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Effect of African Swine Fever Infection on Some Blood Parameters in Pigs from Selected Local Government Areas in Benue State, Nigeria

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Abstract

African swine fever (ASF) is a notifiable highly infectious lethal haemorrhagic diseasein 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 one hundred and eighty seven pigs from various piggries and slaughter houses from selected Local Government Areas in Benue State Nigeria. Thirteen (6.95%) pigs were . 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 negative for ASF were 32.38 ±10.94, 7.49 ±5.32, 8.38 ±10.64/ml. respectively while the values of pigs positive for ASF were 35.50 ±11.35, 10.03 ±4.05 and 9.10 +-6.-25/ml. respectively. It was observed that the mean PCV values (35.50±11.35) of the sero-positive pigs and sero-negative pigs (32.38±10.94) were not significantly different from the normal value; it was also observed that the sero-positive pigs had higher mean WBC count than the normal and also mean seronegative pigs. In conclusion, ASF infection caused increase in the mean values of PCV, RBC and WBC counts.

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

INTRODUCTION

According to 1993 record by Resource Inventory and Management (RIM) cattle, sheep, goats, pigs and poultry form the livestock industry in tropical Africa. The livestock sector accounts for 4.5 to 5% of the gross domestic products (GDP) (Shaw and Hoste, 1987) in Nigerian economy. About one-fifth of the world's pigs is found in the tropics and the production in the tropics is increasing more rapidly than the mid latitude regions (Williamson and Payne, 1984). The swine industry has witnessed an unprecedented increase in production and consumption over the past decade 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 (Rahimi *et. al.,* 2010). The disease that was initially declared in Lagos and

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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. ASF-free statuses in Nigeria were basing their declaration was based on absence of ASF reporting which was not satisfactory (El-Hicheri, 1998). African Swine Fever is known worldwide as a devastating viral disease that has been threatening the pig industry (Ayoade and Adeyemi, 2003). In the middle of July 2013 there was a reported outbreak of ASF on the farm belonging to the Swine Research Institute Otupko Benue state where 55 (63.64%) died and the remaining 36.36% were culled. The organs and blood samples sent to the National Veterinary Research Institute Vom confirmed that it was ASF.

MATERIALS AND METHODS

Study Area

The study was conducted in Benue State which is geographically located in Northern Nigeria. and between latitude 6° 25'N and 8° 8'N, and longitude 7°47'E and 10°'E(Dada *et al.*, ,2010).. It covers an area of 30,955km^2 (Nigeria, 2007). Benue State shares common borders with six other States namely Nassarawa State in the North, Taraba State in the East, Kogi State in the West, and Enugu, Ebonyi and Cross River States in the South and an International border with Cameroon Republic in the Southeast (Nigeria, 2007), It has twenty three Local Governments Areas with several ethnic groups. The LGAs are grouped into Benue North-East, Benue North-West and, Benue South Senatorial Zones. The State capital is situated in Makurdi. The LGAs used for the study are presented in Figure 2

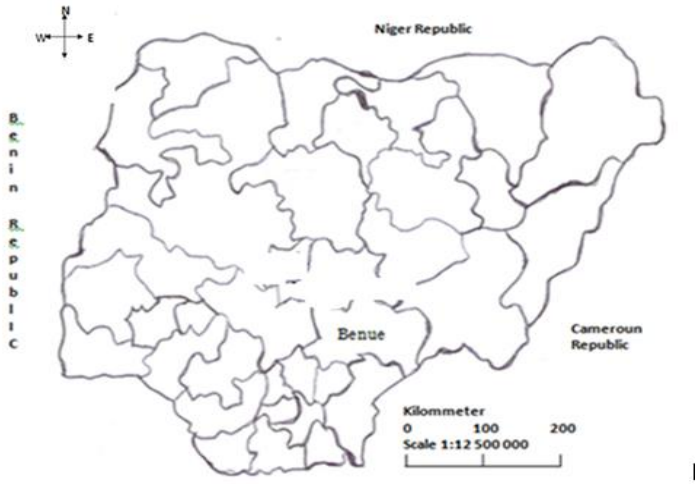

Figure 1: Map of Nigeria showing Benue State used for the study.
Source: Dada *et al.*, (2010)

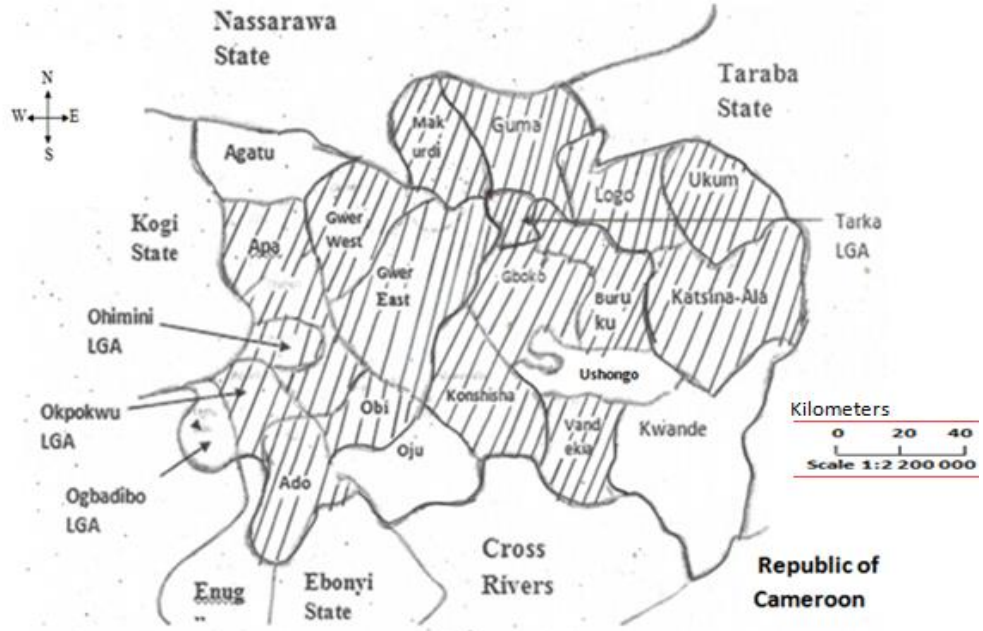

Figure 2: Map of Benue State and Local Government Areas .Shaded areas show the study area (Nigeria 2007).

Study Materials

Consumable materials that were used for the study are serum sample collection bottles, vacuotaner tubes, heparinised tubes, methylated spirit, viral transport medium, needles and syringes (2 ml. and 5 ml.). ELISA kits and result sheets. 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 (H_2SO_4, 0.5m.). Others include adsorbent paper , Glass pipettes for volume of 1-10 ml. power supply, and single channel pipette 1.

The non-consumable materials used for the study are thermometer, weighing ban, cooler (for transporting samples), 96-well microplate reader, manual or automatic wash system, and camera thers are freezer, ELISA recorder and computer, photograph, equipment and printer. Freezer <-10 °C , freezer ≤-70 °C, Fridge 4±30 °C , Tray for horizontal agarose gels, tank, combs, UV transiluminator, and vortex.

Collection of blood samples and **serum preparation**

The blood samples collected are blood from live pigs in various piggeries and slaughter houses. Ten milliliters of blood were collected from the jugular vein of pigs following restraint by an assistant. Five milliliters of the blood were put in an EDTA bottle and processed for haemogramme in the laboratory. The remaining 5ml. of the blood were put in a non-EDTA bottle for serum preparationas recommended by Brown, 1993. In summary the various numbers of blood samples collected from different pigs and places **(i.e** LGAs**)** were six from Ukum, fifteen from Makurdi, ten from Gboko, ten from Buruku, ten from Guma, ten from Gwer East, ten from Gwer West, ten from Tarkaa, ten from Katsina Ala, ten from Konshisha, ten from Logo, ten from Vandekya, thirty two from Oturpo, eight from Apa, seven from Ado, seven from Obi, seven from Okpokwu, and five from Ohimini LGAs

Laboratory hematology and serology

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

The procedure as described by the manufacturers was followed strictly. Briefly, 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/deiodised 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) Serum samples. The ELISA plate had even-numbered microwells that were coated with p32, p62 and p72 ASFV recombinant proteins,while odd-numbered wells were uncoated.

Samples to be tested and controls were added to even and odd- numbered wells with anti-ASFV antibodies with the aid of micropipette, and formed an antigen-antibody complex where antibody was present. 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, no coloration appeared.

The microplates were read at 450nm. ELISA recorder and computer were used to record and result shown by ELISA recorder and computer were printed out. This was followed by the addition of 100 ul of the dilution buffer 14 to each well and tenmicrolitre 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 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 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 red and recorded at 450nm. Result sheets were used in recording the result shown by the ELISA recorder and computer. The results were read as:X 100

Well-O.D result was calculated as follows

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

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

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

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 187 serum samples from 187 different pigs tested, antibodies were detected in 13 (6.95%) i,e 13 (6.95%) were sero-positive (for ELISA). Positive samples were from seven .LGAs. namely Gboko, Tarka, Katsinaala, Otukpo, Ado, Obi and Ohimini. .
Haematological parameters of sampled pigs that were tested for with ASF antibody (for ELISA).are shown in Table 1

Table 1: Seroprevalence of ASF by Local Government Areas of Benue State, Nigeria.

	Local Government Area	Number of Pig Tested	Number of Pigs Positive	Percentage (%) Occurrence
Benue North-West				
1	Makurdi	15	0	0.0
2	Gboko	10	2	20.0
3	Buruku	10	0	0.0
4	Guma	10	0	0.0
5	Gwer East	10	0	0.0
6	Gwer West	10	0	0.0
7	Tarka	10	1	10.0
SubTotal	7	75	3	4.0
Benue North-East				
8	Katsina la	10	2	20.0
9	Konshisha	10	0	0.0
10	Logo	10	0	0.0
11	Ukum	6	0	0.0
12	Vandekia	10	0	0.0
Sub Total	5	46	2	4.3
Benue South				
13	Otukpo	32	2	9.1
14	Apa	8	0	0.0
15	Ado	7	3	42.9
16	Obi	7	2	28.6
17	Okpokwu	7	0	0.0
18	Ohimini	5	1	20.0
Sub Total	6	66	8	12.1
Ground Total	18	187	13	7.0

Chi Square ($\chi 2$) =22.92; P=0.05*

Table 2 : Normal Haematological parameters of pigs (Studdert *et al.*, 2012)

PCV	RBC	WBC	Lymph	Neot	Eos	Baso	Mono
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

Table 3: Mean Haematological parameters of pigs sero-negative in Benue state

PCV	RBC	WBC	Lymph	Neot	Eos	Baso	Mono
32.38 ±10.94	7.488 ±5.321	8.384 ±10.64	51.49 ±18.85	43.33 ±37.54	4.902 ±10.90	0.3963 ±1.346	1.549 ±1.910

Table 4: Mean Haematological parameters of pigs sero-positive in Benue state

PCV	RBC	WBC	Lymph	Neot	Eos	Baso	Mono
35.50 ±11.35	10.03 ±4.045	9.100 ±6.-25	37.77 ±28.27	46.75 ±21.18	12.75 ±17.15	0.1667 ±0.5774	2.583 ±3.088

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

Differential leucocyte counts of pigs infected with African swine fever virus
The mean lymphocyte counts for pigs that were sero-positive for ASF (Table 4) showing mean of 37.77±28.77%, The corresponding mean lymphocytes counts for the sero-negative pigs for ASF (Table 3) was 51.49±18.85% ..

DISCUSSION

Benue State had the detection rate of 7.0 % in this study, though this was observed in seven (7) LGAs out of 18 LGAs sampled. Makurdi LGA recorded zero detection rate.

The mean PCV values of the sero-positive pigs and sero-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 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 pigs had higher mean WBC count than the normal and also mean sero-negative pigs. This may indicate an ongoing progressive responce 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 basophils in the sero-positive pigs were lower than normal values and also lower than the value of sero-negative 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 sero-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).

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

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