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REGULAR ARTICLES

Hematology and biochemical values in equines naturally infected with *Theileria equi* in Nigeria

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Abstract

Equine piroplasmosis (EP) is a tick-borne disease of equines with resultant economic consequences. To date, little information is available regarding the effects of EP on the health of equids in Nigeria. Therefore, this study was carried out to ascertain the effects of natural sub-clinical infection with *T. equi* on the hematology and biochemical profiles of horses and donkeys. Blood and serum samples were subjected to hematology and serum biochemistry analyses, respectively. Data corresponding to PCR-positive (infected) and PCR-negative (uninfected) was computed to ascertain changes in any of the parameters. Hematological significant findings ($P<0.001$) in both horses and donkeys include decreased packed cell volume (PCV), red blood cell (RBC) count, hemoglobin (Hb) concentration, and white blood cell (WBC) counts in the infected group compared to the uninfected group. In horses, the PCV (%) and Hb (g/dL) values were 40.6±1.0 and 13.4±1.7 in the uninfected group compared with 30.8±0.6 and 10.1±0.3 in the infected group respectively and differs significantly ($P<0.001$). Similarly, in the donkeys, the PCV (%) and Hb (g/dL) values were 32.2 ± 0.5 and 10.5 ± 0.2 in the uninfected group compared with 28.5±0.7 and 9.5±0.2 in the infected group respectively with significant difference ($P<0.001$). Additionally, we observed a slight decrease in WBC counts ($\times 10^3/\mu L$) in the infected group in horses (9.9±0.4) and donkeys (10.2±4.3) compared with 10.5±2.0 and 13.0±0.8 from the uninfected respectively. Serum biochemical profiles revealed slight increase in the levels of total proteins principally the globulin fractions in the infected group in both equids. The findings from this study will be helpful in the diagnosis, prognosis, and treatment evaluation of equines in Nigeria.

Keywords Equines · Hematology · Biochemistry · Piroplasmosis · *Theileria equi*

Introduction

The Arewa (West African Barb) are indigenous breeds of horses in Nigeria including their crosses with Sudanese and Arabian Dongola. These horses are mainly found in northern Nigeria and are mainly used for ceremonial ride commonly referred to as durbar and for Polo sport (Garba et al. 2011). Furthermore, the donkeys in Nigeria are mainly working animals transporting goods and farm produce to market (Blench et al. 2003). Equine piroplasmosis (EP) is one of the most important tick-borne diseases affecting health and productivity of working equids. The disease is caused by *Babesia caballi* and *Theileria equi* belonging to the phylum Apicomplexa, order Piroplasmida (Homer et al. 2000). EP is a disease of economic importance preventing the international movement of infected equids especially to countries considered to be disease free (Hirata et al. 2003; Sudan et al. 2015). Horses, donkeys, mules, and zebras are equid species which

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are primarily affected by these piroplasms principally transmitted by ixodid ticks belonging to the genera *Hyalomma*, *Rhipicephalus*, and *Dermacentor* (De Waal 1992; Onyiche et al. 2019). The infection is widespread predominantly in European, Asian, South American, and African continents (Homer et al. 2000).

In endemic areas, the severity of the infection varies from subclinical to life-threatening (Guidi et al. 2015). Equine piroplasms (*B. caballi* and *T. equi*) either singly or in combination infect the equids, causing disease. The clinical severity is higher in *T. equi* compared to *B. caballi* as the latter only affects about 1% of the red blood cells. *Theileria equi* replicates within erythrocytes with parasitemia reaching high levels leading to hemolytic crisis (Ueti et al. 2005). Recovered horses remain chronically infected with no effective prophylactic treatment and may serve as source of infection to competent tick vectors subsequently transmitting the infection (De Waal 1992). On the other hand, infection with *B. caballi* can be treated and sterilized. The clinical picture of EP is variable and frequently nonspecific (Blood and Radostits 1987). Clinical signs in acute form include fever, anorexia, depression, icterus and increased heart and respiratory rates. Additionally, edema, incoordination, and sweating could also be seen (Zobba et al. 2008). The clinical manifestations in chronic infections are variable with weight loss, exercise intolerance, transient fever, and enlarged spleen (Franceschi and Guzzonato 2000; Chhabra et al. 2012). Chronic cases with low levels of parasitemia of EP are more common in donkeys compared with horses (Kumar et al. 2009).

Hematological and biochemical evaluation of the blood is usually carried out in disease diagnosis, substantiation of treatment success and in preventive medicine (Sedlinská et al. 2017). These evaluations are usually carried out on the blood because it is the main transport system in the body and any deviation due to pathogens is reflected as changes in the blood picture (Ihedioha et al. 2004). On the other hand, evaluation of biochemical parameters (blood biochemistry) is useful in determining the extent of disease process, pathological changes in vital organs and early diagnosis of disease (Stockham and Scott 2008). The main observed hematological alteration in horses with EP is reduction in the PCV, RBC count, Hb concentration, and platelet counts (De Waal 1992). On the other hand, hyperbilirubinemia is the most frequent biochemical finding arising due to hemolytic anemia caused by equine piroplasms (Zobba et al. 2008). The pathophysiological events leading to anemia and its accompanying clinical sequellae have been well described (Ambawat et al. 1999; Onyiche et al. 2019). In recent epidemiological studies, attempts have been made to investigate the clinical effects of natural infection with equine piroplasms on some biochemical and hematological parameters (Al-Obaidi et al. 2016; Laus et al. 2015; Camino et al. 2019). Comprehensive attempt to ascertain some of these hemato-chemical aberrations in equids naturally infected with *B. caballi* and *T. equi* in Nigeria is lacking. We recently reported the prevalence and risk factors associated with EP in Nigerian equids using PCR (Onyiche et al. 2020). Our results convincingly suggest that both horses and donkeys including blood-fed ticks collected from these hosts were infected with biological agents (both *T. equi* and *B. caballi*) responsible for EP (Onyiche et al. 2020). The blood and serum samples collected from selected infected equids were further analyzed to ascertain any changes in their hematology and biochemical parameters. This was carried out because knowledge of the clinico-pathological consequences of EP in Nigerian horses and donkeys is vital for effective treatment, prognosis, and control of the disease. Therefore, in this study, we evaluated the hematological and serum biochemical values in horses and donkeys naturally infected with *Theileria equi* in Nigeria.

Materials and methods

Study areas

The North-West region of Nigeria is a semi-arid zone characterized by a savannah type of vegetation. There is a single rainy season (May to October) with mean annual rainfall of 508–1016 mm and dry season (October to April). The daily temperature ranges from 18°C to 45°C with a mean temperature of 27°C. Current estimates put the population heads of horses and donkeys in the country as 206, 212, and 936, 832 respectively (FAO 2019).

Sampling locations and study design

Details of the sampling including study design and sampling locations have been described previously (Onyiche et al. 2020, 2021). Briefly, cross-sectional study was adopted combining both convenient and snowball sampling techniques. Samples were collected within the months of May to September 2017. Due to uneven distribution of equids in the study areas, samples were collected as representatives of these areas as follows: (Kano = 170; Jigawa =127, Katsina = 124, and Kaduna = 47). In total, 281 samples were collected from horses and 187 from donkeys. Flow chart showing the workflow as used in this study is illustrated in Fig. 1.

Blood collections

Prior to blood collection, all animals were physically examined and found to be apparently healthy with no report of clinical disease from both physical examination and testimony from their owners. Nonetheless, some of the animals were found to be infested with ticks mostly at the perineal

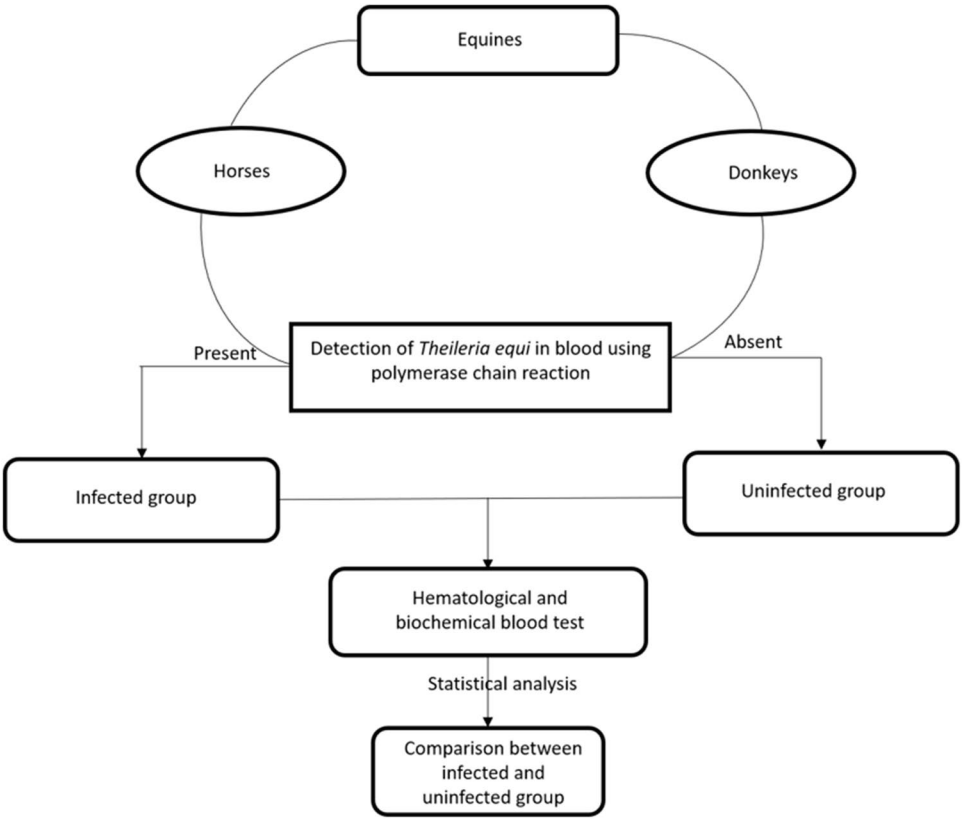

Fig. 1 Flow chart illustrating the study design. All examined equines (both horses and donkeys) were grouped into infected group (based on positive PCR result) and uninfected group (based on negative PCR result)

region. Fifteen millilitres of whole blood was collected from the jugular vein of apparently healthy horses and donkeys of both sexes using 18G hypodermic needle and syringe. Exactly 5 mL were transferred into labelled ethylenediaminetetraacetic acid (K3EDTA) coated tubes for hematological analysis and the remaining 10 mL into sterile tubes without anticoagulant for the exudation of serum. All collected blood samples were maintained at 4°C and transported to the laboratory within 4 h after collection. The plain tubes containing blood without anticoagulant were kept in slanted positions to prevent hemolysis of red blood cells and promote quick exudation of serum. On arrival in the laboratory, the plain tubes containing blood without anticoagulant were centrifuged for 10 mins at 3,000 *g* using a bench centrifuge (Centromix, UK). Separated serum was carefully pipetted into sterile 5 mL cryovials and stored at −20°C for further analysis.

Evaluation of infection status

Blood samples collected from horses and donkeys from all the study locations were screened for *T. equi* and *B. caballi* infections using PCR as described in our previous article (Onyiche et al. 2020). Briefly, total genomic DNA was extracted from FTA card using 5% Chelex 100 resin. Extracted gDNA was stored at −20°C until used for further analysis. Species-specific PCR was carried out for detecting *B. caballi* and *T. equi* DNA in the blood from equines using the following primer pairs for *T. equi* Bec-UF2 (forward): 5′- TCGAAGACGATCAGATACCGTCG-3′ and Equi-R (reverse): 5′- 137 TGCCTTAAACTTCCTTGCGAT-3′ (Alhassan et al. 2005) with expected fragment size of 392 bp, while for *B. caballi*, the primer pairs used are Bec-UF2 (forward): 5′-TCGAAGACGATCAGATACCGTCG-3′ and Cab-R (reverse): 5′-CTCGTTCATGATTTAGAATTGCT-3′, (Alhassan et al. 2005) with expected fragment size of 540 bp. All PCR products were separated on a 1% agarose gel stained with ethidium bromide and visualized under UV trans-illuminator (Gene Genius Imaging System, Syngene, UK). Selected positive amplicons were sent for sequencing in the forward direction (Inqaba Biotechnical (Pty) Ltd., Pretoria, South Africa).

Hematology

The collected blood samples were evaluated for hematology using the automated cell counter (Sysmex XP-300 hematology analyser (Sysmex Corporation, Kobe, Japan) calibrated for veterinary use. The parameters evaluated in the hemogram included erythrogram: packed cell volume (PCV), red blood cell count (RBCs), hemoglobin (Hb) concentration, and red cell indices; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean

corpuscular hemoglobin concentration (MCHC). Leukogram evaluation included the following: white blood cell count (WBCs), lymphocytes, monocytes, granulocytes (neutrophil, eosinophil, and basophil) and platelet count (PLT). We evaluated 212 samples out of the 281 from horses and 85 out of 187 from donkeys. This selection was done randomly from the collected samples to avoid bias.

Serum biochemistry evaluation

Serum samples were tested for biochemical variables. Analytes estimated from the serum includes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), albumin (Alb), globulin, total bilirubin (BT), cholesterol (Chol), triglyceride (TG), urea, and creatinine (Cr). All analysis was carried out using Randox ® Biochemistry kits following standard procedure recommended by the manufacturer. The activities of ALT and AST were determined using the Reitman-Frankel method (Reitman and Frankel 1957). The method of phenolphthalein monophosphate was used to determine the activity of ALP (Babson et al. 1966). Total protein was estimated using the direct Biuret method (Lubran 1978) while albumin was determined by the Bromocresol green method (Doumas et al. 1971). The difference between the serum total protein and the serum albumin gave the serum globulin (Colville 2002). Total bilirubin was estimated using the Jendrassik-Grof method (Doumas et al. 1973). Serum urea was determined using the modified Berthelot-Searcy method (Fawcett and Scott 1960) and creatinine was determined by the modified Jaffe method (Blass et al. 1974). Enzymatic colorimetric method was used to determine the serum cholesterol and triglyceride (Allain et al. 1974). All the above parameters were evaluated for 118 horses and 91 donkeys. All samples selected for evaluation were randomly selected to avoid bias.

Correlation of hemato-biochemical parameters

Due to the influence of host intrinsic factors such as breed variation, sex, and age as well as extrinsic factor such as environmental conditions on hematological and serum biochemical parameters in animal host (Gul et al. 2007), we used parameters selected exclusively from a particular breed (Arewa breed) for horses and (Idabari and their closest relative Auraki) for donkeys. Furthermore, the blood parameters used for the computation were exclusively from equids within 6–12 years of age. Equids (both horses and donkeys) were divided into two groups: infected (PCR-positive) and uninfected (PCR-negative). The third group (apparently healthy uninfected) was used as a standard control. The parameter for the third group was retrieved from published literature evaluating these parameters from apparently healthy animals with no clinical signs of any disease. For hematological analysis, blood samples (n=77) were used in the horses comprising of 50 samples for the infected group and 27 for the uninfected group. For donkeys, 50 samples were used for the analysis comprising of 25 samples for the infected group and 25 for the uninfected group. For serum biochemical parameters, blood samples (n=85) for horses comprising of 41 samples for the infected group and 44 samples for the uninfected group while 73 samples were used for donkeys comprising of 33 samples for the infected group for donkeys and 40 samples for the uninfected group.

Statistical analysis

Data are presented as mean ± standard error. All hematological and serum biochemical parameters used for the analysis were first tested for normality using the Kolmogorov-Smirnov test. Further analysis by comparing the hematological and biochemical parameters between the infected and uninfected groups for each species of equid was carried out using unpaired-sample student t test or Mann-Whitney U test depending on the distribution of the data (symmetry or asymmetry). Two-tailed significance level was set at 0.05 unless otherwise stated at 0.001. All analyses were carried out using GraphPad Prism version 5.

Results

Occurrence of equine piroplasms from the blood of equids

Details of the results on the prevalence of equine piroplasms (both *B. caballi* and *T. equi*) have been published previously (Onyiche et al. 2020). Also, information regarding the ages, sex, breed, and other host intrinsic factors have been published and can be found in our earlier article Onyiche et al. 2020. To avoid the influence of these host factors on the clinical findings, we used results exclusively from animals belonging to a particular age group (6–12 years for both horses and donkeys) and breed for both horses (Arewa; West African Barb) and donkeys (Idabari and Auraki).

Hematological changes in horses

The PCV in *T. equi* infected horses was markedly lower (30.8 ± 0.6) compared with the uninfected group (40.6 ± 1.0) (t = 8.6, df=75, P<0.001) (Table 1). Furthermore, changes in the erythrogram were observed for the Hb concentration with a decrease in the infected group (10.1 ± 0.3) compared with the uninfected group which was comparatively higher (13.4 ± 1.7) (t=7.9, df=75, P<0.001). The RBC count in the infected group was marked lower (6.3 ± 0.1) compared with the uninfected group (8.1 ± 0.2, t=7.34, df=75, P<0.001).

Table 1 Hematological profile in naturally *Theileria equi* infected and non-infected horses

Parameters	Mean ± ***SE*** (95%CI)		Mean ± SD (95%CI)
	Positive (infected) (*n*=50)	Negative non-infected (*n*=27)	Reference value (Schalm et al. 1975)
Packed cell volume (PCV) (%)	30.8 ± 0.6[a] (29.6–32.1) *P* = 0.009	40.6 ± 1.0[b] (38.5–42.7) *P* < 0.0001	41 ± 4.5 (32–53)
Hemoglobin (Hb) (g/dl)	10.1 ± 0.3[a] (9.5–10.6) *P* = 0.0003	13.4 ± 1.7[b] (12.7–14.1) *P* = 0.079	14.4 ± 1.7 (11.0–19.0)
White blood cell count (WBC) (×10^3/µl)	9.9 ± 0.4[a] (9.1–10.7) *P* > 0.10	10.5 ± 2.0[a] (9.6–11.3) *P* = 0.04	9.1 ± 1.8 (5.4–14.3)
Red blood cell count (RBC) (×10^6/µl)	6.3 ± 0.1[b] (5.9–6.6) *P* = 0.0135	8.1 ± 0.2[a] (7.7–8.5) *P* = 0.029	9.0 ± 1.2 (6.8–12.9)
Platelet count (PL) (×10^3/µl)	175.5 ± 7.2[a] (161.0–189.9) *P* > 0.10	175.3 ± 7.3[a] (160.3–190.4) *P* > 0.10	NA
Mean corpuscular volume (MCV) (fl)	50.1 ± 0.5[a] (49.2–51.0) *P* > 0.10	50.4 ± 0.4[a] (49.5–51.3) *P* > 0.10	45.5 ± 4.3 (37.0–58.5)
Mean corpuscular hemoglobin (MCH) (pg)	16.4 ± 0.2[a] (15.9–16.9) *P* = 0.097	16.7 ± 0.2[a] (16.3–17.1) *P* > 0.10	15.9 ± 1.5 (12.3–9.7)
Mean corpuscular hemoglobin concentration (MCHC) (g/dl)	32.4 ± 0.5[a] (31.5–33.3) *P* < 0.0001	33.0 ± 1.2[a] (32.6–33.5) *P* > 0.10	35.2 ± 1.4 (31.0–38.6)
Neutrophil (%)	46.5 ± 2.4[b] (41.8–51.2) *P* > 0.10	42.4 ± 3.3[b] (35.7–49.2) *P* > 0.10	47.5 ± 12.3 (22.6–85.8)
Lymphocytes (×10^3/µl)	46.2 ± 2.3[b] (41.5–50.9) *P* > 0.10	48.8 ± 3.6[b] (41.5–56.2) *P* = 0.0004	35.0 ± 11.2 (15.0–7.0)
Monocytes (%)	4.6 ± 0.3[a] (4.1–5.2) *P* = 0.065	5.6 ± 0.5[a] (4.6–6.5) *P* = 0.059	3.8 ± 2.3 (0–10)
Eosinophil (%)	2.8 ± 0.2[a] (2.4–3.2) *P* = 0.001	3.2 ± 0.4[a] (2.4–3.9) *P* > 0.10	3.1 ± 2.4 (0–10)
Basophil (%)	0.0 ± 0.0[a] (0–0)	0.0 ± 0.0[a] (0–0)	0

NA not available

The *P* value insert in the table is the Kolmogorov-Smirnov normality test

[a, b, c, d]Columns with different superscript differs significantly

Significant level set at $P < 0.001$

Granulocytes like the neutrophils were higher in the infected group (46.5 ± 2.4) compared with the uninfected group (42.4 ± 3.3) (*t*=1.0, *df*=75, *P*=0.32) (Table 1).

Hematological changes in donkeys

Significant differences were observed in the erythrogram of donkeys infected with *T. equi*. The PCV was lower in the infected group (28.5 ± 0.7) compared with the uninfected group (32.2 ± 0.5) (*t*=4.54, *df*=48, *P*<0.001) (Table 2). Similarly, the Hb (*t*=3.59, *df*=48, *P*=0.0008) and RBC (*t*=3.27, *df*=48, *P*=0.0020) counts for the infected groups were lower than that of the uninfected group and varied significantly. The MCHC for the infected group was higher than that of the uninfected group but was not significant (*t* = 9.1, *df*=48, *P*=0.288) (Table 2). The lymphocyte counts for the uninfected group (51.8 ± 4.4) were higher than that of the infected group (41.3 ± 2.9) (*t*=2.00, *df*=48, *P*=0.05) but the opposite was the case for the monocytes where the relative count was higher in the infected group (6.2 ± 0.4) compared with the uninfected group (5.2 ± 0.4) (*t*=1.81, *df*=48, *P*=0.08) (Tables 2).

Serum biochemical changes in horses

No significant changes were observed for the liver function markers in the horses such as AST, ALT, and ALP with similar values in the infected and uninfected groups (*t*=0.59, *df*=71, *P* = 0.55) (Table 3). Similar trend was observed for other markers such as triglyceride and cholesterol. Additionally, the total bilirubin was higher in the infected group (31.9 ± 2.5) compared with the uninfected (28.6 ± 1.9) (*t*=1.09, *df*=83, *P* =0.28) (Table 3).

Serum biochemical changes in donkeys

The AST (*P*=0.78) and ALT (*P*=0.36) levels in the infected group was lower compared to that of the uninfected group

Table 2 Hematological profile in naturally *Theileria equi* infected and non-infected donkeys

Parameters	Mean ± ***SE*** (95%CI)		Reference value (Burden et al. 2016)
	Positive (infected) (*n*=25)	Negative (non-infected) (*n*=25)	
Packed cell volume (PCV) (%)	28.5 ± 0.7^{b} (27.2–29.9) *P* = 0.013	32.2 ± 0.5^{c} (31.2–33.3) *P* = 0.003	33 (27–42)
Hemoglobin (Hb) (g/dl)	9.5 ± 0.2^{a} (9.1–9.9) *P* = 0.088	10.5 ± 0.2^{b} (10.2–10.9) *P* > 0.10	11 (8.9–14.7)
White blood cell count (WBC) (×10^{3}/µl)	10.2^{a} ± 4.3 (8.5–11.9) *P* > 0.10	13.0 ± 0.8^{c} (11.3–14.8) *P* > 0.10	18.3 (16.1–22)
Red blood cell count (RBC) (×10^{6}/µl)	4.9 ± 0.1^{a} (4.6–5.2) *P* = 0.079	5.5 ± 0.1^{c} (5.3–5.8) *P* > 0.10	5.5 (4.4–7.1)
Platelet count (PL) (×10^{3}/µl)	361.6^{a} ± 13.4 (333.7–389.4) *P* > 0.10	361.5^{a} ± 16.8 (326.8–396.1) *P* = 0.08	201 (95–384)
Mean corpuscular volume (MCV) (fl)	53.7^{c} ± 2.6 (48.2–59.1) *P* < 0.0001	57.1 ± 1.9^{c} (52.2–60.9) *P* = 0.0003	60.0 (53–67)
Mean corpuscular hemoglobin (MCH) (pg)	24.6 ± 2.6^{a} (19.2–30.1) *P* < 0.0001	20.7^{a} ± 1.2 (18.3–23.2) *P* < 0.0001	20.6 (17.6–23.1)
Mean corpuscular hemoglobin concentration (MCHC) (g/dl)	34.3 ± 0.9^{c} (32.5–36.1) *P* < 0.0001	33.3 ± 0.3^{c} (32.7–33.8) *P* > 0.10	34 (31–37)
Neutrophil (%)	47.2 ± 2.3^{b} (42.4–51.9) *P* > 0.10	40.4 ± 3.9^{b} (32.2–48.7) *P* = 0.001	38.3 (23–59)
Lymphocytes (×10^{3}/µl)	41.3 ± 2.9^{d} (35.4–47.2) *P* = 0.098	51.8 ± 4.4^{b} (42.7–60.8)* *P* = 0.0003	54 (34–69)
Monocytes (%)	6.2 ± 0.4^{a} (5.5–6.9) *P* > 0.10	5.2 ± 0.4^{a} (4.2–6.0) *P* = 0.075	3.0 (0–7.5)
Eosinophil (%)	3.2 ± 0.4^{b} (2.4–3.9) *P* = 0.021	2.7 ± 0.3^{b} (2.0–3.4) *P* > 0.10	4.0 (0.9–9.1)
Basophil (%)	0.0 ± 0.0^{a} (0–0)	0.0 ± 0.0^{a} (0–0)	0.05 (0–0.5)

NA not available

The *P* value insert in the table is the Kolmogorov-Smirnov normality test

$^{a, b, c, d}$Columns with different superscript differs significantly

Significant level set at *P* < 0.001

with no significant difference between groups. The albumin and globulin levels were also lower in the infected groups compared with the uninfected groups ($P > 0.05$). The total bilirubin levels were higher in the infected group (7.2 ± 0.5) compared with the uninfected group (6.9 ± 0.3) ($t = 0.56$, df = 71, $P = 0.56$) (Table 4). The kidney function markers such as urea (P=0.02) and creatinine (P=0.005) were higher in the uninfected group compared with the infected group and differed significantly (Table 4).

Discussion

The success of any clinical treatment is dependent on proper diagnosis based on adequate clinical examination, hematological and serum biochemical assessment which are indications of biological deviations (Agina et al. 2017). Accurate interpretations of hematological and serum biochemical parameters are sometimes difficult since numerous factors influence these parameters. These include age, sex, breed, season, work, exercise, sub-clinical disease, and health status in horses and donkeys (Melo et al. 2013; Zakari et al. 2016). Due to low number of positive animals (horses = 4, donkeys = 3) associated with infection with *B. caballi* using PCR, it was difficult to carry out any robust assessment of the clinico-pathological alterations associated with the infection in equids in Nigeria. Hence, we excluded it from our analyses to avoid any undue conclusion. To avoid observation that was speculative due to low statistical power, we excluded hematological analysis of *B. caballi* infections due to low number of positive cases of *B. caballi* in donkeys. In a similar study in Italy, Laus et al. (2015) suggested that further analysis should be avoided due to low statistical power.

We observed hematological variations attributed to natural infection with *T. equi* in apparently healthy horses and donkeys. This included reduction in PCV, hemoglobin concentration, and red blood cell count (RBC). This corroborates previous findings on EP in the equids as observed in different countries (De Waal 1992; Camacho et al. 2005; Zobba et al. 2008; Sanusi et al. 2014; Laus et al. 2015).

Table 3 Serum biochemical changes in naturally *Theileria equi* infected and non-infected horses

Parameters	Mean ± ***SE*** (95%CI)		Reference value (Ememe 2015)
	Positive (Infected) (*n*=41)	Negative (non-infected) (*n*=44)	
Aspartate aminotransferase (AST) (IU/L)	78.6 ± 2.0[a] (74.5–82.8) *P* = 0.03	78.5 ± 2.0[a] (74.4-82.6) *P* = 0.07	72.8 ± 8.3 (23–108)
Alanine aminotransferase (ALT) (IU/L)	4.4 ± 0.2[b] (3.9– 4.9) *P* = 0.001	5.1 ± 0.4[b] (4.2–5.9) *P* = 0.0003	6.0 (3.0–15.0)
Alkaline phosphatase (ALP) (IU/L)	116.6 ± 4.4[a] (107.8–125.4) *P* =0.02	116.5 ± 5.0[a] (106.4–126.7) *P* = 0.0004	115.1
Total protein (TP) (g/L)	68.9 ± 1.4[a] (65.9–71.7) *P* > 0.10	67.7 ± 2.1[a] (63.6–71.9) *P* = 0.05	58.8
Albumin (Alb) (g/L)	27.9 ± 0.5[a] (26.8–28.9) *P* > 0.10	28.5 ± 0.9[a] (26.6–30.4) *P* = 0.005	32.7
Globulin (Glb) (g/L)	41.0 ± 1.3[b] (38.4–43.6) *P* > 0.10	39.2 ± 1.5[b] (36.1–42.3) *P* > 0.10	36.1
Total bilirubin (BT) (µmol/L)	31.9 ± 2.5[b] (26.9–37.1) *P* = 0.05	28.6 ± 1.9[b] (24.6–32.5) *P* > 0.10	NA
Cholesterol (Chol) (mmol/l)	2.1 ± 0.09[c] (1.9–2.3) *P* > 0.10	2.1 ± 0.09[c] (1.9–2.3) *P* = 0.002	2.27 (1.2–4.6)
Triglyceride (TG) (mmol/l)	0.5 ± 0.02[a] (0.5–0.6) *P* = 0.01	0.5 ± 0.03[a] (0.4–0.5) *P* = 0.09	0.38 (0.1–0.5)
Urea (Ur) (mmol/l)	6.9 ± 0.3[d] (6.4–7.5) *P* = 0.02	6.7 ± 0.4[d] (5.9–7.5) *P* = 0.09	4.5 (3.5–7.0)
Creatinine (Cr) (µmol/L)	117.2 ± 3.4[b] (110.2–124.3) *P* > 0.10	122.6 ± 6.4[b] (109.7–135.5) *P* <0.0001	133.0 (103–166)

The *P* value insert in the table is the Kolmogorov-Smirnov normality test

[a, b, c, d]Columns with different superscript differs significantly

Significant level set at $P < 0.001$

Despite the changes in these parameters, PCR-positive infected animals were apparently healthy. Previously, in an experimental infection of horses with both *B. caballi* and *T. equi*, the PCV decreased down to 17% without any overt clinical signs (De Waal et al. 1987). Anemia is one of the principal clinical manifestations of EP due to low PCV. The adherence of the parasite antigen to the red blood cells surface leads to intravascular hemolysis of the RBCs. Furthermore, anemia can also be the result of toxic hemolytic factor released by the parasite itself (El-Sherif et al. 2019). Macrocytic hypochromic anemia in equines with EP has been reported by other workers (Zobba et al. 2008; Mahmoud et al. 2016). The probable underlying pathophysiological mechanisms causing hemolysis of red blood cells ultimately leading to anemia has been described elsewhere (Ambawat et al. 1999; Onyiche et al. 2019). Since all the sampled animals were apparently healthy and thus sub-clinically infected with *T. equi*, this infection will probably decrease their work performance most especially in the donkeys and polo horses. This assumption is largely speculative and will require further confirmation.

We observed a decrease in the total white blood cell count between the infected and uninfected group which differs significantly in the donkeys but not in the horses. Similar observation has been made in the donkeys (Laus et al. 2015) and separately in horses (Mahmoud et al. 2016; Osman 2017). Furthermore, we observed a decrease in the agranulocytes (lymphocytes and monocytes) in the infected group compared with the uninfected. Monocytopenia may be due to interruption in granuloma formation as a result of the infection (Territo 2018) while, lymphocytopenia may be due to the effect of the infection on the lymphoid tissues which leads to atrophy and degeneration of the lymphocytes (Squire 1968). Thus, the observed decrease in the lymphocytes as well as monocytes may impair immune response to other insults or pathogens in the affected animals. On the other hand, we observe an increase in relative granulocytes count, mainly the neutrophils in the infected group. One of the characteristics of acute experimental infection of donkeys with *T. equi* is an increase in absolute neutrophil count without these donkeys showing clinical signs (Kumar et al. 2009), corroborating our observation in this study. Nonetheless, more investigations are needed to ascertain whether this alteration are due to natural piroplasms infection or may be due to concomitant subclinical diseases. Furthermore, there was no difference in the platelets count for both the infected

Table 4 Serum biochemical changes in naturally *Theileria equi* infected and non-infected donkeys

Parameters	Mean ± SE (95%CI)		Reference value (Burden et al. 2016)
	Positive (Infected) (*n*=33)	Negative (non-infected) (*n*=40)	
Aspartate aminotransferase (AST) (IU/L)	101.4 ± 7.9[a] (85.2–117.6) *P*<0.0001	108.6 ± 8.8[a] (90.8–126.4) *P* = 0.02	362 (238–536)
Alanine aminotransferase (ALT) (IU/L)	9.3 ± 1.22[d] (6.8–11.8) *P* = 0.012	9.8 ± 0.9[d] (8.1–11.6) *P* > 0.10	NA
Alkaline phosphatase (ALP) (IU/L)	118.0 ± 8.2 (101.3–134.7) *P* > 0.10	127.4 ± 10.4 (106.3–148.5) *P* < 0.0001	152 (98–252)
Total protein (TP) (g/L)	64.9 ± 2.2 (60.6–69.4) *P* > 0.10	66.9 ± 2.6 (61.6–72.2) *P* > 0.10	65 (58–76)
Albumin (Alb) (g/L)	25.9 ± 0.8[b] (24.3–27.5) *P* > 0.10	26.8 ± 0.9[b] (25.0–28.5) *P* = 0.011	26 (21.5–31.6)
Globulin (Glb) (g/L)	39.1 ± 2.1[a] (34.9–43.3) *P* > 0.10	40.1 ± 2.2a (35.6–44.7) *P* > 0.10	38 (32–48)
Total bilirubin (BT) (µmol/L)	7.2 ± 0.5[a] (6.2–8.3) *P* = 0.014	6.9 ± 0.3[a] (6.3–7.5) *P* = 0.06	1.6 (0.1–3.7)
Cholesterol (Chol) (mmol/l)	2.4 ± 0.1c (2.1–2.7) *P* > 0.10	3.0 ± 0.2[d] (2.7–3.3) *P* > 0.10	2.0 (1.4–2.9)
Triglyceride (TG) (mmol/l)	0.9 ± 0.0[c] (0.8–0.9) *P* = 0.07	1.0 ± 0.07[c] (0.9–1.2) *P* > 0.10	1.4 (0.6–2.8)
Urea (U) (mmol/l)	5.2 ± 0.2c (4.7–5.7) *P* = 0.04	6.1 ± 0.3[b] (5.5–6.7) *P* > 0.10	3.2 (1.5–5.2)
Creatinine (Cr) (µmol/L)	96.7 ± 3.2c (90.1–103.3) *P* = 0.005	108.4 ± 3.2[d] (101.9–114.9) *P*= 0.03	87 (53–118)

NA not available

The *P* value insert in the table is the Kolmogorov-Smirnov normality test

[a, b, c, d]Columns with different superscript differs significantly

Significant level set at $P < 0.001$

and uninfected group of equids examined in our study. It is interesting to note that while some authors have reported the thrombocytopenia as one of the hematological observations in both experimental and natural infection (De Waal et al. 1987; Zobba et al. 2008), in this study, we did not observe this feature. It is therefore likely that the strain of the infecting piroplasms could possibly play a role in the hematological observations. The mechanism of thrombocytopenia in equines associated with EP is still poorly understood. Other authors have suggested the probable mechanism of thrombocytopenia due to EP in the horses to be due to localized or systemic disseminated intravascular coagulation, sequestration of platelets in the spleen or due to immune mediated destruction (Boozer and Macintire 2003).

Clinically, EP is associated with some biochemical changes as evident in various independent studies carried out. Variations in these parameters can also be influenced by hydration status, nutrition, exercise, general state of health, and the concurrent presence of other infectious agents (Onyiche et al. 2019). Some biochemical changes associated with natural sub- clinical infection of *T. equi* was observed in this study. Firstly, we observed a slight increase in total bilirubin (TB) in the infected compared with the uninfected group both in the horses and donkeys. This has been widely described in natural infection due to EP parasites in equids which is a consequence of hemolytic anemia (Zobba et al. 2008; Al-Obaidi et al. 2016). Continuous destruction of infected RBC and its removal from the circulation leads to increases in the bilirubin levels in the urine (Alssad 2009). Furthermore, intense exercise due to increased muscular activity could also cause an increase in TB.

Secondly, in the horses, the total protein and globulin fractions were slightly higher in the infected compared to the uninfected group. In contrast, the albumin fraction was slightly lower in the infected compared to uninfected group, although the differences were not significant. Hypoalbuminemia has been previously reported in horses with EP (Zobba et al. 2008). It is well documented that the increase in the total proteins in horses with babesiosis may be due to dehydration as well as due to increase in the γ-globulin concentration (Radostits et al. 1999; Kumar et al. 2002). Albumin is regarded as negative acute phase protein that decreases due to impaired hepatic synthesis or extensive protein degradation in the course of prolonged fever (Dede et al. 2008). Hypoalbuminemia can also result from urinary albumin losses (Kaysen 1998). Since EP is characterized by

fever, it may be responsible for this observation. In the donkeys, the levels of total protein including the albumin and globulin fractions in the infected group were slightly lower compared to the uninfected. Decreased protein profile due to infection with blood parasites occurs because of digestive disturbances as well as decreased production from the liver (Barrera et al. 2010). The slight differences in the observations regarding protein levels in the horses and donkeys chronically infected with *T. equi* remains largely unknown.

Thirdly, we observed no difference in the cholesterol and triglyceride levels in both the infected and uninfected groups in the horses and donkeys naturally infected with *T. equi*. In previous studies, no change was observed between the *T. equi* infected and uninfected horses. The observed values all fall within the normal range (Zaeemi et al. 2016).

Fourthly, no significant differences were observed in the levels of AST, ALT, and ALP in the infected compared to the uninfected group for both horses and donkeys. These parameters are markers of liver and muscle injury. In Malaysia, Al-Obaidi et al. (2016) found no difference in the levels of these enzymes between the control (negative) and subclinically (positive) infected equids, which corroborates our findings. Nevertheless, other workers have observed variations in these parameters in the positive compared with the negative group (Hailat et al. 1997; Camacho et al. 2005). The urea and creatinine levels were all within the reference value in the current study and the slight variations observed between the two groups could be attributed to dehydration as this has been found to influence the levels of these enzymes in the body (Camacho et al. 2005).

The absence of clinical disease and the observation of hematological and biochemical alterations in both horses and donkeys in the study areas confirm the widespread existence of subclinical forms of EP. This phenomenon could be attributed to the marked genetic diversity of the circulating genotypes of *T. equi* in the study area which have been recently reported in some parts of Northern Nigeria (Idoko et al. 2020; Mshelia et al. 2020) as well as in South Africa (Bhoora et al. 2020). It is therefore expedient that an effective control measure is instituted to prevent undesirable effects and loss of economic benefits from international trade/movement of horses and working capacity of donkeys. Some of the control measures that could be instituted include routine surveillance by mass screening of equids, prophylactic treatment of equids and vector control by the use of acaricides.

Conclusion

We assessed hemato-biochemical alterations in apparently healthy horses and donkeys infected with *T. equi*. Despite the marked changes in PCV, RBC, Hb concentrations as well as a decrease in the total white blood cell count between the PCR-positive equids compared with those that are negative, the animals looked apparently healthy. No changes in liver and kidney function markers were found indicating that the probable strain circulating may not be highly pathogenic. Nevertheless, we recommend that further studies be undertaken to ascertain the production performance such as weight gain, work performance, and milk quantity and quality in these groups of animals that were screened and found to be infected with *T. equi*.

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Author contribution TEO and OT conceived and designed the research. TEO conducted experiments. TEO, EI, SAM, IJO, AAB, and OT analyzed data. TEO wrote manuscript draft. EI, SAM, IJO, AAB, and OT critically reviewed the article. OT supervised the study and helped in the acquisition of funding. All authors read and approved the manuscript.

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Data availability All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Permission to carry out the study was approved by the animal research ethics committee of the North-West University South Africa with ethics number NWU-01242-19-S9 in line with the guidelines of the committee.

Conflict of interest The authors declare no competing interests.

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