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PREVALENCE AND ALTERATIONS IN SOME HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN HORSES WITH PIROPLASMOSIS IN PORT HARCOURT POLO CLUB, NIGERIA

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ABSTRACT: *The objective of this study was to investigate equine piroplasmosis in horses from Port Harcourt Polo Club, Nigeria. Forty-one polo horses comprising of different sexes and breeds and aged between 6 months and 10 years were screened for intra erythrocytic protozoan parasites: Theileria equi and Babesia caballi by light microscopy. Ticks (Rhipicephalus species) were seen on mane, tail and perineal region of the horses sampled. Haematological and biochemical evaluations were carried out using standard methods. Light microscopic analysis of Giemsa stained blood smear revealed equine piroplasmosis in 31 of the horses with prevalence of 75.6%. Of these horses, 19 (46.3%) were infected by T. equi, 10 (24.4%) infected by B. caballi and 2(4.9%) infected by both species. 23 (56.1%) males and 8 (19.5%) females were infected. Based on age, 25 (61%) of the infected horses were less than 5 years and 6 (14%) were greater than 5 years. The Argentinean, Sudanese and indigenous (Nigerian) breeds infected were 1 (2.4%), 8(19.5%) and 22 (53.6%) respectively. Haematological analysis revealed decreased packed cell volume, haemoglobin concentration and erythrocyte count compared with normal values. A significant ($P < 0.05$) eosinophilia was observed in infected horses when compared with non-infected horses. Total bilirubin and Alanine aminotransferase were significantly ($P < 0.05$) higher in infected horses. The study showed high level of equine piroplasmosis and some changes in haematological and biochemical parameters in naturally infected Polo horses with T. equi and B. caballi from Port Harcourt Polo Club, Nigeria.*

KEYWORDS: Biochemical, Haematology, Piroplasmosis, Polo Horses, Nigeria

INTRODUCTION

Horses in Nigeria are infected with a wide variety of vector-borne haemoparasites (Pam *et al.*, 2013). Equine piroplasmosis is one of the most important tick-borne diseases, with an economic worldwide impact in the horse industry. Ixodid ticks of the genera *Dermacentor*, *Rhipicephalus*, and *Hyalomma* are able to transmit *T. equi* and *B. caballi* to all equid species, including horses, donkeys, mules, and zebras (Friedhoff *et al.*, 1990; Zobba *et al.*, 2008; Rothschild, 2013). Horses typically persist as carriers of *B. caballi* for one to three years after infection (Guidi *et al.*, 2015) while those infected with *T. equi* remain as carriers for life (Rothschild and Knowles, 2007). Ticks are reservoir of infection because the infection

persists in ticks throughout several generations through transstadial and transovarian transmission (Scoles and Ueti, 2015; Kane, 2016). Equine babesiosis is common in most tropical and subtropical areas than in temperate regions owing to high ambient temperature, humidity and rainfall which sustain tick development in these regions (Motlong *et al*., 2008). Animals in endemic areas mostly survive the infection (OIE, 2009), but acutely affected horses may not survive without treatment (Morrow and Sommardahi, 2014). Symptoms may differ from per acute to chronic forms. The clinical signs include mild to general weakness, depression, rapid or shallow breathing, weight loss, fever, anorexia, anaemia, elevated respiratory and pulse rates, congested mucous membrane, icterus, colic, oedema of the distal limbs, around the head and eyelids, in-coordination and abortion in pregnant mares (Zobba *et al.,* 2008; McFarland, 2016). Massive intravascular destruction of parasitized erythrocytes could result in haemoglobinuria which coincides with presence of the organism in peripheral blood and can last for 8 to 10 days (Taylor *et al.,* 2007). Diagnosis includes detection of intra-erythrocyte parasites in Giemsa-stained blood smears (Shkap *et al*., 1998), which is useful in the acute phase of infection. Latent phase of the infection is characterized by low parasiteamia (Sumbria *et al*., 2015) which is detected using more sensitive diagnostic techniques like the indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and complement fixation test (CFT) (Moretti *et al.,* 2010). Diaminazene aceturate and imidocarb dipropionate are the drugs of choice for treatment of equine piroplasmosis (Morrow and Sommardahl, 2014). The use of acaricides and immediate removal of any tick may help to prevent the infection (Mcfarland, 2016). The aim of the study was to investigate equine piroplasmosis and describe the haematological and biochemical changes in naturally infected Polo horses with *B. caballi* and *T. equi* from Port Harcourt Polo Club, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted in March, 2018 at the Port Harcourt Polo Club Port Harcourt, Rivers State Southern Nigeria. The city of Port Harcourt is located at a coordinate of 4^0 45^1 N, 7^0 00^1E and elevation of 468M (1535 Ft) above sea level. The climate falls within the sub-equatorial climatic belt. It has a mean annual temperature of 30^0C, relative humidity of 80-100% and mean annual rainfall of 2327 mm (Hudges and Richard, 2003).

Study Animal and Management

Forty-one polo horses comprising 15 female and 26 males and aged between 6 months and 10 years were studied. The horses are of three different breeds: Indigenous (25), Argentinean (2) and Sudanese (14). They were housed in standard stable measuring 2.44 m × 3.66 m, made of concrete floor, cement block wall and asbestos roof and well ventilated. The horses were fed wheat bran, sorghum, hay and fresh pasture.

Tick collection and Identification

There was presence of ticks on the mane, tail and perineal region. They were removed with a pair of forceps and preserved in 70% alcohol. The species identification was determined by comparison with standard keys (Keirans and Litwak, 1989).

Sample collection

Five millimeters whole blood was collected through jugular venipuncture and dispensed into tubes with and without potassium ethylene diamine tetra acetic acid (K2EDTA) anticoagulant. The anti-coagulated blood was used for haematological evaluation, while the plain blood was allowed to clot for 30 minutes and then centrifuged for 15 minutes at approximately 1000 x g. The resultant serum was collected and stored at -20^{o}C until used for serum chemistry analysis. **Haematological and Biochemical Analysis**

The anti-coagulated blood was used to determine packed cell volume (PCV), red blood cell counts (RBC), haemoglobin (Hb) concentration, and total and differential white blood cell (WBC) counts by methods described by Cole (1986). Serum was used to determine the values of bilirubin and alanine aminotransferase by method described by Cheesbrough (1991).

Microscopic Detection of Babesia Parasites

Thin blood smears were prepared from the anti-coagulated blood and stained with Giemsa stain at 1:10 dilutions for 60 minutes (Taylor *et al.,* 2007). Within parasitized-erythrocytes, *T. equi* is small pyriform bodies which either appears rounded, amoeboid or Maltese cross, and measure 2 to 3µm in length, while *B. caballi* is large pyriform paired bodies joined at the posterior ends and measure 2 to 5 µm in length (Radostits *et al*., 2007).

Data Analysis

GraphPad Prism 4.0 for Windows (GraphPad Software, San Diego, California, USA) was used for the analysis. The data obtained were either expressed as percentage for prevalence of infection or as mean ± standard deviation (Mean ± SD) for haematological and biochemical data. Student's *t*-test was used to compare the haematological and biochemical parameters between the infected and non-infected horses. Differences were considered significant at $P < 0.05$ level.

Results

Ticks *(Rhipicephalus* species) were seen on mane, tail and perineal region of the horses sampled. Light microscopic analysis of Giemsa stained blood smear revealed equine piroplasmosis in 31 of the horses, 23 males and 8 females with a total prevalent of 75.6%. Based on age, 25 (61%) of the infected horses were less than 5 years and 6 (14%) were greater than 5 years. The Argentinean, Sudanese and indigenous breeds infected were 1 (2.4%), 8(19.5%) and 22 (53.6%) respectively. Of the 31 Giemsa stained piroplasma positive horses, 19(46.3%) were infected by *T. equi*, 10(24.4%) by *B. caballi* and 2(4.9%) by both species. The microscopic appearance of the intra-erythrocytic piroplasm parasites: *T. equi* and *B. caballi*, observed in infected horses are shown in the plate I, II and III. Although there was difference in haematological parameters between the infected and non-infected horses but most values for non-infected horses are not far from those of infected horses and are below the normal values (Table I). The Haematological values in piroplasma-infected horses revealed a decrease PCV (27.4 ± 5.2 %), Hb concentration (9.25 ± 1.9 g/dL) and RBC counts (4.43 ±1.13 x10^{6}/ µL) when compared with normal values of PCV (43.2 ± 0.77%), Hb (15.11± 0.29 g/dL) and RBC (8.69 ± 0.16 x10^{6}/ µL) respectively (Table I). There was a significant increase ($P < 0.05$) in eosinophil count in infected horses compared to the non-

infected horses (Table I). Total bilirubin (6.34 ± 2.0 mg/dL) and alanine aminotransferase (28.9 ± 8.4 IU/L) were significantly ($P < 0.05$) higher in infected horses when compared with 3.36 ± 0.19 mg/dL and 17.6 ± 3.9 IU/L respectively, in non-infected horses.

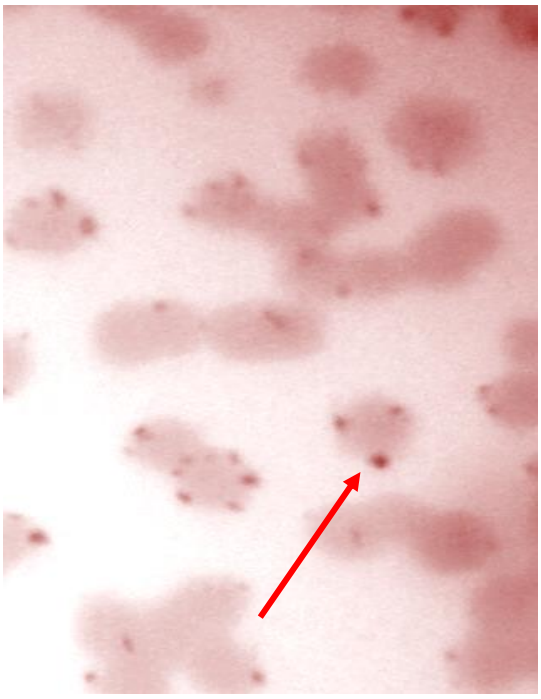

Plate I: Peripheral blood smear showing *Theileria equi* merozoite (arrow) in erythrocytes in Port Harcourt Polo horses, Rivers State, Nigeria.

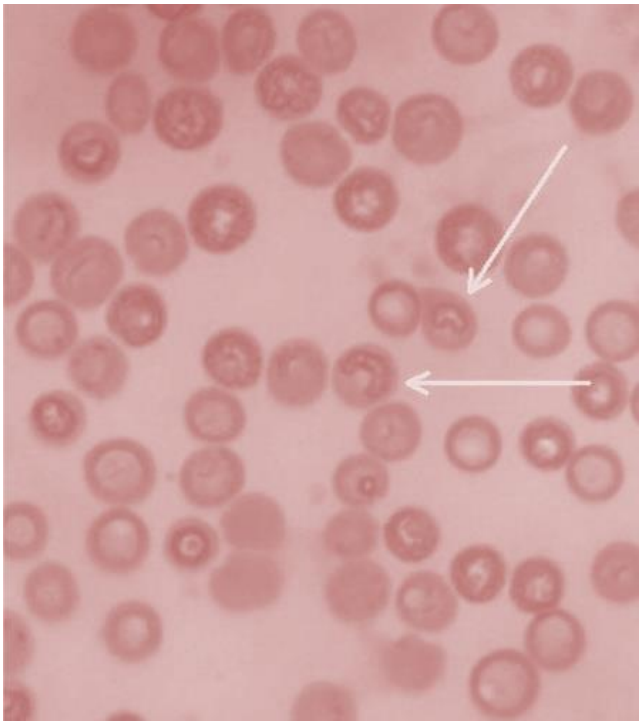

Plate II: Peripheral blood smear showing *Babesia caballi* merozoite (arrow) in erythrocytes in Port Harcourt Polo horses, Rivers State, Nigeria.

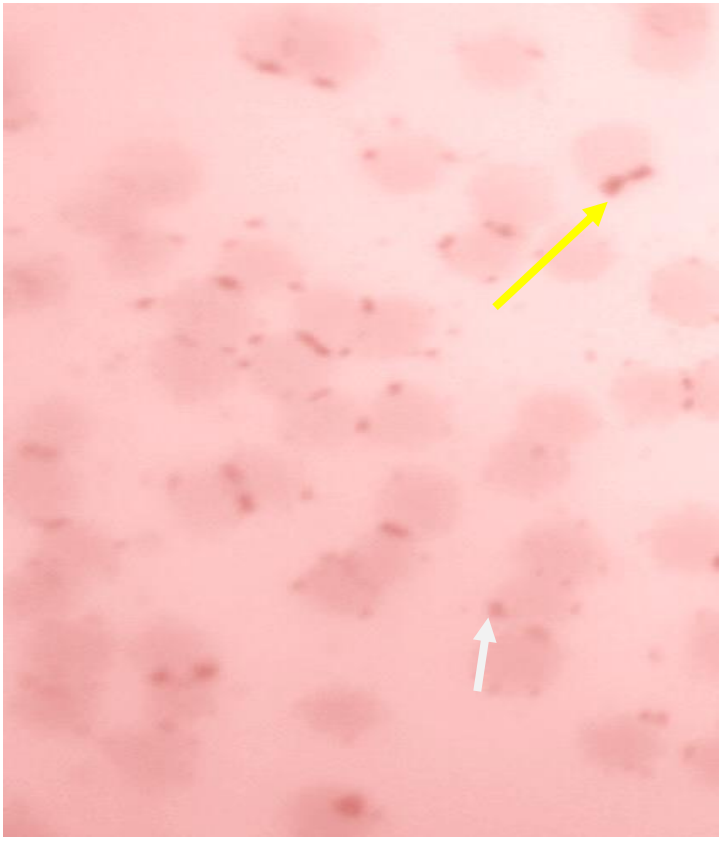

Plate III: Peripheral blood smear showing mixed infection with *B. caballi* (yellow arrow) and *T. equi* (white arrow) in the infected erythrocytes of Polo horses.

Table I: Haematological and some biochemical values of infected and non-infected horses with *T. equi* and/or *B. caballi* in Port Harcourt Polo horses, Rivers State, Nigeria. (Mean ± SD)

	Infected (31)	Non-Infected (10)	Normal *
PCV (%)	27.4 ± 5.2	29. 4 ± 1.8	43.21 ± 0.77
Hb (g/dL)	9.25 ± 1.9	10.2 ± 0.74	15.11 ± 0.29
RBC($x10^6$/µL)	4.43 ± 1.13	4.97 ± 0.84	8.69 ± 0.16
WBC($x10^9$/L)	7.03 ± 2.1	7.86 ± 2.44	9.53 ± 0.31
Neutrophil($x10^9$/µL)	4.31 ± 2.57	4.07 ± 1.3	4.77 ± 0.23
Lymphocytes ($x10^9$/µL)	2.93 ± 0.96	3.55 ± 1.24	4.19 ± 0.19
Eosinophil ($x10^9$/µL)	1.32 ± 1.18 [b]	0.48 ± 0.81 [a]	0.25 ± 0.03
Monocytes ($x10^9$/µL)	0.08 ± 0.09	0.11 ± 0.05	0.03 ± 0.01
Bilirubin (mg/dL)	6.34 ± 2.0[b]	3.36 ± 0.19[a]	3.43 ± 0.13
Alanine aminotransferase (IU/L)	28.9 ± 8.54 [b]	17.6 ± 3.9 [a]	9.44 ± 0.69

Values with different alphabets are significantly ($P < 0.05$) different
**Ihedioha and Agina (2014 and 2015).*

DISCUSSION

This study revealed a high prevalence rate of 75.6% of equine piroplasmosis (EP) due to *T. equi* and *B. caballi* in Port Harcourt Polo club, Rivers State. Garba *et al.* (2011) also observed high prevalence of EP in Nigerian royal horses. Equine piroplasmosis is endemic in Africa (Ibrahim *et al.*, 2011), and is common during rainfall which favours vector multiplication (Tesfie *et al.*, 2018). The study was carried out in March which fell under rainy season. Tick infestation has been shown to be higher during the rainy season than the dry season in tropical climates (Morel, 1989). Outbreaks of babesiosis during such periods of heavy tick infestation have been reported (Rabo *et al.*, 1995). The higher prevalence in male horses than female horses agrees with report of Moheeb *et al.* (2013) who observed a higher prevalence in male than female horses. This is in contrast with the work of Oladipo *et al.* (2015) who observed higher prevalence of equine piroplasmosis in female than male polo horses. All breeds of horses sampled were infected showing that most breeds of horses are susceptible to babesiosis. The horses sampled were either infected by *T. equi* or *B. caballi* and in some cases by both parasites. These agree with the report of Scoles and Ueti (2015) who observed that mixed infections were common in endemic areas. The finding of a higher prevalence for *T. equi* than *B. caballi* is in agreement with OIE (2008) report which stated that *T. equi* is more common and pathogenic than *B. caballi*. It has been stated that persistent infection of *T. equi* is due to sequestration of the organism and immune evasion strategies (Ueti *et al.*, 2012). Scoles and Ueti (2015) also correlated the occurrence of haematological and biochemical changes in horses infected with *B. caballi* and *T. equi*. The decrease in RBC counts was in consistency with the work of Mahmoud *et al.* (2016) which was due to its destruction by piroplasms. Decreased in haemoglobin was due to destruction of RBC. Three mechanisms of haemolysis have been described: mechanically by trophozoite intra-erythrocyte binary fission (Knowles *et al.*, 1994), immune-mediated auto-antibodies directed against components of the membranes of infected and uninfected erythrocytes, and toxicity by haemolytic factors produced by the parasite (Zygne *et al.*, 2007). The increase in eosinophil count observed in the infected horses is in agreement with the results of Ibrahim *et al.* (2005) who reported that parasitic infestations typically induces eosinophilic response. Increased haemolysis by piroplasms might have caused the unconjugated hyperbilirubinaemia (Glader, 2004). Bilirubin is the breakdown product of haemoglobin and diagnostic marker of liver and blood disorders. Increase production of bilirubin may exceed the ability of the liver to conjugate and excrete the pigment (Fevery, 2008). Significant increase in ALT, a liver enzyme was also observed in infected animals as was reported by Zobba *et al.* (2008) who stated that damage to erythrocytes may result in considerable increase in the level of this enzyme. The *Rhipicephalus* species infestation on different parts of the body might have been responsible for transmission of the parasites. De Waal and Potgieter (1987) reported that *Rhipicephalus* species transmits babesia parasites in horses. Prevention is through year-round tick control which involves integrated management including avoidance, treating of premises and horses specifically with acaricides. Ticks are said to attach on their host for 24 hours before disease can be transmitted, hence it is best to check horse regularly and remove ticks immediately after attachment (McFarland, 2016). Ticks thrive in tall grass and wooded area; therefore, pastures should be mowed and bush around fence lines cleared. The horses which were not infected but showed decreased haematological parameters may have latent infection which is not readily detected with Giemsa stain (Mosqueda *et al.*, 2012).

CONCLUSION

The study showed high level of equine piroplasmosis and described some haematological and biochemical changes in naturally infected Polo horses with *B. caballi* and *T. equi*.

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