negative (-VE).

Less than 60% and greater than 50% are considered doubtful (D).

Greater than or equal to 60% are considered positive (+VE)

RESULT

Reactions of antigen –antibody in the wells of ELISA plates

The ELISA recorder and computer helped in recording and results presentation. Antigen-antibody conjugate complexes was formed in wells where horseradish peroxidase (HRP) conjugate fixed as ASF antibodies and this was shown by blue coloration (Figure 2) that became yellow after addition of the stop solution **(**Figure 3**)**. In wells where there was no antigen-antibodies, no coloration appeared. The microplate was read at 450nm.

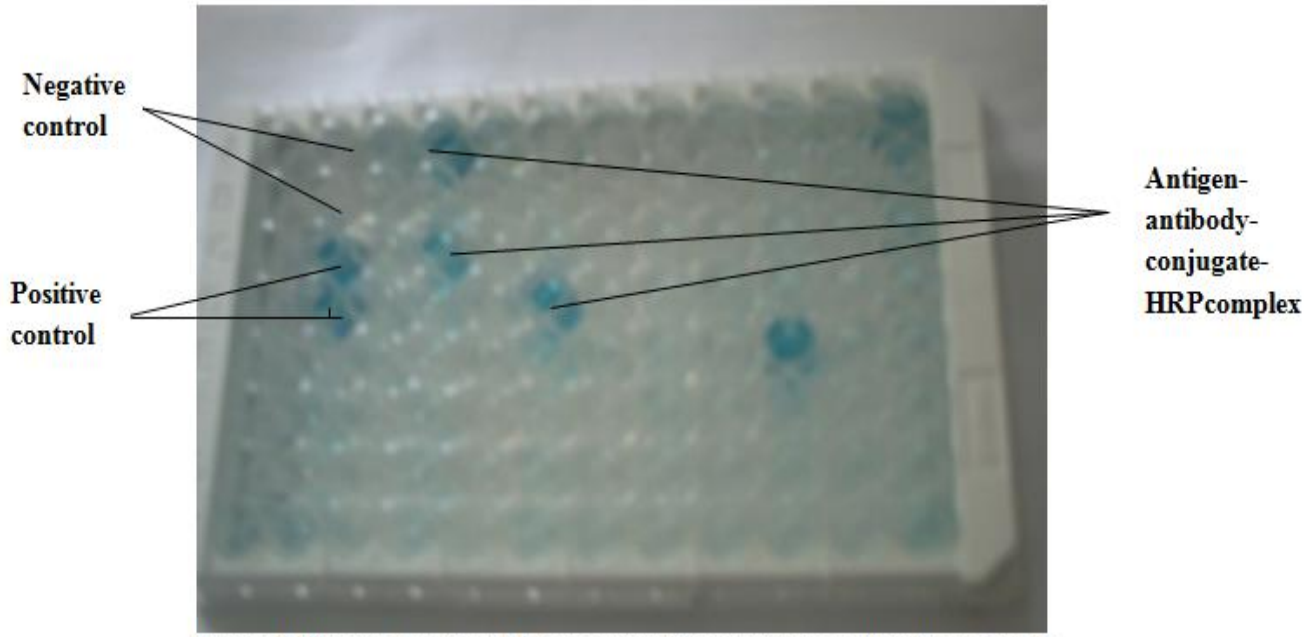


Figure 2.Picture showing African swine fever antigen-antibody-conjugate -HRP complex which is shown (above) by blue coloration.

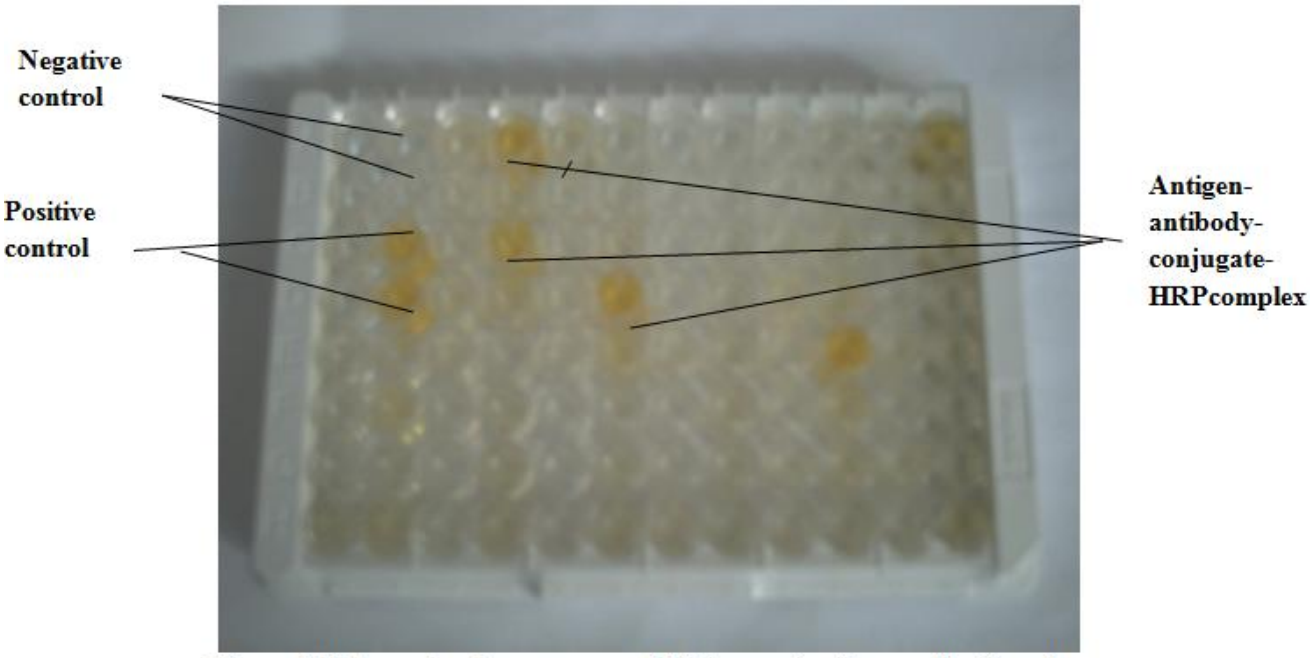


Figure 3.Picture showing presence of African swine fever antibodies, the blue coloration which became yellow after addition of the stop solution.

Table I: ASF antibodies and the S/P% calculated from ELISA result

S/N	Sex	Weight in KG	Breed Trait	S/P%	ASF antibodies
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

DISCUSSION

The results obtained from this study showed that 3(42.86%) sera samples were positive and the remaining 4(57.14 %) were negative by indirect enzyme-linked immunosorbent assays (ELISA) for ASF antibodies in Ado Local Government Area of Benue state of Nigeria. According to Radostits *et al.* (2007), pigs with demonstrable ASF antibody should be considered as chronic carriers of the ASF virus as it is doubtful that true recovery ever occurs, hence, this may mean that 42.86% of the pigs in the study area were chronic carriers of the ASF virus. This has serious implications because according to Rahimi *et al.* (2010), ASF is a notifiable, highly contagious, lethal haemorrhagic disease in domestic pigs. Ayoade and Adeyemi (2003) described ASF as a devastating viral disease currently threatening the pig industry worldwide. The present discovery in Ado LGA of Benue state is a threat to the pig population in the area and could impact on the

economy of the pig owners and on animal protein consumption. This is especially so since the pig population contributes highly to the food security of low income rural and peri-urban population. Pork remains the cheapest source of the meat in Nigeria and food security of the low income earners in rural and semi-urban population in Ado local government area depends largely on pig farming

CONCLUSION AND RECOMMENDATIONS

This study has shown that ASF antibodies are present in pigs in Ado LGA of Benue State of Nigeria and this has confirmed the carrier status of the pigs in the local government area. In order to aid continuous production and maintain health of pig populations in the area, the following steps are suggested as control and preventive measures to eradicate ASF in the LGA and in Nigeria as a whole.

1. Farm equipment such as shovels and wears must not be borrowed or lent out.
2. All garbage or food left overs to be giving to pigs must be cooked. All feed ingredients must be approved by the veterinary authority.
3. Personnel from other pig farms, visitors and butchers should not be allowed into the farm and where it is inevitable biosecurity measures such as foot deep must be put in place.
4. Ticks must be controlled and eradicated by in other to prevent transmission of ASF virus.
5. Product, waste or by product of bush pig or warthog must not be allowed to come in contact with domestic pigs.
6. 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 disease.
7. Pens should be disinfected with strong caustic soda four months before animals are reintroduced.
8. Test and slaughter of animal that test positive for ASF antibody test with compensation
9. The test should be regularly carried out on old breeder pigs and animals staying for very long time so as to eliminate carrier.

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