Prevalence of haemoparasites and influence on haemato-biochemical parameters of polo horses in Ibadan, Nigeria

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Abstract

Parasitic diseases, which can reside inside or outside the host, have a devastating impact on human and animal health worldwide, particularly in developing countries. Little is known about the current haemoparasitic status of polo horses in Ibadan, Nigeria. This study aims to find out the scope and extent of haemoparasites and their associated haematological and serum biochemical changes in polo horses in Ibadan, Nigeria. Blood samples were randomly collected from 52 horses from Ibadan polo stable. Haemoparasites identification, haematology and serum biochemistry parameters were evaluated according to standard procedures. The overall prevalence of haemoparasitic infection in the sampled horses was 13.5%. The haemoparasites found were *Theileria equi* (85.7%) and *Anaplasma phagocytophilum* (14.3%). The haematology result showed that infected horses presented a significant decrease (P<0.05) in PCV, WBC counts and significant elevation (P<0.05) of eosinophils. The serum biochemical values revealed that infected horses had significantly higher (P<0.05) levels of AST and creatinine kinase whereas the glucose level was significantly lower (P<0.05) when compared with the uninfected horses. To reduce the threat of haemoparasitic infection, it is recommended that vector control, as well as prophylactic therapy, be carried out in stables.

Introduction

Parasites either reside inside or outside the host, posing a threat to the health of animals (Ademola *et al.,* 2004; Ola-Fadunsin & Ibitoye, 2017). Horses are susceptible to more than sixty parasites with several of them reported as haemoparasites and helminths (Saeed *et al.,* 2019). Haemoparasites are organisms that live in the bloodstream of vertebrate hosts and are majorly vector-borne in transmission. They destroy the red blood cell which results in anaemia, icterus, anorexia, weight loss and infertility (Farouk *et al.,* 2020). Examples of such haemoparasites include; *Theleria, Babesia, Anaplasma* and *Trypanosoma* species.

In Nigeria, horses are being used in a variety of ways such as polo, crowd control by policemen, racing, festivals, research, pleasure as well as entertainment

(Useh *et al.,* 2005; Ehizibolo *et al.,* 2011). However, polo is currently gaining popularity in southwestern Nigeria with the elites and socialites expressing more interest in the game. This they do by purchasing and importing horses on yearly basis, thereby increasing the population of horses in polo stables and the region (Olusa & Adeyefa, 2009). To ensure maximum performance of polo horses it is, therefore, necessary to constantly monitor their health. The prevalence of haemoparasites in horses has been reported in some states in Nigeria including Kaduna, Bauchi, Plateau, Rivers and Delta (Ehizibolo *et al.,* 2012; Ola-Fadunsin *et al.,* 2018; Eze *et al.,* 2020; Farouk *et al.,* 2020). In addition to trans border transport of horses for shows and performances, the issue of horse haemoparasite control is of worldwide importance. The last survey of haemoparasites of horses carried out in Ibadan, Southwestern Nigeria was over four decades ago (Dipeolu & Oduye, 1976), which is long overdue considering the effects posed by haemoparasites. Therefore, this study aims to determine the current of haemoparasitic and its effects on some haematological and serum biochemical parameters of polo horses in Ibadan southwestern Nigeria.

Materials and Methods
Study area
This study was carried out in Ibadan, southwest, Nigeria. It is the capital city of Oyo state and is in the southeastern part of the state. Ibadan is located on seven hills (average elevation 700 feet) about 100 miles (160km) from the Atlantic coast. The city lies between 7° 05 and 7°25 North and between 32° 40 and 32°55 East. It has tropical wet (March- October) and dry (November-February) seasons. The mean daily temperature is 26.46ºC (NMA, 2017).

Study design
It was a cross-sectional study using a simple random sampling technique. Fifty-two horses stabled in the Ibadan polo club comprising different breeds: local and exotic; ages: young (<4 years), Adult (5-12 years), old (> 12 years) and sex were used for this study. The age of selected horses was determined by their dentition. The horses were provided with fresh pasture, concentrates and hay. Drinking water was given *ad libitum*.

Sample collection
Blood collection was done following proper restraint of each animal through the external jugular vein using a 21 gauge needle fixed to a 10ml syringe. Five millimetres of blood sample was dispensed into

sample bottle containing 1mg ethylenediaminetetra acetic acid (EDTA), and then used for haematological evaluation, while the remaining 5ml was dispensed into a plain bottle, allowed to clot at room temperature and used for serum biochemical determination. These samples were placed on an ice pack and transported immediately to the laboratory.

Parasites Identification
A thin blood smear was prepared on a standard microscope glass slide (75 mm by 25 mm), air dried, fixed in methyl alcohol for 3-5 min, stained in 5% Giemsa stain for 30-45 min in a staining jar and rinsed in buffered distilled water for identification of *Theileria equi* or *Babesia caballi*, microfilaria and trypanosomes as described by Kamani *et al*. (2010). Also, buffy coat was examined for motile parasites as previously (Ehizibolo *et al.,* 2012).

Haematological analysis
The haematological parameters were carried out following as described by Jain (1986). The packed cell volume (PCV) % was determined using a micro haematocrit centrifuge and reader as described by Jain, (1986). Haemoglobin concentration (g/dL) was determined by the method of estimating the concentration of cyanohaemoglobin. Red blood cell (x 10^6/μL) and total white blood cell (x 10^3/μL) counts were determined manually using a Neubaeur haematocytometer. The differential WBC (%) count was determined by identifying 100 WBCs on a blood smear to determine the percentage of each type of WBC.

Serum biochemical analysis
The blood sample was centrifuged at 1008 *g* (3,000 revolutions) per minute for 10 minutes using a table centrifuge (TDL4®, B. Bran Scientific and Instruments Co., England). The concentration of total protein (g/dL), albumin (g/dL), aspartate aminotransferase (AST) (μL), creatinine kinase (μL), blood urea nitrogen (mg/dL), creatinine (mg/dL), and glucose (mg/dL) were determined by spectrophotometry using RANDOX® laboratory reagent kits obtained from RANDOX Laboratories Ltd., Ardmore, United Kingdom. The kits were used according to the manufacturer's directions.

Data analysis
The data collected were subjected to descriptive analysis using GraphPad Prism 7.0 for Windows (GraphPad Software, San Diego, California, USA). The data obtained were either expressed as a percentage for the prevalence of infection (number infected

divided by total sample multiplied by 100) (Spronk *et al.,* 2019) or mean ± standard deviation (Mean ± SD) for haematological and serum biochemical data. The differences in the parameters of infected and non-infected horses were analyzed using a student's t-test at a significant level of (P< 0.05).

Results

The result obtained showed that out of the 52 horses sampled, 7(13.5%) were infected with haemoparasite. The prevalence of haemoparasitic infection in the sampled horses were *Theileria equi* 6 (85.7%) having the highest prevalence followed by *Anaplasma phagocytophilum* 1 (14.3%). For *Theileria equi,* prevalence based on the breed was 4 (10.8%) among exotic breeds while local breed was 2 (13.3%) (Table 1). The prevalence of horses infected with *Theileria equi* based on sex was 2 (11.8%) male and 4(11.4%) female (Table 1). The prevalence of *Theileria equi* based on the age of horses was 4 (30.8%) young, 2 (5.9%) adult and 0 (0%) old (Table 1). For *Anaplasma phagocytophilum,* it was only found in 1(14.3%) horse and distribution based on breed, sex and age was 1(2.7%) exotic breed, 1(5.9%) male and 1(2.9%) adult horse (Table 1). Reference range values (Merck

Manual, 2010) for haematological parameters are presented in Table 2. The mean values and standard deviations (mean ±SD) of haematological parameters of infected horses in this study are as follows; PCV (%): 27.60 ±4.44, Hb concentration(g/dL): 9.60 ±1.89, RBC(x 10^6/µL): 4.76 ±0.90, WBC(x 10^3/µL): 5.17 ±0.24, lymphocytes count(%): 29.33 ±5.42, eosinophils count (%): 9.20 ±3.56, neutrophils count(%): 58.00 ±4.24 and monocytes count(%). 4.66 ±2.58 while mean ±SD values of haematological parameters of non-infected horses were: PCV (%): 31.54 ±4.68, Hb concentration(g/dL): 10.19 ±1.47, RBC (x 10^6/µL): 5.01 ±0.79, WBC(x 10^3/µL): 7.50 ±0.49, lymphocytes count(%): 31.63 ±12.63, eosinophils count (%): 4.02 ±4.44, neutrophils count(%): 61.29 ±12.32 and monocytes count(%): 4.36 ±2.03 (Table 2). There was a significant decrease (P<0.05) in PCV and WBC counts. The eosinophils count of infected horses increased significantly (P<0.05) when compared with non-infected horses (Table 2). Reference range values (Merck Manual, 2010) for serum biochemical parameters are presented in Table 3. The mean values and standard deviations of serum biochemical parameters of infected horses as shown in Table 3 are

Table 1: Prevalence and distribution of haemoparasites of polo horses based on breed, sex and age in the study area

	Number sampled	Number infected	Prevalence
	52	7	13.5%
Distribution		*Theileria equi*	*Anaplasma phagocytophilum*
Breed			
Local	15	2 (13.3%)	0 (0%)
Exotic	37	4 (10.8%)	1 (2.7%)
Sex			
Male	17	2 (11.8%)	1 (5.9%)
Female	35	4 (11.4%)	0 (0%)
Age			
Young (<4yrs)	13	4 (30.8%)	0 (0%)
Adult (5-12yrs)	34	2 (5.9%)	1 (2.9%)
Old (>12yrs)	5	0 (0%)	0 (0%)

Table 2: Influence of parasites on Mean ±SD of haematological parameters

Haematological parameters	Infected	Non-Infected	Reference values
Packed Cell Volume (PCV)%	27.60 ± 4.44[b]	31.54 ± 4.68[b]	27-43
Haemoglobin(Hb) Concentration(g/dl)	9.60 ± 1.89	10.19 ± 1.47	10.1-16.1
Red blood cell (RBC) count (x 10^6/µL)	4.76 ± 0.90	5.01 ± 0.79	6-10.4
White Blood Cell Count (WBC (x 10^3/µL)	5.17 ± 0.24[b]	7.50 ± 0.49[b]	5.6-12.1
Lymphocytes counts (%)	29.33 ± 5.42	31.63 ± 12.63	21-42
Eosinophils count (%)	9.20 ± 3.56[b]	4.02 ± 4.44[b]	0-7
Neutrophils count (%)	58. 00 ± 4.24	61.29 ± 12.32	52-70
Monocytes count (%)	4.66 ± 2.58	4.36 ± 2.03	0-6

P<0.05 is significant

Values within rows with superscript b represent a significant different

Table 3: Influence of parasites on Mean ±SD of serum biochemical parameters

Serum biochemical parameters	Infected	Non- Infected	Reference values
Total Protein (g/dL)	6.51 ± 0.67	6.70 ± 0.61	5.6-7.6
Albumin (g/dL)	2.73 ± 0.24	2.86 ± 0.29	2.6-4.1
Globulin (g/dL)	3.78 ± 0.50	3.84 ± 0.61	2.6-4.0
Aspartate aminotransferase (µL)	334.00 ± 54.23[b]	253.69 ± 50.80[b]	160-412
Creatinine Kinase (µL)	278.50 ± 84.21[b]	194.58 ± 37.51[b]	60-330
BUN (mg/dL)	12.41 ± 0.57	12.48 ± 0.95	11-27
Creatinine (mg/dL)	0.51 ± 0.07	0.52 ± 0.07	0.4-2.2
Glucose (mg/dL)	89.66 ± 31.14[b]	108.30 ± 18.68[b]	62-134

P<0.05 is significant

Values within rows with superscript b represent a significant different

as follows: total protein (g/dL): 6.51 ±0.67, albumin (g/dL): 2.73 ± 0.24, globulin (g/dL): 3.78 ±0.50, AST (µL): 334.00 ± 54.23, creatinine kinase (µL): 278.50 ± 84.21, BUN (mg/dL): 12.41 ± 0.57, creatinine (mg/dL): 0.51 ±0.07 and glucose (mg/dL): 89.66 ± 31.14. For non-infected horses, the mean ± SD values of serum biochemical parameters were: total protein (g/dL): 6.70 ± 0.61, albumin (g/dL): 2.86 ± 0.29, globulin (g/dL): 3.84 ± 0.61, AST (µL): 253.69 ± 50.80, creatinine kinase (µL): 194.58 ± 37.51, BUN (mg/dL): 12.48 ± 0.95, creatinine (mg/dL): 0.52 ± 0.07 and glucose (mg/dL): 108.30 ± 18.68 (Table 3). The serum biochemical values revealed that infected horses had significantly higher (P<0.05) levels of AST and creatinine kinase whereas the glucose level was significantly lower (P<0.05) when compared with the uninfected (Table 3).

Discussion

This study which revealed an overall prevalence of 13.5% of haemoparasites in polo horses in Ibadan is comparable to 13% by Farouk *et al*. (2020) in polo Horses in Zaria, Nigeria. However, this prevalence is lower (53.83%) than the report of Eze *et al.* (2020) in Port Harcourt and Abraka polo fields in the South-South. These disparities could be linked to geographical changes, which impact the distribution of the vectors. *Theileria equi* had the highest prevalence of 85.7%. This finding is similar to earlier reports by Dipeolu & Oduye, (1976) and those obtained in other parts of Nigeria (Eze *et al.,* 2020; Farouk *et al.,* 2020). A high prevalence of *Theileria equi* in female horses was observed in this study. This result supports the findings of Eze *et al.* (2020) who reported a high prevalence in females than males which could be due to the stress of cyclical hormonal changes in females that possibly suppressed their immune system (Ademola & Onyiche 2013). Polo players typically retain more females in their stables because they are easier to control. However, fewer males are kept due to their aggressiveness in terms of their temperament. Moreover, the prevalence of *Theileria equi* in this study decreased with increasing age, which could be a result of immunity acquired from a previous infection. This finding is similar to the report of Oladipo *et al*. (2015) report on the prevalence of equine piroplasmosis but in contrary to what was reported by Eze *et al*. (2020) on *Theileria equi* status of horses. The present study showed that exotic breeds had a higher prevalence of *Theileria equi* than the local breeds contrary to the work of Farouk *et al*. (2020) who observed a high prevalence in local breeds compared to exotic breeds. Constant exposure to infections and the development of immunity against haemoparasites might be responsible for lower prevalence in local breeds. In addition, more attention to the management of exotic breeds gives fewer chances of exposure to vectors and develops none or less immunity, resulting in the frequent occurrence of haemoparasites (Siddiki *et al*., 2010). *Anaplasma phagocytophilum* was found only in one horse in the present study. This could be due to the presence of bacteria-infested ticks on the horse, which act as ectoparasites.

The variations observed in the haematological and serum biochemical parameters of horses in our study compared to reference range values (Merck Manual, 2010) could be attributed to environmental and nutritional factors (Jain, 1986). The anaemia observed in infected horses when compared with non-infected horses in this study is similar to the report of Ehizibolo *et al*. (2012) in horses in some states of Northern Nigeria but contrary to the findings of Farouk *et al*. (2020) in Zaria who observed no significant decrease on the haematological parameters. The majority of blood protozoan parasites are known to cause anaemia by inducing erythrophagocytosis. Anaemia is a good indicator of the severity of haemoparasitic infection (Adejinmi *et al*., 2004). As a result, all haemoparasites are linked to a considerable

decrease in mean PCV in infected animals. The observed reduction in WBC counts of infected horses in the present study could be linked to stress which often leads to immune suppression (Egbe-Nwiyi *et al.,* 2018). The significant eosinophilia observed in infected horses compared with non-infected horses is consistent with the findings of Ibrahim *et al*. (2005) and Ememe *et al*. (2019) in equine piroplasmosis, where it is believed that parasite infections often cause an eosinophilic reaction. The current study also showed a significant increase in aspartate aminotransferase (AST) in infected horses. A similar result was reported by Zaeemi *et al*. (2016), Pasolini *et al*. (2018), Ahmadi *et al*. (2020) in equines. Elevations in the serum levels of AST is attributed to reduced flow of blood to the liver leading to centrilobular necrosis (Zobba *et al.,* 2008). Also, AST is elevated in hepatocytes. Any increase in this enzyme in the blood is due to damage to hepatocytes or their membrane (Zygner *et al.,* 2011).

The elevation of creatinine kinase (CK) in infected horses is synonymous with mild damage of the muscles. In addition, an increase in CK can also be due to hemolysis (Latimer, 2011). The elevated creatinine kinase is in agreement with Diana *et al*. (2007) and Ahmadi *et al*. (2020) but disagrees with Takeet *et al*. (2009), who reported no alterations in serum enzyme activity in two horses infected with theileriosis. The slight hypocalcaemia observed might be attributed to starvation, malabsorption and hepatic depletion. This agrees with the reports of Nel *et al*. (2004) and Hussein (2019).

In conclusion, this study has established the presence of haemoparasites in polo horses with some alterations in the haematological and serum biochemical values. Given the importance of horses in the study area, it is suggested that vector control as well as prophylactic treatment should be carried out in stables to limit the menace of haemoparasitic infection.

Conflict of Interest
The authors declare that there is no conflict of interest.

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