NIGERIAN VETERINARY JOURNAL

ISSN 0331-3026

Nig. Vet. J., September 2022 Vol 43 (3): 1 - 15.

https://dx.doi.org/10.4314/nvj.v43i3.1 **ORIGINAL ARTICLE**

COMPARATIVE BIOCHEMICAL CHANGES INDUCED BY EXPERIMENTAL INFECTION OF *TRYPANOSOMA CONGOLENSE* AND *TRYPANOSOMA BRUCEI* IN WEST AFRICAN DWARF (WAD) SHEEP.

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SUMMARY

Trypanosomosis is still a major impediment to livestock production in most tropical environments despite various efforts geared towards eradicating the disease. Small ruminants are known to be fully susceptible to pathogenic trypanosome infections, however, studies in small ruminants are still scanty compared to large ruminants. Comparative pathogenicity and biochemical changes in twenty West African Dwarf (WAD) sheep aged between 8-10 months were investigated. Sheep were randomly assigned into groups A and B and experimentally infected with either *Trypanosoma congolense* or *Trypanosoma brucei*. Both species of trypanosomes caused significant but varying degrees of alterations in the biochemical parameters studied. There was a consistent and significant increase ($P<0.05$) in the levels of total proteins, globulin, fibrinogen, urea, creatinine, sodium, bicarbonate, inorganic phosphate and chloride from day 21 post infection in both groups, and these were sustained till the experiment was terminated. Similarly, the levels of albumin, cholesterol and albumin/globulin ratio progressively decreased ($p<0.05$) post infection starting from day 28, whereas potassium levels remained unchanged throughout post-infection period. There was a strong positive correlation between total protein and urea in groups A and B ($r = 0.937$ and $r = 0.908$), respectively as well as between total protein and creatinine in groups A and B ($r = 0.937$ and $r = 0.908$), respectively. Generally, significantly higher biochemical alterations ($p<0.05$) in *T. congolense*-infected sheep. It can thus be concluded that trypanosome infection in sheep could lead to significant pathological and functional disorders in vital organs including liver and kidney which may have resulted in the biochemical alterations observed; these being influenced by individual trypanosome species.

Keywords: Plasma biochemical changes, ovine Trypanosomosis, experimental infection.

INTRODUCTION

Small ruminants are an important component of the socio-economic life of rural dwellers particularly in Nigeria where they contribute significantly to livelihood. Animal trypanosomosis is known to cause impediment to livestock production and productivity with a wide distribution in Africa, Asia and Latin America (Krammer 1986; Murray and Dexter 1988; Katunguka-Rwakishaya *et al.,* 1992; Luckins 1992; Van den Bossche 2000; Sam-Wobo *et al.,* 2010; Dede *et al.,* 2013; Ode *et al.,* 2017). The disease is reported to cause serious health problem and humongous economic losses in Nigeria (Olaniyi *et al.,* 2001; Yanan *et al.,* 2007; Fatihu *et al.,* 2009; Anyaegbunam and Okafor, 2013; Isaac *et al.,* 2017). The disease in ruminants is caused by several species of trypanosomes including *T. congolense, T. vivax* and *T. brucei* (Silva *et al.,* 1999) with various species and strain of the parasite having great influence on the severity and course of the infection (Connor and Bossche 2004; Auty *et al.,* 2015; Bakari *et al.,* 2017). Many studies have shown that small ruminants particularly sheep are fully susceptible to pathogenic trypanosome infections (Ogunsanmi *et al.,* 1994; Omotainse *et al.,* 2000; Olaniyi *et al.,* 2001; Katanguka-Rwakishaya *et al.,* 2003; Dinka and Abebe 2005; Yanan *et al.,* 2007; Sow *et al.,* 2014; Odeniran *et al.,* 2018; Oyewusi *et al.,* 2019) and such infection had been associated with huge economic losses (Dinka and Abebe, 2005; Kalu *et al.,* 2001; Isaac *et al.,* 2017). In addition, the possibility of small ruminants serving as a potential reservoir for other animals had been reported (Waiswa *et al.,* 2003; Dinka and Abebe 2005, Ngayo 2005; Oyewusi *et al.,* 2019). However, despite several attempts over many decades to control and eradicate animal trypanosomosis in Nigeria, it still persists as a major animal health challenge causing severe morbidity and mortality in animals (Sonibare *et al.,* 2016; Isaac *et al.,* 2017; Odeniran *et al.,* 2018). Biochemical evaluation is an important diagnosis of trypanosomosis of the functional state of various body systems and organs (Awobode, 2006). Alterations in biochemical parameters which develop sequel to trypanosome infection depend on the species of the trypanosome and its virulence (Anosa 1988; Addisu *et al.,* 2017; Bakari *et al.,* 2017) as well as susceptibility of the host and the period of infection during which the samples are taken (Akinseye *et al.,* 2020). Serum or plasma biochemical changes during trypanosome infection involving single trypanosome infection had been previously studied in small ruminants (Katanguka-Rwakishaya *et al.,* 1997; Omotainse *et al.,* 2000; Biryomumaisho *et al.,* 2003; Katanguka-Rwakishaya *et al.,* 2003; Ogunsanmi *et al.,* 2003; Sanni, *et al.,* 2013), nonetheless, varying and conflicting reports on serum biochemical changes were reported in different studies for most of these parameters. Hitherto, no consistent pattern of change in the levels of fibrinogen has been detected in trypanosome infection in sheep and only a few reports exist on the level of metabolites during trypanosome infections in small ruminants. Therefore, the aim of this study is to investigate biochemical alterations in WAD sheep experimentally infected with either *T. congolense* or *T. brucei* with a view to having an in-depth knowledge of the biochemical changes following trypanosome infection and compare the pathogenicity of the two major trypanosome species infecting ruminants in Nigeria.

MATERIALS AND METHODS

Experimental Animals

Twenty (10 males and 10 females) apparently healthy young WAD Sheep aged between 8-10 months and weighing averagely 20Kg were used for this study. They were sourced from a local market at Odeda and the nearby villages in Ogun State and kept in the small animal unit of the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Nigeria. Animals were housed in a standard fly-proof pen with normal room temperature and relative humidity. They were put on wood shaving bedding and were conditioned for two weeks during which time they were treated with 20% oxytetracycline injection (kepro® product, Holland) at 50mgkg^{-1} body weight, 2.5% panacur oral suspension (Panacur® Milton Keynes, UK) at a dosage of 5mgkg^{-1} body weight, intramuscular Diminazene aceturate injection (Berenil®, Hoechst, Germany) at a dosage of 3.5mgkg^{-1} body weight and a tick bath against external parasites using Diazinon (DIAZINTOL®, Animal Care, Nig. Ltd.) at a concentration of 2mlL-3 of water. For proper identification all the sheep were ear-tagged. Animals were given grass and supplemented with formulated sheep ration (500g per day), while water, salt lick were supplied *ad libitum*.

Experimental Design

Animals were divided into groups A & B randomly, with each group consisting of 5 males and 5 females were housed in 2 separate fly-proof houses. After 49 days of infection, survivors were treated with Diaminazine aceturate (Berenil®, Hoechst, Germany) at dosage of 7.0 mgkg^{-1} body weights. Animals were screened for trypanosome infection by wet mount technique (Uilenberg *et al.*, 1998). During the last week of acclimatization, the animals were bled twice for baseline (control) data before the commencement of the experiment. The results served as values for Day 0.

Infection with Trypanosome

The two species of trypanosomes (*Trypanosoma congolense* (GT/04/NITR/128) and *Trypanosoma brucei* (MK/04/NITR/6) used in this study were obtained from Liquid Nitrogen Bank of the National Institute for Trypanosomosis Research (NITR) Vom, Nigeria. The parasites were passaged severally in albino mice prior to use. Individual animal in group A was inoculated intraperitoneally with 3.6x10^{-6} *T. congolense* while group B was inoculated with 3.6x10^{-6}*T. brucei* in 2.5ml of sterile normal saline.

Parasitology

Animals that were inoculated with the parasites and were confirmed positive for trypanosomes by wet mount technique as described by Uilenberg *et al.*, (1998). Blood (5ml) collected from each animal on weekly basis by jugular venipuncture for 7weeks into anticoagulant and plain bottles for plasma and serum, respectfully. The plasma and serum samples were kept at -20^{o}C until analyzed.

Biochemical Technique

Plasma total protein, globulin, cholesterol, albumin and urea, were measured spectrophotometrically using standard commercial test kits (RANDOX®

Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom) using manufacturer's guide.. The total protein and creatinine were analyzed spectrophotometrically using cromatext test kits (Cromatex®, Barcelona, Spain) according to the manufacturer's prescription. Fibrinogen concentration was determined as described by Coles (1986). Serum sodium and potassium were determined with a photometer (Corning model 400, Corning Scientific Limited, England), while bicarbonate, chloride and inorganic phosphate were measured using the method of Jain (1996). Plasma Protein: Fibrinogen (PP: F) ratio was calculated using the formula of Stockham and Scott (2008).

Statistical Analysis

Results were subjected to statistical analysis to test significant differences between the two groups. Analysis of variance was used for all statistical calculations using GraghPad prism 7.3 software package (GraphPad software Inc., San Diego, CA). Correlations were performed using the correlation coefficient (r) on a minitab programme. Results were presented as mean± standard error of mean (SEM) and $p< 0.05$ was considered significant.

Ethical Approval

All applicable international, national, and or institutional guideline for the care and use of animals were duly followed. Approval was obtained from institutional animal care and use research ethics committee of the Federal University of Agriculture, Abeokuta, Nigeria.

RESULTS

Changes in plasma protein concentration

Both species of trypanosome used in this study caused severe alterations in the plasma proteins namely total protein, albumin and globulin in the experimental animals. There were significant changes ($p<0.005$) in plasma protein concentrations in both groups of the infected sheep. Total protein concentrations remain stable from Day 0 till Day 14 post-infection (pi), however, on Day 21 pi, a significant increase ($p<0.05$) was recorded from 6.8 ± 0.1 g/dl and 6.5 ± 0.3 g/dl at Day 21 pi to 8.8 ± 0.4 g/dl and 8.7 ± 0.2 g/dl on Day 49 pi in group A and group B, respectively (Table 1). Similarly, plasma globulin followed the same pattern. The plasma fibrinogen concentration also recorded a significant progressive increase ($p<0.05$) from Day 21 pi (Table 1), whereas albumin showed a significant increase ($p>0.01$) from day 28 pi (Table 1). Plasma protein: Fibrinogen (PP: F) ratio was 8.6 for groups A and B on Day 49 pi. Generally, *T. congolense*-infected sheep (Group A) appeared to have a more significant increase or decrease ($p 0<0.05$) in plasma protein concentrations throughout the period of experiment.

Changes in Plasma Cholesterol concentration

The mean plasma cholesterol concentration in the two groups showed a significant decrease between Day 14 and 21 pi (Table 1) followed

by a slight stability followed by a decrease on Day 35 and 49 pi.

Table 1: Plasma Biochemical changes in sheep experimentally infected with *T. congolense* (Group A) and *T. brucei* (Group B) (Mean±SEM; n=20)

Parameters \ Days Post Infection	0	7	14	21	28	35	42	49
Total Protein (g/dL)								
Group A	5.4±0.2 ^{a}	5.3±0.1^{a}	5.6±0.1^{a}	6.8±0.1^{b}	7.9±0.3^{c}	8.2±0.1^{d}	8.4±0.3^{d}	8.7±o.4^{d}
Group B	5.5±0.2 ^{a}	5.3±0.2 ^{a}	5.5±0.2^{a}	6.5±0.1^{b}	7.8±0.2^{c}	8.1±0.3 ^{d}	8.3±0.2^{d}	8.7±0.2^{d}
Albumin (g/dL)								
Group A	3.4±1.8^{a}	3.3±0.3^{a}	3.5±0.2^{a}	3.2±0.2^{a}	2.8±0.1^{b}	2.7±9.2^{c}	2.2±0.2^{d}	3.1±0.4^{c}
Group B	3.1±0.3^{a}	3.1±1.2^{a}	3.3±0.3^{a}	3.3±0.3^{a}	2.5±0.1^{b}	2.6±0.3^{c}	2.1±0.3^{d}	3.3±0.2^{c}
Globulin (g/dL)								
Group A	4.0±0.4^{a}	4.1±0.2^{ab}	4.2±0.3^{b}	4.7±0.3^{c}	4.9±1.d^{c}	5.1±0.2^{d}	6.3±0.3^{d}	5.5±0.2^{d}
Group B	4.2±0.2^{a}	4.0±0.1^{ab}	4.4±0.3^{ab}	4.5±0.2^{c}	5.1±0.1^{d}	5.4±0.3^{d}	5.7±0.4^{d}	5.3±0.7^{d}
Albumin/Globulin ratio								
Group A	0.8±0.1^{a}	0.7±0.3^{a}	0.7±0.2^{a}	0.6±0.8^{ab}	0.5±0.1^{b}	0.4±0.1^{c}	0.4±0.1^{c}	0.4±0.2^{c}
Group B	0.8±0.1^{a}	0.8±0.1^{a}	0.7±0.4^{a}	0.6±1.3^{ab}	0.5±0.2^{b}	0.4±0.2^{c}	0.4±0.1^{c}	0.4±0.1^{c}
Total Cholesterol(mg/dL)								
Group A	68.6±2.3^{a}	68.8± 1.5^{a}	65.9±1.8^{a}	63.5±1.6^{ab}	60.8±1.6^{b}	58.8±2.4^{c}	56.6±2.1^{d}	55.5±1.3^{d}
Group B	68.5±1.5^{a}	68.6±0.7^{a}	64.8±1.2^{a}	62.7±2.3^{ab}	61.5±1.8^{b}	57.8±2.8^{c}	56.7±3.3^{d}	56.4±1.4^{d}
Creatinine (mg/dL)								
Group A	0.8±0.2^{a}	0.7±0.1^{a}	0.9±0.2^{b}	1.0±0.2^{b}	1.0±0.2^{c}	1.2±0.2^{c}	1.5±1.1^{d}	1.6±0.2^{d}
Group B	0.7±0.1^{a}	0.7±0.1^{a}	0.9±0.1^{b}	0.9±0.7^{b}	0.9±0.3^{c}	1.0±0..2^{c}	1.1±1.2^{d}	1.3±0.2^{d}

Urea (mg/dL)								
Group A	15.0±0.1 [a]	16.2±0.1[a]	22.8±0.2 [b]	25.1±8.8 [c]	26.9±0.1[c]	27.8±7.3 [c]	39.6±0.2 [d]	43.4±0.2 [d]
Group B	14.6±0.2 [a]	15.8±6.1[a]	20.3±0.1 [b]	20.7±0.1 [c]	24.8±8.3 [c]	26.1±8.5 [c]	32.2±0.2 [d]	35.0±8.8 [d]
Fibrinogen(g/dL)								
Group A	0.4±0.2[a]	0.5±0.2[a]	0.4±0.1[a]	0.6±0.2[b]	0.6±0.2[b]	0.7±0.2[c]	0.8±0.2[d]	0.9±0.4[e]
Group B	0.5±0.1[a]	0.4±0.1[a]	0.5±0.2[a]	0.6±0.3[b]	0.6±0.1[b]	0.7 ±0.2[c]	0.8±0.2[d]	0.9±0.3[e]

Values on the same row with different superscripts differ significantly ($P<0.05$ or $p<0.001$)

Changes in metabolites

The plasma urea and creatinine showed consistent and significant increase ($p<0.05$) starting from Day 14 pi in both groups (Table 1). There was a strong positive correlation between total protein and urea in both groups of infected sheep ($r = 0.937$ for group A and $r = 0.908$ for group B), respectively as well as between total protein and creatinine in both groups ($r = 0.937$ for group A and $r = 0.908$ for group B), respectively.

Changes in Electrolytes

Changes in the electrolytes were as shown in Table 2. The values of HCO^{3-}, PO_4^{2+} and Na^+ were maintained in both groups till Day 28 pi when a steady but significant increase ($P<0.05$) was observed. No significant change ($P>0.05$) in K^+ level in both groups even though slight changes in values were observed (Table2).

Table 2: Serum Electrolytes changes in sheep experimentally infected with T. congolense (group A) and T. brucei (Group B (Mean±SEM;n=20)

Days Post Infection / **Parameters**	0	7	14	21	28	35	42	49
Potassium (K^+)(Mmol/L)								
Group A	4.6±0.7^a	4.7±0.9^a	4.4±1.2^a	4.5±0.4^a	4.2±0.8^a	4.3±1.7^a	4.3±1.8^a	4.2±1.4^a
Group B	4.5±1.7^a	4.6±0.3^a	4.4±0.7^a	4.4±0.4^a	4.3±0.5^a	4.2±1.5^a	4.3±1.7^a	4.2±1.3^a
Sodium (N^+) (Mmol/L)								
Group A	131.2±1.4^a	132.5±1.3^a	133.1±1.5^a	136.2±1.9^{ab}	138.7±1.8^b	144.4±3.1^c	147.1±2.5^d	148.3±1.9^d
Group B	132.2±1.3^a	132.1±2.2^a	131.7±1.4^a	135.6±1.7^{ab}	137.8±1.6^b	142.2±1.6^c	144.7±1.3^d	146.6±1.5^d
Bicarbonate (HCO^{3-}) (Mmol/L)								
Group A	21.4±2.2^a	21.5±1.4^a	21.7±3.6^a	21.8±2.7^a	24.7±2.5^b	26.5±4.6^c	26.9±6.4^c	27.3±3.2^d
Group B	21.8±2.7^a	20.8±1.3^a	21.2±3.4^a	20.8±3.4^a	24.2±4.8^b	23.8±2.4^b	25.5±2.5^c	25.7±3.7^c
Chloride (Cl^-) (Mmol/L)								
Group A	102.3±2.3^a	102.5±2.5^a	100.6±2.8^a	102.7±1.2^a	103.9±1.5^b	104.7±1.2^b	105.7±3.1^c	107.8±4.2^d
Group B	103.9±2.2^a	102.4±1.2^a	102.6±1.7^a	102.7±1.1^a	104.8±1.7^b	104.7±1.4^b	104.9±3.9^b	106.8±5.1^c
Phosphate (PO_4^{2-})(Mmol/L)								
Group A	4.3±0.4^a	4.1±0.4^a	4.2±0.2^a	4.3±0.3^a	4.7±0.2^b	5.3±0.3^c	6.5±1.2^d	7.4±2.1^d
Group B	4.4±0.5^a	4.2±0.1^a	4.2±0.2^a	4.4±0.3^a	4.4±0.2^b	5.2±0.4^c	6.2±0.5^d	6.9±1.4^d

Values on the same row with different superscripts differ significantly ($P<0.05$ or $p<0.001$)

DISCUSSION

The present study investigated the biochemical changes induced by infection of *T. congolense* or *T. brucei* infection and compared the pathogenicity of the two trypanosomes in WAD sheep. Biochemical assessment of the body fluids is a measure of functional state of the various body organs (Akinseye *et al.,* 2020). Biochemical alterations in the blood induced by trypanosome infections have been reported to depend on the species of the parasite, its virulence, susceptibility of the host and the period of infection during which the samples were collected (Anosa 1988).

Several biochemical alterations indicative of pathological and functional disorders were observed in the present study. The results showed significant ($P<0.05$) alterations in the plasma proteins studied in both groups of infected sheep. There was marked hyperproteinaemia which is concomitant decrease in albumin: globulin ratio. This result is consistence with previous reports of katunguka-Kwakishaya *et al.,* (1992) and Biryomumaisho *et al.,* (2003). The total protein increases have been reported to be due to increased IgM sub-fraction which is primarily involved in the immune response in the host, for the control of the infection (Biryomumaisho *et al.,* 2003). In the present study, there was a remarkable hypoalbuminaemia, hypoalbuminaemia had previously been reported to occur during trypanosome infection (Ogunsanmi *et al.,* 2003; Katunguka-Kwakishaya *et al.,* 1992) and this had been suggested to be due to increased catabolism of albumin and uptake by the trypanosome as well as haemodilution (Anosa, 1988). However, Katunguwa-Kwakishaya *et al.,* (1992) suggested that it could largely be due to haemodilution. Hypoalbuminaemia had also been reported to occur as a result of decreased protein synthesis sequel to hepatic dysfunction (Addisu *et al.,* 2017). It was observed that the group infected with *T. congolense* suffered much more reduction in plasma albumin than *T. brucei*-infected group. In this study, globulin increased disproportionately to albumin, this had been reported in trypanosome infection of domestic animals (Anosa 1988). Hyperglobulinaemia in trypanosomosis had been attributed to phenomena of antigenic variation and polyclonal activation in trypanosomes which might have led to generation of large amounts of antibodies in form of 1gM in the infected host (Borst 2002).

In this study, there was a significant decrease in the levels of cholesterol in both groups of infected sheep following the infection. This observation concurs with the study of Katunguka-Rwakishaya *et al.,* (1997) in *T. congolense*-infected Scottish Black-head sheep. Several studies have reported varying degree of decreases in serum phospholipids and cholesterol in trypanosomes infections in domestic animals (Katunguka-Rwakishaya *et al.,* 1992, 1997; Biryomumaisho *et al.,* 2003; Taiwo *et al.,* 2003; Adamu *et al.,* 2008; Dagnachew *et al.,* 2014; Bakari *et al.,* 2017). Trypanosomes have been reported to require cholesterol for growth and membrane synthesis (Black and Vanderweed 1989; Biryomumaisho *et al.,* 2003), and since the blood-stream forms of trypanosomes are not capable of synthesizing cholesterol required for membrane synthesis and growth, hence they acquire it from the host (Biryomumaisho *et al.,* 2003; Taiwo *et al.,* 2003). This could account for the reason why more and rapid reductions with time were observed in cholesterol values in *T.congolense*-infected sheep than *T. brucei* a tissue invader which moves to tissue in the

course of the infection. Decreased phospholipids and cholesterol levels in trypanosome infections had also been attributed to impaired synthesis of these substances which could in turn be the result of insufficient hepatocellular respiration as a result of hypoxia and subsequent reduction in the release of cholesterol from the liver in the trypanosome infected animals (Bartley, 1989; Adamu *et al.,* 2008). The above phenomenon had been suggested to aggravate the neurological disorders often associated with trypanosome infections since cholesterol is vital in cell signaling in neuronal synapses formation (Biryomumaisho *et al.,* 2003). In addition, the rapidly growing numbers of trypanosomes post-infection in sheep require some lipids to support their growth (Taiwo *et al.,* 2003). In the present study, the plasma fibrinogen showed a significant progressive increase ($p<0.05$) in concentration from Day 14 pi. This is similar to the findings of Dagnachew *et al.,* (2014) in *T. vivax*-infected cattle. Hyperfibrinogenaemia had been reported mainly in dehydration and acute inflammatory conditions (Coles 1986). Hyperfibrigenaemia in this study could probably have occurred as a result of inflammatory reaction because Plasma Protein: Fibrinogen (PP: F) ratio was less 10 in the two groups on Day 42 and 49 pi. Fibrinogen is an acute phase protein; it is therefore, plausible to suggest that the increase in its consumption is proportional to the degree of inflammation as earlier reported (Taylor and Authie 2004, Sow *et al.,* 2014). Meanwhile, normal fibrinogen levels had been reported in trypanosome infection of dogs (Omotainse *et al.,* 1994), *T. rhodesiense* infection of cattle (Anosa 1988) and *T. congolense* and *T. brucei* infections of cattle (Addisu *et al.,* 2017). On the other hand, hypofibrinogenaemia was reported to occur in human *T. rhodesiense* infection and in *T. congolense*-infected cattle (Akinseye *et al.,* 2020). In this study, plasma urea and creatinine values showed a consistent and significant increase ($p<0.05$) starting from Day 14 pi in both groups. The increases in the levels of urea and creatinine in the course of the infection observed in this study are similar to results of (Abenga and Anosa 2007) concerning *T. gambiense* infection in vervet monkeys. An elevated level of creatinine was also observed in human infected with *T. b. gambiense* (Awobode 2006), in pigs with *T. brucei* infection (Adamu *et al.,* 2009), in sheep infected with *T. congolense* (Adamu *et al.,* 2008) and in cattle during trypanosomosis (Bakari *et al.,* 2017). Serum urea had been reported to increase in acute and chronic intrinsic renal disease (Abenga and Anosa, 2007, Anosa, 1988) and characterized by decreased effective circulating blood volume with decreased renal perfusion, in postrenal obstruction of urine flow, and in high protein intake states, while increase in serum creatinine had been reportedly observed in any renal functional impairment (Anosa 1988). The present study showed that there was a strong positive correlation between total protein and urea in both groups of infected sheep ($r = 0.937$ for group A and $r = 0.908$ for group B), as well as between total protein and creatinine in both groups ($r = 0.937$ for group A and $r = 0.908$ for group B). This indicated that with increasing levels of total protein, there was progressive increase in the levels of urea and creatinine in the infected sheep. The increase had been linked with hepatic and renal dysfunction (Anosa 1988; Abenga and Anosa 2007) associated with intrinsic renal lesions, decreased perfusion of the kidney, or obstruction of the lower urinary tract leading to renal injury and associated glomerular

dysfunction during the course of the infection (Omeje and Anene 2012). The increases in blood urea and creatinine levels have also been attributed to fever and tissue damage resulting from inflammatory reaction as suggested by Finco (1989). In the present study, renal dysfunction is likely to be the major factor, since plasma urea and creatinine values showed a significant increase in the course of the infection indicating kidney damage and inability to flush out urea and creatinine leading to progressive accumulation of these products. The mean bicarbonate levels showed progressive elevation throughout the period of infection. This observation does not agree with the report of Goodwin and Guy (1973) who reported a decrease in serum bicarbonate in *T. brucei*-infected rabbits. Increased serum bicarbonate had been attributed to the generation of bicarbonate ions by the kidney which was reported to increase during trypanosome infection (Calson 1989). The sharp drop in the bicarbonate levels during the later stages of infection as observed in this study is suggested to result from acidosis associated with anaemia, renal malfunction and release of toxic metabolites such as free fatty acids by trypanosomes as previously reported (Anosa and Isoun 1976). Potassium level slightly decreased in the course of the infection while chloride increased significantly. These observations agreed with the reports of Raisinghani *et al.* (1981) and Goodwin and Guy (1973) in *T. evansi*- and *T. brucei*-infected camels and rabbits, respectively. The hyperchloraemia associated with hypokalaemia and hypernatraemia may be related to renal dysfunction as suggested by Zilva and Pannalli (1984) and Calson (1989).

CONCLUSIONS

It is therefore concluded that *T. congolence* and *T. brucei* infections in WAD sheep caused severe biochemical alterations associated with the pathological and inflammatory changes in body organs. It was apparent from the study that *T. congolense*-infected sheep suffered more alteration than those infected with *T. brucei*. This study calls for further work on other trypanosome species to determine whether differences in pathogenicity occur in infections with other trypanosome species.

REFERENCES

ABENGA, J.N. and ANOSA, V.O. (2007): Serum biochemical changes in experimental Gambian trypanosomosis. II. Assessing hepatic and renal dysfunction. *Turk. J. Vet. Sci.,* 31: 293–296.

ADAMU, S., IGE, A.A., JATAU, I.D., NEILS, J.S., USEH, N.M., BISALLA, M., IBRAHIM, N.D.G., NOK, A.J. and ESIEVO, K.A.N. (2008): Changes in the serum profiles of lipids and cholesterol in sheep experimental model of acute African trypanosomosis. *African Journal of Biotechnology*, 7: 2090–2098.

ADAMU S., BARDE, N., ABENGA, J.N., USER, J.N., IBRAHIM, N.D.G. and ESIEVO, K.A.N. (2009): Experimental *Trypanosoma brucei* infection-induced changes in the serum profiles of lipids and cholesterol and the implications in pigs, *Journal of Cell and Animal Biology*, 3: 15–20.

ADDISU, A., BELAY, F. and EYOB, E. (2017): Review on Serum Biochemical Changes in Ruminants Infected with Major Trypanosome Species. *Int. J. Adv. Res. Biol. Sci.*, 4: 26–35. DOI:

http://dx.doi.org/10.22192/ijarbs.2017.04.10.006.

AKINSEYE, O.R., MUSTAPHA, A. and ANGELA, A.N. (2020): Biochemical indicators in trypanosomiasis infections. *J. Anal. Pharm Res.,* 9: 11–14.DOI: 10.15406/japlr.2020.09.00346

ANOSA, V.O. (1998): Haematological and biochemical changes in human and animal trypanosomisis. Part II. *Reve Elev Med Vet Pays Trop.*, 41: 151–164.

ANOSA, V.O. and ISOUN, T.T. (1976): Serum proteins, blood and plasma volumes in experimental *Trypanosoma vivax i*nfection of sheep and goats. *Trop. Anim. Hlth. Prod.*, 8: 11–19.

ANYAEGBUNAM, L.C. and OKAFOR, O.J. (2013): Trypanosomiasis in Red Sokoto and West African Dwarf goats at Ikpa abattoir Nsukka, Enugu state, *Nigeria. J. Entomol. Zool. Stud.*, 1: 35–39.

AUTY, H., TORR, S.J., MICHOEL, T., JAYARAMAN, S. and MORRISON, L.J. (2015): Cattle trypanosomosis: the diversity of trypanosomes and implications for disease epidemiology and control trypanosome species of relevance to cattle. *Rev Sci Tech Off Int Epiz.*, 34:587–98.

AWOBODE, H.O. (2006): The biochemical changes induced by natural human African trypanosome infections. *African J Biotechnol.*, 5:738–742.

BAKARI, S.M., OFORI, J.A., KUSI, K.A., ANING, G.K., AWANDARE, G.A., CARRINGTON, M. and GWIRA, T.M. (2017): Serum biochemical parameters and cytokine profiles associated with natural African trypanosome infections in cattle *Parasites & Vectors,* 10:312. DOI: 10.1186/s13071-017-2255-9.

BARTLEY, J.C. (1989): Lipid metabolism and its diseases. In: Clinical Biochemistry of domestic animals. 4th ed. (Kanako, J.J. Edt.). Academic Press Inc. San diego, California. pp106–114.

BIRYOMUMAISHO, S., KATANGUKA-RWAKISHAYA, E. and RUBAIRE-AKIIKI, C. (2003): Serum biochemical changes in experimental *Trypanosoma congolense* and *Trypanosoma brucei* infection in Small East African goats. *Veterinaski Archives,* 73(3): 167–180.

BLACK, S.J and VANDERWEED, V. (1989): Serum lipoproteins are required for multiplication of *Trypanosoma brucei brucei* under axenic culture conditions. *Mol. Biochem. Parasitol.* 37: 65–72.

BORST, P. (2002): Antigenic variation and allelic exclusion. *Cell,* 109: 5–8.

CALSON, G.P. (1989): Fluid, electrolyte and acid-base balance. 4th ed. Kanako, J.J. eds. Clincal biochemistry of domestic animals. San Diego, Carlifonia, Press Inc., 678–743.

COLES, E.H. (1986): Veterinary Clinical Pathology. 4th edition.Philadelphia, W.B. Saunders Company, 22–23.

CONNOR, R.J. and BOSSCHE, P.V.D. (2004): African Animal Trypanosomoses. In: Infectious Diseases of Livestock, Coetzer, J. A. W. and Tustin R. C. (Eds.), Oxford University Press southern Africa, Cape Town, 251–296.

DAGNACHEWA, S, TEREFEA, G., ABEBE, G., DAVE, J., BARRY, D.J. and GODDEERIS, B.M. (2014): Comparative biochemical changes in young Zebu cattle experimentally infected with *Trypanosoma vivax* from tsetse infested and non-tsetse infested areas of Northwest Ethiopia *Vet. Parasitol.*. 205: 451–459.

DEDE, P.M., DADAH, A.J., SHAIDA, S.S., KOJAH, B.F., BABA, U., OKOH, E.O. and MAMMAN, M. (2013): In: 32nd International Scientific Council for Trypanosomiasis Research and Control (ISCTRC): September 8–12, Khartoum, Sudan, 1:04.

DINKA, H. and ABEBE, G. (2015): Small ruminants trypanosomosis in the Southwest of Ethiopia. Small Ruminant Research, 57(2-3): 239 – 243

FATIHU, Y.M., ADAMU, S., GIGINYA, D.N., IBRAHIM, N.D.G., EDUVIE, O.L. and ESIEVO, K.A.N. (2009): The effect of experimental *Trypanosoma vivax* infection on thyroid gland in Zebu bulls. *Veterinarski Arhiv,* 79 (5) 429–437

FINCO, D.R. (1989) Kidney functions. In: Clinical biochemistry of domestic animals. Kaneko, J.J. edt., 4th edition. San Diego, California, Press Inc., 496–537

GOODWIN, L.G. and GUY, M.W. (1973): Tissue fluid in rabbits infected with *Trypanosoma (Trypanozoon) brucei.* Parasitology, 66, 499–513

ISAAC, C., OHIOLEI, J.A., EBHODAGHE, F., IGBINOSA, I.B. and EZE, A.A. (2017): Animal African Trypanosomiasis in Nigeria: A long way from elimination/eradication. *Acta Tropica,* 176, 323–331.

JAIN, N.C. (1996): Schalm´s Veterinary Hematology, 5th Edition, Lea & Febinger, Philadelphia, USA, 208–224.

KALU, A.U., OBOEGBULEM, S.I. and UZOUKWU, M. (2001): Trypanosomosis in small ruminants maintained by low riverine tsetse population in central Nigeria. *Small Rumin. Res.,* 4: 109 –115.

KATANGUKA-RWAKISHAY, E., MURRAY, M. and HOLMES, P.H. (1997): The pathophysiology of ovine trypanosomosis; haematological and biochemical changes. *Vet. Parasitology,* 45: 17–32.

KATANGUKA-RWAKISHAYA, E., MURRAY, M. and HOLMES, P.H. (1992): The pathophysiology of *Trypanosoma congolense* in two breeds of sheep, Scottish Blackface and Finn Dorset. *Vet. Parasitology,* 68: 215–225.

KRAMMER, J.W. (1986): Incidence of trypnosomiasis in the West African Dwarf sheep and goat in Nsukka, Eastern Nigeria. *Bull. Epizoot. Dis. Afr.,* 14: 423–428.

LUCKINS, A.G. (1992): Trypanosomosis in small ruminants – A major constraint to livestock production? *Br. Vet. J*ournal, 148: 471–473.

MURRAY, M. and DEXTER, T.M. (1988): Anaemia in bovine Trypanosomiasis. A. review. *Acta Trop.,* 45: 389-432.

NGAYO, M.O., NJIRU, Z.K., KENYA, E.U., MULNVI, G.M., OSIR, EO. and MASIGA, D.K.. (2005): Detection of trypanosomes in small ruminant and

pigs in Western Kenya: Important reservoir in the epidemiology of sleeping sickness? *Kinetoplastid Bio Dis.*, 4: 5–10

ODE , S., ADAMU, M., TAIOE, M., THEKISOE, O., ADAMU, S. and SAROR, D. (2017): Molecular occurrence of trypanosomes, erythrocyte and serum sialic acid concentrations of Muturu and Bunaji cattle in Benue state, Nigeria. *Vet. Parasitology*, 242: 10–13.

ODENIRAN, P.O., ADEMOLA, S.O., MACLEOD, E.T. and WEBURN, S.C. (2018): Bovine and Small Ruminants Trypanosomiasis in Nigeria- A Review. Veterinary Parasitology: *Regional studies and Review*, 1: 5–13.

OGUNSANMI, A.O., AKPAVIE, S.O.AND ANOSA, V.O. (1994): Serum biochemical changes in West African Dwarf sheep experimentally infected with *Trypanosoma brucei*. *Revue Elev. Med. Vet. Pays trop.*, 47(2): 195–200.

OLANIYI, M.O., TAIWO, V.O. and OGUNSANMI, A.O. (2001): Heamatology and Dynamics of Erythrocyte Membrane Sialic Acid Concentration during Experimental *Trypanosoma congolense and T. brucei* infections of sheep. *Journal Applied Animal Research*, 20(1): 57–64. https://doi.org./10.1080/07121192001.9706737.

OMEJE, J.N. and ANENE, B.M. (2012): Comparative serum biochemical changes induced by experimental infection of *Trypanosoma brucei* and *T. congolense* in pigs. *Vet. Parasitol.*, 190: 368–374.

OMOTAINSE, S.O., KALEJAIYE, J.O., DEDE, P. and DADA, A.J. (2004): The current status of tsetse and animal trypanosomosis in Nigeria. *Vom J. Vet.. Sc.*, 1, 1–7.

OMOTAINSE, S.O., KALEJAIYE, J.O., ECHE, T. and HALID, I. (2001): The Prevalence of Animal Trypanosomiasis in Yamaltu Deba LGA of Gombe State, Nigeria. *African J. of clin. Exp. microbiol.*, 2(2): 10–12.

OMOTAINSE, S.O., ATAWODI, S., EDEGHERE, H. and ODUWOYE, L. (2000): Some biochemical changes in ovine *Trypanosoma vivax* infection. *Afr.j. Clin. Exp. Microbiol.*, 1(2):103–107.

OYEWUSI, I.K., TAKEET, M.I., OYEWUSI, J.A., TALABI, A.O. and OTESILE, E.B. (2019): Molecular detection and haematological evaluation of trypanosomosis in naturally infected sheep in Abeokuta, Nigeria. Alexandria Journal of Vet. Sci. 63(1):129–134. DOI: 10.5455/ajvs.67270.

RAISINGHANI, P.M., LODHA, K.R., BHATA, J.S. and DWARAKANATH, P.K. (1981): Variation in haematological and serum electrolyte levels during first 20 bouts of experimental Surra in camels. *India J. Anim. Sci.* 51: 724–29.

SADIQUE, N.A., ADEJINMI, J.O.and ARIRI, H. (2001): Haematological and plasma protein values of Zebu cattle in trypanosome-endemic zone. Tropical Animal Production Investment 4:219–223.

SAM-WOBO, S.O., IGENEZOA, A.J.,

IDOWU, O.A., OTESILE, .E.B, EKPO, U.F. and KEHINDE, O.O. (2010): Bovine Trypanosomosis and its impact on cattle in derived savanna areas of Ogun State, Nigeria. *Publ. Health Epidemiol.* 1: 43–47.

SANNI, T., ONASANYA, G., ADEFENWA, M., YAKUBU, A., IKEOBI, C., ADEBAMBO, O., TALABI, A., OZOJE, M., WHETO, M., TAKEET, M., PETERS, S., DONATO, M.B., THOMAS, B. and IMUMORIN, I. (2013): Molecular Diagnosis of Subclinical African *Trypanosoma vivax* Infection and Association with Physiological Indices and Serum Metabolites in Extensively Managed Goats in the Tropics. *Open Journal of Veterinary Medicine*, 3(1): 39–45 doi: 10.4236/ojvm.2013.31007.

SILVA, R.A.M., RAMIREZ, L., SOUZA, S.S., ORTIZ, A.G., PEREIRA, S.R. and DUVILA, A.M.R. (1999): Haematology of natural bovine trypanosomosis in the Brazilian Pantanal and Bolivian wetlands.*Vet. Parasitol.* 85: 87–93.

SONIBARE, A.O., FAMUYIDE, I.M., TAKEET, M.I., OYEWUSI, I.K., EGBETADE, A.O., ABAPKA, S.A.V., ADENIRAN, O.A., SOLANKE, A.O. (2016): Diminazene-resistant *Trypanosoma vivax* in West African dwarf lamb, South-West, Nigeria: a case report. *Niger. J. Parasitol.,* 37: 62–65

SOW, A., ZABRÉ, M., MOUICHE, M., KOUAMO, J., KALANDI, M. and BATHILY, A. *et al.* (2014):Investigation of biochemical parameters in Burkinabese local small ruminants breed naturally infected with trypanosomosis. *Int. J. Biochem. Res. Rev.* 4: 666–79.

STOCKHAM, S.L. and SCOTT, M.A. (2008): Proteins. In: Fundamentals of Veterinary clinical Pathology. 2nd edition. Blackwell Publishing, USA, 369–413.

TAIWO, V.O., OLANIYI, M.O. and OGUNSANMI, A.O. (2003): Comparative plasma biochemical changes and susceptibility of erythrocytes to in vitro peroxidation during experimental *Trypanosoma congolense* and *T. brucei* infections in sheep. *J. Israel Vet. Med. Assoc.* 58 (4):1–7.

TAYLOR, K. and AUTHIE, M.E.L. (2004): Blood. In: The Trypanosomiasis. lan Maudlin, Peter H. Holmes and Michael A. Miles edit.. CABI Publishing Oxfordshire, UK. 338–350.

UILENBERG.G. and BOYT, W.P. (1998): Food and Agriculture: A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis, Food and Agriculture Organization of the United Nations

VAN-DEN BOSSCHE, P., SHUMBA, W. and MAKHAMBERA, P. (2000): The distribution and epidemiology of bovine trypasonomosis in Malawi. *Vet. Parasitol.* 88: 163–176.

WAISWA, C., OLAHO-MUKAMI, W. and KATUNGUKA-RWAKISHAYA, E. (2003): Domestic animals reservoirs for sleeping sickness in three endemic foci in South-eastern Uganda. Ann. Trop. Med. Parasitol., 97: 149–155.

YANAN, E.G., EVUTI, M.A., SHAMAKI, B.U., SUGUN, M.Y., DONGKUM, C.1. and OGUNSAN, E.A. (2007): Epidemiology of small ruminant trypanosomosis in some communities in Jos East, Plateau State: A Guinea

Savana Zone, North-Central Nigeria. *J. Protozool. Res.*, 17: 44–47.

ZILVA, J.F. and PANNALL, P.R. (1984): Clinical chemistry in diagnosis and treatment. 4th edition. London, Lloyd-Luke Medical Books Ltd. 184p