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Detection of African Swine Fever Antibody in Pigs in some Local Government Areas of Nasarawa State, Nigeria

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Abstract

Investigation of Africanswine fever (ASF) antibody in pig population was carried out on serum samples collected from 127 different pigs in Akwanga, Keffi, Awe and Keana local government areas(LGAs)of Nasarawa State of Nigeria by indirect Enzyme-linked immunosorbent assays (ELISA).One of the samples (2.00%)tested positive, and 49(98%) tested negative out of the 50 (100%) serum samples collected from Awe LGA. Serum samples from different pigs from Keana, Akwanga and Keffi LGAs were all negative for the test.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 management and all animal that is positive for ASF antibody test should be destroyed with adequate compensation are suggested most especially in a situation where there is no much report about the disease in Nasarawa state before the disease spread to other animals.

Keywords: African swine fever, serum, Antibody, Awe, Nasarawa state

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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). Thedisease is caused by African swine fever virus (ASFV) which is an enveloped double stranded deoxyribonucleic acid (DNA) virus, belonging to genusAsfivirus. 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). A member of the family is a double stranded DNA virus that measures 200-220nm in diameter. 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°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).

Different versions of serological tests are available but the most commonly used is the Enzyme-linked Immunosorbent Assay (ELISA) (OIE, 2012; Cubillos et al., 2013). However, in acute disease, the death may occur before the animal may produce antibodies, and serologicl testing may fail to detect the disease in an early stage (Penrith *et al.,* 2009; OIE, 2012).It is used to evaluate antibody in serum or fluid from tissues. ELISA is mostly used to detect the disease in endemic areas or where there is low virulent isolates. (OIE, 2008, 2012).Enzyme-linked immunosorbent assays (ELISA) test has been developed for long time for a variety of antibodies against the highly antigenic ASF viral proteins p30, p54, and p72. More than 90% of infected pigs can be detected by this method. The test is highly specific and sensitive and is used as a screening test on a large scale and pigs with demonstrable antibody should be considered as

chronic carriers of the virus as it is doubtful that true recovery ever occurs (Radostits *et, al;* 2007)
.
This work is justified because there is no enough information on ASFanti body level in Nasarawa State of Nigeria. If swine production is affected by ASF in Nasarawa State, the pork consumers and other people that come to buy from the Southern part of Nigeria are likely to suffer from meat shortage. It is therefore important to assess the ASFanti body status of pigs in the area so that control and preventive measures can be carried out so as to safeguarded the pig population in Nasarawa State from the disease.

MATERIALS AND METHODS

The materials that were used include serum sample collection bottles, vacuotanertubes, heparinised tubes, thermometer, methylated spirit, weighing ban, 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

NasarawaState is located in the Middle-Belt .Latitudes 080-35’N and Longitudes 08°–10.2^{o} E. Land Area is 27,116.8 square kilometers (approximate landmass) (Dada *et, al;* 2010).It is bounded in the north by Kaduna state, in the west by the Abuja Federal Capital Territory, in the south by Kogi and Benue States and in the east by Taraba and Plateau States. The state has thirteen LGAs. The state capital is Lafia.(Dada *et, al;*2010).
Samples were collected from pig population from both commercially managed and traditionally managed pigs in Akwanga, Keffi, Awe and Keana LGAs of Nasarawa State of Nigeria. Akwanga LGA is located in Latitudes 8.54N and Longitudes 8.24E .Keffi LGA is located in Latitudes 8.51N and Longitudes 7.47E. Both LGAs are in Nasarawa north senatorial zone. Awe LGA is located in Latitudes 8.09N and Longitudes 9.08E Keana LGA is located in Latitudes 8.09N and Longitudes 8.52E. Both Awe and Keana Local Government area in Nasarawa south senatorial zone. The laboratory work was carried out at the National Animal Production Research Institute (NAPRI) Shika Zaria, Department of Veterinary Medicine Laboratory,andDepartment 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 quantity of 5 to 10 ml of blood samples collected from different pigs from various numbers of pigs in each LGAs as follows: 15 from Akwanga, 10fromKeffi,50 fromAweand52from Keana of Nasarawa 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 vacationer 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.

Detection of anti-African Swine fever antibodies in porcine serum and plasma samples or blood filter samples

The ELISA plate had even-numbered microwells that were coated with p32, p62 and p72 ASFV recombinant proteins, and 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 it form an antigen-antibody complex. 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 helped 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/deiodised water.

Testing of serum samples

All reagents were allowed to come to room temperature (21° 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 wellsTable 1)

Table 1Arrangement of serum samples in plate

	No Ag 1	Ag 2	No Ag3	Ag 4	No Ag5	Ag 6	No Ag 7	Ag 8	No Ag9	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

Plate map and how Samples are deposited in duplicate in adjacent Evenandodd-numbered wells. No Ag=Uncoated wellAg=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,
10ulof 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° C (± 5°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° C (± 5°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 ± 2min at 21° C (± 5°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 netas value of Zero

Interpretation
S/P percentage (S/p%) was calculated $Net\ OD\ sample\ S/P\% = X100$

Samples with S/p% with less than or equal to 50% are considered negative and those greater than or equal to 60% are considered positive.

RESULTS

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 (Plate II). In wells where there was no antigen-antibodies, no coloration appeared.

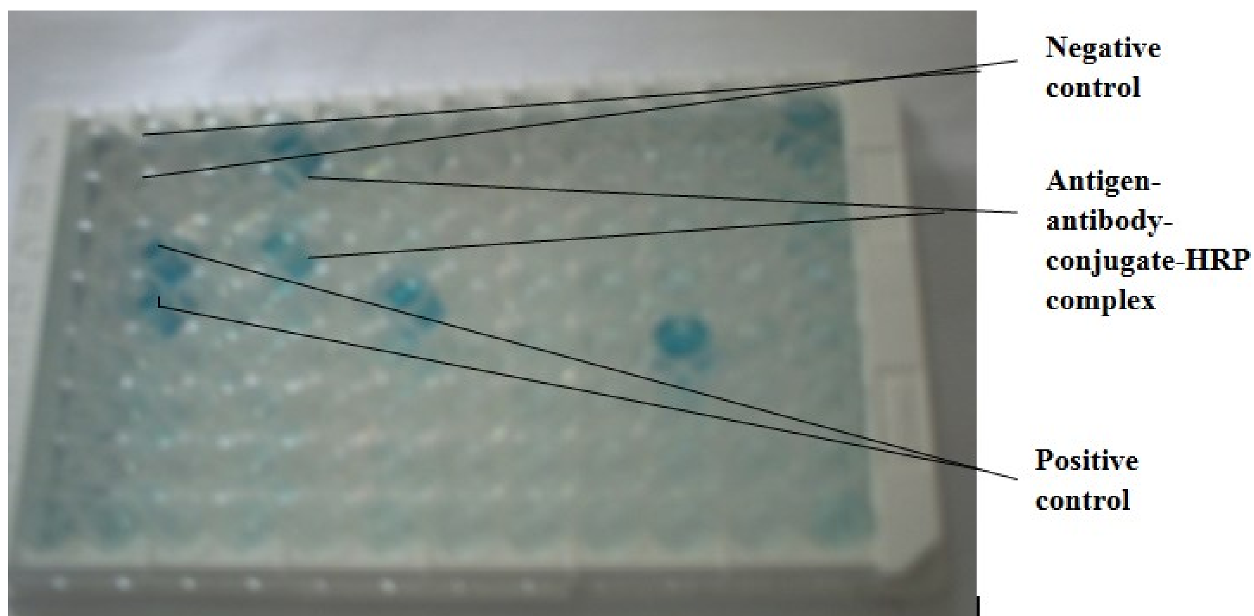

Plate1 African swine fever antigen-antibody-conjugate-HRP Complex which is shown by blue coloration.

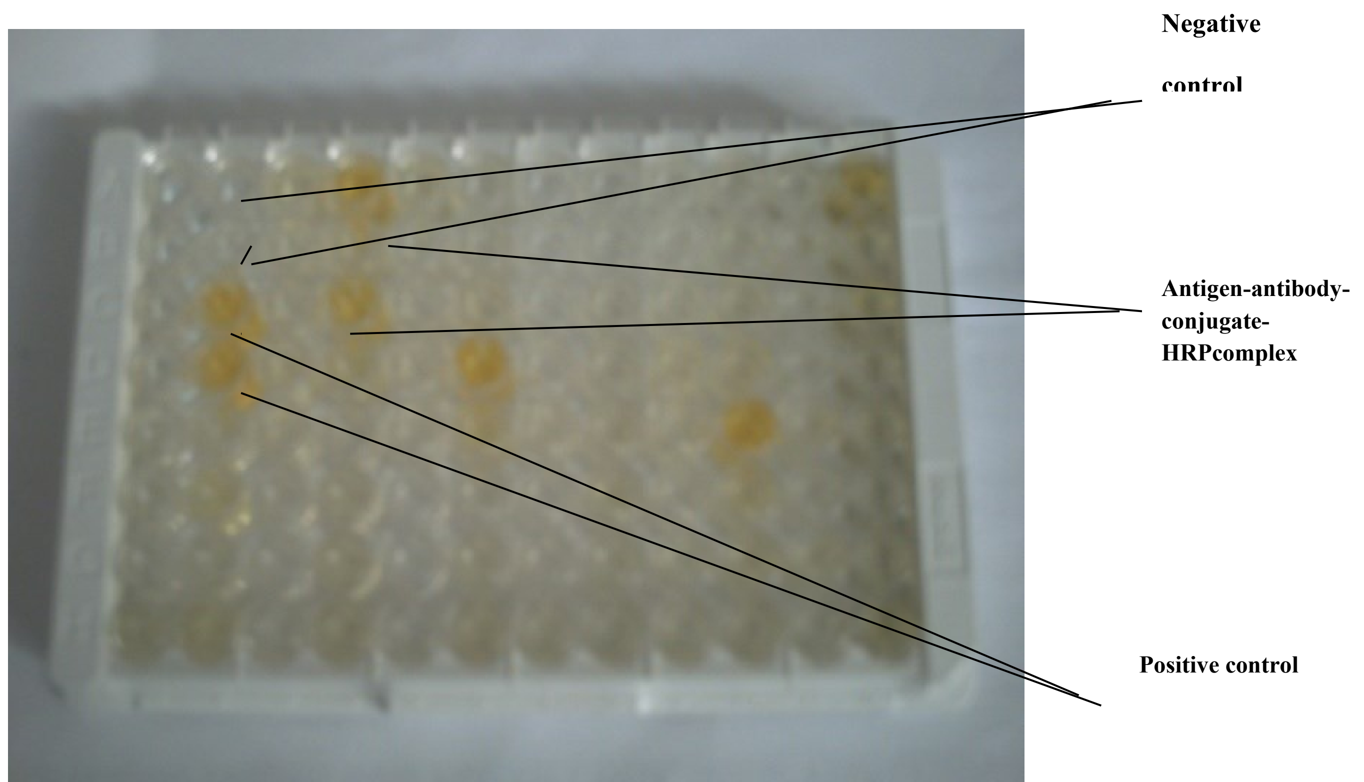

Plate II Presence of African swine fever antibodies in the serum, the blue solution which become yellow after addition of the stop solution.

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 results are as shown on tables 2,3,5 and 5.

Table 2 :Information about of the animals from Akwanga LGA and the S/P% as calculated from ELISA result

S/N	Sex	Weight in KG	Brd	S/P%	ASF antibodies
1	F	80	Lwcrs	11.44	-
2	M	70	Lwcrs	11.05	-
3	M	55	Lwcrs	12.97	-
4	F	55	Lwcrs	10.04	-
5	M	55	Lwcrs	16.83	-
6	M	65	Lwcrs	07.50	-
7	M	30	Lwcrs	26.04	-
8	M	25	Lwcrs	07.71	-
9	F	45	Lwcrs	13.59	-
10	F	55	Lwcrs	13.50	-
11	M	45	Lwcrs	20.82	-
12	F	55	Lwcrs	13.11	-
13	M	50	Lwcrs	19.02	-
14	F	35	Lwcrs	24.76	-
15	F	45	Lwcrs	29.98	-

Table 3 : Information about of the animals from : Keffi LGA and the S/P% as
As calculated from ELISA result

S/N	Sex	Weight in KG	Brd	S/P%	ASF antibodies
1	F	50	Lwcrss	10.23	-
2	M	25	Lwcrss	8.993	-
3	F	25	Lwc	8.939	-
4	F	25	Lwc	5.600	-
5	M	15	Lwc	8.454	-
6	F	20	Lwc	5.870	-
7	M	45	Lwc	14.65	-
8	M	15	Lwc	11.36	-
9	M	25	Lwc	11.16	-
10	M	15	Lwc	11.52	-

Table 4: Information about of the animals from: Awe LGA and the S/P%
As calculated from ELISA result

S/N	Sex	Weight in KG	Brd	S/P%	ASF antibodies
1	F	65	Lw	13.10	-
2	F	70	Lw	19.30	-
3	F	60	Lw	09.44	-
4	F	70	Lw	21.94	-
5	F	75	Lw	19.40	-
6	F	80	Lw	14.47	-
7	F	70	Lw	59.74	-
8	F	70	Lw	18.80	-
9	F	65	Lw	13.10	-
10	F	60	Lw	12.94	-
11	F	50	Lw	11.47	-
12	F	50	Lw	12.37	-
13	M	45	Lw	16.20	-
14	F	75	Lw	08.74	-
15	F	50	Lw	13.34	-
16	F	45	Lc	94.55	+
17	F	45	Lc	20.10	-
18	F	50	Lc	08.34	-
19	F	65	Lw	09.84	-
20	F	65	Lw	11.07	-
21	M	60	Cross	09.02	-
22	M	60	Lw	07.44	-
23	M	60	Lw	09.77	-
24	M	60	Lw	09.84	-
25	M	70	Lw	15.17	-
26	M	60	Lw	12.04	-
27	M	55	Lc	11.24	-
28	F	50	Lc	11.54	-
29	F	70	Lc	14.30	-
30	F	70	Lw	11.77	-

Table 4 : Awe **LGA continues**

S/N	Sex	Weight in KG	Brd	S/P%	ASF antibodies
31	F	60	Lw	15.47	-
32	F	70	Lw	18.37	-
33	F	70	Lw	18.37	-
34	F	55	Lc	15.94	-
35	F	50	Lc	14.74	-
36	F	65	Lw	12.87	-
37	F	60	Cross	47.88	-
38	F	60	Cross	14.94	-
39	F	60	Cross	18.77	-
40	F	65	Lw	11.27	-
41	F	50	Lc	17.34	-
42	F	50	Lc	12.24	-
43	F	45	Lc	16.37	-
44	F	50	Lc	17.80	-
45	F	60	Lw	27.10	-
46	F	60	Lw	16.76	-
47	F	60	Lw	18.82	-
48	F	65	Lw	13.88	-
49	F	60	Lw	14.91	-
50	F	65	Lw	13.46	-

Table 5: Information about of the animals from :Keana LGA and theS/P%
As calculated from ELISA result

S/N	Sex	Weight in KG	Brd	S/P%	ASF antibodies
1	M	60	LW	54.03	-
2	M	55	LW	13.15	-
3	M	55	LW	11.44	-
4	F	55	LC	09.55	-
5	F	55	LC	19.96	-
6	F	100	LC	22.74	-
7	F	65	LC	34.79	-
8	F	60	Cros	32.32	-
9	M	65	Lw	17.07	-
10	F	55	Lw	22.19	-
11	M	55	Lw	07.24	-
12	F	55	Lw	19.31	-
13	F	55	LC	15.11	-
14	F	50	Lw	20.85	-
15	F	50	Lw	32.70	-
16	F	50	Lw	07.52	-
17	M	55	Lw	07.32	-
18	M	60	Lw	16.90	-
19	M	50	Lw	15.01	-
20	M	55	Lw	25.11	-
21	M	50	Lw	13.53	-
22	M	48	Lw	15.87	-
23	F	60	Lw	14.15	-
24	F	50	Lw	21.47	-
25	F	60	Lw	17.65	-
26	M	50	Lw	09.93	-

Table5 :Keana LGA continues

S/N	Sex	Weight in KG	Brd	S/P%	ASF antibodies
27	M	60	Lw	10.17	-
28	M	60	Lw	08.28	-
29	M	50	Lw	06.56	-
30	F	50	Lw	29.02	-
31	F	50	Lw	24.11	-
32	M	70	Lw	18.79	-
33	M	60	Lw	11.27	-
34	M	50	Lw	19.78	-
35	M	50	Lw	15.12	-
36	F	70	Lw	09.89	-
37	F	60	Lw	23.05	-
38	F	50	Lw	21.12	-
39	F	60	Lw	15.52	-
40	F	50	Lw	09.82	-
41	F	100	Lw	21.83	-
42	F	60	Lw	11.35	-
43	F	50	Lw	18.19	-
44	F	60	Lw	07.19	-
45	F	65	Lw	19.48	-
46	F	60	Lw	10.26	-
47	M	60	Lw	22.26	-
48	M	90	Lw	09.99	-
49	F	100	Lw	06.71	-
50	M	85	Lw	12.36	-
51	M	50	Lw	17.27	-
52	F	50	Lw	06.40	-

Lw: Large white breeds
Lw/cr: Large white crossbreeds
Cros: Cross Between local breeds and other undefined breeds.
LC: Local breeds
M: Male
F: Female

Table 6: Summary of the result of Indirect Antibody Enzyme-linked immunosorbent Assays Test

Serial no	Local Government	Number of pigs bled	Number (and +ve)	Number(and% -ve)
1	Akwanga	15	0(0)	15(100)
2	Keffi	10	0(0)	10(100)
3	Awe	50	1(2.00)	49(98.0)
4	Keana	52	0(0)	52(100)
Total		127	1(0.79)	126(99.21)

DISCUSSION

The study agreed with OIE 2008, 20012 that ELISA is mostly used to detect the disease in endemic areas or where there is low virulent isolates. (OIE, 2008, 2012). This is so because there was no report of outbreak during the study. This study has shown that African swine fever antibody is present in some pigs in Nasarawa state particularly Awe LGA . 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 1% of the pigs in Awe LGA of Nasarawa 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 are present in some pig population inAwe LGA of Nasarawa state of Nigeria and this has confirmed the carrier status of the pigs in the LGAs.

1)Biosecurity measures such as not sharing farm equipment such as shovels with other farms, use of foot deep on the entrance of the farm and cooking all garbage 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 since ticks can transmit the virus.

3) Awareness campaign is very important to pig farmers and other members of the public about the disease.

4) The movement of pigs from one part of the country to another should be controlled

5) Pig pens should be disinfected strong solution of caustic soda 4 months before use.

6) Quarantine, compulsory slaughter of infected and contact animals and other animals at risk with adequate compensation to the owners will help in eradication of the disease.

REFERENCES

Ayoade, G.O. and Adeyemi, I.G. (2003).African swine fever: an overview Pigtrop the information center about pig production and pork meat commodity chains in developing countries. RevueÃ‰lev.MÃ©d, Paystrop.2003, 56 (3-4): 129-134

Brown Babera (1980).Hematology Principles and procedures 3rd Edition Massechussets.

Dada Fredrick OA, JibrinGarba Musa and Ijeoma Adanne (2010).Secondary school Atlas. Nigerian South West Geo-political Zonal /Region Published by Macmillan Nigeria Fourth Edition. Pp16-17

Ekwe, N. F., and Wilkinson, P.J. (2000). Pigtrop - the information center about pig production and pork meat commodity chains in developing countries. Comparison of genomes of African swine fever virus isolates from Cameroon, other African countries and andEurope*RevueÃ‰lev.MÃ©d, Paystrop, 2000, 53 (3) : 229-236*

EU (European Union. (2010). News Reports African Swine Fever outbreaks and epidemic 2010 Internal control measures for African Swine Fever; OIE. www.Pig health.com/ASFhtm copyright Pig Disease information centre 2010 Pig Health.com.

El-Hicheri, Khaled.., (1998).Emergency assistance on control and eradication of an outbreak of African swine fever in western Nigeria. Report of fao consultancy mission to Nigeria. Food and Agricultural Organization of United Nations Rome, December 1998.

Jallo, H. (2000). Vision and Actions for a Greater Kaduna state; he MakarfiEra. Vol 1:5-8

Merchant, I.A. and Parcker, R.A. (2005) Veterinary Bacteriology and VirologyCBS Publishers and Distributors 4596/ I-A,11Daryaganji, New Delhi,=110002 (India)SevethEditionPp703

Office international des epizooties (OIE). (2008). African swine fever. In: Manual of diagnostic tests and vaccines for terrestrial animal mammals, birds and bees [http://www.oie.int/eng/norms /mm anual/2008/pdf/2.08.01_ASF.pdf].

Office international des epizooties (OIE). (2012). WAHID interface animal helath information African swine fever [athttp://web.oie.int/wahis/public.php] site visited on.

Otesile, E.B., Ajuwape, A.T.P., Odemuyiwa, S.O., Akpavie, S.O., Olaifa, A. K. and Odaibo, G.N. (2005).An Outbreak of African swine fever in Nigeria Revue Élev.Méd.vét. Pays trop., 2005, 58 (1-2): 21-26

Penrith, M L., Vosloo, W., Jori, F. and bastos, A. D. S. (2009). Review of African swine fever. Transmission spread and control. *Journal of South African veterinary Association,* 80(2): 58-62

Radostit, O.M, Gay C.C, Hinchcliff .K. W and Constable P.D (2007). VETERINARY MEDIC-INE A textbook of the diseases of cattle, horses, sheep, pigs and goats 10th ,Edition Saunders LtdISBN: 978-0-7020-2777-2 Pp 1167-1173

Rahimi, Pooneh, Sohrabi Amir, AshrafielanJavadetEdalat Rosita, Alamdari Mehran Masoudi Ohammadhossein, Mostofi Saied, and AzadmaneshKayhan (2010).Emergence of African Swine Fever Virus, Northwestern EMERGING INFECTIOUS DISEASES Iran Pp1946.

Sarma, Dilip. Kumar. (2012). *A Textbook of Veterinary Virology and Viral Disease* 3rd Revised Edition Asfaviridae. Published by Mrs Usaha Raj Kumar for Kalyani Publishers, -New Delhi-Noida 100 002 Pp139-142