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AFRICAN SWINE FEVER ANTIBODY DETECTION AND STATUS PIGS IN SABON GARI LOCAL GOVERNMENT AREA OF KADUNA STATE, NIGERIA

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ABSTRACT

Investigation of African Swine Fever antibody in pig population was carried out on serum samples collected from 20 different pigs in Sabon Gari local government area of Kaduna state of Nigeria by indirect Enzyme-linked immunosorbent assays (ELISA) as described by ID.vet Innovative Diagnostics IDvet,310, rue Louis Pasteur-Grabels-FRANCE).Two of the samples (10%) tested positive and 18 (90 %) tested negative for the test. Since pigs with demonstrable antibody should be considered as chronic carriers of the virus because it is doubtful that true recovery ever occurs. Control measures such as good managements, biosecurity measures, tick control with acaricides and also slaughter of animal that test positive for ASF antibody test with adequate compensation are suggested.

Keywords: African swine fever, Antibody, serum, Sabon Gari , Kaduna State.

INTRODUCTION

African swine fever (ASF) is a 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 measuring 200 – 220nm in diameter. The virus belongs to the genus Asfivirus. Maintenance and transmission of ASFV involve cycling of virus between soft ticks of the genus *Ornitodorus* 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 AFSV vary in their ability to cause disease, but there is one serotype of the virus detectable by blood antibody test (EU, 2010). 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). 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). The dried virus is not destroyed by exposure to 40^{0}C in 15 days. It is preserved in 0.5% phenol in 50% per cent glycerin mixture at room temperature for 536 days. It is quite stable and since it remains viable for 11 days at room temperature, 15 weeks in carcasses, 5 months in processed hams, and 6 months in bone marrow. (Merchant and Parker, 2005).

The usual route of an ASF infection in pig is via the oral route mouth or the upper respiratory system. The ASF virus provokes proliferation of lymphocytes and reticular cells. After four days, degeneration becomes more prominent than proliferation (Ayoade and Adeyemi, 2003). If infection is acquired via the respiratory tract, virus replicates first in pharyngeal tonsils and lymph nodes draining the nasal mucosa before being disseminated rapidly throughout the body via a primary viremia in which the virus is associated with both erythrocyte and leuckcytes. A generalized infection follows with titers up to 10^{9} ID 50 ml of blood or/gram of tissue. Consequently, all secretions and excretions contain large amounts of infectious virus (Murphy *et, al.,* 1999). The virus invades through the tonsils and respiratory tract and replicates in the lymphoid tissues of the nasopharynx prior to the occurrence of a generalized viremia, which can occur within 48-72 hours of infection. Infectivity and contact transmission develops at this time and continues for at least 7 days (Radostits *et, al;* 2007).

This work is justified because there is scarcity of information on African swine fever antibody level Sabon Gari local Government area of Kaduna state of Nigeria .The local government is the main source of pigs in Kaduna north senatorial Zone in Kaduna state of Nigeria. This means that if swine production is affected by ASF in the local Government, the pork consumers and other people that come to buy even all the way from the southeast and Niger Delta 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 so that the pig population in the area should be safeguarded.

MATERIALS AND METHODS

The materials that were used include serum sample collection bottles, vacuotaner tubes, heparinised tubes, thermometer, methylated spirit, weighing band, viral transport medium, needles and syringes (2 ml and 5 ml) cooler (for transporting samples), mono or multi-channel micropipettes tips capable of delivering 10 ul, 100 ul, and 200 ul, dispensable tips,96-well microplate reader, distilled water, manual or automatic wash system, ASF antigen, slides, cover slip, Giemsa powder, Methanol (99%). Glycerol, cotton gauze, disinfectant, detergent, microheamatocrit capillary tube (plain), Natt-Herrick` diluents, camera, Vials, deep freezer, ELISA kits, ELISA recorder and computer, and result sheets, Markers, The ID.vet Innovative Diagnostics kit. component for detection of ASFV antibody include Microplates coated with p32, p62, p72 ASFV recumbent proteins (biwelformal), concentrated conjugate (10X), positive control, negative control, dilution buffer 14, dilution buffer 3, wash concentrate (20X), stop solution (H2SO4, 0.5m) **.** Freezer <-10C, Freezer ≤-70 C, Fridge 4±3 C, Photograph camera printer, and power supply.

Study Area

Samples were collected from pig population from both commercially managed and traditionally managed pigs in Sabon Gari Local Government area (LGA) **of** Kaduna state of Nigeria.. . The global location of the state is between Longitude 06^{o} and 11^{o} 3' north and 8^{o}E of the equator. The state occupies an area of approximately 48,732.2 square kilometers. Kaduna state has 23 local governments (Jallo, 2000). The 23 Local Governments Council are grouped into Kaduna North, Kaduna Central and Kaduna South senatorial zones. Sabon Gari LGA is under Kaduna North South senatorial zones. The state capital is Kaduna. The Laboratory work was carried out in the National Animal Production Research Institute (NAPRI) Chika Zaria, Department of Veterinary Medicine Laboratory, and Department of Public health and Preventive Medicine Laboratory, Ahmadu Bello University, Zaria, Kaduna State Nigeria.

Activity before the collection of samples for analysis

The following were taken from live animals: The breed and sex of each animal were recorded, the weights of the animals were taken mostly with the use of weighing band. 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).

Sample collection

A total number of 5 to 10 ml of blood samples collected from 20 pigs from Sabon Gari LGA of Kaduna state of Nigeria. The blood was collected mainly from each live animals or animals during slaughter through the jugular vein in two different tubes (one tube containing anticoagulant and vacuotaner tube without anticoagulant). All the samples collected were placed in a cooler and covered with ice pack.

Antibody detection Test

The detection of antibody against ASF was carried out by indirect enzyme-linked immunosorbent assays (ELISA) as described by ID.vet Innovative Diagnostics (IDvet,310, rue Louis Pasteur-Grabels-FRANCE).(It is an indirect ELISA 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.After washing, an anti-multi- species horseradish peroxidase (HRP) conjugate was 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 helped in recording, and result sheets for recording all the results were 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/deiodised 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)

Arrangement of 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.10ul 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

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

$$S/P\% = \frac{\text{Net OD sample}}{\text{Net OD PC}} \times 100$$

Samples with a S/p%

With less than or equal to 50% are considered negative .

Less than 60% and greater than 50% are considered doubtful.

Greater than or equal to 60% are considered positive

RESULT

Reactions of antigen –antibody in the wells of ELISA plates

Antigen-antibody conjugate complexes was formed in wells where horseradish peroxidase (HRP) conjugate fixed as ASF antibodies and this was shown by blue coloration (Plate 1) that became yellow after addition of the stop solution **(**PlateII **)**. In wells where there was no antigen-antibodies, no coloration appeared.

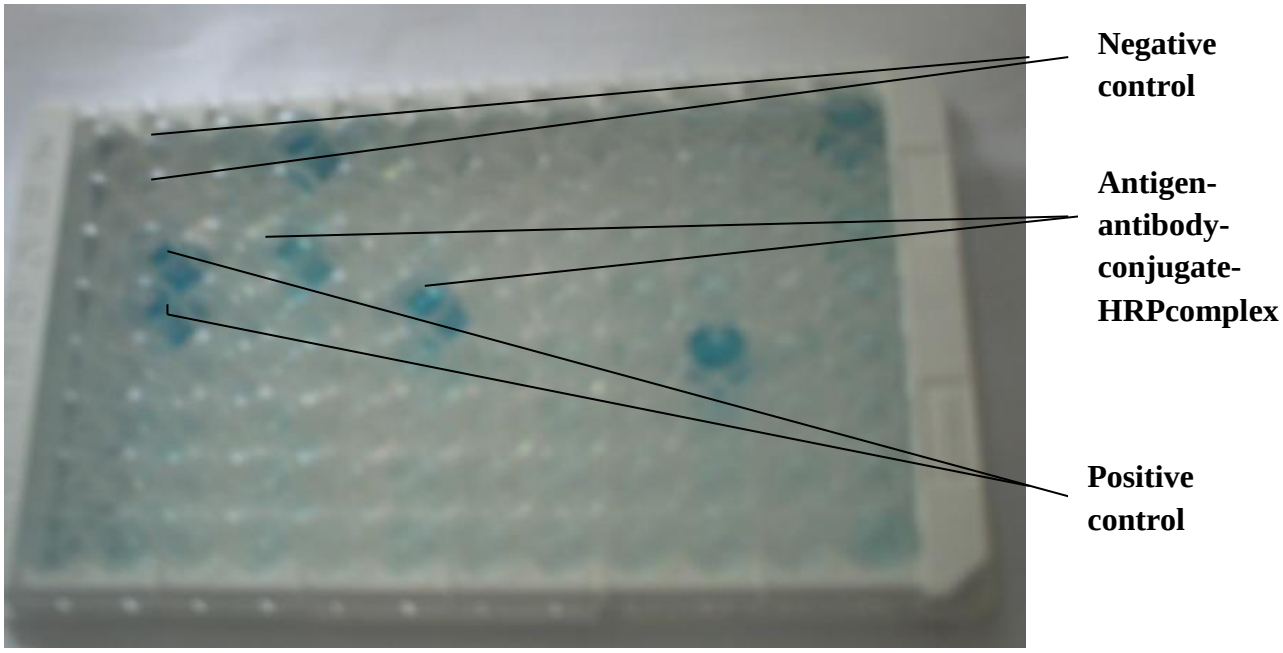
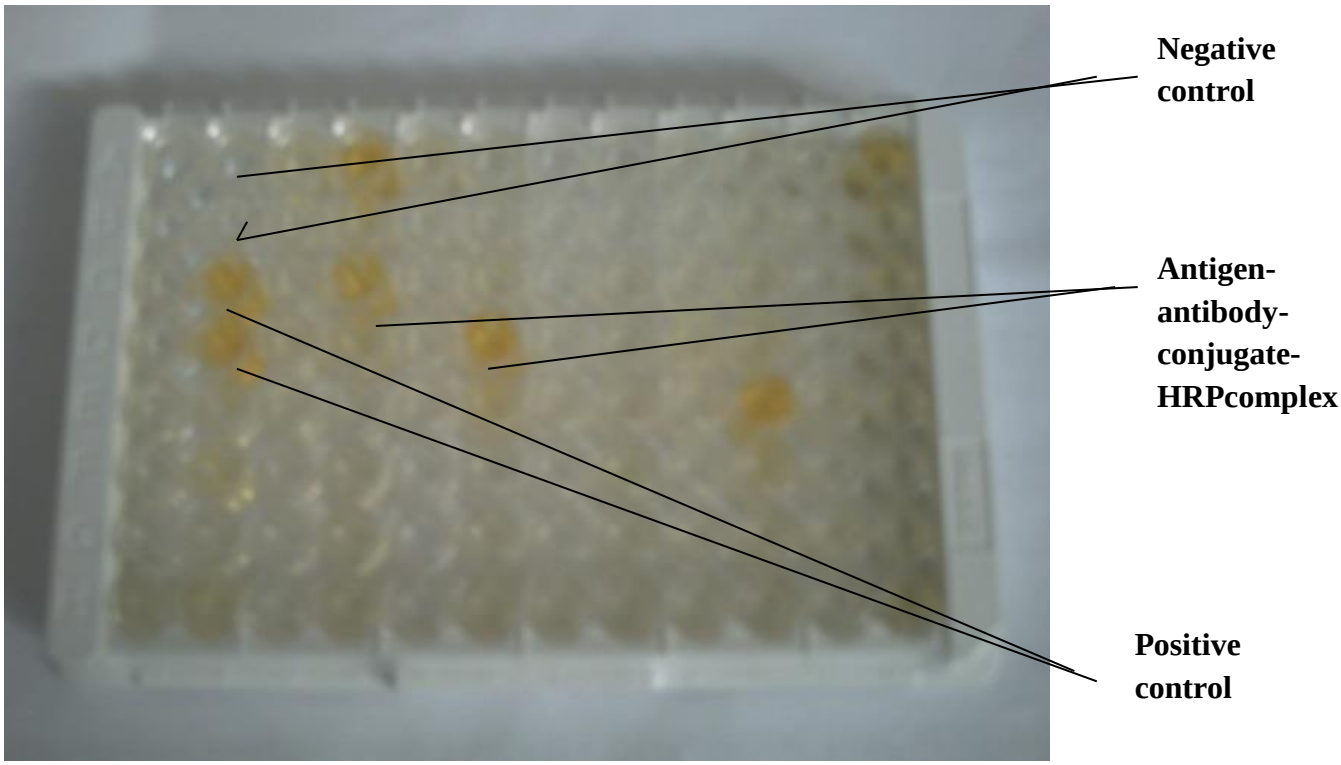


Plate1 Picture showingAfrican swine fever antigen-antibody-conjugate-HRP

Complex which is shown (above) by blue coloration.



PlateII Picture showing presence of African swine fever antibodies in the serum, the blue solution which become yellow after addition of the stop solution.

The microplate was read at 450nm.The ELISA recorder and computer helped in recording, and result sheets for recording all the result shown by the ELISA recorder and computer.

The indirect antibody ELISA test for ASF confirmed that 2(10%) of the samples collected from Sabon Gari LGA were positive and 18 (90%) were negative (see Table I) ..

Table I : Showing the information about of the animals from Sabon Gari Local Government area and the S/P% calculated from ELISA result

S/N	Sex	Weight in KG	Breed Trait	S/P%	ASF antibodies
1	**F**	**35**	**Lw/cr**	**10.04**	**-**
2	**M**	**25**	**Lwcrss**	**42.93**	**-**
3	**M**	**55**	**Lw/cr**	**22.18**	**-**
4	**F**	**40**	**Lw/cr**	**16.32**	**-**

5	**F**	**55**	**Lw/cr**	**21.79**	**-**
6	**M**	**50**	**Lw/cr**	**16.94**	**-**
7	**F**	**75**	**Lw/cr**	**169.73**	**+**
8	**M**	**70**	**Lw/cr**	**61.54**	**+**
9	**F**	**40**	**Lw/cr**	**28.51**	**-**
10	**M**	**65**	**Lw/cr**	**22.35**	**-**
11	**M**	**60**	**Lw/cr**	**17.747**	**-**
12	**M**	**35**	**Cros**	**8.28**	**-**
13	**F**	**75**	**Cros**	**6.044**	**-**
14	**M**	**35**	**Cros**	**5.974**	**-**
15	**F**	**75**	**Cros**	**15.616**	**-**
16	**M**	**55**	**Cros**	**6.847**	**-**
17	**M**	**50**	**Cros**	**4.751**	**-**
18	**M**	**50**	**Cros**	**8.529**	**-**
19	**F**	**45**	**Cros**	**16.035**	**-**
20	**M**	**120**	**Cros**	**6.917**	**-**

Lw/cr: Large white cross

Cros: Cross : Between local breeds and other undefined breeds.

DISCUSSION

This study has shown that African swine fever antibody is present in some pigs in in Sabon Gari LGA **of** Kaduna states of Nigeria. If we are to consider 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 at least 10% of the pigs in Sabon Gari Local Government area of Kaduna are carriers of African swine fever virus. This has serious implication because according to Rahimi *et, al;* 2010 African swine fever (ASF) is a notifiable, highly lethal hemorrhagic disease in domestic pigs. This disease can be a very frustrating disease to most pig farmers and this can lead to decrease in animal protein production and consumption.

CONCLUSION

This study has shown that ASF antibodies is present in pig population in Sabon Gari LGA of Kaduna state of Nigeria and this has confirmed the carrier status of the pigs in the LGA .

RECOMMENDATIONS

According to Hicheri, 1998, **'**The pig population contribute highly to the food security of low income rural and peri-urban population. Pork remains the cheapest source of meat in Nigeria. The following steps should be taking to eradicate ASF in in Sabon Gari LGA of Kaduna.

1) Biosecurity measures such as control of personnel from other pig farms, visitors, butchers and provision of foot deep .Farm equipment such as shovels and wears must not be lent out to other farms .Cooking all gabages or food left overs to be giving to pigs. The use of blood meal made from blood of pigs for other pig feed should be discouraged.
2) Soft ticks of the genus *Ornitodorus* must be controlled. This will help in controlling the ticks that transmit the virus. Domestic pigs should not be allowed to have contact with the wild pigs (warthogs, bush pigs, and giant forest boars) since this will help in braking the cycling of virus between soft ticks of the genus *Ornitodorus* and wild pigs.
3) Prohibition of movement of pigs from one part of the country to another that is free of the disease unless the animals are confirmed by veterinarians to be free from the virus.
4) Contaminated site can remain infective for period exceeding 3 months (Radstits et al 2007). Pig pens should be disinfected with strong solution of caustic soda 4 months before animals are reintroduced.
5) Quarantine, compulsory slaughter of infected and contact animals and other animals at risk with adequate compensation to the owners.

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