HAEMATOLOGICAL AND SERUM BIOCHEMICAL FINDINGS ASSOCIATED WITH NATURAL TRYPANOSOME INFECTIONS IN NIGERIAN HORSES

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Abstract

This study evaluated the haematological and serum biochemical findings associated with natural trypanosome infections in Nigerian horses. The study population were trade horses presented for sale and slaughter at the Obollo Afor horse Lairage/Slaughter slab, Udenu Local Government Area, Enugu State, Nigeria. The horse Lairage/Slaughter slab was visited once a week for 6 months during which period a total of 207 horses that were bought for slaughter were clinically examined and blood collected from them for parasitological examination, haematology and serum biochemistry determinations. Out of the 207 horses studied, 5 (2.4%) were infected with trypanosomes, and the trypanosome species involved was Trypanosoma brucei in all the 5 cases. The mean packed cell volume, red blood cell count, haemoglobin concentration, total leukocyte, neutrophil and eosinophil counts, serum protein and albumin levels of the trypanosome infected horses (TIH) was significantly ($p < 0.05$) lower than that of the non-trypanosome infected apparently healthy horses (AHH). The mean erythrocyte corpuscular volume, monocyte and platelet counts, erythrocyte sedimentation rate, serum alanine aminotransferase and aspartate aminotransferase activities were significantly ($p < 0.05$) higher than that of the AHH. There was however no significant ($p > 0.05$) differences between the TIH and AHH in their mean erythrocyte mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, lymphocyte and basophil counts, serum alkaline phosphatase activity, and total cholesterol, creatinine and total bilirubin levels.

Key words: Trypanosomosis, Haematology, Serum biochemistry, Nigerian horses

Introduction

African trypanosomosis (Nagana) is a very important disease of animals and humans characterized by anaemia, dullness, cachexia, corneal opacity, enlargement of the superficial lymph nodes, oedema of the ventral abdomen and reproductive disorders (Taylor & Authie, 2004; Radostits *et al.*, 2007). The disease is caused by organisms of the genus *Trypanosoma* and transmitted cyclically by tse-tse fly (*Glossina* spp) or mechanically by other biting flies such as *Tabanus spp.* and *Stomoxys calcitran* (Radostits *et al.*, 2007). Trypanosomosis is one of the major health constraints and obstacle to livestock development and agricultural production in the tropics. The evaluation of the haematological and serum biochemical profile is of importance in animals and humans because the blood is the major transporter of substances in the body, and any deviations from normal caused by derangement of metabolic processes, invasion of the body by pathogens, deprivation, stress and other forms of injury/insult commonly translate to changes in the haematological and serum biochemical parameters. Such evaluations are important because of their predictive value of pathological changes in vital internal organs of the body, thus haematological and serum biochemical assays are indispensably important in arriving at a diagnosis, making a prognosis and assessing the efficacy of therapy and the toxicity of drugs and chemical substances (Schalm *et al.*, 1975; Ihedioha, 2004; Stockham & Scott, 2008). Nigerian horses, which are a group made up of the mixed Arewa breeds and their crosses with Arabian, Dongola and Sudanese breeds, are used mainly for draft, transport, sports, ceremonial exhibitions, research purposes, and as food (meat) (Ihedioha & Agina, 2013 & 2014). Horses, like most other domestic animals are susceptible to trypanosome infections (Anosa, 1988a & b; Taylor & Authie, 2004). There had been several reports of haematological and serum biochemical abnormalities associated with African trypanosomosis in several animals and humans (Anosa 1988a & b; Taylor & Authie, 2004), but very little information in available literature on trypanosome infection in horses (Kihurani et al., 1994; Mekuria et al., 2010; DaSilva et al., 2011) and none in Nigerian horses specifically. The objective of this present study was to evaluate the haematological and serum biochemical findings associated with natural trypanosome infections in Nigerian horses.

Materials and Methods

This study was a cross-sectional disease surveillance survey. The study population was Nigerian horses sold for slaughter at Obollor Afor Horse Lairage/Slaughter slab, Udenu Local Government Area, Enugu State, Nigeria. A total of 207 of these horses were evaluated for the study. These 207 were horses bought and billed for slaughter on the days of research visit to the Obollo Afor Horse Lairage/Slaughter slab – the research visits were made once a week for a period of six months (Feb. – August 2012). All horses bought and slaughtered on each day of visit were evaluated. Each of the horses was physically examined, and blood samples collected for parasitological, haematology and serum biochemistry evaluations. A wet mount of each horse's blood sample was made immediately upon blood sample collection to check for trypanosomes. Blood samples of trypanosome infected horses (TIH) were retained for further evaluation, and for each horse that was found to be positive for trypanosomes, blood from six other non-trypanosome infected apparently healthy horses (AHH) were also retained to serve as control. Blood for haematology and further parasitological examination were dispensed into sample bottles containing ethylene diamine tetra acetic acid (EDTA) anticoagulant, while the blood samples for serum biochemical assay were dispensed into clean plain glass test tubes and allowed to stand for 30 minutes at room temperature to clot. Serum for the biochemical assays was thereafter separated from clot by centrifugation. All the further parasitological, haematological and biochemical determinations were done immediately after blood sample collection. Blood film for wet mount trypanosome detection and thin blood film for trypanosome identification was done following standard procedures (Hoare, 1972; Taylor & Authie, 2004). The packed cell volume (PCV) was determined by the microhaematocrit method, the haemoglobin concentration (HbC) by the cyanomethemoglobin method, and the red blood cell (RBC) counts and total leukocyte counts (TLC) by haemocytometer method. Blood smear for differential leukocyte count were stained following the Leishman technique and enumerated by the battlement counting method. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae. The erythrocyte sedimentation rate (ESR) was determined by the Wintrobe method (Schalm et al., 1975; Thrall & Weiser, 2002). The platelet count (PC) was done by the Rees and Ecker direct counting method (Brown, 1976). The serum biochemistry determinations (alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities, total bilirubin, total proteins, albumin, globulin, urea, creatinine and total cholesterol) were done following standard procedures (Burtis et al., 2008), using Quimica Clinica Aplicada (QCA) test kits (QCA, Spain). Data generated from the study was subjected to appropriate statistics using SPSS statistical package. The differences between the haematological and serum biochemistry parameters of the trypanosome-infected horses and apparently healthy horses were compared using student's t - test. Significant difference was accepted at $p<0.05$.

Results, Discussion and Conclusion

Out of the 207 horses evaluated, 5 (2.4%) were trypanosome infected, and the trypanosome species involved was identified morphologically as *Trypanosoma brucei* in all the 5 cases. The 2.4% occurrence recorded in this study is relatively lower than the 10.7% reported by Mekuria et al. (2010) in a similar cross-sectional study, and far lower than the 45.7% and 33.3% reported in outbreaks by Kihurani et al. (1994) and DaSilva et al. (2011), respectively. The relatively lower percentage occurrence recorded in this study may be due to the very high usage of trypanocides in our environment. The finding in this study that the trypanosome responsible for infections is *T. brucei* contrasts with the reports of Mekuria et al. (2010) who reported *T. congolense* and *T. vivax* and that of DaSilva et al. (2011) who reported *T. vivax* alone, but is in agreement with the reports of Kihurani et al. (1994) who reported *T. brucei* and other species. The mean PCV, RBC count, HbC, TLC, segmented neutrophil and eosinophil counts of the trypanosome infected horses (TIH) were significantly ($p < 0.05$) lower than those of the apparently healthy horses (AHH), while the MCV, absolute monocyte and platelet counts, and ESR of the TIH were significantly ($p < 0.05$) higher than those of AHH (Table 1). However, the MCH, MCHC and basophil and lymphocyte counts of the TIH did not significantly ($p > 0.05$) differ from those of AHH (Table 1). The findings of a significantly low PCV, RBC count and HbC, and TLC associated with neutropenia, eosinopenia, monocytosis, and a higher ESR in the horses with trypanosomosis are in agreement with the reports of Anosa (1988a), Kihurani *et al.* (1994) and Da Silva *et al.* (2011). The significantly lower TLC recorded in this study due to lowered neutrophil,

Table 1. Haematological and serum biochemical profile of trypanosome infected horses compared to apparently healthy horses.

Haematological and Serum biochemical parameters	Means ± standard error	
	Trypanosome infected horses (n = 5)	Apparently healthy horses (n = 30)
Packed cell volume (%)	13.17 ± 2.95 [a]	42.70 ± 0.83 [b]
Red blood cell count (10^6/µl)	2.10 ± 0.54 [a]	8.34 ± 0.21 [b]
Haemoglobin concentration (g/dl)	4.09 ± 1.23 [a]	15.03 ± 0.32 [b]
Mean corpuscular volume (fl)	63.65 ± 1.85 [a]	49.91 ± 0.51 [b]
Mean corpuscular haemoglobin (pg)	19.11 ± 0.84	17.86 ± 0.41
Mean corpuscular haemoglobin conc. (g/dl)	30.15 ± 2.20	35.27 ± 0.68
Total leukocyte count (10^3/µl)	6.46 ± 0.70 [a]	9.60 ± 0.33 [b]
Band neutrophils (10^3/µl)	0.45 ± 0.10	0.29 ± 0.05
Segmented Neutrophils (10^3/µl)	2.55 ± 1.51 [a]	4.84 ± 0.27 [b]
Lymphocytes (10^3/µl)	3.27 ± 0.46	4.18 ± 0.20
Monocytes (10^3/µl)	0.15 ± 0.04 [a]	0.03 ± 0.01 [b]
Eosinophils (10^3/µl)	0.03 ± 0.02 [a]	0.24 ± 0.04 [b]
Basophils (10^3/µl)	0.01 ± 0.01	0.01 ± 0.01
Platelets (10^3/µl)	490.00 ± 21.18 [a]	321.56 ± 15.18 [b]
Erythocyte sedimentation rate (mm/20 min)	50.65 ± 4.77 [a]	27.76 ± 3.38 [b]
Alanine amino transferase (IU/L)	46.35 ± 14.07 [a]	10.02 ± 0.73 [b]
Aspartate aminotransferase (IU/L)	104.99 ± 2.32 [a]	93.23 ± 1.05 [b]
Alkaline phosphatase (IU/L)	84.54 ± 19.95	72.34 ± 5.46
Total protein (g/dl)	6.13 ± 0.05 [a]	6.85 ± 0.06 [b]
Albumin (g/dl)	1.67 ± 0.20 [a]	3.36 ± 0.04 [b]
Globulin (g/dl)	4.46 ± 0.15 [a]	3.49 ± 0.07 [b]
Total cholesterol (mg/dl)	112.21 ± 1.59	109.69 ± 3.06
Total bilirubin (mg/dl)	2.16 ± 0.10	3.39 ± 0.14
Creatinine (mg/dl)	1.04 ± 0.27	1.29 ± 0.05
Urea (mg/dl)	51.03 ± 1.21 [a]	30.78 ± 0.86 [b]

[a b] Different alphabetical superscripts in a row indicate significant difference between the means of a parameter between trypanosome infected horses and apparently healthy horses ($p < 0.05$)

lymphocyte and eosinophil numbers could be attributed to bone marrow hypoplasia (Stockham & Scott, 2008). The monocytosis observed could be due to the fact that monocyte precursors are not commonly affected by bone marrow hypoplasia in trypanosomosis (Anosa, 1988a). The anaemia recorded in the TIH may be due to dyshaemopoiesis, bone marrow hypoplasia and/or red blood cells haemolysis (Anosa, 1988a). The mean serum ALT and AST activities and serum globulin and urea levels of the TIH were significantly ($p < 0.05$) higher than those of the AHH, while the mean serum total protein and albumin levels of the TIH were significantly ($p < 0.05$) lower than those of the AHH (Table 1). There were no significant differences ($p > 0.05$) between the mean serum ALP activity, serum total cholesterol, creatinine and total bilirubin levels of the TIH when compared with those of the AHH (Table 1). The significantly higher serum ALT and AST activities recorded for the TIH could be due to liver damage by the parasites and their toxic metabolites (Anosa, 1988b). Also, the significantly higher serum urea levels recorded for the TIH could be attributed to renal insufficiency and/or kidney damage caused by the toxic metabolites of the parasites (Anosa, 1988b; Kihurani *et al.*, 1994). The significantly higher globulin levels recorded for the TIH may be due to increased production of beta and gamma globulins which is usually attributed to antigenic variation-induced chronic antigenic challenge (Kihuran et al. 1994; Taylor & Authie, 2004). The significantly lower serum total protein recorded for the TIH in this present study contrasts with the reports of Kihurani *et al.*, (1994) and Da Silva *et al.* (2011), who reported significantly higher serum total protein levels in TIH. Based on the results of this study, it was concluded that natural trypanosome infections in Nigerian horses is mainly by *T. brucei*, and is characterized by macrocytic normochromic anaemia, significantly lower TLC, neutropaenia, cosinopaenia and monocytosis, significantly lower serum total protein and albumin levels, and significantly higher serum ALT and AST activities and urea levels.

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