Some Biochemical Perturbations May Modify the Understanding of Trypanotolerance in the West African Dwarf Sheep Infected With *Trypanosoma brucei brucei* and *Trypanosoma congolense*

Davinson C Anyogu [ID], Shodeinde VO Shoyinka and John I Ihedioha

Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria.

ABSTRACT: Trypanosomes are single-celled protozoa that cause severe diseases in both humans and livestock in sub-Saharan African countries. The disease in the West African Dwarf (WAD) sheep is often neglected due to the issue of trypanotolerance. The current study is aimed to evaluate some biochemical changes in this breed that may modify the understanding of trypanotolerance. Fifteen WAD sheep were assigned into 3 groups (A, B, and C). Baseline (day 0) values of the parameters assayed were obtained before groups A and B were infected with *Trypanosoma brucei brucei* and *Trypanosoma congolense*, respectively, by intraperitoneal inoculation with 10^6 trypanosomes per animal. Standard procedures using Quimica Clinica Applicada (Spain) and Randox (UK) test kits were used to evaluate serum levels of AST, ALT, ALP, total protein, albumin, total cholesterol, urea, and creatinine on days 0, 14, 28, 42, 56, and 70 post infection. The infections caused sustained pyrexia, hypoproteinaemia, hypocholesterolaemia, weight loss, hepatitis, and mortalities although parasitaemia was greatly controlled especially in the *T congolense* infected rams. The findings suggest that the WAD rams are not just passive reservoirs of trypanosomes for human and animal infections, but experience active host-parasite interactions with huge price for resilience, biochemically.

KEYWORDS: Parasite, serum biochemistry, trypanosomiasis, trypanotolerance, West African Dwarf sheep

Introduction

Trypanosomes are unicellular parasitic protozoa belonging to the Trypanosoma Genus and Family Trypanosomatidae.[1] The parasites live and thrive in the blood and other body fluids of vertebrate hosts, and some of them cause disease known as trypanosomiasis, leading to significant morbidity and mortality in both man and animals with enormous economic losses.[2] *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambianse* cause acute (haemolymphatic) and chronic (meningoencephalitic) forms of human African trypanosomiasis (HAT), respectively, whereas *Trypanosoma brucei brucei*, the third member of the *Trypanosoma brucei* group together with *Trypanosoma congolense* and *Trypanosoma vivax* are the causative agents of 'Nagana' or African animal trypanosomiasis (AAT) in a wide range of livestock.[3]

The Djallonke or West African Dwarf (WAD) sheep, as they are often referred to, inhabit the area south of latitude 14° N including the coastal areas of west and central Africa. These include Nigeria, Dahomey, Ghana, Ivory Coast, Guinea, Senegal, Cameroon, Gabon, Congo, and Southern Mali. They are also found in Angola and Botswana. The WAD sheep are known for their adaptation to tropical hot and humid environment of West Africa, and they are also considered tolerant to trypanosomiasis.[4,5] The WAD sheep (Djallonke) and goats, as well as the Taurine cattle (*Bos taurus* or N'Dama), which entered Africa from the near east around 5000 BCE and 7000 BCE, respectively, developed innate tolerance to African animal trypanosomosis (AAT), probably as a result of natural selection pressures.[6-8] The innate ability of these livestock breeds to survive and remain productive under AAT challenge, with very low mortality and without the use of trypanocidal drugs, is referred to as trypanotolerance. It was estimated that approximately 32% of sheep and 47% of goats in West and Central Africa are trypanotolerant, and this is so because the WAD breeds are predominant in these regions.[9] In N'Dama cattle specifically, trypanotolerance was shown as a better ability to control both parasitaemia and, more importantly, anaemia, and this permits the host to remain productive under disease challenge.[7]

Trypanosomiasis is known to cause anaemia, hypoproteinemia, leukocytosis, immunosuppression, hypoglycemia, and changes in serum enzyme and cholesterol levels.[10] However, these changes have not been well evaluated in the WAD sheep and goats and N'Dama cattle breeds partly because of the presumptions associated with trypanotolerance.

The present study evaluated some biochemical changes in WAD rams infected separately with *T brucei brucei* and *T congolense* to elucidate what trypanotolerance may preclude and widen its understanding or limit overemphasis as well as highlight targets for therapeutic intervention in the so-called trypanotolerant breeds.

Materials and Methods

Animals

Fifteen sexually mature WAD rams, weighing between 7 and 13 kg, and aged 1 to 1½ years were used for the study. They

were procured from local breeders. They were allowed to acclimatize for 3 weeks, during which they were dewormed with albendazole at 10 mg/kg body weight (b.w.) per os and ivermectin at 1 mL/50 kg b.w. By buffy coat examination, those already infected with trypanosomes or any other haemoparasite were screened out and excluded from the study. The rams were thereafter assigned into 3 groups (A, B, & C) of 5 rams each. Group A rams were infected with *T brucei brucei*, group B rams were infected with *T congolense* and group C served as the uninfected control. The trypanosomes were sourced from the Nigerian Institute for Trypanosome Research (NITR, Nigeria). Infection of groups A and B was by intraperitoneal injection of 10^6 trypanosomes per head. The rams were placed on fresh forage and drinking water ad libitum. Baseline values of the mean body weight, rectal temperature, and serum biochemical assays were determined before infecting groups A and B. The infected animals and the control were monitored for 70 days.

Ethical approval

The study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, University of Nigeria.

Parasitaemia

Blood samples of the infected groups were examined for parasitaemia daily until parasitaemia was established, then the level of parasitaemia were measured on days 7, 14, 21, 28, 35, 42, 56, and 70 post infection (PI) by buffy coat scoring method.[11]

Biochemical analyses

On days 0, 14, 28, 42, 56, and 70 PI, 3 mL of blood was collected from the external jugular vein of the rams into clean tubes and allowed to clot followed by centrifugation at 3000 rpm for 10 minutes to obtain clear serum samples for biochemical analyses.

The biochemical parameters assayed in serum were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activity, total protein, albumin, total cholesterol, urea, and creatinine. All the biochemical assays were done using Diatek Biochemistry Analyzer (Wuxi Hiwell Diatek Instruments Co Ltd, China). The test kits used for the assay of serum AST, ALT, ALP, total cholesterol, and urea were sourced from Quimica Clinica Applicada (QCA), Spain, while the ones used for the assay of serum levels of total protein, albumin, and creatinine were sourced from Randox Laboratories Ltd, UK.

The serum AST and ALT activity were assayed based on the Reitman-Frankel colorimetric method,[12] while assay of the ALP activity was based on the phenolphthalein monophosphate method.[13-15] The serum total protein levels were determined by the direct biuret method,[16] while the serum albumin levels were determined based on the bromocresol green
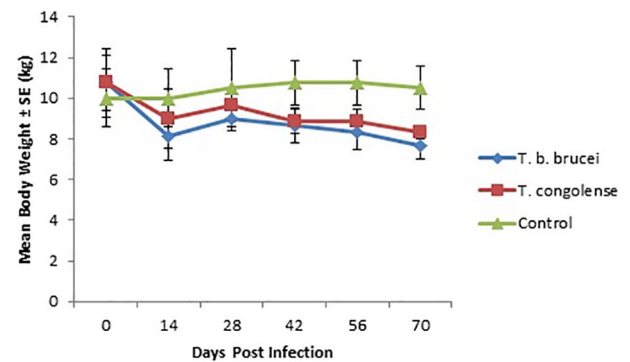
Figure 1. Graph showing the mean body weight (kg) of WAD rams infected with *T brucei brucei* and *T congolense.*

method.[17,18] The serum globulin level for each sample was calculated by subtracting the serum albumin level from total protein level. The serum total cholesterol was determined based on the enzymatic colorimetric method,[19] while the serum level urea was determined based on the Berthelot-Searcy method.[20]

Histopathology

Rams that died due to the infection were thoroughly necropsied and tissue samples were generously collected and fixed in 10% neutral buffered formalin. The tissues were routinely processed, sectioned at 5 μm thickness and stained with haematoxylin and eosin (H&E). However, focus was mainly on the liver tissue because it is the site for the synthesis of most the biochemical elements evaluated in the study.

Statistics

Data generated from the study were subjected to 1-way analysis of variance using SPSS version 21. The level of parasitaemia was analysed by Student *t* test. The variant means were separated post hoc using the least significant difference (LSD) method. The level of significance was accepted at probability $P < .05$.

Results

Physiological parameters and parasitaemia

There was no significant ($P > .05$) changes in the mean body weights of the rams except on day 70 PI on which *T brucei brucei* infected rams had significantly ($P < .05$) lower mean body weight compared with the control (Figure 1).

The rectal temperature of the infected groups were significantly ($P < .05$) higher than the control from day 14 to the end of the experiment (day 70), with *T congolense* producing relatively higher temperature elevations than *T brucei brucei* (Figure 2).

Parasitaemia was first detected on day 5 PI in both *T brucei brucei* and *T congolense* infected rams and by day 6 PI all the infected rams had become parasitaemic. Three peaks of parasitaemia were observed in *T brucei brucei* on days 14, 35, and 56 PI. *T congolense* peaked on day 14 PI after which no sharp
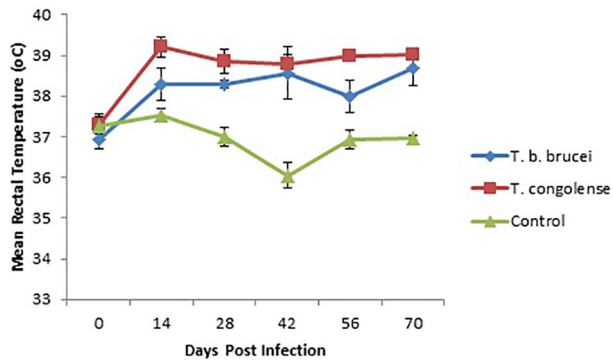
Figure 2. Graph showing the mean rectal temperature (°C) of WAD rams infected with *T brucei brucei* and *T congolense.*
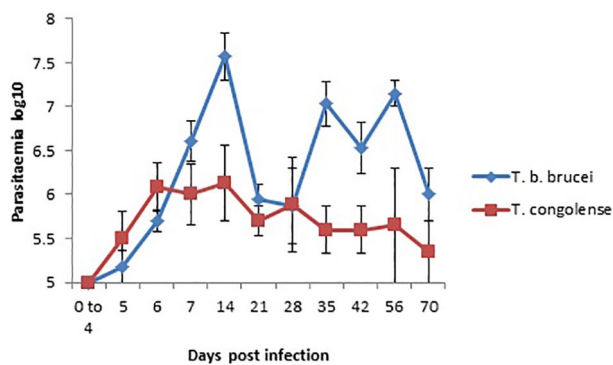
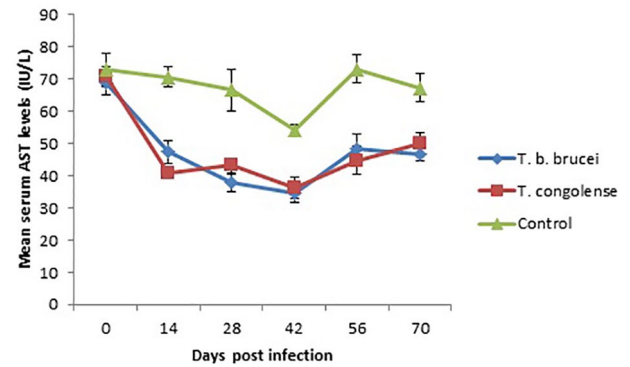**Figure 3.** Graph showing the mean log10 parasitaemia of WAD rams infected with *T brucei brucei* and *T congolense.*

Figure 4. Graph showing the mean serum AST activity (IU/L) of WAD rams infected with *T brucei brucei* and *T congolense.*
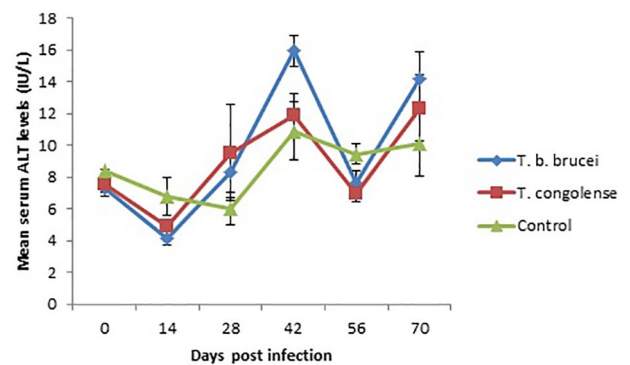
Figure 5. Graph showing the mean serum ALT activity (IU/L) of WAD rams infected with *T brucei brucei* and *T congolense.*

Figure 6. Graph showing the mean serum ALP activity (IU/L) of WAD rams infected with *T brucei brucei* and *T congolense.*

fluctuations were observed. Also, *T brucei brucei* produced significantly ($P < .05$) higher levels of parasitaemia compared with *T. congolense* especially on the peak days (Figure 3).

Biochemical perturbations in T brucei brucei and T congolense infections in the rams

There were significantly ($P < .05$) lower levels of AST activity in the infected groups on days 14, 28, 42, and 70 PI compared with the control (Figure 4). Serum activity of ALT was significantly ($P < .05$) higher in *T brucei brucei* infected rams than the control on day 42 PI while *T congolense* was significantly ($P < .05$) lower than the control on day 56 PI (Figure 5). No significant ($P > .05$) variations were observed in the serum activity of ALP in both the infected rams and uninfected control (Figure 6).
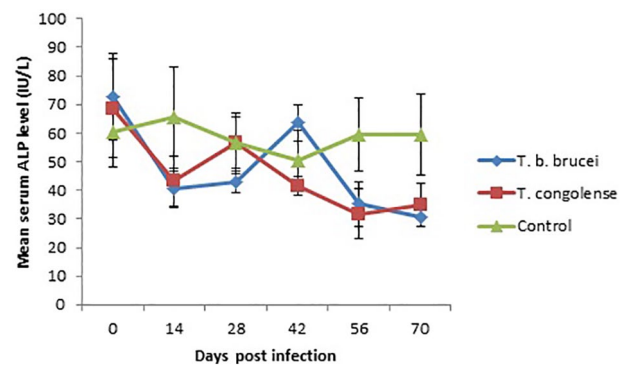
Serum total protein level was significantly ($P < .05$) higher in *T congolense* infected rams than in *T brucei brucei* infected rams on day 42 PI and was also significantly ($P < .05$) higher than both *T brucei brucei* infected rams and the uninfected control on day 70 PI, whereas *T brucei brucei* infected rams had significantly ($P < .05$) lower total protein level than the uninfected control on day 70 PI (Figure 7). Serum albumin levels of *T brucei brucei* infected rams were significantly ($P < .05$) lower than the uninfected control on days 14, 28, 42, 56, and 70 PI, while that of *T congolense* was significantly ($P < .05$) lower than the uninfected
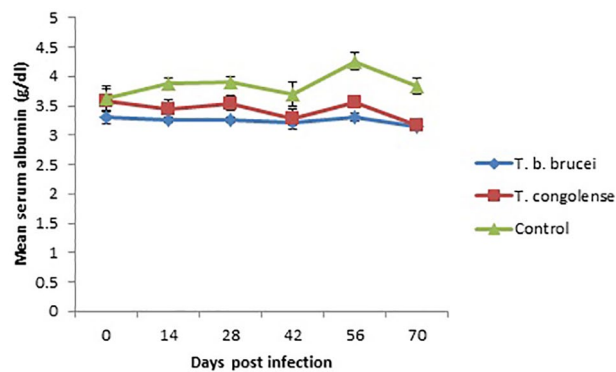
control on days 14, 28, 56, and 70 PI (Figure 8). Serum globulin level of *T congolense* infected rams was significantly ($P < .05$) higher than those of *T brucei brucei* infected rams as well as the uninfected control on days 42 and 70 PI (Figure 9).

Serum total cholesterol level of *T brucei brucei* infected rams was significantly ($P < .05$) lower than the uninfected control on days 14, 28, 56, and 70 PI. While that of *T congolense* infected rams was significantly ($P < .05$) lower than the uninfected control on days 14, 56, and 70 PI (Figure 10).

Serum urea level of *T congolense* infected rams were significantly ($P < .05$) higher than *T brucei brucei* infected rams on day 28 PI and significantly ($P < .05$) higher than the uninfected
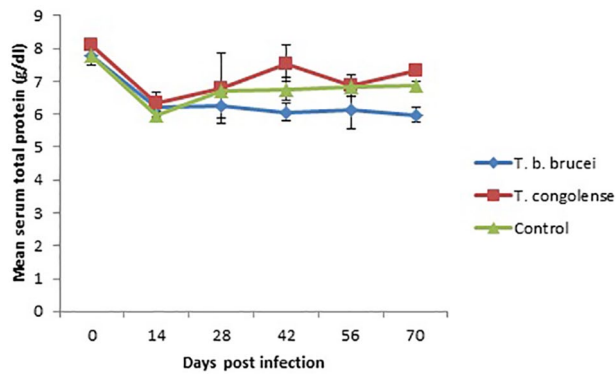
Figure 7. Graph showing the mean serum total protein level (g/dL) of WAD rams infected with *T brucei brucei* and *T congolense.*

Figure 8. Graph showing the mean serum albumin level (g/dL) of WAD rams infected with *T brucei brucei* and *T congolense.*
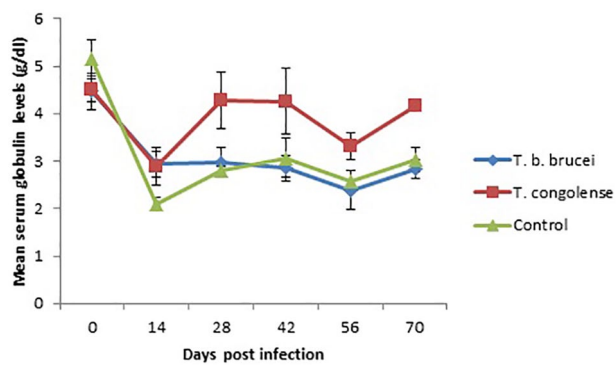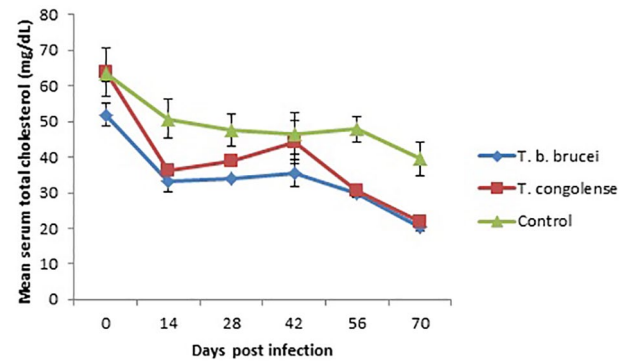

Figure 9. Graph showing the mean serum globulin level (g/dL) of WAD rams infected with *T brucei brucei* and *T congolense.*

Figure 10. Graph showing the mean serum total cholesterol level (mg/dL) of WAD rams infected with *T brucei brucei* and *T congolense.*

Figure 11. Bar graph showing the mean serum urea level (mg/dL) of WAD rams infected with *T brucei brucei* and *T congolense.*
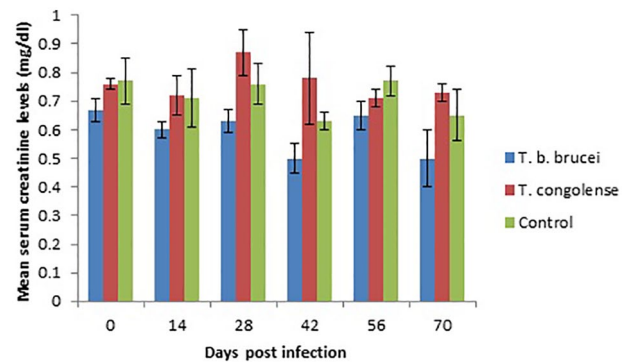
Figure 12. Bar graph showing the mean serum creatinine level (mg/dL) of WAD rams infected with *T brucei brucei* and *T congolense.*
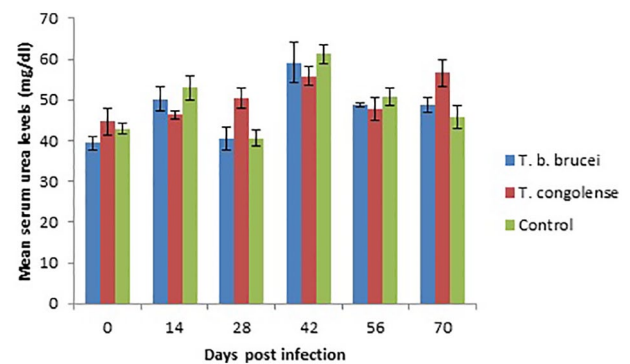
control on days 28 and 70 PI (Figure 11). There were no significant ($P > .05$) variations in the serum creatinine levels of the infected and uninfected rams excepts on day 28 PI on which the serum creatinine level of *T congolense* infected rams was significantly higher than that of *T brucei brucei* infected rams (Figure 12).

Histopathology

Mortality occurred in the *T congolense* infected rams on days 14 and 66 PI and in *T brucei brucei* infected rams on days 14 and 50 PI. The liver tissue of *T brucei brucei* infected rams showed severe multifocal random hepatocellular necrosis in the form of

uncapsulated microgranulomas with massive infiltration of mononuclear inflammatory cells (Figure 13). The liver of the *T congolense* infected rams also showed mild coagulative hepatocellular necrosis and perivascular inflammation in the portal area (Figure 14). There were other organs with significant lesions but they were not relevant to the focus of the research.

Discussion

From the results of our study, the body weight of the infected rams did not drop significantly and this agrees with the reported observations in trypanotolerant breeds.[6-8] However, the significant drop in the body weight on day 70 PI in the
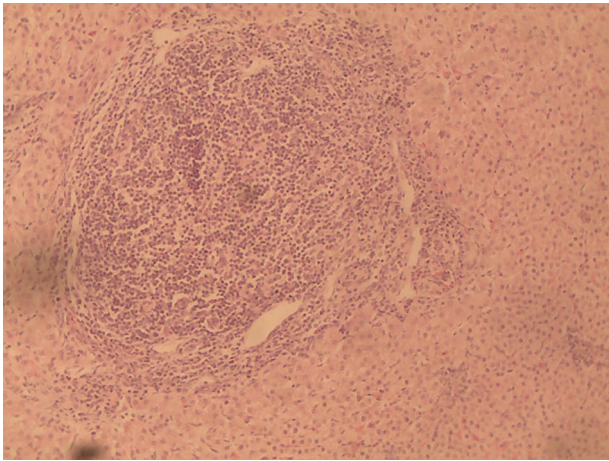
Figure 13. Liver section of WAD ram infected with *T brucei brucei* showing large focal uncapsulated microgranuloma comprising of necrotised hepatocytes and infiltrated mononuclear inflammatory cells. H&E stain ×100.

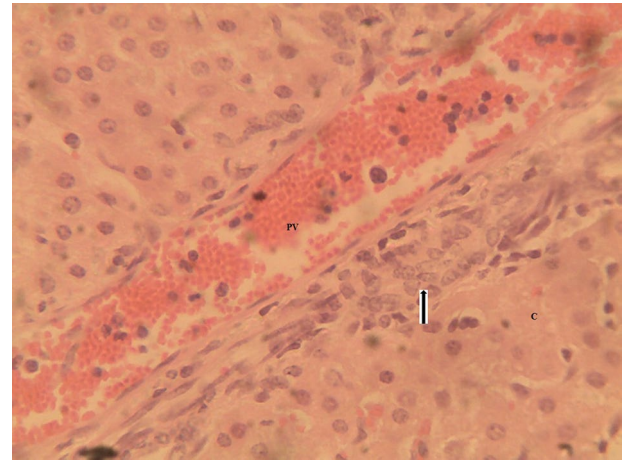**Figure 14.** Liver section of WAD ram infected with *T congolense* showing mild infiltration of mononuclear inflammatory cells (arrow) around the portal vein (PV) with mild coagulative hepatocellular necrosis (C). H&E stain ×400.

T brucei brucei infected group may suggest that weight loss may eventually occur in tolerant animals chronically infected with highly *virulent* strains of *T brucei brucei*.

The significant rise in rectal temperature in the infected animals is consistent with trypanosome infection and *T congolense* appears to produce higher level of pyrexia than *T brucei brucei* probably due to its affinity for the microvasculatures of the brain.[21]

The prepatent period of 5 to 6 days for intraperitoneal inoculation of *T brucei brucei* and *T congolense* in our study compares well with experimental infections in mice in which the mean prepatent period was 3.8 days for *T brucei brucei* and 6.5 days for *T congolense*.[22] Also, in our study, *T brucei brucei* produced higher level (4.0×10^7 trypanosomes/mL of blood) and more fluctuating peaks of parasitaemia than *T congolense* (1.3×10^6 trypanosomes/mL of blood). The understanding of trypano-tolerance includes the ability of the tolerant breeds to control parasitaemia. This is very evident in our study because very much higher levels of parasitaemia, up to 1×10^9 trypanosomes/mL of blood, are seen in rodent models and other very susceptible animals.[23] It appears that parasitaemia was better controlled in the *T congolense* infected WAD rams than in *T brucei brucei* infected ones. Variations in parasitaemia within same species or breed of animals may depend on the strain and pathogenicity of the trypanosome isolate.

Significant reductions were observed in the serum AST activity of the infected rams. Also, significant reduction was observed in the serum ALT activity of *T congolense* infected rams on day 56 PI, but that of *T brucei brucei* was significantly higher than the control on day 42 PI. Furthermore, no variations were seen in the serum ALP levels of the infected rams. These finding differ moderately from serum enzymes activity reported in other susceptible animals. Increases in the serum activity of AST, ALT, and ALP have been reported in *T brucei brucei* infection, and were attributed to liver injury caused by the parasite. However, in *T congolense* infection, the serum enzymes activity remained unchanged.[10,24] Reductions in serum AST and ALT observed in our study may be attributed to inhibition of their cofactor (vitamin B$_6$) following losses of total protein and reductions in hepatic synthesis due to hypo-proteinemia.[25] Although the significant increase in ALT in *T brucei brucei* infected rams on day 42 PI may be due to liver damage induced by the tissue invasive nature of the parasite, this did not affect serum AST probably because AST is a less specific marker for liver damage and has a shorter half-life.[26]

Total proteins and gamma globulins increase while serum albumin decreases in several trypanosomes infections.[27] In the present study, serum total protein and globulin levels significantly increased only in the *T congolense* infected rams, but serum total protein level of *T brucei brucei* infected rams was significantly lower than those of both *T congolense* infected rams and the uninfected control. Decrease in serum total protein in *T brucei brucei* infection has also been reported in boars.[28] However, significant decrease in serum albumin level in the infected groups in our study agrees with the findings in other susceptible species.[27] The degree of hypoalbuminaemia was related to the level of parasitaemia and/or severity of the disease and occurrence of haemodilution.[29]

Significant reductions in serum total cholesterol levels were observed in the infected rams. This has also been reported in susceptible goat breeds.[30] It has been reported that the bloodstream forms of trypanosomes are unable to synthesize cholesterol although they require it for growth and synthesis of their membranes and this could contribute to lowering of the serum levels of lipids and cholesterol in infected hosts.[31]

Serum urea level was significantly higher in *T congolense* infected rams on days 28 and 70 PI. Also, serum creatinine level of the infected groups did not vary significantly with the uninfected control. High urea levels were reported in cattle infected with *T congolense* and it occurred early when parasitaemia was

high and returned to normal level in chronic trypanosomiasis.[32] It appears that excessive protein catabolism caused by fever contributes to increased blood urea nitrogen. In the present study, both serum total protein and pyrexia were more pronounced in the *T congolense* infected rams. Moreover, trypanosome infection in the WAD sheep may not be associated with significant muscle or kidney damage that may increase serum creatinine level as reported in the more susceptible species.[24]

The histopathological changes in the liver in our study, especially in the *T brucei brucei* infected group may be responsible for the significantly higher level of serum ALT activity observed in this group. The liver lesions may have been triggered by massive invasion of trypanosomes into the liver parenchyma with attendant inflammatory response and hepatocellular necrosis.

Conclusions

The results of the present study revealed significant clinical and biochemical perturbations in the so-called trypanotolerant WAD rams ranging from pyrexia, hypoproteinaemia, and hypocholesterolaemia to weight loss, liver damage, and death. These findings suggest that the WAD rams are not passive reservoirs of trypanosomes for human and animal infections,[33,34] but also suffer significantly from the infection where the disease surveillance, control of the insect vector, as well as therapeutic interventions are neglected while trypanotolerance is overemphasized.

Author Contributions

D.C.A., V.S.O.S., and J.I.I. conceived and designed the experiments. D.C.A. and J.I.I. performed the laboratory work, analysed the data, and wrote the first draft of the manuscript. D.C.A., V.S.O.S., and J.I.I. contributed to the writing of the manuscript, agreed with manuscript results and conclusions, jointly developed the structure and arguments for the article, made critical revisions and approved final version, and reviewed and approved the final manuscript.

ORCID iD

Davinson C Anyogu (iD) https://orcid.org/0000-0003-2205-5400

REFERENCES

1. Cayla M, Rojas F, Silvester E, Venter F, Matthews KR. African trypanosomes. *Parasit Vectors*. 2019;12:190.
2. D'leteren GMD, Authie E, Wissooq N, Murray M. Trypanotolerance, an option for sustainable livestock production in areas at risk from trypanosomosis. *Res Sci Tech*. 1998;17:154-175.
3. Stijlemans B, De Baetselier P, Magez S, Van Ginderachter JA, De Trez C. African trypanosomiasis-associated anemia: the contribution of the interplay between parasites and the mononuclear phagocyte system. *Front Immunol*. 2018;9:218. doi:10.3389/fimmu.2018.00218.
4. Osaer S, Goossens B, Jeffcoate I, Jaitner J, Kora S, Holmes P. Effects of *Trypanosoma congolense* and nutritional supplements on establishment and outcome of pregnancy in trypanotolerant Djallonké ewes. *Anim Reprod Sci*. 1998;51:97-109.
5. Osaer S, Akinbamijo OO, Goossens B. Some biochemical changes following *Trypanosoma congolense* infection in Djallonke ewe lambs and breeding ewes fed on two levels of nutrition. *Acta Trop*. 2000;75:229-241.
6. Dolan RB. *Trypanotolerance in Orma Boran Cattle on Galana Ranch in Kenya*. International Livestock Centre for Africa (ILCA) Consultancy Report. Nairobi, Kenya: ILCA; 1993.
7. Naessens J. Bovine trypanotolerance: a natural ability to prevent severe anaemia and haemophagocytic syndrome? *Int J Parasitol*. 2006;36:521-528.
8. Muigai AWT, Hanotte O. The origin of African sheep: archaeological and genetic perspectives. *Afr Archaeol Rev*. 2013;30:39-50.
9. Geerts S, Osaer S, Goossens B, Faye D. Trypanotolerance in small ruminants of sub-Saharan Africa. *Trends Parasitol*. 2009;25:132-138.
10. Awekew A, Fetene B, Eshetu E. Review on serum biochemical changes in ruminants infected with major trypanosome species. *Int J Adv Res Biol Sci*. 2017;4:26-35.
11. Herbert WJ, Lumsden WHR. *Trypanosoma brucei*: a rapid 'matching' method for estimating the host's parasitaemia. *Exp Parasitol*. 1976;40:427-431.
12. Reitman S, Frankel S. A Colorimetric method for determination of serum glutamic, oxaloacetic and glutamic pyruvic transaminase. *Am J Clin Pathol*. 1957;28:56-62.
13. Klein B, Read PA, Babson Al. Rapid method for the quantitative determination of serum alkaline phosphatase. *Clin Chem*. 1960;6:26-275.
14. Babson AL, Greeley SJ, Coleman CM, Philips GE. Phenolphthalein monophosphate as a substrate for serum alkaline phosphatase. *Clin Chem*. 1966;12:482-490.
15. Colville J. Blood chemistry. In: Hendrix CM, ed. *Laboratory Procedures for Veterinary Technicians*. 4th ed. St. Louis, MO: Mosby; 2002: 75-103.
16. Lubran MM. The measurement of total serum proteins by the biuret method. *Ann Clin Lab Sci*. 1978;8:106-110.
17. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta*. 1971;31:87-96.
18. Doumas BT, Peters T Jr. Serum and urine albumin a progress report on the measurement and clinical significance. *Clin Chim Acta*. 1997;258:3-20.
19. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total cholesterol. *Clin Chem*. 1974;20:470-475.
20. Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. *J Clin Pathol*. 1960;13:156-159.
21. Fry MM, McGavin MD. Bone marrow, blood cells, and the lymphatic system. In: Zachary JF, McGavin MD, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Elsevier; 2012:719-720.
22. Ndungu K, Thungu D, Wamwiri F, et al. Route of inoculation influences *Trypanosoma congolense* and *Trypanosoma brucei brucei* virulence in Swiss white mice. *PLoS ONE*. 2019;14:e0218441.
23. Gitonga PK, Ndung'u K, Murilla GA, et al. Differential virulence and tsetse fly transmissibility of *Trypanosoma congolense* and *Trypanosoma brucei* strains. *Onderstepoort J Vet Res*. 2017;84:a1412.
24. Egbe-Nwiyi TN, Igbokwe IO, Onyeyili PA. Pathogenicity of *Trypanosoma congolense* infection following oral calcium chloride administration in rats. *African J Biomed Res*. 2005;8:197-201.
25. York MJ. *A Comprehensive Guide to Toxicology in Nonclinical Drug Development* (ed. Faqi AS). 2nd ed. Amsterdam, The Netherlands: Elsevier; 2017: 325-374.
26. Yang X, Schnackenberg LK, Shi Q, Salminen WF. Hepatic toxicity biomarkers. In: Gupta RC, ed. *Biomarkers in Toxicology*. Waltham, MA: Academic Press; 2014:241-260.
27. Herrera HM, Alessi AC, Marques LC, et al. Experimental *Trypanosoma evansi* infection in South American coati (Nasuanasua): haematological, biochemical and histological changes. *Acta Trop*. 2002;81:203-210.
28. Otesile E, Fagbemi BO, Adeyemo OJ. The effect of *Trypanosoma brucei* infection on serum biochemical parameters in boars on different planes of dietary energy. *Vet Parasitol*. 1991;40:207-216.
29. Katunguka-Rwakishaya E, Murray M, Holmes PH. The pathophysiology of ovine trypanosomosis: haematological and blood biochemical changes. *Vet Parasitol*. 1992;45:17-32.
30. Biryomumaisho S, Katunguka-Rwakishaya E, Rubaire-Akiiki CM. Serum biochemical changes in experimental *Trypanosoma congolense* and *Trypanosoma brucei* infections in East African sheep. *Vet Arch*. 2003;3:167-180.
31. Green HP, Portela MPM, Lugli EB, Raper J. Evidence for a *Trypanosoma brucei* lipoprotein scavenger receptor. *J Biol Chem*. 2003;278:422-427.
32. Anosa VO. Haematological and biochemical changes in human and animal trypanosomiasis. Part II. *Rev Elev Med Vet Pays Trop*. 1988;41:151-164.
33. Mahmaud MM, Elmalik KH. Trypanosomiasis: goats as a possible reservoir of *Trypanosoma congolense* in the Republic of Sudan. *Trop Anim Health Prod*. 1977;9:167-170.
34. Scott CM, Frezil J, Toudic A, Gregory DG. The sheep as a potential reservoir of human trypanosomiasis in the Republic of the Congo. *Trans R Soc Trop Med Hyg*. 1983;77:391-401.