



Haematological Changes Associated with Porcine Haemoparasitic Infections in Ibadan, Oyo State, Nigeria

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SUMMARY

The study was carried out between January and July 2016. Blood samples were obtained from 153 pigs by venipuncture and jugular severance at slaughter. The blood samples were examined for all known hemoparasites detectable by light microscopic examination. Haematimetric indices, complete blood cell count and leukocyte differentials were determined. The level of parasitaemia and changes in blood indices were subjected to statistical analysis across seasons. *Trypanosoma brucei* and *Eperythrozoon suis* were the only hemoparasites detected in the blood of pigs during the period of sampling. The prevalence of haemoparasitic infections in sampled pigs was 5.23%. *T. brucei* contributed 3.9% while *E. suis* contributed 1.31% to the prevalence. Anaemia (PCV<32) was a consistent and significant finding in all parasitemic samples. *Eperythrozoon suis* caused more severe anaemia (20 ± 9.89) when compared with *Trypanosoma brucei* (27 ± 3.03). The anaemia caused by *E. suis* was mostly microcytic normochromic while *T. brucei* mostly caused normocytic normochromic anaemia. Mild leucopenia was observed in eperythrozoonosis while a moderate lymphocytosis was observed in *T. brucei* infections. It was observed that in spite of intense chemoprophylaxis and other control measures employed, we still have persistent infections with *Eperythrozoon* sp and *Trypanosomes* in our pig population. Further studies should be carried out to detect the possibility of drug resistance by some of these circulating hemoparasites in the pig industry. Attempts should also be made to control the vectors of these parasites which are usually abundant during the rainy season and may be responsible for the higher prevalence recorded during this period.

Key words: Porcine, Infection, Haemoparasites, Haematological changes, Nigeria.

INTRODUCTION

Pigs are one of the sources of animal proteins (Olayeni *et al.*, 2006) and are majorly kept for meat (pork) and lard (fats) production. Other sources of animal protein include beef, milk, poultry, eggs, fish and game animals. Of these sources, pork represents one of the fastest means of increasing animal protein supply due to the fact that pigs grow at a speedy rate, can convert food waste to valuable products and they are more prolific than cattle, sheep and goats (Ajieh and Okwuolu, 2015). Pork is a very important source of protein. Many developing countries, as well as Nigeria, are facing a shortage of dietary protein. The negative consequence of this shortage is on the rise with increasing population and urbanization (Okpor, 1999).

The pig is not only a source of protein, it also serves as an investment alternative and additional income especially in the rural areas (M.K Ajala *et al* 2007). Despite the benefits of pig production, there are several limitations which include; losses due to disease which could present in different forms; inadequate feeding in terms of quality and quantity; housing problems and overcrowding; taboo such as in most parts of the northern states and some parts of the south western states where people are discouraged from eating pork due to religious and cultural reasons, hence, a drawback to pig production (Blench, 2000). Diseases affecting pigs are mostly parasitic (Ironkwe and Amefule, 2008).

Parasites of pigs cause major economic losses globally to both the pig and pork industries. This usually stems from reduced feed conversion and poor weight gain (Joaching and Dulmar, 2001; Boes *et al.*, 2000). The most prevalent blood parasites of pigs are; *Trypanosoma* spp, *Eperythrozoon* spp, *Babesia* spp and *Anaplasma* sp. (Finelle, 1973; Levine, 1985).

Trypanosomosis is a parasitic infection caused by the introduction of *Trypanosoma* species such as *T. simiae*, *T. brucei*, *T. evansi* and *T. congolense* by tsetse flies' bites into blood circulation. The trypanosomes then flow freely in the plasma and continually stimulate immune response by changing its surface coat antigens, causing intermittent fever. Clinical signs include development of chancre at the site of tsetse fly bite within 5 to 15 days after fly bite, intermittent fever, anaemia and weight loss (Abenga, 2014). Necropsy findings are not specific, in acute cases, extensive petechiation of the serosal membranes (Morris *et al*, 1981), swollen lymph nodes and spleen (Losos, 1986) while in chronic cases, swollen lymph nodes, serous atrophy of fat and anaemia are seen (Losos and Ikede, 1972).

Eperythrozoonosis is a rickettsial infection caused by *Eperythrozoon suis* which is transmitted mechanically by arthropods. The main method of transmission in pigs is through lice (Claxton and Kumesh, 1975). The parasites attach to the surface of erythrocytes and cause deformity and damage to the erythrocytes. The clinical signs in pigs are fever, staggering or paralysis, pale mucous membranes, emaciation and jaundice (Hsu 1986). "Reproductive failure and weakness in piglets (Gwaltney, 1995). The infestation of these parasites is inimical to the production of pig and invariably causes reduction of availability of pork and lard and by extension result in protein depletion from sources that depend on pig. The purpose of this study is to evaluate the haematological alterations associated with porcine haemoparasitic infections caused by Trypanosomosis and Eperythrozoonosis which would aid rapid diagnosis of the infections and stimulate their effective control.

MATERIAL AND METHODS

Study site

The study was carried out in Ibadan, Oyo state. Ibadan is the largest city in West Africa and second largest in Africa with an estimated population of over 2,550,593. Ibadan city lies on longitude 3°5' East of Greenwich meridian and latitude 7°23' North of the Equator (Filani *et al.*, 1994). Animals slaughtered in Bodija abattoir (sample site) alone accounts for 65.93% of the total animals slaughtered in Oyo state (Abiola, 1995).

Sample collection

Blood samples were collected at the Bodija Municipal abattoir from 153 pigs. 3mls of blood were collected from severed jugular veins of each animal into ethylene diamine tetra-acetic acid (EDTA) bottles. The samples were then transported in a vacuum flask with ice packs to Veterinary Clinical Pathology Laboratory, Faculty of Veterinary Medicine for haematological analyses within one hour of collection.

Haematological parameters

Red blood cell count

This was done according to the method of David B. Fankhauser, 2003, with little modifications. 1ml of red blood cell diluent was poured into a dilution bottle, 10 microlitres of blood was drawn with the aid of a micropipette and added to the dilution bottle containing the diluent. 3-5 drops of this mixture was dispensed to fill the Neubaur's counting chamber and allowed to settle for 3 seconds. Then it was viewed under the light microscope, all red cells in 80 small squares were counted and the figure multiplied by 10,000.

White blood cell count

About 1ml of Turk's solution was dispensed into dilution bottles according to Pagana K. D. *et al.*, 1997 with little modifications, 50 microlitres of blood was pipetted and added to the dilution bottles. The mixtures were allowed to mix and thereafter dispensed onto the Neubaur's counting chamber. Under the light microscope, on the Neubaur's counting

chamber, all white blood cells in 64 large squares were counted and the figure multiplied by 50.

Platelet count

The same method with WBC count was used but the platelets were counted in 64 large squares and multiplied by 1000 (Sarma, 1990).

Leucocyte differentials

A differential determines the percentage of each of the five types of mature white blood cells. The five different types of white blood cells include neutrophil, basophil, lymphocyte, eosinophil and monocytes. The manual method of using differential counter was employed to count each cell type on a stained slide under the light microscope at magnification of 1000 (Stamminger *et al.*, 2002).

Packed cell volume (PCV)

Heparinized capillary tubes were inserted into each sample bottle containing anticoagulated blood and allowed to fill to two-thirds by capillary action. The capillary tubes were sealed with plasticene to avoid spillage. They were arranged in the micro centrifuge and the centrifuge was allowed to spin at 3,000 revolutions per minutes for 5 minutes. After 5 minutes, the packed cell value (PCV) of each blood sample was read with micro haematocrit reader by placing the capillary tube on the reader. The junction between red cell and plasticene was positioned on the lowest line (black) on the reader while the topmost end of the plasma was adjusted to the uppermost line on the reader and the middle line of the reader was adjusted just below the buffy coat in order to determine the PCV (David B. Fankhauser, 2003).

Haemoglobin concentration

Using the Sahlis apparatus graduated tube, 20ml of HCl was poured into the tube (using lower meniscus). 20 microlitres of blood was pipetted from the sample bottles using the micropipette and poured into the

Sahlis apparatus tube containing normal HCl. This mixture was left to react for 5 minutes. After 5 minutes, the mixture was then compared with the standard Sahlis comparator in terms of colour. If the colours do not tally, the mixture was diluted with distilled water till the colours matched (Schalm et al., 1975).

Haematimetric indices

Mean corpuscular volume: This is the average volume of individual erythrocyte in a blood sample. The MCV for each blood sample was calculated using the formula (Sarma, 1990).

$$\text{MCV} = \frac{\text{PCV} (\%) \times 10}{\text{RBC (million/ul)}}$$

Mean corpuscular haemoglobin concentration

This is the average weight of haemoglobin content in a red blood cell and it was calculated using: $\text{MCHC} = \frac{\text{Haemoglobin concentration (g/dl)} \times 100}{\text{PCV} (\%)}$

Smear preparation

With the use of an applicator stick, a drop of the blood sample was placed on the frosted side of a clean glass slide and a spreader was used to disperse out the drop of blood over the slide length forming a feathered end. The aim is to produce a monolayer, where the cells are spaced far enough apart to be differentiated and counted. The smears were air dried, and thereafter immersed briefly for about 3 minutes in methanol for fixing. After fixation, the slide was stained with Giemsa stain and allowed to stain for 30 minutes. Then, the slides were rinsed with water and allowed to dry before viewing under the microscope (Roger S. Riley et al., 1999).

Wet mounts

A drop of blood was made on a slide, covered with a cover slip and then viewed under the microscope for the presence of

moving trypanosomes within the blood. (Woo 1970).

Buffy coat

The capillary tubes after being spun were cut just below the buffy coat and the content (buffy coat) was poured onto a clean slide and covered with a coverslip. These were then examined under the microscope at various magnifications to detect the presence of trypanosomes (Paris et al., 1982; Murray et al., 1983).

Statistical analysis

The data collected in this study was statistically analysed to test significance differences using the paired student's t-test (JCF De Winter, 2013).

RESULTS

Table I, 5.23% of the total blood samples collected was positive for haemoparasites (Trypanosomes and Eperythrozoon) while 94.77% did not contain haemoparasites.

Table II, 4.53% of the total blood samples collected had haemoparasites and were anaemic, 0.65% were parasitaemic but did not show anaemia, 36.60% of the total blood samples were anaemic but aparasitemic, while 58.17% were non-anaemic and aparasitemic.

Table III, Anaemia was observed in haemoparasitic infections caused by *Eperythrozoon suis* and *Trypanosoma brucei*. Eperythrozoonosis caused a more severe anaemia. The anaemia caused by *E.suis* is microcytic anaemia while that caused by *T. brucei* is normocytic normochromic anaemia. Mild leucocytosis was observed in Trypanosomosis while a moderate leucopenia was seen in Eperythrozoonosis, mild neutropenia was observed in Trypanosomosis. Severe thrombocytopenia was observed in haemoparasitic infection caused by *Eperythrozoon suis* and *Trypanosoma brucei*. Values with homozygous superscripts are not significantly different ($P < 0.05$).

TABLE I: Prevalence of haemoparasitic infection

Parasitemic samples	Aparasitemic samples
5.23%	94.77%

TABLE II: Relationship between anaemia and haemoparasitism

Non-anaemic aparasitemic	Anaemic aparasitemic	Anaemic parasitemic	Non-anaemic parasitemic
58.17%	36.60%	4.58%	0.65%

TABLE III: Effects of type of haemoparasites on haematological parameters

Erythrocyte parameter	<i>Trypanosoma brucei</i>	<i>Eperythrozoon suis</i>	P VALUE
Prevalence	3.9%	1.3%	
PCV	27.0 ± 3.03 ^a	20 ± 9.89 ^a	0.605
HB	8.25 ± 1.68 ^a	11.5 ± 0.42 ^a	0.207
RBC count	4.45 ± 0.40 ^a	4.96 ± 0.76 ^a	0.479
MCV	60.62 ± 3.55 ^a	42.36 ± 26.47 ^a	0.574
MCHC	30.60 ± 5.67 ^a	66.13 ± 34.85 ^a	0.419
WBC count	24025 ± 16369.5 ^a	8300 ± 282.84 ^a	0.399
Lymphocytes	69.5 ± 34.65 ^a	33.0 ± 3.84 ^a	0.630
Neutrophils	27.5 ± 31.82 ^a	36.5 ± 12.02 ^a	0.820
Eosinophils	1.5 ± 0.70 ^a	4 ± 2.82 ^a	0.344
Monocytes	1.5 ± 2.12 ^a	1.5 ± 0.70 ^a	1.0
Platelets	86633.33 ± 9399.5	103175 ± 134102.8	0.483

TABLE IV: Effect of the presence of parasites on haematological parameters

	PCV	HB	RBC	MCV	MCHC	WBC	LYM	NEU	MON	EOS	PT
Positive	25.25 ^a ± 5.57	9.06 ^a ± 2.07	4.58 ^a ± 0.50	56.06 ^a ± 13.44	39.48 ^a ± 21.60	19487.50 ^a ± 15964.42	61.38 ^a ± 29.64	28.50 ^a ± 17.93	1.50 ^a ± 0.93	2.25 ^a ± 1.67	90768.75 ^a ± 94543
Negative	31.13 ^b ± 8.54	7.05 ^a ± 2.39	4.99 ^a ± 1.06	61.65 ^a ± 10.80	23.80 ^b ± 7.72	7787.50 ^a ± 2367.60	48.88 ^a ± 4.49	47.63 ^b ± 4.83	1.88 ^a ± 0.83	1.75 ^a ± 0.89	7925 ^b ± 8543
P value	0.014	0.072	0.414	0.115	0.035	0.075	0.22	0.010	0.351	0.553	0.037

Table IV, Anaemia was observed in both parasitemic and aparasitemic samples. The mean PCV value of the parasitemic samples was significantly lower ($P < 0.05$) than values obtained in the aparasitemic samples. In the aparasitemic samples, there was a macrocytic hypochromic anaemia while mean values of HB, RBC, and MCV of the parasitemic samples are not significantly different from those obtained in the aparasitemic sample. Severe thrombocytopenia was also

observed in aparasitemic samples as well as a mild lymphopenia.

Values with heterozygous superscripts are significantly different ($P < 0.05$).

4.58% of the total blood sample was parasitemic and showed anaemia, while 0.65% were parasitemic but did not show anaemia, a greater percentage of the blood sample were non-anaemic and aparasitemic.

DISCUSSION

Haemoparasites were detected in 8 blood samples (5.23%) of the total blood samples while 145 blood samples (94.77%) did not contain haemoparasites. The infection rate of haemoparasitism in this study is generally low (5.23%), this is in agreement with findings from Ademola and Onyiche (2013) who recorded 4.9% haemoparasitism amongst sampled pigs. Omeje (2014) also

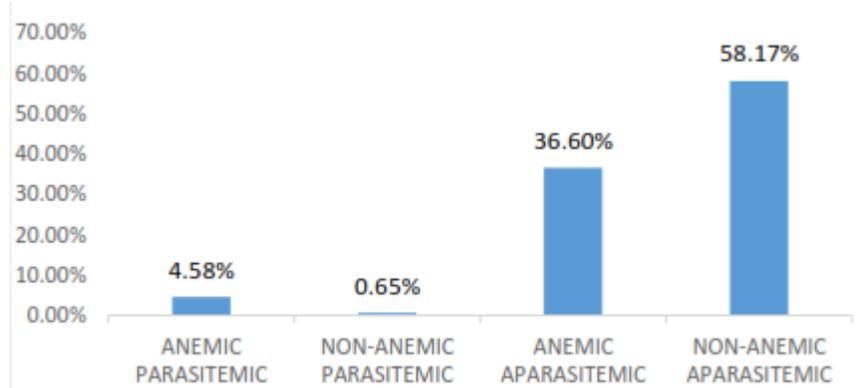


FIGURE I: Relationship of anaemia and haemoparasitism

reported that the overall prevalence of porcine trypanosomosis in Enugu North Senatorial Zone was 5.2%. These low infection rates could be as a result of the fats deposit in the subcutaneous tissue layer of the pigs which makes it difficult for the insect vectors to penetrate through the skin in their biting and sucking mode of feeding (Gagman *et al.*, 2014) as well as regular chemoprophylaxis against haemoparasites. Gagman *et al.* (2014) recorded a higher prevalence of 17.29 % infection rate amongst sampled pigs in Jos and this could be as a result of prevalence of insect vectors of haemoparasitism or irregular chemoprophylactic treatment against haemoparasitism in Jos (Usip, 2014). Dipeolu *et al.* (1982) reported that 81% of local pigs were positive for blood parasites while only 41% exotic pigs had haemoparasites, probably because

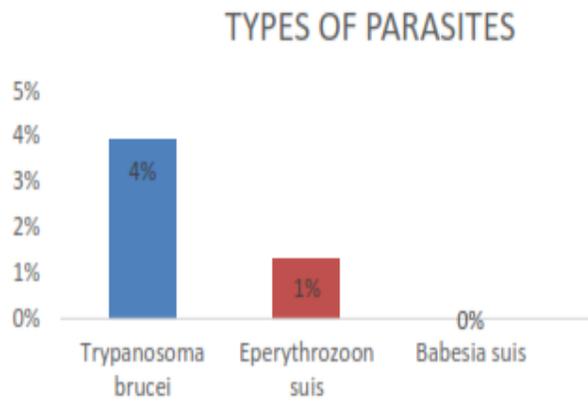


FIGURE II: Prevalence of haemoparasitism and types of haemoparasite encountered

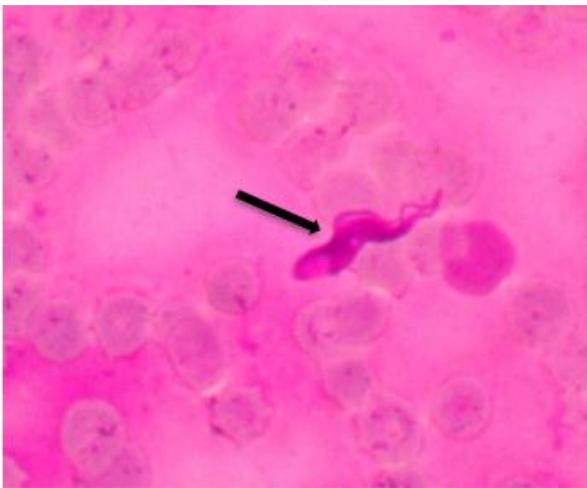


FIGURE III: Photomicrograph of Giemsa stained blood smear showing presence of *Trypanosoma brucei* (arrow) in the plasma. X1000

of pig trypanosomosis was caused by *T. brucei* and there was no evidence of *T. simiae*. Omeje (2014) demonstrated *T. brucei* was the most prevalent haemoparasite with 84.9% infection rate, while *T. congolense* caused an infection rate of 15.1%. No record of mixed infection was observed amongst the sampled pigs. Omeje, (2014) also reported that no mixed infection was seen amongst examined pigs.

The high incidence of haemoparasitism in the tropics could be as result of favourable environmental conditions that promote the survival and proliferation of the arthropods vectors responsible of haemoparasites (Adejinmi *et al.*, 2004).

they were reared intensively The parasites detected in this study were *Trypanosoma brucei* and *Eperythrozoon suis* and is not in accordance with reports from Dipeolu *et al.* (1982), that the prevalent blood parasites of pigs in Ibadan were *Eperythrozoon suis* and *Babesia* sp. *Eperythrozoon suis* was reported by Usip (2014) to be the most prevalent haemoparasite (31.9%) in Uyo and this may be due to increased insect vectors (*Haematopinus suis*) of transmission in that area. *Trypanosoma simiae* was not observed in this study unlike in Ademola and Onyiche's findings which recorded that 3.5 % of the 4.9 % positive for haemoparasitism was caused by *Trypanosoma simiae*. Omeke (1994) also observed that 23.9%

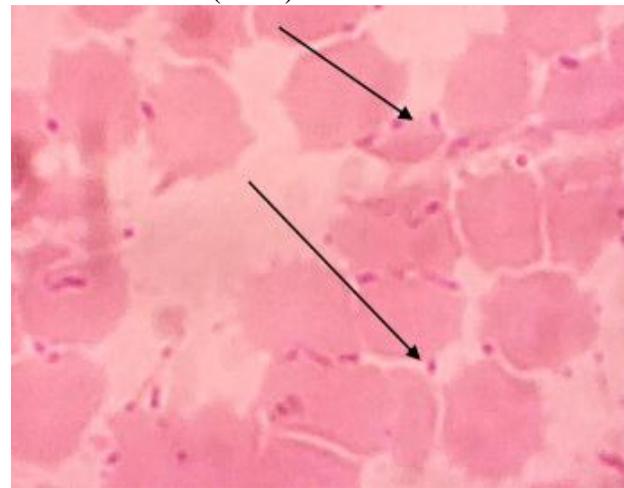


FIGURE IV: Photomicrograph of a Giemsa-stained blood smear showing the presence of *Eperythrozoon suis* (arrows) attached to the margins of red blood cells. X1000

Anaemia was a consistent and significant finding in parasitemic samples. Anaemia was observed in both parasitemic and aparasitemic samples. The mean PCV value of the parasitemic samples was significantly lower ($P < 0.05$) than values obtained in the aparasitemic samples. In the aparasitemic samples, there was macrocytic hypochromic anaemia while in the parasitaemic samples, the anaemia observed was normocytic normochromic. Severe thrombocytopenia was also observed in aparasitemic samples as well as a mild lymphopenia. Haemoparasitism is a cause of anaemia in the parasitemic samples while other causes such as ectoparasitism, gastrointestinal parasites or even starvation/malnutrition could be

responsible for anaemia in the aparasitemic samples. Adejinmi *et al.*, (2004) reported anaemia as a reliable indicator for the severity of haemoparasitic infections.

4.58% of the total blood sample was parasitemic and showed anaemia, while 0.65% were parasitemic but did not show anaemia, this could be due to a low parasitaemia or dehydration (Antonides *et al.*, 2016), 36.60% of the total blood samples were anaemic but aparasitaemic and this could be as a result of other causes of anaemia such as malnutrition (Jennings 1976), stress or other infectious agents besides haemoparasites (Obi and Anosa, 1980), a greater percentage (58.17%) of the blood sample were non-anaemic and aparasitemic. *Eperythrozoon suis* caused more severe anaemia (20 ± 9.89) compared with *Trypanosoma brucei* (27 ± 3.03). The anaemia observed in eperythrozoonosis is microcytic normochromic anaemia while that in trypanosomosis is normocytic normochromic anaemia, this is in agreement with findings from Obi and Anosa (1980), who reported the MCV of cattle, horses and sheep to be normal in chronic infections of trypanosomosis despite the persistence of anaemia and also a normal mean corpuscular haemoglobin concentration (MCHC) in *T. brucei* infections of mice and rabbits. Mild leucocytosis was observed in trypanosomosis while a moderate leucopenia was seen in Eperythrozoonosis, mild neutropenia was observed in trypanosomosis.

Severe thrombocytopenia was observed in haemoparasitic infection caused by *Eperythrozoon suis* and *Trypanosoma brucei*.

In conclusion, haemoparasites such as *T. brucei* and *E. suis* are still in circulation plaguing the pig industry and anaemia is the commonest clinical presentation of these conditions. However, efforts should be made to prevent and control these conditions through various standard approaches including the control of external parasites which are vectors of transmission and

correct diagnoses are expedient for correct and effective treatments.

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