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Serum Proteins, Thyroid Hormones and Alkaline Phosphatase Concentrations in Acute Experimental *Trypanosoma congolense* Infection in *Yankasa* Sheep Immunomodulated with Levamisole

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Abstract: Serum proteins, thyroid hormones and alkaline phosphatase concentrations were measured in *Yankasa* sheep experimentally infected with *T. congolense*. Parasitemia occurred in the *T. congolense* infected sheep immunomodulated with levamisole two days earlier than the infected group without immunodulation.Packed cell volume decreased significantly($p<0.05$) in the infected groups with and without immunomodulation when compared to the controls from the first week of infection up to the end of the experiment. Serum total protein, albumin, Thyroxine (T4) and Triiodothyronine (T3) decreased significantly ($p<0.05$) in the infected sheep with and without immunodulation when compared to the controls. Serum Thyrotopin (TSH) and Alkaline Phosphate (AP) did not alter significantly ($p>0.05$) in the infected sheep with and without immunomodulation when compared to the controls throughout the period of the experiment. In general, levamisole administration did not appear to alter the infection when compared to the infected group without immunomodulation.

Key words: *Trypanosoma congolense*, *Yankasa* sheep, immunomodulation, serum proteins, thyroid hormones, alkaline phosphatase

INTRODUCTION

Trypanosomosis (Trypanosomiasis) is an important disease of man and animals. The disease has long been recognized as a major constraint to livestock and/or beef production in tropical Africa. The disease is transmitted by tse-tse flies (*Glossina*) and is endemic in many parts of tropical Africa, including Nigeria. Economic losses resulting from death of affected animals, stunting and debilitation occur. It is known that patho-physiological alterations occur in the cellular and plasma components of blood during infection (Makinde *et al*., 1991). African trypanosome infections are generally characterized by haematological and serum biochemical aberrations, the severity of which are often determined by the strain of infecting trypanosomes and the host (Anosa, 1988a; b). Immunosupression is a frequent accompaniment of African trypanosome infections. Levamisole is also known to be an antihelminthic and a non-specific immunopotentiator (Abath *et al*., 1988).

Information on protein, thyroid hormones and alkaline phosphatase changes is lacking in trypanosome infected animals immunomodulated with levamisole. Therefore such studies are carried out in experimentally infected *Yankasa* sheep.

MATERIALS AND METHODS

Experimental animals: Seventeen *Yankasa* sheep of mixed sexes and aged between nine months to one year were used for the study. The sheep were acclimatized for three weeks in a fly proof pen. They were dewormed with Ivermection (Kepromec®, Holland) and treated with Oxytetracyline Long Acting (Tetroxy LA®, Bimeda, Holland). They were fed grass hay, groundnut hay, corn bran and wheat bran. Feed and water were provided *ad libitum*. During acclimatization, the animals were subjected to routine handling such as collection of blood samples.

Parasite: *Trypanosoma congolence* (NITR/Zonkwa) isolated from a pure natural infection of a cattle herd in Zonkwa, Kaduna State and obtained from the National Institute for Trypanosomiasis Research was used for the

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study. The parasites were cryopreserved in Liquid Nitrogen from where they were subpassaged into donor albino rats before use.

Experimental design: The sheep were divided into three groups based on their Packed Cell Volumes (PCV). Group 1 consists of 5 animals and served as the uninfected control groups. Group 2 consisting of six animals were infected with 2×10^6 Trypanosomes via jugular venepuncture using a 5 mL syringe and 22 gauge needle. Group 3 consists of 6 animals and infected as in Group 2 but were also administered with levamisole (Farvet, Holland) at 2.5 mg kg^{-1} subcutaneously every week throughout the length of the experiment.

Infection: A donor sheep was infected via jugular venepuncture with the blood of the infected rats at high parasitaemia was allowed to rise in the infected donor sheep. Trypanosomes from the blood of the infected sheep were then counted in a haemocytomer before infection of Group 2 and 3.

Sample collection and analyses: Rectal temperature was measured every morning using a rectal thermometer. Blood for haematology was collected via jugular venepuncture. Blood was collected into Ethylene Diamine Tetraacetic Acid (EDTA) bottles. Blood for serum was obtained into sterile test tubes and centrifuged at 1500 g for 5 min. Serum was then harvested into labelled sterile tubes and stored at-20°C until used. Packed cell volume was determined using the standard microhaematocrit method. Parasitemia was determined by the buffy coat/phase contrast technique, which provides an estimate of the number of parasites per ml of blood. The scoring system is based on a log scale such that 1 = 0^2-10^3 trypanosomes, 2= 10^3-10^4, 3 = 5×10^3-5×10^4, 4 = 10^4-5×10^5, 5 = $>5\times10^5$ and 6 = $>5\times10^6$. Thin blood smears were stained by Giemsa (Jain, 1986; Paris *et al*., 1982). Serum Thyroxine (T4), Triiodothyronine (T3) and Thyrotropin (TSH) were measured weekly by Enzyme Linked Immunosorbent Assay (Dialab®, Austria). Total proteins were measured by the Biuret method (Reinhold, 1953) and serum albumin was measured by the Bromocresol Green Method. Data were analysed using Analysis of Variance (ANOVA) to determine the level of significance.

RESULTS

General course of infection: Parasitamia in *T. congolense* infected animals immunomodulated with levamisole appeared three days post infection whereas in

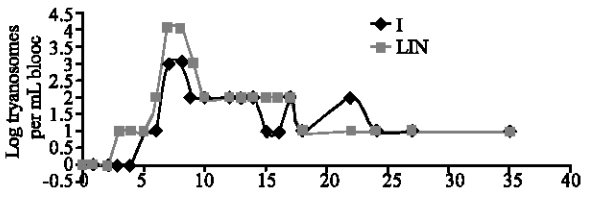

Fig. 1: Mean parasitemia of sheep infected with (I) and T congolense infected and immonumodulated with Levamisole (LIN)

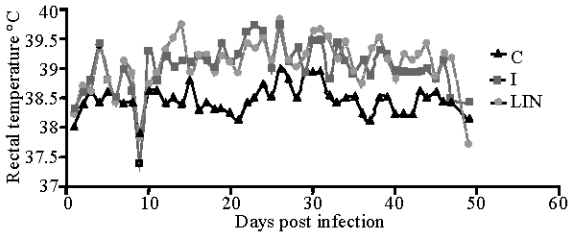

Fig. 2: Mean rectal temperature of sheep infected with *T. congolense* (I), infected with T congolense and immunomodulated with Levamisole (LIN) and Control sheep (C)

Group 2 animals became parasitemic at 5 days post infection (Fig. 1). Average rectal temperatures 4 days post infection (pi) were 38.4±0.6, 39.4±0.5 and 39.4±0.4 in Groups 1, 2 and 3, respectively. The temperature stabilized on day 48 p.i to 38.1±06, 38.4±0.6 and 38.6±0.5 in Groups 1, 2 and 3, respectively. The period of high parasitemia tended to coincide with fever.

Packed cell volume: The packed cell volume decreased from preinfection values of 30.2±3.4 and 30.0±2.2 to 25.0±3.5 and 26.4±3.9 in the *T. congolense* infected ad *T. congolense* infected and immunomodulated with levamisole, respectively. The PCV decreased significantly ($p<0.05$) in the infected groups with and without immunomodulation with levamisole. The PCV values continued to decrease in the infected groups and were 21.6±1.8 and 22.8±2.3 in the *T. congolense* infected and *T. congolense* infected and immunomodulated with levamisole, respectively by the 5th week of infection. Values of PCV in the control group were relatively constant throughout the experimental period (Fig. 2 and Table 1).

Serum proteins: Serum Total Protein (TP) concentration increased during the course of infection in Groups 2 and 3 ($p<0.05$). Mean concentrations at 6 weeks post infection were 70.0±1.6, 81.5±2.7 and 87.2±2.0 g L^{-1} in Groups 1, 2 and 3, respectively (Fig. 3). Serum albumin

Table 1: Mean (S.D) TSH concentration (mIU/L) in *T. congolense* infected (In), *T congolense* infected and immunomodulated with Levamisole (Lin) and control sheep ©

	Time (Weeks)							
Group	-7	0	1	2	3	4	5	6
C	2.58 ±0.16	2.52±0.13	2.74±0.24	2.78±0.19	2.68±0.13	2.78±0.15	2.82±0.08	2.82±0.08
In	2.52±0.15	2.53±0.15	2.68±0.15	2.65±0.16	2.58±0.19	2.68±0.15	2.90±0.26	2.68±0.15
Lin	2.56±0.16	2.58±0.12	2.65±0.23	2.61±0.21	2.65±0.19	2.66±0.15	2.96±0.19	2.63±0.15

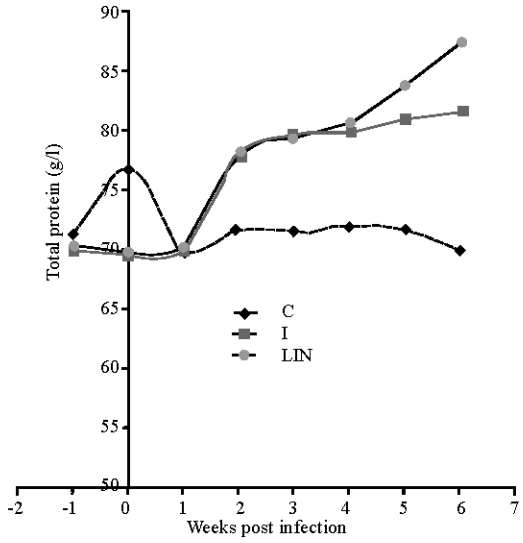

Fig. 3: Mean total protein (g L^{-1}) in *T. congolense* Infected (I), *T. congolense* infected and immunodulated with Levamisole (LIN) and Control sheep (C)

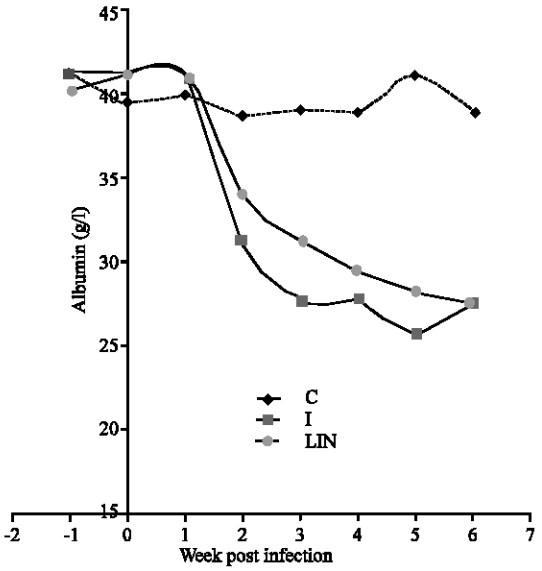

Fig. 4: Mean albumin (g L^{-1}) in *T. congolense* Infected (I), *T. congolense* infected and immunodulated with levamisole (LIN) and Control sheep (C)

concentration decreased in the course of the infection in Groups 2 and 3 (Fig. 4). Average albumin concentration

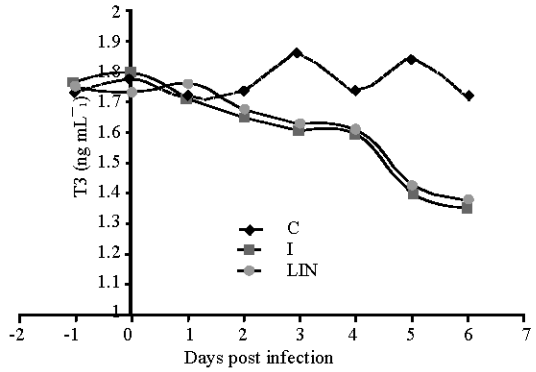

Fig. 5: Mean T3 (ng mL^{-1}) in *T. congolense* Infected Sheep (I), *T. congolense* infected and immunodulated with Levamisole (LIN) and Control sheep (C)

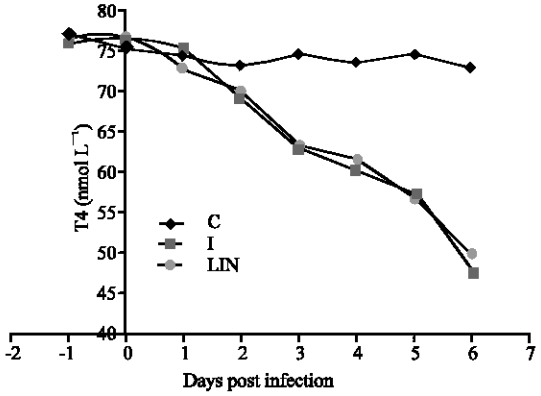

Fig. 6: Mean T4 (nmol L^{-1}) in *T. congolense* Infected Sheep (I), *T. congolense* infected and immunodulated with Levamisole (LIN) and Control sheep (C)

was lower in the infected animals than in control animals, from week 3 p.i onwards ($p<0.05$). At 6 weeks p.i, albumin concentration was 39.0±1.2, 27.6±1.6 and 27.5±1.9 in Groups 1, 2 and 3, respectively.

Alkaline phosphatase: There was no statistically significant difference ($p>0.05$) in alkaline phosphate concentration from pre-infection to the end of the experimental period in Groups 1, 2 and 3 (Table 2).

Table 2: Mean (S.D) Alkaline Phosphate concentration (mIU/L) in *T. congolense* infected (In), *T congolense* infected and immunomodulated with Levamisole (Lin) and control sheep ©

	Time (Weeks)							
Group	-7	0	1	2	3	4	5	6
C	60.8 ±10.7	58.8±13.7	66.4±11.5	51.2±5.3	56.0±12.8	59.2±5.5	63.6±3.8	53.6±3.6
In	60.0±8.3	75.6±5.3	52.0±5.1	53.8±11.8	57.1±8.6	58.6±11.0	59.8±6.2	67.0±6.5
Lin	65.7±15.0	56.3±4.8	60.5±8.9	71.5±9.5	57.5± 7.1	58.6±7.8	59.5±6.7	61.5±5.8

Serum T3: Serum T3 concentration decreased significantly ($p<0.05$) from the pre-infection value in Groups 2 and 3 from 5 weeks p.i onwards (Fig. 5).

Serum T4: Serum T4 concentrations was 76.8±1.9, 76.0±4.0 and 76.0±4.1 nmol/l pre-infection (Fig. 6). The concentrations at 6 weeks p.i were 72.6±7.4, 47.8±6.5 and 50.0±9.5 in Groups 1, 2 and 3, respectively. There was a statistically significant difference ($p<0.05$) between the control and infected groups.

Serum TSH: Serum TSH concentration was not statistically different ($p>0.05$) between the control and infected groups during the course of the infection (Fig. 7).

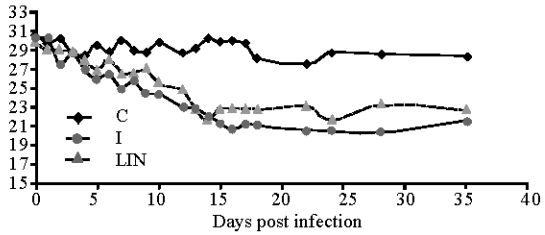

Fig. 7: Mean packed cell volume (%) in *T. Congolense* Infected sheep (I), *T. Congolense* infected and immunodulated with Levamisole (LIN) and Control sheep (C)

DISCUSSION

Parasitemia in the *T. congolense* infected animals immonomodulated with levamisole occurred 2 days earlier than in the infected group without immunomodulation. This is in consonance with the report that treatment of some breeds of mice infected with *T. congolense* with levamisole increased parasitemia and moratality (Libeau and Pirder, 1981). In contrast to our observations, levamisole was reported to reduce the peak of parasitemia in *T. cruzi* infected mice and had no apparent effect on the mortality rate (Abath *et al*., 1988). However, in our study levamisole did not appear to have any effect on the mortality rate as all the infected animals survived the experimental period. Anaemia is the principal pathological feature of animal trypanosomiases (Saror, 1979). The drop observed in PCV of the infected animals is similar to the observations in *T. vivax* infected goats (Saror, 1979) and *T. congolense* infected goats (Akinbamijo *et al*., 1992).

Serum total protein concentration increase in the infected animals agrees with the report of Kavnguka *et al*. (1993) in goats infected with *T. vivax*. However, (Anosa and Isoun. (1976) reported a lowered total protein concentration in *T. congolense* infected Scottish Blackface sheep. Increased total protein concentration could be attributable to gammaglobulinaemia, which is a prominent feature of trypanosomosis, primarily due to increase in IgM levels (Dam *et al*., 1988). The decreased albumin concentration in infected animals observed in our study is in tandem with the observations in *T. vivax* infected West African Dwarf goats (Katunguka *et al*., 1995) and *T. congolense* infected Scottish Black sheep (Mohammad, 1992).

Serum alkaline phosphatase was not significantly different in both the infected and control animals throughout the course of the infection. A remarkable drop in alkaline phosphatase was however reported in *T. godfreyi* infected piglets (Abenga and Anosa, 2004) *T. b.gambiense* infected monkeys (Arowolo *et al*., 1988) and *T. brucei* infected rabbits (Kovach *et al*., 1992). Levamisole is known to inhibit the activity of alkaline phosphatases in man (Mutayoba *et al*., 1998).

The decreases in serum T3, T4 and TSH concentrations observed during the course of infection in the infected animals with and without immunomodulation is in agreement with decreases in serum T3 and T4 in *T. vivax* infected West African Dwarf goats (Katumguka *et al*., 1995). TSH is known to induce enlargement and proliferation of thyroid epithelium with increased secretion of thyroid hormone into the blood. The pituitary gland of *T. congolense* infected goats was reported to show partly degranulated basophils with fewer secretory acini in the medulla and slight hypertrophy of the acidophils in the cortical zones of the adenohypophysis. These pituitary lesions may probably explain why there is no negative feedback increase in TSH following the lowered T4 concentration observed in our study.

CONCLUSION

In conclusion, immonomodulation of *T. congolense* infection in sheep at the regime we used does not appear

to have altered the course of the infection. However, the early parasitemia detected in infected animals immonomodulated with levamisole needs to be further investigated. Although the net effect of non-specific immunopotentiators seems to depend on several factors which include host immune state, severity of infection, dose and timing of drug administration (Abath *et al*., 1988).

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