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Effects of African Swine Fever infection on blood of Pigs from Selected Local Government Areas in Nasarawa State, Nigeria

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Abstract
African Swine Fever (ASF) is a notifiable highly infectious lethal haemorrhagic disease in domestic pigs. Indirect enzyme-linked immunosorbent assays (ELISA). was carried out to detect antibodies against ASF virus and haematological investigations were also carried out on blood samples collected from 127 pigs from various piggeries and slaughter houses from selected Local Government Areas in Nassarawa State Nigeria. 1(0.79%) *pig was positive to ELISA haematological investigations consisting of packed cell volume (PCV), red blood cell count (RBC), and white blood cell count (WBC). The mean PCV, RBC and WBC values of pigs sero-negative for ASF were 32.64±6.509, 12.97± 3.653 and 10.30±5.397/ml respectively while the values of pig sero-positive for ASF was 32.80± 0.00 , 13.00±0.00 and 20.60±0.00/ml respectively. It was observed that the PCV values (*32.800± 0.00*) of the sero-positive pig and sero-negative pigs (*32.64±6.509*) were not significantly different from the normal value; it was also observed that the sero-positive pig had higher mean WBC count than the normal and also mean sero-negative pigs. There was increase in the mean values of PCV, RBC and WBC count caused by African Swine Fever infection due to response to the disease infection.*

Keywords: African swine fever, Blood parameters, packed cell volume, red blood cell count Nassarawa State

INTRODUCTION
The livestock industry in tropical Africa consists of mainly cattle, sheep, goats, pigs and poultry (Resource Inventory and Management, 1993). The livestock sector accounts for 4.5 to 5% of the gross domestic products (GDP) in Nigerian economy (Shaw and Hoste, 1987). Approximately one-fifth of the world's pigs are found in the tropics and the production in the tropics is increasing more rapidly than the mid latitude regions (Williamson and Payne, 1984). According to 1993 record by Resource Inventory and Management (RIM) cattle, sheep, goats, pigs and poultry form the livestock industry in tropical Africa.The swine industry has witnessed an unprecedented increase in production and consumption over the past decades and this situation is likely to continue. African swine fever (ASF) is a notifiable, highly contagious, lethal haemorrhagic disease in domestic pigs (Rahimi *et al.,* 2010). The African swine fever virus (ASFV) is an enveloped double stranded DNA virus, and is the only known DNA arbovirus. Maintenance and transmission of ASFV involve cycling of the virus between soft ticks of the genus *Ornithodoros* and wild pigs (warthogs, bush pigs, and giant forest boars) (Rahimi *et al.,*2010). The virus can be acquired through ingestion of contaminated feed

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(Rahimi *et. al.*, 2010). The disease that was initially declared in Lagos and Ogun States has now been reported in almost all the Southern and Middle Belt States of Nigeria (El-Hicheri, 1998). In certain areas, the major losses were restricted almost entirely to the poor rural pig-owners. In certain areas most of the declared uninfected States. The declaration ASF-free statuses in Nigeria were based on absence of ASF reporting which was not satisfactory (El-Hicheri, 1998). The disease has been described by Ayoade and Adeyemi, (2003) as a devastating viral disease that has been threatening the pig industry worldwide. Mortality of 125,000 was recorded in 9 States of the Federal Republic of Nigeria namely Lagos, Ogun, Kaduna, Benue, Enugu, Akwa Ibom, Rivers, Plateau and Delta States (El-Hicheri, 1998).

MATERIALS AND METHODS

Study Area

The study was conducted in Nassarawa State which is geographically located in Northern Nigeria. and located in latitudes of central position 080^{o}n -35^{o}n and longitudes of central position 08^{o} E – 10.2^{o} E. Land area is 27,116.8 square kilometers (Dada *et al.*,2010). It shares common borders in the north with Kaduna State, in the west with the Abuja Federal Capital Territory, in the south with Kogi and Benue States and in the east with Taraba and Plateau States. According to Dange, (2010) the State has thirteen Local Government Areas (LGAs). The LGAs are grouped into Nassarawa North, Nassarawa West and, Nassarawa South Senatorial Zones. The State capital is situated in Lafia. The LGAs used for the study are shown in Figure 1.

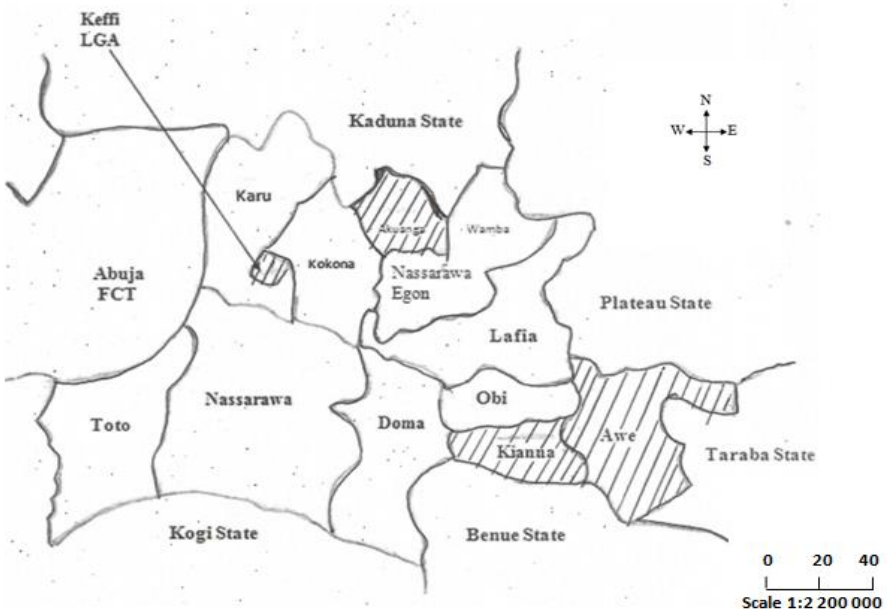

Figure 1 Map of Nassarawa State and Local Government Areas Shaded used for the study areas
Source: Dange, 2010

Collection of blood samples and serum preparation

The blood samples collected was from live pigs in various piggeries and slaughter houses. Ten milliliters of blood was collected from the jugular vein of pigs following restraint by an assistant. 5mlls of the blood was put in an EDTA bottle and processed for haemogramme in the laboratory. The remaining 5ml. of blood was put in a non-EDTA bottle for serum preparation as recommended by Brown, (1993). The various numbers of blood samples collected from different pigs and places **(**i.e**.** LGAs**)** were 15 from Akwanga, 10 from Keffi, 50

from Awe, and 52 from Kiaana LGAs from January to December 2017. The samples were collected from the various LGAs by conveniences.

Laboratory hematology and serology

The blood samples containing anticoagulant were used for hematology while those for sera were allowed to clot and then centrifuged at 1500 g for 15 minutes at -20 °C until examined. Both samples were kept in a cooler **(**in ice-pack**)** to the laboratory for analysis. The serology used was enzyme-linked immunosorbent assays **(**ELISA**).** The ELISA was as carried out as recommended by the manufacturer called The French company in 2013 called ID.vet **(**Innovative Diagnostics**).**

The procedures as described by the manufacturers was followed strictly. The wash concentrate (20X) was brought to room temperature and then mixed thoroughly to ensure that it was completely solubilised. The solution 1X was prepared by diluting the wash concentrate (20X) to 1/20 in distilled water. All reagents were allowed to come to room temperature (16 - 26^{o} C) before use and reagents were homogenized by inversion or vortex. Each sample was deposited twice (adjacently in even and odd-numbered wells). The ELISA plate had even-numbered microwells that were coated with p32, p62 and p72 ASFV recombinant proteins, The odd-numbered wells were uncoated. Samples to be tested and controls were added to even and odd- numbered wells with anti-ASFV antibodies using micropipette, formation of agglutination shall be taken as positive result. After washing, an anti-multi- species horse radish peroxidase (HRP) conjugate was added to the wells. It fixed to the antibodies, forming an antigen-antibody-conjugate-HRP in the presence of antibodies, into blue solutions which became yellow after addition of the stop solution. In the absence of antibodies, was no coloration,

The microplates were red at 450nm. ELISA recorder and computer were used to record and result shown were printed out. This was followed by the addition of 100 ul of the dilution buffer 14 to each well and 10 microlitre of the negative control to wells A1, B1 and A2, and B2 while 10 ul of the positive control were added to wells C1, D1 and C2, D2 and 10ul of each sample to be tested were added 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 with 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 with of wash 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 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 microplates O.D was recorded at 450nm as shown by the ELISA and computer. The recorded result were calculated as follows

$$(S/p\%) = \frac{\text{Net OD sample}}{\text{net OD PC}} X\ 100$$

O.D net = O.Deven - O.D odd well
S/P percentage (S/p%) was calculated
Samples with an S/p%: Less than or equal to 60% were considered negative;
Greater than or equal to 60% were considered positive

RESULTS

Out of the total number of 127 serum samples tested, antibodies was detected in 1(0.79%) ie 1(2.0%) was sero-positive (for ELISA) out of 50 samples from Awe Local Government Area The sero-negative samples were 15 from Akwanga, 52 from Kiaana and 10 from Keffi LGAs.Haematological parameters of sampled pigs that were tested for with ASF antibody (for ELISA).are shown in Table 1

Table 1: Seroprevalence of ASF based on selected Local Government Areas of Nassarawa State, Nigeria.

Senatorial District	Local Government Area	Number of Pigs Tested	Percentage (%) Occurrence
Nassarawa West	Keffi	10	0.0
Nassarawa North	Akwanga	15	0.0
Nassarawa South	Awe	50	2.0
	Keana	52	0.0
Total	4	127	0.8

Chi Square ($\chi 2$) =6.279; P=0.616

Table 2 : Haematological parameters of pigs that are normal with sero-negative and sero-positive in Nasarawa state s

	PCV	RBC	WBC	Lymph	Neot	Eos	Baso	Mono
Nomal parameters	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– 2.0
Mean sero-negative	32.64± 6.509	12.97± 3.653	10.30± 5.397	61.50± 7.564	6.18± 11.42	1.632± 2.191	0.440± 1.247	0.000± 2.659
Mean sero-positive	32.800± 0.00	13.00 ±0.00	20.60 ± 0.00	57.00 ± 0.00	38.00 ±0.00	0.00 ±0.00	0.00 ±0.00	5.00 ±0.00

RBC_____ Red blood cell count
WBC_____ white blood cell count
PCV_____ Packed cell volume in %
Lym_____Lymphocytes in %
Neu_____ Neutrophils
Eos_____ Eosinophils
Bas_____ Basophils
Mon_____ monocytes

The mean lymphocytes counts for the sero-negative pigs for ASF (Table 2) was 61.50±7.564% .The lymphocyte counts for pig that was sero-positive (Table 2) with the value of 57.00±0.00%,

DISCUSSION

Nassarawa state had the detection rate of 0.79% in this study, though this was observed in one LGA out of 4 LGAs sampled..

The PCV values of the sero-positive pig and the mean PCV seronegative pigs were not significantly different from the normal value even though there was higher detection rate of antibodies to ASFV infection. This might indicate the stage of the disease at which the blood samples were collected. Since the values were at the normal it may indicate an ongoing initial heamorhagic syndrome as reported by Radostits *et al* (2007) and Zimmaerman *et al* (2012).
Considering the differential of total WBC of pigs examined it was observed that the sero-positive pig had higher WBC count than the normal and also mean seronegative . 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 value of the basophils in the sero-positive pig was lower than normal values and also lower than the value of seronegative pigs .This indicated ASFV infection. Considering the mean monocytes count the value for sero-positive was much higher than normal and also higher than mean values of seronegative 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).

CONCLUSION

The study showed that ASF caused an increase in the mean values of PCV, RBC and WBC values in pigs. .Whenever haematological parameters of pigs are taken and there is high packed cell volume. red blood cell count and white blood cell count, ASF should be suspected and ELISA should be carried out to confirm the infection. Whenever haematological parameters of pigs are taken and there is high PCV, RBC and WBC sera of pigs should be tested for ASF antibodies by ELISA method

the following actions are necessary in other :to prevent the spread of ASF

(i) Biosecurity measures such as control of people and provision of foot deep so as to prevent the spread of the virus .

(ii) Movement of pigs from one part of the country to another should be well monitored and must be animals are confirmed to be free from the virus by veterinarians .

(iii) Affected pens should be disinfected with strong solution of caustic soda not less than 4 months before new pigs are reintroduced.

(iv) There is need to quarantine all in coming animals and compulsory slaughter of infected and contact animals and other animals at risk. with adequate compensation.

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