https://dx.doi.org/10.4314/dujopas.v9i2b.28
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 9 No. 2b June 2023*

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

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Abstract
Indirect Enzyme-linked immunosorbent assays (ELISA) was carried out to detect antibodies against African Swine Fever (ASF) virus and haematological investigations were also carried out on blood samples collected from 299 pigs from selected Local Government Areas in Kaduna State Nigeria. The serum samples from 10 pigs (3.34%) were positive by ELISA haematological investigations consisted of packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC). The mean PCV, RBC and WBC values of pigs negative for ASF were 34.84±9.611, 6.299±6.093 9.953±5.565/ml respectively while the values of pigs positive for ASF were 32.30±6.464, 5.160±1.182 and 9.740±4.37 /ml respectively. It was observed that the mean PCV values of the sero-positive pigs and seronegative pigs 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, African Swine Fever infection caused increase in the mean values of PCV, RBC and WBC count.

Keywords: African Swine Fever, Blood, ELISA, Kaduna State

Introduction
Cattle, sheep, goats, pigs and poultry are the main livestock industry in tropical Africa (Resource Inventory and Management 1993) About 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). 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*. Maintenace 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).The virus can be acquired through ingestion of contaminated feed (Rahimi *et al.,*2010). Apart from the outbreak in 1973, nothing suggests that the ASF epizootic was declared in Nigeria before 1997 (El-Hicheri, 1998). African Swine Fever 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. ASF-free statuses in Nigeria were basing their declaration only on the absence of ASF reporting which was not satisfactory (El-Hicheri, 1998). African Swine Fever is a devastating viral disease that

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has been threatening the pig industry worldwide (Ayoade and Adeyemi, 2003). Death of pigs (125,000) due to ASF has been reported in following states of the Federal Republic of Nigeria: Lagos, Ogun, Kaduna, Benue, Enugu, Akwa Ibom, Rivers, Plateau and Delta States (El-Hicheri, 1998).

Materials and methods

Study Area

Kaduna State shares borders in the North West part of Nigeria with Zamfara States, Katsina and Kano States in the North in the West with Niger State, the East with Bauchi and Plateau States and in the South with Nassarawa State and Federal capital Territory Abuja. The global location of the State is between Longitude 06° and 11° 3' north of the Equator. The State occupies an area of approximately 48,732.2 square kilometers.(Jallo, 2000).Kaduna State has 23 Local Ggovernments Areas (Jallo, 2000). The 23 Local Governments Areas are grouped into Kaduna North, Kaduna Central and Kaduna South Senatorial Zones. The State capital is Kaduna. The LGAS used for the study in Kaduna State with the LGAS are shown in figure 1.

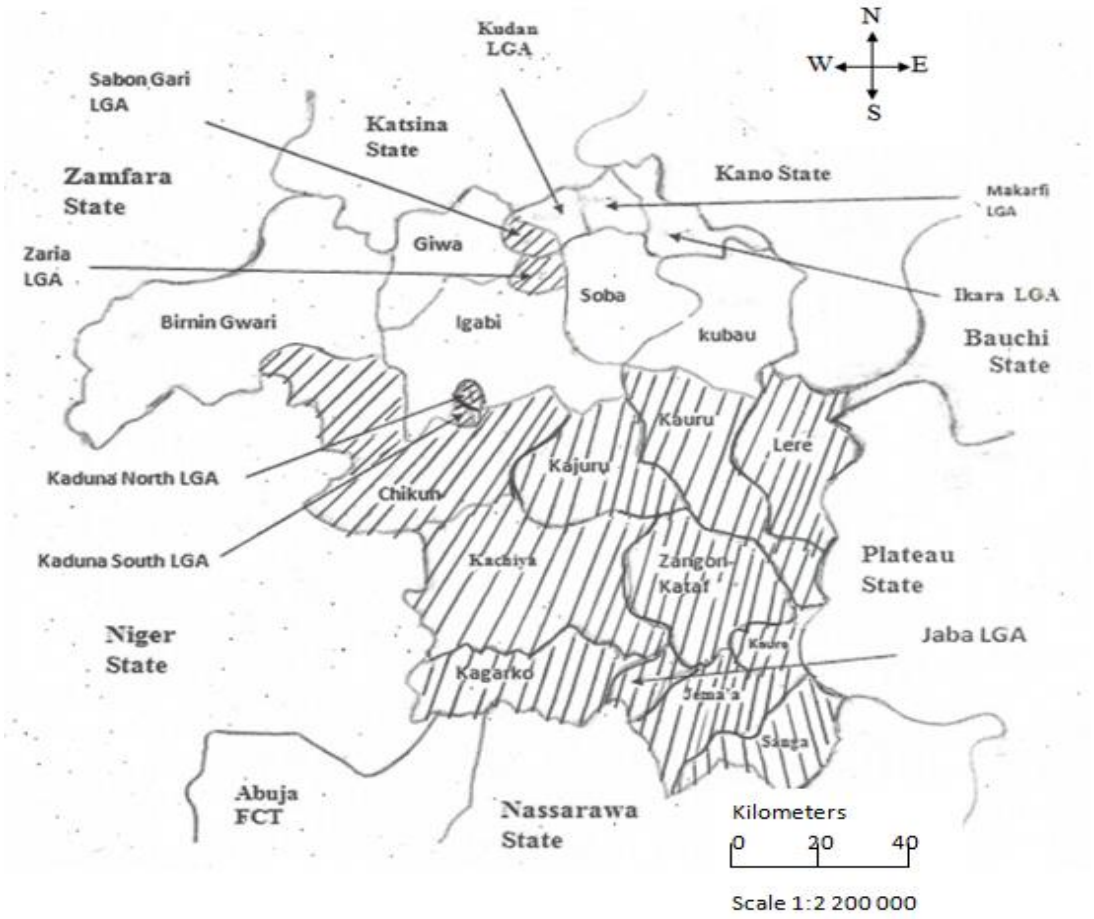

Figure 1 Map of Kaduna State showing study areas

Blood samples collection, serum preparation

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 preparation.

Table1 Seroprevalence of ASF by Senatorial districts and Local Government Areas of Kaduna State, Nigeria.

Senatorial District	Local Government Area	Number of Pigs tested	Number of Pigs positive	Percentage (%) Occurrence
Kaduna North				
1	SabonGari	20	2	10.0
2	Zaria	55	0	0.0
3	Lere	18	2	16.7
Sub Total	3	93	4	4.3
KadunaCentral				
4	Kaduna North	11	0	0.0
5	Kaduna South	15	0	0.0
6	Chiukun	15	0	0.0
7	Kajuru	19	0	0.0
SubTotal	4	60	0	0.0
Kaduna South				
8	Kachiya	25	2	8.0
9	ZangonKataf	10	0	0.0
10	Jaba	18	1	5,6
11	Jama'a	19	1	5.3
12	Kagarko	31	0	0.0
13	Kaura	15	1	6.7
14	Kauru	10	0	0.0
15	Sanga	18	1	5.6
SubTotal	8	146	6	4.1
Ground total	15	299	10	3.3

Laboratory hematology and serology

The blood containing anticoagulant were used for hematology while those for sera were allowed to clot and then centrifuged at 1500 g for 10-15 minutes for the purpose of separation of the serum and stored at -20^{O}C until examined. Both samples were kept in a cooler **(**in ice-pack**)** 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 ID.vet Innovative Diagnostics 2013 from France. The mean values were calculated with the confidence interval (CI).

Results

Out of the total number of 299 serum samples, antibodies were detected in 10 pigs or 3.34% were sero-positive for ELISA .Positive samples were from 7 Local Governments Areas (.LGAs.) which are Sabon Gari, Saminaka Kachiya Jaba Jama'a Kaura and Sanga LGAs. Haematological Parameters of sampled pigs that were tested for with African Swine Fever antibody (for ELISA).are shown in Tables 2.

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

	PCV	RBC	WBC	Lym	Neu	Eso	Bas	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 seronegative	34.84± 9.611	6.299± 6.093	9.953± 5.565	64.75± 11.51	30.86± 8.807	1.237± 1.319	0.1498± 0.4447	1.355± 1.268
Mean seropositive	32.30± 6.464	5.160± 1.182	9.740± 4.-37	59.70± 16.23	51.30± 9.922	1.500±1.2 69	0.200± 0.6325	1.800± 1.135

Wt----------Weight in Kilogram
RBC----------Red blood cell count in $x10^{12}$IL
WBC----------white blood cell count in $x10^{9}$IL
PCV----------Packed cell volume in %
Lym---------- Lymphocytes in %
Neu---------- Neutrophils in %
Eso---------- Esonophils in$x\ 10^{9}$IL
Bas---------- Basophils in$x10^{9}$IL
Mon---------- Monophils in $x10^{9}$IL

Differential leucocyte counts of pigs infected with African swine fever virus

The lymphocyte counts for pigs that were seropositive for ASF (Table 3) showing mean of 59.70±16.23%, The corresponding lymphocytes counts for the seronegative pigs (Table 2) was 64.75±11.51%.

The overall detection of antibodies to ASFV in Kaduna State was 3.3%. This may indicate that African Swine Fever infection is presently endemic in the State. Its maintainance may be due to frequent contact with infected pigs by borrowing boar for breeding puorpose or through infected inanimate objects such as boots, vehicles, or used syringesor visitors for of pig industries especially butchers (Adenaike *et al.,* 2016)

Discussion

Kaduna State had the detection rate of 3.3%. in this study, though this was observed in 7 LGAs out of 23 LGAs sampled.. The mean PCV values of the sero-positive pigs and seronegative pigs were not significantly different from the normal value even though there was higher detection rate of antibodies to ASFV infection. The seronegative groups had than lower normal PCV while the sero-positive groups had less 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 Zimmaerman *et al (*2012) and Radostits et al (2007).

Considering the differential of total WBC of pigs examined it was observed that both the sero-positive and seronegative pigs had lower mean WBC count than the normal. and also mean seronegative pigs had higher WBC than sero-positive in pigs. In an experimental infection of pigs with the ASFV (Dixon et al 2000) cited by Karalyan et *al (*2012) reported neutropenia and lymphopenia in the later stage of the disease.

The mean value of the basophils in the sero-positive pigs were lower than normal values but lower than the value of seronegative pigs .This indicated ASFV infection. Considering the mean monocytes count the value for sero-positive was higher than normal but lower 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 African swine fever made the mean values of packed cell volume. red blood cell count and white blood cell count to be at the lower range of the normal values in the pigs. Experimental infections of various breeds of pigs of different ages by African swine fever virus on different breeds of pigs and different age and sex need to be carried out were facilities are available

Haematological parameters may not be a reliable source of suspecting pigs to be a carrier of ASF since the parameter can vary based on level of stage of the viral infection ,However any suspected pig should be tested for ASF antibodies by ELISA method and the following action should be carried out:

1) Biosecurity measures such as control of personnel from other pig farms, visitors, butchers and provision of foot deep (Zimmaerman *et al* 2012).

2) 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 (Zimmaerman *et al* 2012).

3 Contaminated pig pens should be disinfected with strong solution of caustic soda 4 months before animals are reintroduced .since the site can remain infective for period exceeding 3 months (Radostits *et al* 2007).

4) Quarantine, compulsory slaughter of infected and contact animals and other animals at risk with adequate compensation to the owners (Radostits *et al* 2007 and Zimmaerman *et al* 2012)..

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