Levamisole immunomodulation of *Trypanosoma congoense* infection in sheep led to early appearance of parasitemia

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ABSTRACT
Parasitemia and rectal temperatures were determined in Yankasa sheep experimentally infected with *Trypanosoma congoense* and immunomodulated with levamisole. Group A (6 sheep) and group B (6 sheep) were infected with $2 \times 10^6$ *T. congoense* parasites. In addition Group B sheep were each administered with 2.5 mg/kg levamisole hydrochloride subcutaneously on the day of infection and later weekly throughout the experimental period. Group C (5 sheep) served as the uninfected controls. Parasitemia in the *T. congoense* infected immunomodulated group appeared 2 days earlier than the group with *T. congoense* infection only and the parasitemic level was also higher in the infected immunomodulated group at the first parasitic peak. Rectal temperatures were significantly ($p < 0.001$) higher in the infected groups when compared the control but were not significantly different ($p > 0.05$) between the infected groups. It appears that levamisole immunomodulation of *T. congoense* sheep enhanced the early appearance of parasitemia but was not associated with mortality as all the infected animals survived the experimental period which lasted 6 weeks.

Key words: *Trypanosoma congoense*, sheep, levamisole

INTRODUCTION

African trypanosomosis (trypanosomiasis) caused by the protozoan parasites, trypanosomes is one of the most important vector borne diseases of human and livestock in tropical Africa. Trypanosomosis is an important constraint, if not the most important constraint to livestock and mixed crop-livestock farming in tropical Africa (Kristjanson et al., 1999). Protozoan parasites such as trypanosomes can increase the susceptibility of a host to secondary bacterial infection by suppression of the immune system (Tizard, 2000). Besides causing disease, the trypanosomes are also held responsible for producing a state of severe immunosuppression, which renders the infected host more susceptible to secondary bacterial infections and produce poor immune responses to bacterial and viral vaccines (Holmes, 1980).

Trypanosome induced immunosuppression was first reported by Goodwin (1970); Goodwin et al. (1972) in studies on the response of *Trypanosoma brucei* infected mice and rabbits to heterologous red cells. In has been shown that administration of endotoxin (Singer et al., 1964), polyribonucleotides (Herman and Baron, 1971), BCG and *Cryptosporidium parvum* (Murray and Morrison, 1979) prior to or at the same time with *T. congoense*, *T. brucei rhodesiense* and *T. brucei* challenge significantly increased the survival time of infected mice and the increased survival time was associated with prolonged prepatent period, a delay in the time taken to reach the first parasitic peak and a reduction in the level of parasitemia. In recent years, a number of substances that appeared to have the potentials as immunomodulatory drugs have been identified. One of these, tetrahydro-6-phenylimidazo-thiazole (levamisole), a synthetic broad spectrum antihelminthic first introduced by Thieopont et al. (1966) has been shown to have immunomodulating effect (Symoens and
Rosenthal, 1977). There are few reports available on the use of levamisole as an immunomodulator in animal trypanosomosis. Libeau and Pinder (1981), treated *T. congolense* infected mice with levamisole throughout the experimental period and concluded that the drug enhanced parasitemia and increased mortality in 3 of the 4 strains of mice infected. However, Abath et al. (1988) treated *T. cruzi* infected mice with levamisole and reported that the drug reduced the peak of parasitemia. There appears to be no report on the effects of levamisole administration in trypanosomosis of sheep. This necessitated the study with the aim of determining the onset and level of parasitemia and rectal temperatures in *T. congolense* Yankasa sheep.

**MATERIALS AND METHODS**

**Animals**

Seventeen Yankasa sheep of mixed sexes and aged between 9 months and 1 year were purchased from Kafur market in Katsina State, an apparently tsetse-free area and used for the study. The sheep were housed and acclimatized for 3 weeks. They were fed corn bran, groundnut hay, *Digitaria* hay and water was given *ad libitum*. During, acclimatization, they were screened for blood and intestinal parasites. They were treated against parasites and secondary bacterial infections with oxytetracycline long acting (Tet oxy LA®, Bimed, Holland) at 20 mg/kg body weight by deep intramuscular injection and Ivermectin (Keprovec®, Holland) at 200 µg/kg body weight by subcutaneous injection. Blood samples were collected to obtain baseline values. The animals were identified by neck tags and grouped at random into 3 based on their packed cell volumes (PCV) and treatments. Group A (*T. congolense* infected) of 6 animals, Group B (*T. congolense* infected, immunomodulated sheep group) of 6 animals and Group C (uninfected control group) of 5 animals. Group B animals were each administered with 2.5 mg/kg body weight levamisole (Farvet®, Holland) subcutaneously on the day of infection and later weekly throughout the experimental period.

**Parasite**

The *T. congolense* (Zonkwa isolate) used in this study was isolated from a pure natural infection in a cattle herd in Zonkwa, Kaduna State, by N.I.T.R. (National Institute for Trypanosomiasis Research). The parasite was obtained from the latter as cryopreserved material in liquid nitrogen from where they were subpassaged into donor albino rats.

**Infection**

The donor sheep was inoculated with the blood of rats previously infected with *T. congolense* via jugular venipuncture. Parasitemia was monitored daily. Parasitemia peaked at the seventh day post infection. A 2 ml blood containing approximately 2 x 10⁶ parasites was obtained from the donor sheep via jugular venipuncture using a 5 ml syringe and a 22G needle was used to infect each animal in Groups A and B using heparin as the anticoagulant. The level of parasitemia in the infected animals was monitored daily by wet mount and hematocrit centrifugation technique (HCT).

**Clinical examination**

All experimental animals were monitored daily for clinical signs. Rectal temperatures (°C) were determined daily in the morning between 0800 and 0900 hours by the use of a rectal thermometer.

**Sample collection**

Five ml of blood was collected from all animals daily via jugular venipuncture using a 5 ml syringe and 22G needle. Two ml was obtained into clean, labeled, disodium ethylene diamine tetraacetic acid (Na-EDTA) tubes for detection of parasitemia.

**Parasitemia**

Parasitemia was estimated at each sampling using the hematocrit centrifuge test as described by
Woo (1969) and presented below as +, ++, ++++, +++++.

+ ===== Up to 10 trypanosomes in buffy coat and plasma layer seen per field.
++ ===== 15 to 20 trypanosomes in buffy coat and plasma layer seen per field.
+++ ===== Numerous trypanosomes in buffy coat and plasma layer per field.
++++ = Massive trypanosomes in buffy coat and plasma layer per field.

Sample analyses

Results were analyzed using GraphPad prism version 4.0 for Windows from GraphPad software, San Diego, California, U.S.A. (www.graphpad.com) to test the level of significance between the means obtained from test groups compared to the control value of p < 0.05. Tukey post hoc test was used to compare differences between groups if overall mean p < 0.05.

RESULTS AND DISCUSSION

The infected animals showed varying clinical signs which include pale mucous membranes, weakness and rough hair coats. In general, the infected animals in the T. congolense infected, immunomodulated group looked clinically better than the T. congolense infected group. However, all the infected animals in both groups survived the experimental period.

The T. congolense infected and levamisole immunomodulated group became parasitemic on day 3 post infection but appeared 2 days later in the group with T. congolense infection only. Parasitemia rose and peaked on day 7 (++++) and 8 (+++ in both infected groups. The level of parasitemia remained high in both groups but gradually fell to ++ on day 10 post infection up to the end of the experimental period. The level of parasitemia in the infected, immunomodulated group was also higher between days 6 and 8 post infection. This agrees with the report of Libeau and Pinder (1981) who observed that levamisole treatment of 4 inbred strains of mice infected with T. congolense resulted in enhanced parasitemia in 3 strains but contrasts with the work of Abath et al. (1988) who reported that levamisole treatment in T. cruzi infected mice reduced the peak of parasitemia.

The increase in the peak of parasitemia observed in our study was not associated with increased mortality as all the infected sheep survived the experimental period. This agrees with the report that no apparent effect on mortality was observed in levamisole treated T. cruzi infected mice (Abath et al., 1988). In contrast however, an increased mortality in all the 4 inbred strains of mice infected with T. congolense was observed (Libeau and Pinder, 1981).

The pre-infection mean rectal temperatures were 38.3 ± 0.6, 38.2 ± 0.4 and 38.0 ± 0.5 °C in groups A, B and C respectively. The mean rectal temperature values rose to 39.4 ± 0.5 and 39.4 ± 0.5 °C on day 4 in groups A and B respectively but later dropped on days 5 and 6 post infection. The values rose again to 39.0 ± 0.8 and 39.1 ± 0.9 °C on day 7 post infection in groups A and B respectively. The mean values on day 13 post infection were 39.0 ± 0.5, 39.5 ± 0.7 and 38.5 ± 0.4 °C in groups A, B and C respectively. There was a statistically significant difference (p < 0.001) in the mean rectal temperatures of groups A and B when compared to group C (control). However, there was no statistically significant difference (p > 0.05) between the mean rectal temperatures of infected group A and B throughout the experimental period. The increased rectal temperature observed in the infected groups was intermittent and different from those of the control. The pyrexia observed in the infected groups is similar to the observations of Valli et al. (1978) in T. congolense infected calves with a peak within 5 to 6 days post infection and corresponded with parasitemia.
The early appearance and higher level of parasitemia observed in the *T. congolense* infected immunomodulated group did not appear to affect the rectal temperature profile when compared to the infected group without immunomodulation. The pyrexia recorded in our study in the immunomodulated group was intermittent and similar to the infected group without immunomodulation. In conclusion, it appears that levamisole immunomodulation of *T. congolense* infected sheep led to an early and higher level of parasitemia although did not appear to affect the rectal temperature profile between the infected groups with and without immunomodulation. The early appearance and higher level of parasitemia was also not associated with mortality.

![Graph showing level of parasitemia in T. congolense infected sheep](image.png)

**Fig. 1.** Level of parasitemia in *T. congolense* infected sheep
Fig. 2. Rectal temperature (°C) of *T. congoense* infected sheep

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