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Effect of immunomodulation with levamisole on the course and pathogenesis of acute experimental Trypanosoma congolense infection in sheep

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The effect of immunomodulation with levamisole on the pathogenesis and course of acute experimental Trypanosoma congolense infection in sheep was studied. Eighteen Yankasa sheep were divided into three groups: Group A- six (T. congolense infected), Group B- seven (T. congolense infected, immunomodulated) and Group C- five (uninfected controls). Groups A and B sheep were each infected with approximately 2×10⁶ trypanosomes. In addition, group B sheep were each administered 2.5 mg/kg levamisole hydrochloride subcutaneously on the day of infection and later weekly throughout the experimental period. Clinical signs, appearance and level of parasitemia, PCV, rectal temperatures, total and differential leukocyte counts and body weights were monitored. Clinical signs observed were pale mucous membranes (anaemia), weakness and rough hair coats which were milder in group B sheep. Group B sheep became parasitemic two days earlier than group A and maintained a higher parasitemia at the first peak. The mean PCV decreased significantly (p<0.001) in groups A and B when compared to the controls. Mean rectal temperature decreased significantly (p<0.001) in the infected groups when compared to the controls. Mean total leukocyte counts increased significantly (p<0.001) in group B when compared to groups A and C. Absolute lymphocyte counts increased significantly in group B (p<0.001) when compared to groups A and C. There was no statistically significant difference (p>0.05) in the absolute values of neutrophils, monocytes and eosinophils in all the groups throughout the experimental period. Also there was no statistically significant difference (p>0.05) in the mean body weights in all the groups. The immunomodulation with levamisole of acute experimental T. congolense infection in sheep appears to have led to an early onset of parasitemia with a leukocytosis and a lymphocytosis.

Key words: Sheep, trypanosomosis, immunomodulation, levamisole.

INTRODUCTION

Trypanosomosis in domestic livestock negatively impacts on food production and economic growth in many parts of the world, particularly in sub-Saharan Africa (Taylor, 1998). 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 infections and produce poor immune response to bacterial and viral vaccines (Holmes, 1980). Trypanosome induced immunosuppression was first recorded in studies on the response of Trypanosoma brucei infected mice and rabbits to heterologous red cells (Goodwin, 1970; Goodwin et al., 1972). Immunosuppression has also been demonstrated in humans infected with trypanosomes after inoculation with bacterial vaccines and domestic animals with bacterial and viral vaccines (Holmes et al. 1974; MacKenzie et al., 1975; Scott et al., 1977). In the domestic animals, it has been demonstrated that sheep infected with Trypanosoma congolense showed a diminished

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modulation with levamisole on the pathogenesis and appearance and level of parasitemia, packed cell volume course of acute experimental aim of this study is to determine the effect of immunomodulatory drugs. One of these, tetrahydro-6-phenylimidazo-thiazole (levamisole), was initially a synthetic broad spectrum antihelminthic introduced in veterinary practice. Levamisole is the levorotatory form of tetramisole, a synthetic antihelminthic first described by Thienpont et al. (1966). Since 1971, tetramisole (a stearic-isomer of levamisole-hydrochloride) has been recognized as an immunomodulating drug, which augmented the protective effect of Brucella vaccine in mice (Symoens and Rosenthal, 1977). Levamisole has recently attracted more attention owing to its use as an immunomodulator, in supporting anticarcogenic drugs, in the treatment of skin diseases and in improving weight gain in animals (Chadwick et al., 1980; Fox et al., 1985; Sanjaya et al., 1999). It is an immunomodulating drug, which exerts an immunostimulant action in different animal species when administered at repeated doses of 2.5 mg/kg (Stellata et al., 2004). 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 three of the four strains of mice infected. However, Abath et al. (1988) treated Trypanosomosis cruzi infected mice with levamisole and reported that the drug reduced the peak of parasitemia but had no apparent effect on mortality or histopathological findings. All these studies were in murine trypanosomosis and there are apparently no recorded studies in trypanosomiasis of the economically more important domestic livestock species. The aim of this study is to determine the effect of immunomodulation with levamisole on the pathogenesis and course of acute experimental T. congolense infection in sheep with the following objectives: to determine the appearance and level of parasitemia, packed cell volume (PCV), total and differential leukocyte counts, rectal temperatures, total and changes in body weight of T. congolense infected sheep immunomodulated with levamisole.

MATERIALS AND METHODS

Experimental animals

Eighteen 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. During this period they were screened against any infections. The sheep were treated with Ivermectin (KEPROMEC®, Holland) and Oxytetracycline Long Acting (TETROXY LA®, Bimeda, Holland). They were fed grass hay, groundnut hay, and wheat and corn bran. Feed and water were provided ad libitum. During acclimatization, blood samples were collected to obtain baseline values.

Parasite

T. congolense (NITR/Zonkwa) isolated from a pure natural infection of cattle herd in Zonkwa, Kaduna State and obtained from the National Institute for Trypanosomiasis Research (NITR, Kaduna) was used for the study. The parasites were cryopreserved in liquid Nitrogen from where they were sub-passaged into donor albino rats before use. At a very high parasitemia, the experimental donor sheep was infected with the blood from the rats sacrificed under chloroform anaesthesia. The parasites were studied in the Department of Veterinary Parasitology and Entomology, A.B.U. Zaria.

Experimental design

The sheep were divided into three groups based on their mean packed cell volumes (PCV). Group A of six sheep (T. congolense infected), Group B consisting of seven sheep (T. congolense infected and immunomodulated) and Group C consisting of five animals (uninfected controls). All sheep in groups A and B were each infected with about 2 x 10⁶ trypanosomes via jugular venepuncture with blood from a previously infected donor sheep. Group B sheep were each administered levamisole (LEVAJECT 100, Farvet®, Holland) at 2.5 mg/kg subcutaneously on the day of infection and later every week throughout the experimental period.

Sample collection

Blood (3 ml) was collected daily for PCV and parasitemia into disodium ethylene diamene tetracetic acid (Na-EDTA) tubes via jugular venepuncture.

Rectal temperature

Daily morning rectal temperatures were determined by the use of a rectal thermometer.

Packed Cell Volume (PCV)

PCV was determined using the standard microcapillary method as described by Coles (1986).

Parasitemia

Parasitemia was estimated at each sampling using the haematocrit centrifuge test (Woo, 1969) and reported as +, ++,

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

Total and differential white blood cell count

Total white blood cell count was determined by the haemocytometer method. Thin blood smears stained with Giemsa were...
Figure 1. Parasitemic score in T. congolense infected (A) and T. congolense infected, immunomodulated sheep (B).

used for morphological identification of white blood cells and differential leukocyte count as described by Coles (1986).

Body weight

Weight (kg) of animals was determined by the use of a weighing machine (Hana®, China).

Statistical analyses

Results were analysed 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

Clinical observation

Infected animals showed varying clinical signs which include pale mucous membranes, weakness and rough hair coats. In general, Group B sheep looked clinically better than Group A. All the infected animals in both groups survived the experimental period.

Parasitemia

The T. congolense infected sheep in the levamisole immunomodulated group (Group B) became parasitemic three days post infection (p.i.). The sheep that were infected with T. congolense (Group A) became parasitemic 5 days p.i. Parasitemia rose and peaked on day 7 (++++) and 8 (+++) in Group B and Group A respectively. The level of parasitemia remained high in both groups but gradually fell to ++ on day 10 p.i. The ++ level continued in both groups up to day 14 p.i. It gradually dropped to + on days 15 and 16 p.i. in Group A and was ++ in Group B up to day 17 p.i. The level of parasitemia then dropped to + in both groups up to the end of the experimental period (Figure 1).

Packed cell volume

The pre-infection mean packed cell volume (PCV) values were 30.2 ± 3.4, 30.0 ± 2.2 and 30.6 ± 4.6% in groups A, B and C respectively. The mean PCV values fell gradually on day 7 post infection to 25.0 ± 3.5 and 26.4 ± 3.9% in groups A and B respectively. The decrease in mean PCV values in groups A and B was significantly different (p< 0.05) from the values of group C (control). The mean PCV values in groups A and B continued
to fall on day 14 post infection were 22.0 ± 3.9 and 21.6 ± 3.6% in groups A and B respectively. The mean PCV values rose a little to 21.6 ± 1.8 % and 22.8 ± 2.3% in groups A and B respectively on day 35 post infection. There was a statistically significant difference (p<0.001) in the mean PCV values of groups A and B when compared to group C (control). There was no statistically significant difference (p>0.05) in the mean PCV values of groups A and B throughout the experimental period. The mean PCV values in group C (control) remained relatively constant throughout the period of the experiment (Figure 3).

Rectal temperature

The pre- infection mean rectal temperatures were 38.3 ± 0.6, 38.2 ± 0.4 and 38.0 ± 0.5 ºC in groups A, B and C respectively. The mean rectal temperature values rose to 39.4 ± 0.5 and 39.4 ± 0.5ºC on day 4 in groups A and B respectively but later dropped on days 5 and 6 post infection. The mean values on day 13 post infection were 39.0 ± 0.5, 39.5 ± 0.7 and 38.5 ± 0.4ºC in groups A, B and C respectively. The mean rectal temperatures on day 24 post infection were 39.6 ± 0.6 and 39.5 ± 0.6ºC in groups A and B respectively. The mean rectal temperatures continued to remain high in the infected groups and on day 44 post infection were 39.0 ± 0.6 and 39.4 ± 0.5ºC in groups A and B respectively. The mean rectal temperature values in group C remained relatively constant throughout the experimental period. 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 groups A and B throughout the experimental period (Figure 2).

Total white blood cell count

The mean total white blood cell count (WBC) was 7.7, 7.8 and 8.1 (x 10^3) at 13 days pre infection in group A, B and C respectively. The mean WBC count dropped the next day in group A to 9.9 but was still high (11.2) in group B. The mean total WBC counts was statistically significant (p<0.001) in group B from the first week post infection when compared to group C (control). Mean total WBC counts were not statistically significant (p> 0.05) between groups A and C (control) throughout the experimental period but there was a statistically significant difference (p<0.001) between groups B and C (Figure 4). However, there was also a statistically significant difference (p<0.001) between groups A and B. In general there was a leukocytosis in group B when compared to values of group A and C (control).
**Figure 3.** Mean (±S.E.M.) PCV% of *T. congolense* infected (A), *T. congolense* infected, immunomodulated (B) and control sheep (C).

**Figure 4.** Mean (±S.E.M.) total leukocyte count (×10⁹/L) of *T. congolense* infected (A), *T. congolense* infected, immunomodulated (B) and control sheep (C).
Absolute lymphocyte counts

The absolute lymphocyte counts 13 days pre-infection were 4.6, 5.1 and 6.5 \((x10^3/L)\) in groups A, B and C respectively. The mean absolute lymphocyte counts in group B was higher than values of group A and C (control) from the second week of infection up to the end of the experimental period. Absolute lymphocyte counts in group B was statistically significant \((p<0.001)\) when compared to values of group C (control). Absolute lymphocyte counts of group A was not statistically significant \((p>0.05)\) from group C (control). The absolute lymphocyte counts between groups A and B was statistically significant \((p<0.001)\). There was generally a lymphocytosis in group B from the second week post infection up to the end of the experimental period.

Absolute neutrophil count

The absolute neutrophil counts were 2.9, 2.6 and 2.3 \((x10^3/L)\) on day 13 pre-infection in groups A, B and C (control) respectively. The values were 2.9, 2.5 and 2.1 \((x10^3/L)\) in groups A, B and C on the day of infection. On day 35 post infection, the absolute neutrophil counts were 2.8, 4.3 and 3.0 \((x10^3/L)\) in groups A, B and C respectively. There was no statistically significant difference \((p>0.05)\) between the absolute neutrophil counts of the infected groups (A and B) and control (C).

Absolute monocyte counts

The absolute monocyte counts were 0.01, 0 and 0.01 \((x10^3/L)\) on day 13 pre-infection in groups A, B and C respectively. Absolute monocyte counts were 0.06, 0.08 and 0.05 \((x10^3/L)\) on day 15 post infection in groups A, B and C respectively. Absolute monocyte counts from then fluctuated slightly in all the groups up to the end of the experiment. There was no statistically significant difference \((p>0.05)\) in the absolute monocyte counts in all the groups throughout the experimental period.

Absolute eosinophil counts

The absolute eosinophil counts were 0.86, 0.11 and 0.09 \((x10^3/L)\) on day 13 pre-infection in groups A, B and C respectively. Absolute eosinophil counts then dropped to 0.29, 0.01 and 0.08 \((x10^3/L)\) on day 15 post infection in groups A, B and C respectively. The absolute eosinophil counts then continued to remain low with minor fluctuations up to the end of the experiment. The absolute eosinophil counts in both infected groups A and B were 0 \((x10^3/L)\). There was no statistically significant difference \((p>0.05)\) in the absolute eosinophil counts in all the groups throughout the experimental period.

Body weight

The mean body weights (kg) were 16.5 ± 1.9, 16.1 ± 1.1 and 15.6 ± 1.5 kg in groups A, B and C (control) respectively. The mean weight of the animals on day 17 post infection rose in group A to 17.2 ± 1.5 kg but dropped in group B animals to 16.0 ± 1.3 kg. The mean weights at day 35 post infection increased to 17.7 ± 2.1, 17.1 ± 1.2 and 18.6 ± 2.6 kg in groups A, B and C respectively (Figure 5). The mean weights decreased on day 41 post infection to 16.3 ± 1.9 and 15.6 ± 1.5 kg in groups A and B respectively but increased to 17.8 ± 2.2 kg in group C (control). There was no statistically significant difference \((p>0.05)\) in the mean weights of the infected animals in groups A and B from the control group (C).

DISCUSSION

In this study, parasitemia in the \(T. congolense\) infected and levamisole immunomodulated group appeared two days earlier than the group with \(T. congolense\) infection only. The level of parasitemia in the infected, immunomodulated group was also higher. This agrees with the report of Libeau and Pinder (1981) who concluded that levamisole treatment of some breeds of mice infected with \(T. congolense\) resulted in enhanced parasitemia and mortality. However, this is in contrast with the observations that levamisole treatment in \(T. cruzi\) infected mice reduced the peak of parasitemia and had no apparent effect on mortality rate (Abath et al., 1988). Although in our study, a higher parasitemia was recorded in the infected immunomodulated group in our study, there was no recorded mortality in the \(T. congolense\) infected sheep with and without immunomodulation with levamisole throughout the experimental period. It has also been shown that administration of endotoxin (Singer et al., 1964), polynucleotides (Herman and Baron, 1971), BCG and \(C. parvum\) (Murray and Morrison, 1979) prior to or at the same time as challenge with \(T. congolense\), \(T. rhodesiense\) or \(T. brucei\) 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 parasitemic peak and a reduction in the level of parasitemia. The absence of mortality in our study may be related the strain of the organism and the host.

The increased rectal temperature observed in the infected groups was different from those of the control. However, there was no statistically significant difference between the infected groups. The pyrexia observed in the infected groups is similar to the observations in \(T. congolense\) infected calves with a peak within 5 to 6 days post infection which corresponded with parasitemia (Valli et al., 1978). 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. Administration of therapeutic
doses of levamisole in man has also been reported to be associated with fever (Gupta and Gupta, 2003).

Leukocytosis observed in the $T. congolense$ infected immunomodulated group when compared to the $T. congolense$ infected group and control. The observed leukocytosis was due to a lymphocytosis with neutrophil counts similar to the control. The observed increase in leukocyte counts may be due to the cholinergic effect of levamisole on leukocytes and its ability to effect the maturation of immature leukocytes and stimulate T cell differentiation (Wauwe and Janssen, 1991) and levamisole is also known to increase protein and nucleic acid synthesis in resting lymphocytes (Bourne et al., 1978). This is similar to leukocytosis characterized by neutropenia and lymphocytosis reported in $T. congolense$ infected West African Dwarf goats (Adah et al., 1993) but in our study, the neutrophil counts were within normal range. However, Adah et al. (1993) reported a contrasting finding in the same study in $T. congolense$ infected Red Sokoto goats with leucopenia characterized by neutrophilia and lymphopenia. A leukocytosis was also observed in $T. congolense$ infected sheep but only in the chronic stage (Goosens et al., 1998). Another contrasting observation was the report of Valera et al. (2005) who reported a leukocytosis in $T. vivax$ infected sheep occurring from the first week of infection up to the 90 days experimental period. In our study, there were no variations in the neutrophil, monocyte and eosinophil counts between the infected animals and the control.

The significant drop in PCV observed in this study in the $T. congolense$ infected sheep is similar to the observations in $T. congolense$ infected sheep and goats (Goosens et al., 1998), $T. vivax$ infected goats (Saror, 1979), $T. congolense$ infected goats (Adah et al., 1993) and $T. vivax$ infected sheep (Valera et al., 2005). The PCV in the $T. congolense$ infected, immunomodulated sheep also decreased as observed in the $T. congolense$ infected sheep. This shows that levamisole immunomodulation of $T. congolense$ infected sheep does not appear to alter the PCV despite an early and higher level of parasitemia observed in the immunomodulated group. Anaemia has been reported to be the principal pathological feature of animal trypanosomiasis (Saror, 1979) and several factors have been incriminated in pathogenesis which includes the removal of erythrocyte surface sialic acids by trypanosome derived sialidase rendering them more prone to phagocytosis (Esievo et al., 1982) and the generation of free fatty acids by dead autolysing trypanosomes including stearic, linoleic, palmitic and oleic acids which are cytotoxic to trypanosomes and haemolytic in vitro have also been incriminated in the pathogenesis of anaemia (Assoku et al., 1977).

In this study, no significant changes in body weight were observed in all the infected groups when compared to the controls. This contrasts to the observation that administration of immunomodulatory dose of levamisole to piglets at birth has been reported to increase their weight at weaning (Sanjaya et al., 1999). It has been found that weight changes in trypanosomosis are markedly influenced by the levels of protein intake and high intake allows infected animals to grow at the same rate as uninfected controls providing energy intake is adequate whilst low energy levels can exacerbate the adverse effects of trypanosomosis on body weight.
(Holmes et al., 2000). In another study, Gomez et al. (1994) have shown that protein deprivation resulted in higher parasitemia and mortality in mice infected with T. cruzi. The observed non significant changes in weight between the infected groups and the control sheep used in this study may have been due to the fact that the animals were on a very good plain of nutrition with high quality protein in the diet and may also be attributable to the fact that it was an acute study.

Conclusion

The results of the present study appear to indicate that immunomodulation of T. congolense infection in sheep led to an early and higher level of parasitemia at the first peak. There was also a marked leukocytosis due to a lymphocytosis.

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REFERENCES


