



Complement Levels and Haemato-Biochemical Parameters as Indices of Trypanotolerance in Nigerian Goats Experimentally Infected with *Trypanosoma congolense*

KUMSHE, H.A.^{1*}, MBAYA, A. W.², OTESILE, E.B.³, SON IBARE A.O.³
ALAKA, O.O.⁴ AND OLADOSU, L.A.⁵

¹Department of Veterinary Medicine, University of Maiduguri, Nigeria; ²Department of Veterinary Microbiology and Parasitology, University of Maiduguri, Nigeria; ³Department of Veterinary Medicine, University of Agriculture, Abeokuta, Nigeria; ⁴Department of Veterinary Pathology, University of Ibadan, Nigeria; ⁵Department of Veterinary Medicine, University of Ibadan, Nigeria.

*Corresponding Author: husseinkumshe@yahoo.com, Tel: + 2348036411956

SUMMARY

Complement levels and haemato-biochemical parameters in West African Dwarf (WAD) and Borno White (BW) goats experimentally infected with *Trypanosoma congolense* were investigated. Parasitaemia was established in both breeds of goats by day 7 post-infection. Peak parasitaemia of $7.5 \times 10^3/\mu\text{L}$ for WAD goats was attained by day 14 post-infection while, in the BW goats, parasitaemia of $18 \times 10^3/\mu\text{L}$ was attained by day 19 post-infection. This was characterized by anaemia, leucopenia, hypocomplementaemia and depletion of C3 level and increased levels of total serum protein and globulin. There was a significant ($p < 0.05$) decline in packed cell volume (PCV), total haemolytic complement (CH50) values among BW when compared to WAD goats. The perceived relative trypanotolerance of WAD goats when compared to BW goats can be attributed among other things, to higher levels of C3, CH50 units, total proteins and PCV in WAD goats.

KEY WORDS: Trypanosomosis, Trypanotolerance, Complement, Haemato-biochemical parameters, Nigerian goats.

INTRODUCTION

Nearly half of the small ruminants in the humid zone of Africa are found in Nigeria of which, the population of goat is estimated at about 34.5 million and a provider of up to 30% of national meat supply (Jabbar, 1988; RIM, 1992., Shaib et al., 1997). About 99.97% of Nigerian goats are traditionally managed and are therefore, constantly threatened by diseases (RIM, 1992). Of all the livestock diseases, African trypanosomosis is one of the major constraints on animal production (D' Ieteren et al., 1998; D' Ieteren and Kimani, 2001). Trypanosomosis is a disease caused by haemoprotozoa of the genus *Trypanosoma* and is transmitted by the insect vector of the genus *Glossina* (Radostitis, et al., 2000). The major pathogenic species of trypanosomes that affect livestock are *Trypanosoma vivax*, *T. congolense*, *T. brucei*, *T. evansi* and *T. Simiae* (Soulsby, 1982). Although, the prevalence of trypanosomosis in small ruminants is usually lower than in large ruminants, the effect is however, devastating (Gererts et al., 2000). According to breed, the West African Dwarf Goat (WAD) was reported to be most trypanotolerant than the Red Sokoto breed (Adah, 1991). Although, the mechanism of trypanotolerance is poorly understood, trypanolytic antibodies have been identified as important in the control of successive waves of parasitaemia (Palling et al., 1991). In addition, some host factors and serum components have been associated with trypanotolerance (Otesile et al., 1991). Most of the previous works in this

regard were done in man and large ruminants (Adah, 1991; Doko et al., 1999). Comparatively, there appears to be a dearth of information on the indices of trypanotolerance in goats during trypanosome infections. Thus, in this study, trypanotolerant markers such as red blood cell parameters, serum complements and protein concentrations were determined in trypanotolerant West African Dwarf (WAD) and trypanosusceptible Borno White (BW) goats following experimental *Trypanosoma congolense* infection.

MATERIALS AND METHODS

Experimental animals

Seven West African Dwarf (WAD) and seven Borno White (BW) adult male goats aged between 1-1½ years were obtained from Ibadan and Maiduguri, Nigeria. The animals were housed in the experimental Animal Breeding House of the Department of Veterinary Medicine, University of Ibadan, Nigeria. They were treated against endo- and ectoparasites with ivermectin at 0.2mg/kg body weight subcutaneously, and intramuscularly, with tetracycline at 25mg/kg against blood rickettsial organisms for five consecutive days. They were also treated intramuscularly, with one standard single dose (7.0mg/kg) of diminazene aceturate (Berenil®) against trypanosomosis and piroplasmiasis and later vaccinated against Peste des Petits Ruminant (PPR) using PPRV vaccine (NVRI, Vom, Nigeria). They were allowed 2 months acclimatization period during which, they were fed freshly cut grass supplemented with wheat bran, groundnut husk and cowpea leaves. Salt lick and water was provided ad-libitum. One week to the commencement of the experiment blood and faecal samples were collected every two days and screened for parasites. The Ethics Committee of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria, approved the study. Strict compliance with EC Directive 86/609/EEC for animal experimentation was observed.

Source of trypanosomes

Trypanosoma congolense (strain 2295) used in the study was obtained from Nigeria Institute for Trypanosomiasis Research (NITR), Vom, Nigeria. It was originally isolated from a cow in Karu Local Government Area of Kaduna State, Nigeria in April 2011. It was stored in liquid nitrogen, sub-inoculated into donor albino mice, and transported to the Laboratory of the Department of Veterinary Medicine, University of Ibadan, Nigeria where, the study was conducted.

Experimental design

The goats were randomly, divided into four groups (A - D) and infected as follows: Group A comprised of five West African Dwarf goats. They were each, infected with 1ml of infected blood containing 107 *Trypanosoma congolense* through the jugular vein. Group B comprised of two West African Dwarf goats, which served as uninfected control. Meanwhile Group C comprising of five Borno White goats was infected with 1ml of infected blood containing 107 *Trypanosoma congolense* through the jugular vein and Group D comprising of two Borno White goats served as uninfected control.

Sample collection

To establish base line data, 8mls of blood was collected from each animal of which, 2mls was immediately dispensed into a Bijou bottle containing disodium ethylene diamine tetracetic acid (EDTA) solution as anticoagulant. The remaining 6mls of blood was dispensed into plain test-tube for serum. Samples were labelled appropriately and transported to the laboratory on ice pack. The animals were sampled twice weekly.

Estimation of parasitaemia and haematological parameters

Parasitaemia was estimated according to standard methods (Herbert and Lumsden, 1976). Packed cell volume (PCV) red blood cell count (RBC), total (WBC) and differential

white blood cell counts were analysed using autohaemolyzer (Auto haematology Analyser (Mindray) Bc 2800 Vet.).

Preparation of goat anti-rabbit haemolysin

To raise haemolysin against goat red cells standard procedures were used with slight modifications. Briefly, two adult chinchilla rabbits weighing between 1.8 to 2 kg were inoculated intravenously with 1ml of goat RBC membranes (containing 2 mg suspension) in isotonic saline solution (Dodge et al., 1963). Intravenous injections of RBC membranes were repeated at a 24 hourly interval with 11 injections administered. Seventy-two hours after the last injection, blood collected from the rabbits was allowed to clot for one hour at room temperature. Serum containing haemolysin was collected by centrifugation at 5,000-x g for 10 minutes at 4oC. Haemolysins from the two rabbits were pooled together, dispensed in 0.5ml aliquots into sterilized Bijou bottles, and immediately stored at -20oC until used.

Preparation of anti-C3 sera

Anti-C3 serum was prepared according to standard techniques (Hudson and Hay, 1980). Briefly, goat serum was activated with zymoson by mixing 13.5 mg/ ml serum and incubated at 37oC for 30 minutes. The zymoson C3 complex was washed six times in barbitione buffered saline (pH 8.6). Freund's incomplete adjuvant (FIA) was added and contents thoroughly mixed. Appropriately, 0.2ml of zymoson – C3/FIA mixture was injected subcutaneously to each of the four sites of an adult chinchilla rabbit namely; the right and left shoulder regions and right and left rump regions. This is equivalent to a total of 0.8 ml of C3/FIA emulsion per rabbit. Each of the two rabbits was immunized three times at 10 days interval (on days 0, 10 and 20). Seven days after the last immunization with zymoson C3/ FIA emulsion, blood from the rabbits were collected through the marginal ear veins and Anti-C3 serum was collected and dispensed in 0.5ml aliquots and stored at -20oC until used.

Determination of serum levels of complement C3

Serum levels of complement C3 was determined by single radial immunodiffusion (RID) technique (Tabel, 1982). The levels were expressed in relation to the level in a standard reference pool of apparently healthy goat serum.

Estimation of total haemolytic complement CH50 levels

A micro method of the standard haemolytic assay was used for the determination of total serum complement (CH50) level (Ogundele, 1988). Briefly, 50µl of 1:50 initially titrated diluted complement, which, gave CH50, was pipetted into 84 wells of U-shaped bottom micro titre plates and 50µl of 5 x 10⁸ cells/ml of washed sheep red cell was added into each well. The plates were incubated at 37oC for one hour with constant shaking at 10 minutes interval after which, the sensitized red cell suspension was washed three times with Tris-Saline buffer and supernatant solution decanted. The volume of sensitized RBC was adjusted to 50µl with Tris-saline buffer and 50µl of 40 percent fresh goat serum (FGS) added into each test well and gently shaken to ensure even suspension of erythrocytes with FGS (giving a final reaction concentration of 20 percent FGS). Positive and negative control wells were set up. The plates were incubated for one hour at 37oC with constant shaking at 10 minutes intervals and later spun at 200 x g for 10 minutes. The supernatant was pipetted and transferred into flat bottom micro titre plates. The optical density (OD) reading of the released haemoglobin was read at 541nm. The degree of lysis (which reflects complement activity) was calculated and converted into CH50.

Estimation of serum globulin concentration

Serum globulin concentration was determined by a modification of the zinc sulphate turbidity (ZST) technique (Otesile, 1985). The turbidity

reading was found by subtracting the reading of the “control” tube from that of the “test” tube.

Estimation of total protein

The total serum protein was estimated by standard method (Toro and Ackerman, 1985). Optical density (OD) readings were done at 340 nm wavelength in a spectrometer after zeroing the equipment with the negative control. Concentration of total serum protein was calculated using standard formulae.

RESULTS

Parasitaemia was established in both breeds of goats by day 7 post-infection. Peak parasitaemia for WAD ($7.5 \times 10^3/\mu\text{L}$) goats was attained by day 14 post-infection while, in the BW ($18 \times 10^3/\mu\text{L}$) goats, it was attained by day 19 post-infection.

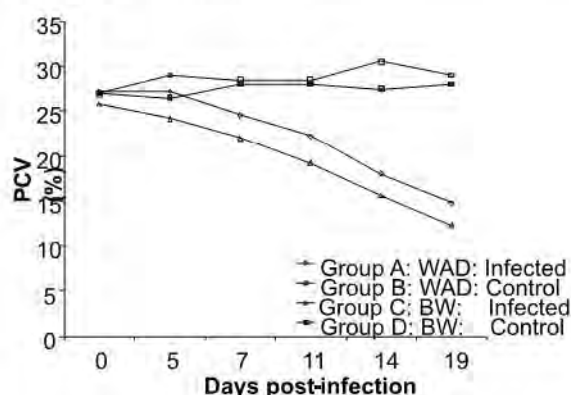
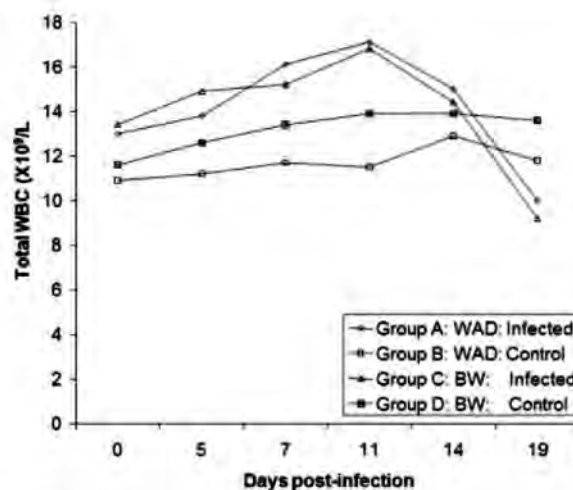


Fig. 1: Change in PCV (%) for West African Dwarf (WAD) and Borno White (BW) goats infected with *T. congolense*

A progressive leukocytosis was the initial response to infection in both breeds up till day 11 post-infection when a steady decline began to occur (Fig. 2).



total WBC count ($\times 10^9/\text{L}$) for West African Dwarf (WAD) and Borno White (BW) goats infected with *T. congolense*

The values increased significantly ($p < 0.05$) from $13.6 \pm 1.6\%$ (WAD) and $13.4 \pm 1.8\%$ (BW) to $17.1 \pm 0.5\%$ and $16.8 \pm 0.4\%$ by day 11 post-infection, respectively. Thereafter, the values declined to $10.0 \pm 3.3\%$ for WAD and 9.2 ± 1.2 for BW, with significant ($p < 0.05$) variation between breeds.

The mean relative complement C3 concentration for WAD and BW goats infected with *T. congolense* is presented in Fig. 3. A fall in relative C3 concentration was observed in all infected goats by day 5 post-infection. Thereafter, there was a rapid depletion of C3 concentration leading to the lowest level by day 19 post-infection. The mean decline of relative C3 in infected goats of both breeds compared to their controls was found to be significantly ($p < 0.05$) different from days 5 to 19 post-infection. Relative C3 of infected WAD goats declined from a pre-infection level of 143% to 48.6% by day 19 post-infection, representing a decline of 66.6%. Mean relative C3 concentration of infected BW

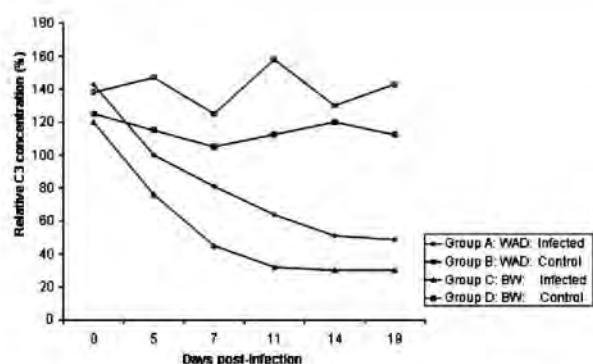


Fig. 3: Change in C3 concentration for West African Dwarf (WAD) and Borno White (BW) goats infected with *T. congolense*

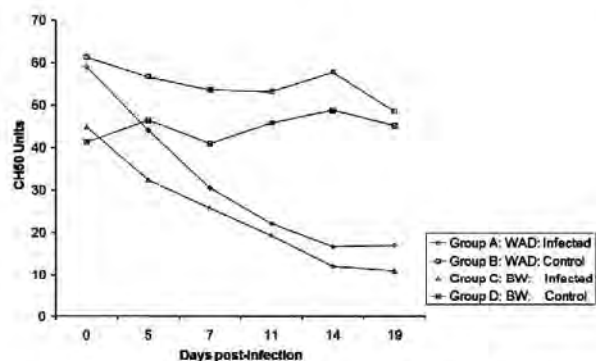


Fig. 4: Change in (CH50 units) level for West African Dwarf (WAD) and Borno White (BW) goats infected with *T. congolense*

goats declined from a pre-infection value of 120% to 30% by day 19 post-infection representing 75.0% decline. Controls however, maintained fairly, constant C3 levels throughout the experiment.

The mean CH50 of infected WAD goats declined from a pre-infection level of $58.9 \pm 1.4\%$ to $17.0 \pm 2.2\%$ by day 19 post-infection (drop of 71%) whereas, in BW goats, CH50 dropped (76%) more significantly ($p < 0.05$) than in WAD goats. Similarly, the controls had fairly, constant values (Fig. 4).

The mean total serum protein (TSP) values for WAD and BW goats infected with *T. congolense* are presented in Figure 5. Mean TSP values of infected WAD goats increased by 32.4% from a pre-infection value of $5.0 \pm$

0.2g/dl to $7.4 \pm 0.2 \text{ g/dl}$ by day 14 post-infection. Similarly, infected BW goats, TSP values rose by 32.9% from a pre-infection value of ($5.0 \pm 0.2 \text{ g/dl}$ to $7.3 \pm 0.8 \text{ g/dl}$) by day 14 post-infection.

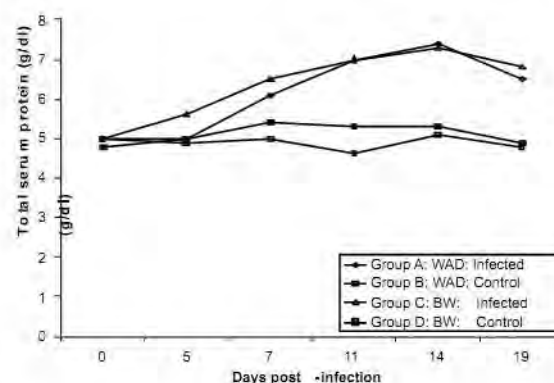


Fig. 5: Change in serum protein concentration (g/dl) for WAD and BW goats infected with *T. congolense*.

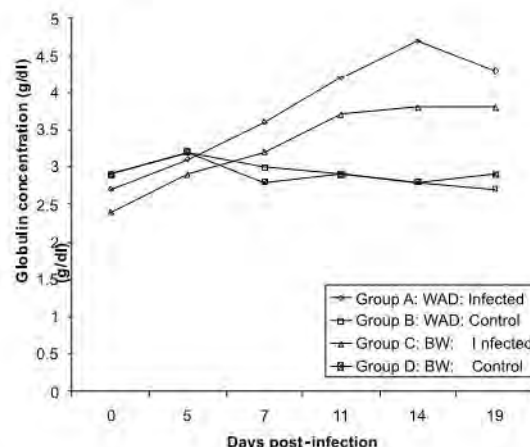


Fig. 6: Change in globulin concentration (g/dl) for WAD and BW goats infected with *T. congolense*

The mean serum globulin concentration for WAD and BW goats infected with *T. congolense* are presented in Figure 6. A gradual increase in globulin concentration was observed as from days 5 to 14 post-infection in both breeds of goats. The mean globulin concentration in infected WAD goats increased by 42.5% from a pre-infection value of 2.7 ± 0.9 g/dl to 4.7 ± 0.1 g/dl by day 14 post-infection. In BW goats, it rose by 36.8% from a pre-infection value of 2.4 ± 0.2 g/dl to 3.8 ± 0.6 g/dl by day 14 post-infection.

DISCUSSION

A direct relationship between parasitaemia and anaemia was observed in all infected goats. This observation is in agreement with several authors (Anosa, 1988; Mbaya et al., 2009). Although all infected goats were anaemic by day 7 post-infection, a decline of 42.9% PCV was observed among infected WAD goats by day 19 post-infection whereas, in corresponding BW goats, PCV declined by 52.3% from pre-infection values. One mortality among BW was observed with a PCV of 9% by day 19 post-infection. As control of anaemia is a major, attribute of trypanotolerance, it is therefore, apparent that WAD goats were more trypanotolerant than BW goats (ILRAD, 1993). The mean serum levels of complement C3 in *T. congolense* infected goats were rapidly depleted as the infection progressed. This may partially, be explained by increased consumption of this component considering the fact that it is an important candidate for improved generalized defence mechanism and may be responsible for the in-vivo control of parasitaemia and immunosuppression widely reported in trypanosome infected animals. This finding however, disagrees with earlier reports (Authie and Pobel, 1990; Malu and Tabel, 1986), where they reported increase in circulating C3 levels which later returned to normal values in the course of the infection.

Depletion of serum C3 concentration is more

severe in BW goats (75%) compared to WAD (66.6%) depletion. This confirms the role of C3 complement as an index of trypanosusceptibility status of BW goats compared to the WAD goats. Hypocomplementaemia as reflected by a decrease in the haemolytic complement activities (CH50 level) in all the *T. congolense* infected goats are obvious in this study. A depressed level of haemolytic complement in sheep infected with *T. congolense* and West African Dwarf short horn cattle with *T. brucei* have been reported respectively (Doko et al, 1999; Tabel, 1982). In this study, the hypocomplementaemia status was significantly ($p < 0.05$) higher in BW than in WAD goats. It is therefore, possible that haemolytic complement levels play an important role in the genesis of trypanotolerance.

The phenomenon of hypocomplementaemia observed in this experiment can be attributed to massive consumption of complement because of persistent parasitaemia leading to the formation of antigen-antibody complexes; however, hypocomplementaemia as caused directly or indirectly by the parasite is of considerable significance in the disease syndrome. Elevated total serum proteins and globulins observed in this study agree with similar findings in sheep and goats infected with *T. vivax* (Anosa and Isoun, 1976). It is therefore, concluded that the perceived relative trypanotolerance of WAD when compared to BW goats can be attributed among other things to higher level of C3, CH50 units, total proteins and PCV. This therefore, means that the WAD will thrive better than the BW goats in tsetse infested areas and in areas where mechanical transmission of trypanosomosis is maintained by haematophagous arthropod vectors. This work is important because it is the first of its kind to be conducted between these breeds in Nigeria. However, to take this work to the next level, the molecular markers in the gene sequence that is responsible for trypanotolerance in the WAD goats needs to be

identified in order to facilitate cross breeding between trypanotolerant WAD and trypanosusceptible BW goats.

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