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Serum biochemical parameters of Yankasa sheep experimentally infected with *Trypanosoma evansi* and treated with diminazene aceturate (Berenil®)

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Abstract

Blood glucose, serum total protein, and liver transaminases were determined in Yankasa sheep experimentally infected with *Trypanosoma evansi* (*T. evansi*) and treated with diminazene aceturate (Berenil®). A total of 30 animals were divided into 6 groups (A to F) (n=5). Animals from each group were either uninfected or infected with *T. evansi* and treated with Berenil®. Infection of the infected groups (A, C and E) was done via intravenous inoculation of *T. evansi,* while the infected group C and E were treated with Berenil® at 3.5 and 7 mg/kg BW (single dose), respectively, by day 16 post-infection (PI). The infected groups had a pre-patent period of 8 days, with similar levels of parasitaemia of 4.7 ± 0.27. In group A, the mean parasite count rose significantly ($p < 0.05$) to 72.8 ± 1.07 by day 12 PI and continued to a peak value of 250.6 ± 1.98 by day 28 PI. In groups C and E, the initial parasitaemia rose significantly ($p < 0.05$) to a peak count of 80.8 ± 1.12 and 78.2 ± 1.11 by day 12 PI, following treatment with 3.5 and 7.0 mg/kg BW of Berenil®, by day 20 PI, respectively, and was completely eliminated by day 9 and 5 post-treatment (PT), respectively. The biochemical analysis showed that, from day 8 PI, the infected sheep experienced significant ($p < 0.05$) increases in alkaline phosphatase, aspartate and alanine aminotransferase, creatinine and urea; with decreases in blood glucose and total protein. However, these changes reverted to their pre-infection values, by day 28 PI in all the affected animals following treatment with both doses of Berenil®. It is therefore, concluded that the two doses of Berenil® (3.5 mg/kg and 7.0 mg/kg) were effective in the treatment of the disease but 7.0 mg/kg cleared the parasitaemia faster.

Keywords: Biochemical, Diminazene aceturate, Experimental, *Trypanosoma evansi*, Yankasa sheep

Introduction

Disease is the major singular entity limiting livestock production in Nigeria (Lamorde, 1996), leading to a shortage in animal protein available for the populace. Parasitic diseases, particularly those associated with anaemia are known to cause several disease conditions including reproductive loss (Allam *et al.,*

2014, Adeyeye *et al*., 2016) and behavioral changes (Risso *et al.,* 2015) in livestock. Trypanosomosis is one of such parasitic disease. Clinically, the effects of trypanosomosis on animals range from intermittent fever, anoxia, nasal discharge, lacrimation, diarrhea or dark hard feces, anaemia, immunosuppression, depression with the inability to rise, pyrexia directly associated with parasitaemia, paleness of mucous membrane, rapid pulse beat (Dargantes *et al.,* 2005; Bezerra *et al.,* 2008). Others include retarded growth, roughness of haircoats, enlargement of peripheral lymph nodes, low milk production, low meat quality, and weight loss as well as infertility, abortion, stillbirth and depressed reproductive performance and reduced capacity to work leading to morbidity and mortality in the absence of treatment (Batista *et al.,* 2012; Silva *et al.,* 2013; Wada *et al.,* 2016, Wada *et al.,* 2020).

Trypanosoma evansi naturally affects camels, horses, and donkeys (Al-Rawashdeh *et al.,* 2000). Consequently, the herding together of camels with other domestic animals may pose a serious threat to these animals. As a result of this, *T. evansi* is assuming greater economic importance among other animals, particularly sheep, where acute and chronic diseases have been reported (Audu *et al.,* 1999).

The field control of animal trypanosomosis has over the years relied on two broad strategies: using chemotherapeutic agents on infected animals and vector control. The chemotherapeutic approach is used much more widely than vector control because it is easier to kill the trypanosomes than the flies (WHO, 1998). Chemotherapy, by stopping the multiplication of the trypanosomes, helps the immune system to overcome the infection (Osman *et al.,* 1992). Chemotherapeutic drugs are toxic to the trypanosomes and often have a similar disruptive effect on the cells of the host (Jennings *et al.,* 1977), and are therefore always used with care and at the recommended dose level only (Homeida *et al*., 1981). The most widely and preferred drug used as curative trypanocide against *Trypanosoma evansi* is diminazene aceturate (Tuntasuvan *et al.,* 2003). Other drugs that can be used include isometamidium chloride (both curative and preventive), cymelarsan (so far, only recommended for curative treatment of camels), suramin, and quinapyramine (curative and/or preventive) (Dia & Desquesnes, 2004). Although, extensive work has been done on other animal trypanosomosis, but with little on infection due to *T. evansi* especially in Yankasa breed of sheep. Therefore, this research was designed to evaluate the performance of Yankasa breed of sheep infected with *T. evansi*, considering the difference in its severity of syndromes with respect to animal breed and geographical region, as well as to compare the effects of two different doses of diminazene aceturate (Berenil®) on the infected animal.

Materials and Methods

Study location

The study was conducted at the Department of Veterinary Medicine, University of Maiduguri, the capital of Borno State, which lies in the northeastern geopolitical zone of Nigeria. The state is situated within the semi-arid zone of West Africa. It lies on latitude 11^{O} 5'N and 13^{O} 5'E. It has a total area of 72,609 square km, with temperature ranging from 35-40^{o}C for most of the year

Experimental animals

A total of thirty (30) adult Yankasa breed of sheep (12-18 months of age and mean body weight of 30.22 ± 2.5 kg) were procured and used for this study. The animals were kept in fly-proof pens and acclimatized for 2 weeks. During this period, they were screened for haemoparasites and helminths by collecting blood and faecal samples for examination, respectively. They were fed twice a day with hay and concentrate supplements while salt lick and water were given *ad libitum*.

Source of parasite

Trypanasoma evansi used in this study was obtained from the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University Zaria, Kaduna State, Nigeria. The organism was initially isolated from the blood of naturally infected camel in Kano State Abattoir and maintained by serial passage in albino rats until used.

Experimental infection of animals

Six (6) adult albino rats were sourced and used as donors. They were screened and certified negative for haemoparasites using thin blood smears. The rats were then inoculated intraperitoneally with 0.5 ml of *T. evansi* parasitaemic blood to multiply the parasites. At 8 days post inoculation, parasitaemia was established and become patents. The donor rats were bled via the tail vein into a petri dish diluted with phosphate-buffered saline glucose (pH 7.4). Each sheep in groups A, C and E were inoculated through the jugular intravenous route with 0.5ml of blood containing 1.0×10^{6} *Trypanosoma evansi* as quantified using serial dilution as reported by Herbert and Lumsden (1976).

Source of experimental drug

Diminazene aceturate used in this study was manufactured by Interchemie Werken "De Adelaar" B.V. Metaalweg and venry, Holland. It was marked under the trade name Berenil® and administered at the dose rate of 3.5 and 7.0mg/kg body weight by deep intramuscular (IM) route.

Experimental design

The 30 adult Yankasa breed of sheep (mean body weight 30.22 ± 2.5 kg) were randomly divided into six groups (A to F) of five animals each and labelled as follows:

Group A (n=5) was intravenously inoculated with 0.5ml of *Trypanosoma evansi* but untreated control.

Group B (n=5) was uninfected and untreated control.

Group C (n=5) was inoculated with 0.5ml of *Trypanosoma evansi* and treated with diminazene aceturate (Berenil®) at a dose rate of 3.5mg/kg body weight (single dose) by day 16 post-infection (PI).

Group D (n=5) was uninfected but treated with diminazene aceturate at a dose rate of 3.5mg/kg body weight (single dose) by day 16 PI.

Group E (n=5) was infected with 0.5ml of *Trypanosoma evansi* but treated with diminazene aceturate at a dose rate of 7.0mg/kg body weight by day 16 PI.

Group F (n=5) was uninfected but treated with diminazene aceturate at a dose rate of 7.0mg/kg body weight (single dose) by day 16 PI.

Monitoring of experimental animals

After infection, all the sheep infected with *T. evansi* were closely monitored for clinical signs of trypanosomosis such as pyrexia, palor of visible mucous membranes, anorexia, depression, lacrimation, nasal discharge, and enlargement of the lymph node.

Estimation of parasitemia

Two millilitres of blood were collected from the jugular vein into sample bottles containing EDTA. This blood was used to estimate parasitemia and determine glucose and total plasma protein. Parasitemia was determined using wet mount and hematocrit centrifugation techniques for the detection of trypanosomes as described by Woo (1969) and Murray et al. (1977). The number of parasites was estimated following the method described by Herbert & Lumbsden (1976).

Treatment of infected animals

The treatment commenced on day 16th at the peak of parasitaemia (> 45 × 10^3/uL), when the PCV was low in all the infected groups.

Blood sample collection

About 3 ml of blood samples were obtained from the experimental animals via jugular vein, and dispensed into non-heparinized sample bottles. Blood samples collected were properly labelled according to each group and allowed to clot before centrifuging at 3000 rpm for 10 minutes to extract serum. Serum samples were stored until used for serum biochemical analysis.

Determination of serum biochemical parameters

Serum biochemical parameters including blood glucose, total protein, alanine amino transferase, aspartate amino transferase and alkaline phosphatase, creatinine and blood urea nitrogen were all determined using the procedure as documented by Esievo (2017).

Statistical analysis

Data generated were analyzed using analysis of varians (ANOVA) using GraphPad Instant (2009). Turkey - Kramer multiple comparison test was used to compared the differnces within and between groups. Results obtained were expressed as mean ± standard deviation (SD) and values of $P<0.5$ were considered significant.

Results

The mean parasite counts of the sheep experimentally infected with *T. evansi* and treated with diminazene aceturate and their controls are presented in Figure 1. All the infected groups (A, C, and E) had a pre-patent period of 8 days PI with a uniform parasitaemia of 4.7 ± 0.27. In group A, the mean parasitaemia count of 4.7 ± 0.27 by day 8 PI, rose significantly ($p < 0.05$) to 100.2 ± by day 16 PI and continued without abatement to a peak value of 250.6 ± 1.98 by day 28 PI. At these points, the mucous membranes (ocular and buccal) of the animals in this group became pale. They became extremely weak, disinclined to move, anorectic and recumbent. They had to be humanly euthanatized at this point (Day 28 PI) to avoid painful death in accordance with international guidelines of using uninfected/uninfected animals for biomedical research. At this point (Day 28 PI), it was deemed that all the infected untreated animals had died of the infection. In group C, the initial parasitaemia of 4.7 ± 0.27, which occurred by 8 PI, reached a significant (p

< 0.05) peak count of 80.8 ± 1.12 by day 12 PI, following treatment with Berenil® at 3.5m g/kg BW by day 16 PI, parasitaemia dropped significantly (p < 0.05) to 20 ± 0.56 by day 20 PI, until it was completely eliminated by day 24 PI or by day 9 post-treatment (PT). In group E, the initial parasitaemia count of 4.7 ± 0.27 which occurred by day 8 PI, reached a peak count of 78.2 ± 1.11 by day 12 PI, following treatment with 7.0 mg/kg BW of Berenil®, by day 16 PI, parasitaemia was completely eliminated by day 20 PI or by day 5 PT. No death or relapse parasitaemia was encountered for 90 days after monitoring parasitaemia in the treated groups (C and E) respectively. In groups B, D, and F, no parasitaemia was detected.

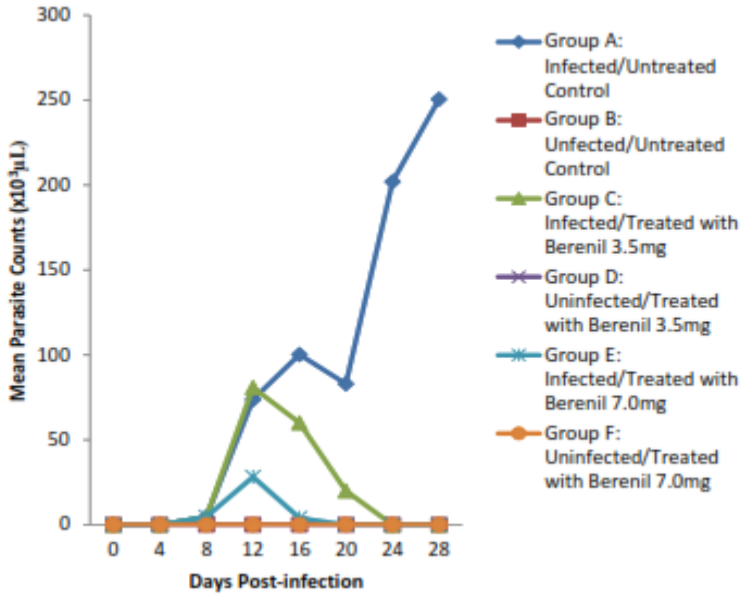

Figure 1: Mean parasite counts (x10^3/µL) of Yankasa breed of sheep Experimentally infected with *T. evansi* and treated with two different doses of Berenil®

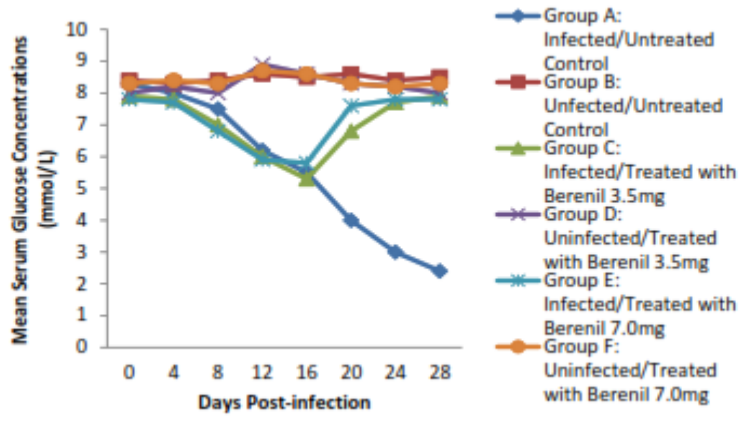

Figure 2: Mean serum glucose concentrations (mmol/L) of Yankasa breed of sheep experimentally infected with *T. evansi* and treated with two different doses of Berenil®

The mean serum glucose level of sheep is presented in Figure 2. In group A, the pre-infection value of 8.3 ± 0.36 experienced a continuous but significant (p < 0.05) decline to 2.2 ± 0.19 by day 28 PI. In groups B, D and F, their pre-infection value of 8.4 ± 0.36, 8.0 ±0.35 and 8.3 ± 0.36 respectively all remained fairly constant (p > 0.05) throughout the study. In group C the pre-infection value of 7.9 ± 0.35 declined significantly (p < 0.05) to 5.3 ± 0.29 by day 16 PI. Following treatment with 3.5mg/kg BW of Berenil® by day 16 PI, the pre-infection value was attained by day 28 PI or by day 13 PT. In group E with a pre-infection value of 7.8 ± 0.35, experienced a significant decline (P < 0.05) to 5.8 ± 0.30 by day 16 PI. Following treatment with 7.0 mg of Berenil® by day 16 PI, its pre-infection value was attained by day 24 PI or by day 9 PT.

The mean serum alanine aminotransferase activity of sheep is presented in Figure 3. In group A the pre-infection value of 25.2 ± 0.63 increased significantly (p < 0.05) without abating to a value as high as 72.4 ± 1.06 by day 28 PI. Meanwhile, in groups B, D and F, their pre-infection values of 25.0 ± 0.63, 25.7 ± 0.63 and 25.3 ± 0.63, respectively all remained fairly constant (p > 0.05) throughout the study. In group C, its pre-infection value of 25.6 ± 0.63 increased significantly (p < 0.05) to 58.8 ± 0.96 by day 16 PI. Following treatment with 3.5mg/kg BW of Berenil®, the value declined significantly (p < 0.05), thereby attaining its pre-infection value by day 28 PI or by day 13 PT. In group E, its pre-infection value of 25.4 ± 0.63 increased significantly (P < 0.05) to 59.9 ± 0.97 by day 16 PI.

Following treatment with 7.0mg/kg BW of Berenil® by day 16 (PI.), the value began to decline significantly (P < 0.05), thereby attaining its pre-infection value by day 24 PI or by day 9 PT.

The mean serum aspartate aminotransferase activity of Yankasa sheep is presented in Figure 4. In group A, its pre-infection value of 47.2 ± 0.86 increased significantly (p < 0.05) and unabatedly to 78.8 ± 1.11 by day 28 PI. For groups B, D and F, and their pre-

infection values of 47.3 ± 0.86, 47.2 ± 0.86 and 47.2 ± 0.86, respectively, all remained fairly constant ($p > 0.05$) throughout the study. Meanwhile, in group C, the pre-infection value of 47.0 ± 0.86, increased significantly ($p < 0.05$) to 49.8 ± 0.88 by day 16 PI. Following treatment with 3.5mg/kg BW of Berenil® by day 16 PI, the values began to decline significantly ($p < 0.05$), thereby attaining its pre-infection value by day 28 PI or by day 13 PT. For group E, its pre-infection value of 47.6 ± 0.86 increased significantly ($p < 0.05$) to 55.0 ± 0.93 by day 12 PI. However, following treatment with 7.0 mg/kg BW of Berenil®, the values began to decline significantly ($p < 0.05$), thereby attaining their pre-infection value by day 24 PI or day 9 PT. Mean Serum Alkaline Phosphatase (iu/L) activity of Yankasa breed of sheep experimentally infected with *T. evansi* and treated with two different doses of berenil® and their controls.

The mean serum alkaline phosphatase activity of the sheep and their controls are presented in Figure 5. In group A, the pre-infection value of 54.3 ± 0.92 rose significantly ($p < 0.05$) and continued to rise without abating to 92.2 ± 1.20 by day 28 PI. However, in groups B, D and F, all their pre-infection values remained fairly constant ($p > 0.05$) throughout the experiment. In group C, the pre-infection value of 54.2 ± 0.92 rose significantly ($p < 0.05$) to 80.0 ± 1.12 by day 16 PI. Following treatment with 3.5mg/kg BW of Berenil® by day 16 PI, the value began to decline significantly ($p < 0.05$), thereby attaining its pre-infection value by day 24 PI or by day 9 PT. In group E, the pre-infection value of 54.2 ± 0.92 rose significantly ($p < 0.05$) to 79.0 ± 1.11 by day 16 PI. sheep experimentally infected with *T. evansi* and treated with two different doses of berenil® and their controls. The mean serum total protein of the sheep is presented in Figure 6. In group A, the pre-infection value of 46.0 ± 0.85 decreased significantly ($p < 0.05$) and continued so, to 16.3 ± 0.50 by day 28 PI. However, in groups B, D and F with their pre-infection values of 42.2 ± 0.85, 42.0 ± 0.81 and 45.6 ± 0.84 all remained fairly constant ($p > 0.05$) throughout the

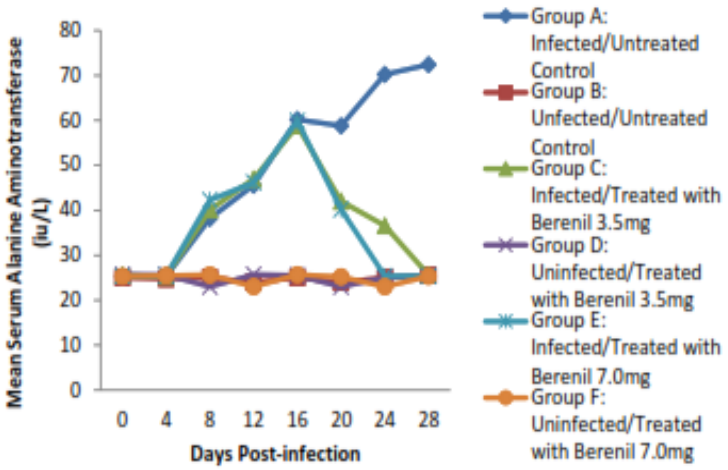

Figure 3: Mean serum alanine aminotransferase (iu/L) of Yankasa breed of sheep experimentally infected with *T. evansi* and treated with two different doses of Berenil®

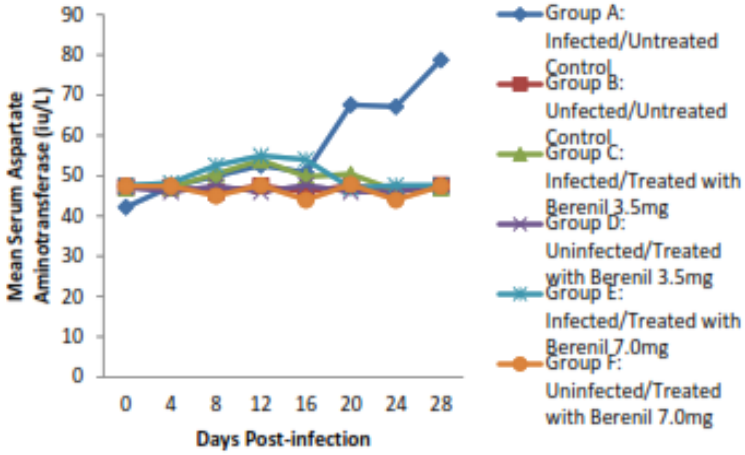

Figure 4: Mean serum aspartate aminotransferase (iu/L) of Yankasa breed of sheep experimentally infected with *T. evansi* and treated with two different doses of Berenil®

Following treatment with 7.0mg/kg BW of Berenil® by day 16 PI, the value declined significantly ($p < 0.05$) to its pre-infection value by day 24 PI or by day 9 PT.

Mean serum total protein (g/dl) of Yankasa breed of study. In group C, the pre-infection value of 46.0 ± 0.85, decreased significantly ($p < 0.05$) to 22.0 ± 0.59 by day 16 PI. Following treatment with 3.5mg/kg BW of Berenil®, by day 16 PI, the declined value began to appreciate significantly ($p < 0.05$) to its pre-infection value by day 28 PI or by day13 PT. In group E, its pre-infection value of 42.0 ± 0.81 decreased significantly ($p < 0.05$) to 22.8 ± 0.60 by day 16 PI. Following treatment with 7.0mg/kg BW of Berenil®, its pre-

infection value was attained by day 24 PI or by day 24 PT.

The mean serum creatinine level of the sheep is presented in Figure 7. In group A, its pre-infection value of 48.2 ± 0.87 increased significantly ($p < 0.05$) to 104 ± 1.27 by day 28 PI. In groups B, D, F, their pre-infection values of 48.3 ± 0.87, 48.3 ± 0.87 and 48.6 ± 0.87, respectively, all remained fairly constant, ($p > 0.05$) respectively throughout the experiment. In group C, its pre-infection value of 48.0 ± 0.87 increased significantly ($p < 0.05$) to 74.0 ± 1.08 by day 16 PI. Following treatment with 3.5mg/kg BW of Berenil®, the value began to amend by day 20 PI significantly ($p < 0.05$), thereby attaining its pre-infection value by day 28 PI or by day 13 PT. In group E, its pre-infection value of 48.3 ± 0.87 increased significantly ($p < 0.05$) to 73.2 ± 1.07 by day 16 PI.

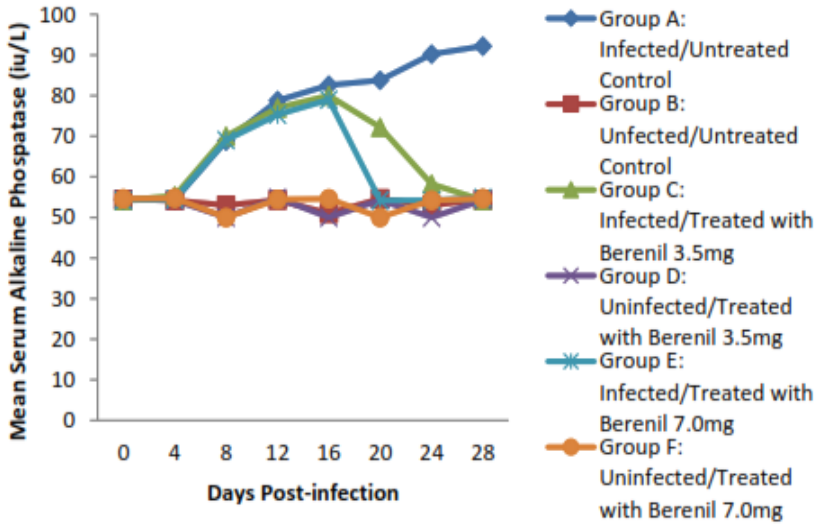

Figure 5: Mean serum alkaline phosphatase (iu/L) of Yankasa sheep experimentally infected with *T. evansi* and treated with two different doses of Berenil®

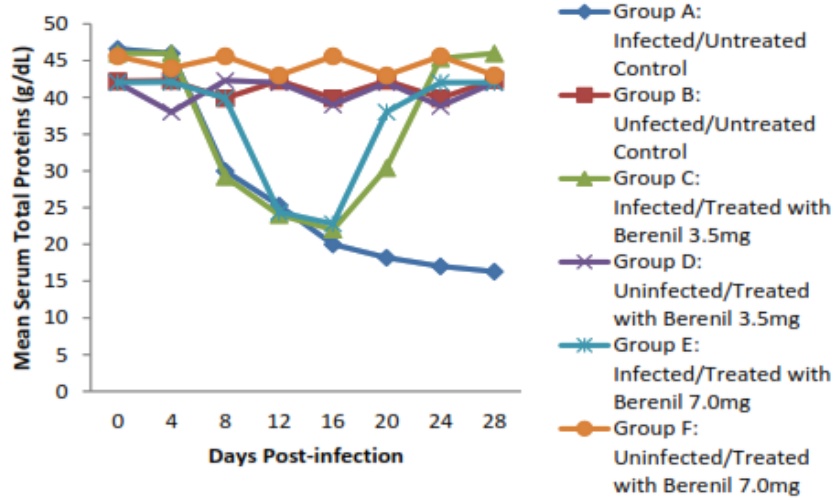

Figure 6: Mean serum total proteins (g/dL) of Yankasa breed of sheep experimentally infected with *T. evansi* and treated with two different doses of Berenil®

Following treatment with 7.0mg/kg BW of Berenil® by day 16 PI, the values began to amend significantly ($p < 0.05$), thereby attaining their pre-infection value by day 24 PI or by day 9 PT.

The mean serum urea concentration of sheep is presented in Figure 8. In group A, the pre-infection value of 7.0 ± 0.33 continued to rise ($p < 0.05$) unabatedly to 53.2 ± 0.91 by day 28 PI. In groups B, D and F, their pre-infection value of 7.2 ± 0.33, 7.5± 0.34 and 7.5± 0.33, respectively, all remained fairly constant ($p > 0.05$) throughout the study. In group C, the pre-infection value of 7.4 ± 0.34 rose significantly ($p < 0.05$) to 33.3 ± 0.72 by day 16 PI. Following treatment with 3.5mg/kg BW of Berenil® by day 16 PI, the value amended to its pre-infection value by day 28 PI or by day 13 PT. In group E, its pre-infection value of 7.0 ± 0.34 rose significantly ($p < 0.05$) to 35.0 ± 0.74 by day 16 PI. Following treatment with 7.0mg/kg BW of Berenil®, by day 16 PI, the pre-infection value was attained by day 24 PI or by day 9 PT.

Discussion

The observed clinical features in this study were dullness, emaciation, loss of weight, staggering gait, recumbency and anaemia which were also recorded by Losos (1980) in cattle and by Stephen (1986) in horses.

In this study, fluctuation of parasitaemia was observed generally, among the *T. evansi* infected groups of sheep. Fluctuations of parasitaemia are known features of trypanosomosis commonly caused by antigenic variation (Nwosu and Ikeme, 1992; Mbaya *et al.,* 2009). The ability of the host to limit the peak and number of each wave of parasitaemia is however, dependent on whether the infection is acute, sub-acute or chronic (Katunguka-Rwakishaya *et al.,* 1992) and this may explain the reason why following treatment by day 16 PI. The fall in total protein concentrations in infected sheep in this study is in agreement with the reports of Losos & Ikede (1972) and Audu *et al.* (1999) for *T. evansi* infection in different domestic animals. The low protein levels may be adduced to loss of blood associated with anaemia, it could also be a result of increased protein breakdown or urea loss and haemodilution (Bisalla *et al.,* 2007).

The results of this study showed that from day 4 PI, the sheep in groups (A, C and E) experienced increased concentrations of alkaline phosphatase, aspartate aminotransferase and creatinine. While on the other hand, they experienced a decrease in concomitant serum glucose levels, similarly reported by Alireza *et al*. (2011) in dromedary camels (*C. dromedaris*) sub-clinically infected with *T.evansi*. In this study, biochemical changes were however modulated to their pre-infection values before day 28 PI in the infected sheep treated with Berenil® 3.5mg/kg BW being less effective or more effective in sheep treated with 7.0mg/kg BW of Berenil® (group E). The elevation of alkaline phosphatase and aspartate amino transferase were suggestive of liver damage with the resultant release of the enzyme into circulation. These enzymes increased as the infection progressed without abating. Similarly, these have been reported in *T. brucei* infection of gazelles (Mbaya *et al.,* 2008), *T. brucei gambiense* infection of baboons (*Papio anubis*) (Mbaya *et al.,* 2009), *T. evansi* infection of dogs (Aquino *et al.,* 2002) and donkeys (Cadioli *et al.,* 2006).

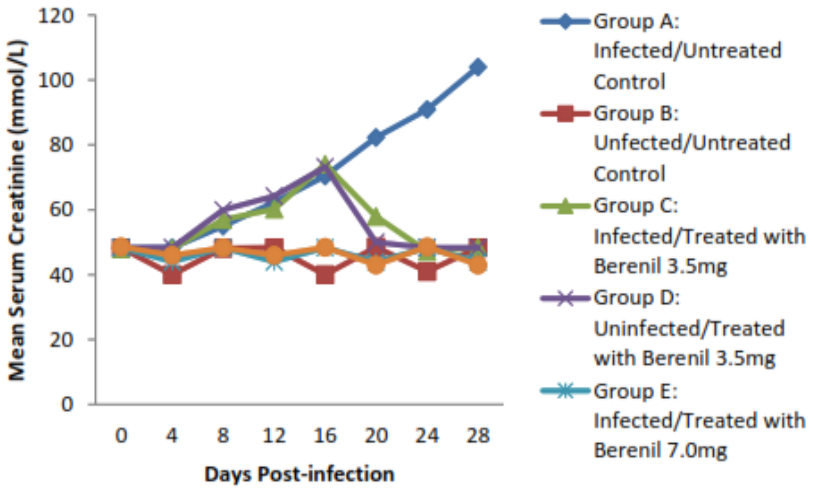

Figure 7: Mean serum creatinine (mmol/L) of Yankasa breed of sheep experimentally infected with *T. evansi* and treated with two different doses of Berenil®

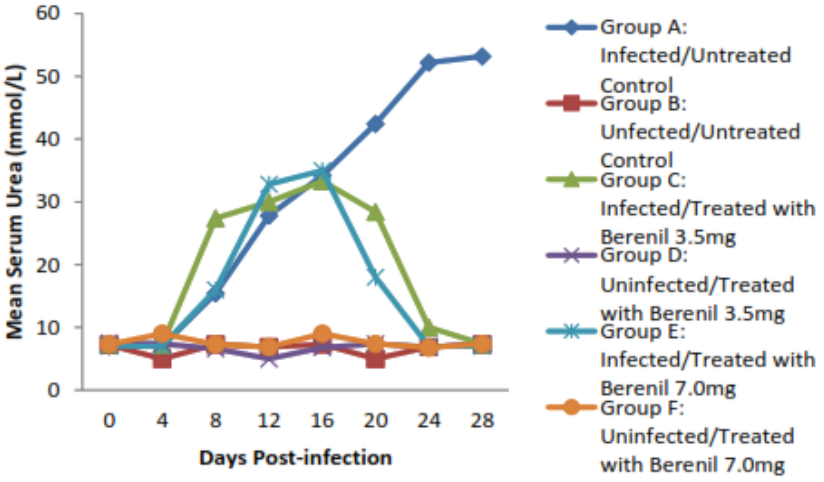

Figure 8: Mean serum urea (mmol/L) concentration of Yankasa breed of sheep experimentally infected with *T. evansi* and treated with two different doses of Berenil®

The elevation of creatinine levels in sheep infected with *T. evansi* may be due to renal dysfunction earlier reported by Mbaya *et al*. (2008). The retention of creatinine in the body showed that the kidneys were severely affected, thereby, failing to excrete these catabolic products. Similarly, high levels of creatinine particularly in the infected but treated sheep might be attributed to muscle wasting experienced by the experimentally infected sheep during the course of the infection.

Hypoglycaemia observed in the infected control continued without abating as the infection

progressed. Sequel to this, the sheep experienced profound weakness. This might probably be associated with the high energy demand in the sheep during high parasitaemia, impaired glucose release from the gluconeogenic pathways, as well as host consumption of large quantities of glucose according to Igbokwe (1994). *Trypanosoma evansi* metabolizes glucose to produce 4-hydroxyl-4-methyl α-ketogluterate which is inhibitory to the tricarboxylic acid cycle (TCA) in the mitochondria, leading to severe energy deficit in the host (Mbaya *et al.,* 2008). This therefore suggests that the TCA cycle and oxidative phosphorylation might have been inhibited, leading to its total failure to generate energy from energy-rich compounds (Igbokwe, 1994; Mbaya *et al.,* 2008). Ninety percent of the energy available in glucose is released when pyruvate is oxidized to CO_2 and H_2O through the TCA cycle and electron transport chain (Conn and Stumpf, 1976). This might have been the reason for the profound weakness experienced among the sheep in group A. These effects were reversed after treatment with the two different doses of Berenil® (3.5 and 7.0 mg/kg BW). From the result of this study, it is concluded that both the two doses of Berenil® (3.5mg/kg and 7.0mg/kg) were effective in the treatment of the disease under experimental conditions but 7.0mg/kg clear the disease faster, thus reversing the negative effect of the parasite on all the studied serum biochemical parameters.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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