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Effect of Concurrent *Trypanosoma brucei* Infection on Haemonchosis in Red Sokoto (Maradi) Goats

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ABSTRACT

The effect of concurrent *Trypanosoma brucei* infection on caprine haemonchosis was investigated in Red Sokoto (Maradi) goats infected with either *Haemonchus contortus* alone or concurrently with *Trypanosoma brucei*. The goats infected with *H. contortus* alone manifested clinical disease that was mild and without mortality. On the other hand, concurrent infection with *T.brucei* resulted in severe clinical disease characterized by pyrexia, pallor of visible mucous membranes, anorexia, loss of body weight, diarrhoea and death of all the animals 18 - 24 days post infection. The concurrent infection with *T. brucei* also resulted in shorter prepatent period (16 ± 0.5 days) and the establishment of a greater number (137 ± 24) of adult *Haemonchus* worms per animal than in those infected with only *H. contortus* alone with prepatent period of 18 ± 0 days and 98 ± 26 adult worms per animal. Reduction in the packed cell volume, increased erythrocyte sedimentation rate, persistent monocytosis and leucopenia characterised by lymphopenia and neutropenia were associated with the concurrent infection while *Haemonchus* infection alone resulted in persistent eosinophilia. The results of the study show that a concurrent trypanosome infection may potentiate the clinical severity of an otherwise sub-clinical infection with *H. contortus* in Red Sokoto goats through reduced prepatent period, increased worm burden and faecal egg output coupled with significantly greater anaemia and loss of body weight. It is suggested that in the tsetse or trypanosomosis endemic areas like southern Nigeria, grazing animals likely to harbour sub-clinical *H. contortus* infections may need to be prophylactically treated against concurrent trypanosome infection in order to maintain optimal productivity.

Key words: Concurrent infection, haemonchosis, trypanosomosis, goats

INTRODUCTION

Haemonchus species are highly prevalent and widespread in Nigeria (Chiejina, 1986; Nwosu *et a*l., 1996a, b). They are the most important parasites involved in field outbreaks of helminthosis in small ruminants in the country (Schillhorn van Veen, 1973, 1978; Anosa, 1977; Fabiyi *et al*., 1979). However, field and experimental evidence have shown that several factors including the breed of host (Preston and Allonby, 1978) and the presence of concurrent infections (Christensen *et al*., 1987) modulate haemonchosis in animals. In Nigeria, trypanosome species are often the most common concurrent parasites encountered in small ruminants suffering from helminthiasis (Schillhorn van Veen, 1974; Fakae and Chiejina, 1993). Field studies in Mozambique, Southern Africa (Spetch, 1982) suggested that concurrent infection of small ruminants with trypanosomes increased their susceptibility to infection with nematode species.

Among the small ruminant stock in Nigeria, goats are much more important in meat production than sheep as a result of the wide acceptability of goat meat by the people (Omeke, 1988). The Red Sokoto (Maradi) goat breed is widely distributed in Nigeria. They are predominantly owned by the nomads and are prone to concurrent infections with several parasite species during nomadic migrations. In this paper, the effects of concurrent infection with *Trypanosoma brucei brucei* on haemonchosis in Red Sokoto (Maradi) goats are described.

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MATERIALS AND METHODS

Experimental animals

Fourteen male Red Sokoto (Maradi) goats aged 6 - 8 months and weighing 8 - 9 kg each were used for this study. At the time of acquisition, the goats were clinically healthy but were each treated with Morantel^{R} (Banminth, Pfizer Plc., Nigeria) at the rate of 25 mg/kg, and Niclosamide^{R} (Up John Plc., Nigeria) at the rate of 100 mg/kg against helminths while Berenil^{R} (Diminazene aceturate, Faewerke, A. G. Germany) at 3.5 mg/kg, was used against blood protozoan parasites endemic in Nigeria. All treatments were completed within the first week of acquisition. They were fed daily on fresh hand-cut grasses (mainly *Panicum maximum*) and legumes (mainly *Centrosema pubescens and Stylosanthes guinensis*) supplemented with maize and bean husks. Water was provided *ad libitum*. The goats were allowed to acclimatize for 8 weeks in fly-proof pens with concrete floors before the commencement of experimental infection.

Experimental parasites

Haemonchus

Pure cultures of infective larvae of *Haemonchus contortus* were obtained by slightly macerating gravid female *H. contortus* freshly collected from the abomasum of naturally infected Red Sokoto goats. The macerated worms were made into a paste with sterile cow faeces and cultured routinely. Infective larvae recovered by the modified Baermann's technique (Anon, 1977) were used in oral infection of the experimental goats.

Trypanosomes

The Mkar strain of *Trypanosoma brucei brucei* (MKA/84/NITR/6) was used for this study. The isolation, identification and storage of the parasites were previously described (Nwosu and Ikeme, 1992). Prior to infection of the experimental animals, the parasites were passaged in a healthy goat. The experimental animals were infected through the jugular vein with goat blood containing trypanosomes.

Experimental protocol

Pre- and post-infection faecal and blood samples were collected from the goats before six of the experimental goats were each concurrently infected with 500 infective larvae of *H. contortus* and 10^4 *Trypanosoma brucei brucei* (Group I). Another 4 goats received only the same amount of *Haemonchus* infection (Group II) while the remaining 4 goats were used as uninfected controls (Group III).

Parasitological studies

Haemonchus eggs were concentrated by floatation using saturated sodium chloride solution and counted by the modified McMaster technique (Anon, 1977). The gastrointestinal tracts of goats that died or were sacrificed at the end of the study were routinely examined at necropsy for worms (Hansen and Perry, 1990).

The initial detection of trypanosomes was done by the microhaematocrit method while the degree of parasitaemia was determined with the improved Neubauer haemocytometer (Brown and Losos, 1977; Murray *et al*., 1983).

Clinical and haematological examinations

Routine clinical examination of all the experimental animals was carried out on daily basis. Live body weights were determined using a trade spring balance (Salter, Great Britain). Blood samples for haematological examinations were collected every two days through the jugular vein into vacoutainer tubes with EDTA as anticoagulant. The packed cell volume (PCV) was determined by the microhaematocrit method, the erythrocyte sedimentation rate (ESR) by the Wintrobe method, total white blood cell (WBC) counts by the improved Neaubauer haemocytometer method and differential WBC by counting and differentiating 100 WBC (Schalm *et al*., 1995).

Statistical analysis

The data obtained from the study were summarized as means ± standard deviations and differences between the means determined by the Student's *t*-test at the 5% level of significance (GraphPad, 2000).

RESULTS

Clinical observations

A severe clinical disease characterized by pyrexia, pallor of visible mucous membranes, progressive depression, loss of body weight, focal alopecia, intermittent loss of appetite, pasty diarrhoea and death of all the infected animals after 18 - 24 days was observed in the goats infected concurrently with *H. contortus* and *T. b. brucei* (Table 1). One animal in this group exhibited a paddling movement of the limbs with blood oozing from the ears and anus a few hours before death. In contrast, animals suffering from haemonchosis alone manifested only a mild form of the disease (from day 16 post infection.) that was not associated with pyrexia or nervous signs and all the animals survived to the end of the observation period. The uninfected control goats remained very active and apparently healthy throughout the entire period of the study. Compared to the uninfected controls, the concurrently infected goats showed significant reductions ($p<0.05$) in live body weight from day 10 post infection. (Fig. 1). Similar reduction was not observed ($p>0.05$) in the group infected with *H. contortus* alone throughout the period of observation.

Table 1. Prepatency, survival period and worm burden of Red Sokoto (Maradi) goats infected with *Haemonchus contortus* alone or concurrently with *Trypanosoma brucei*

Treatment groups	No. of animals	Prepatent period (days)	Survival period (days)	Worm recovery	
				Mean ± SD	Range
Group 1 (*H. contortus* + *T. brucei*	6	16 ± 0.5[a]	18 - 24	137 ± 24[a]	99 - 162
Group II (*H. contortus* alone	4	18 ± 0.0[a]	>24	98 ± 26[b]	67 - 128
Group III (Uninfected control)	4	0	>24	0	0

Parasitological findings

Trypanosma brucei parasitaemia was first detected in the infected goats after 2 - 4 days of inoculation with the parasite. Trypanosome counts rose sharply to a peak of 2.5×10^8/ml on day 6 post infection. Thereafter, the parasites were detected intermittently and by day 10 post infection, no trypanosomes were detectable from the blood of all the infected goats (Fig. 2). Prepatent period for *Haemonchus* species was similar in both single and concurrently infected groups but egg counts remained generally higher in animals with concurrent infection throughout the study (Fig. 2). Mean worm burden was significantly ($p<0.05$) greater in the concurrently infected group than in the group infected with *Haemonchus* species alone (Table 1).

Haematological changes

Significant ($p<0.05$) reduction in PCV was observed in the concurrently infected goats but no significant ($p>0.05$) variation occurred in the mean PCV of those infected with *Haemonchus* species alone and the uninfected controls (Fig. 3). The reduction in PCV was rapid initially in the concurrently infected group but as trypanosomes were no longer detectable in circulation PCV reduction became gradual. Compared to the uninfected control group, the ESR was significantly ($p<0.05$) increased in both infected groups but the increase was earlier and higher in the concurrently infected group than in those infected with *H. contortus* alone (Fig. 1).

The total leucocyte counts of the concurrently infected goats remained normal until day 6 post infection from when it progressively declined throughout the remaining part of the study (Fig. 3). The leucocyte counts for goats infected with *Haemonchus* species alone did not show any significant ($p>0.05$) changes throughout the study period. Lymphocyte populations became gradually reduced from day 6 post infection in the concurrently infected goats (Fig. 4). In the goats suffering from haemonchosis alone, lymphocyte numbers remained normal throughout the study except for a slight reduction from day 14 post infection. Neutrophil numbers in the concurrently infected goats

were gradually reduced from day 6 post infection to the end of the study. On the other hand, in the goats infected with *Haemonchus* species alone, neutrophil numbers remained normal throughout the study.

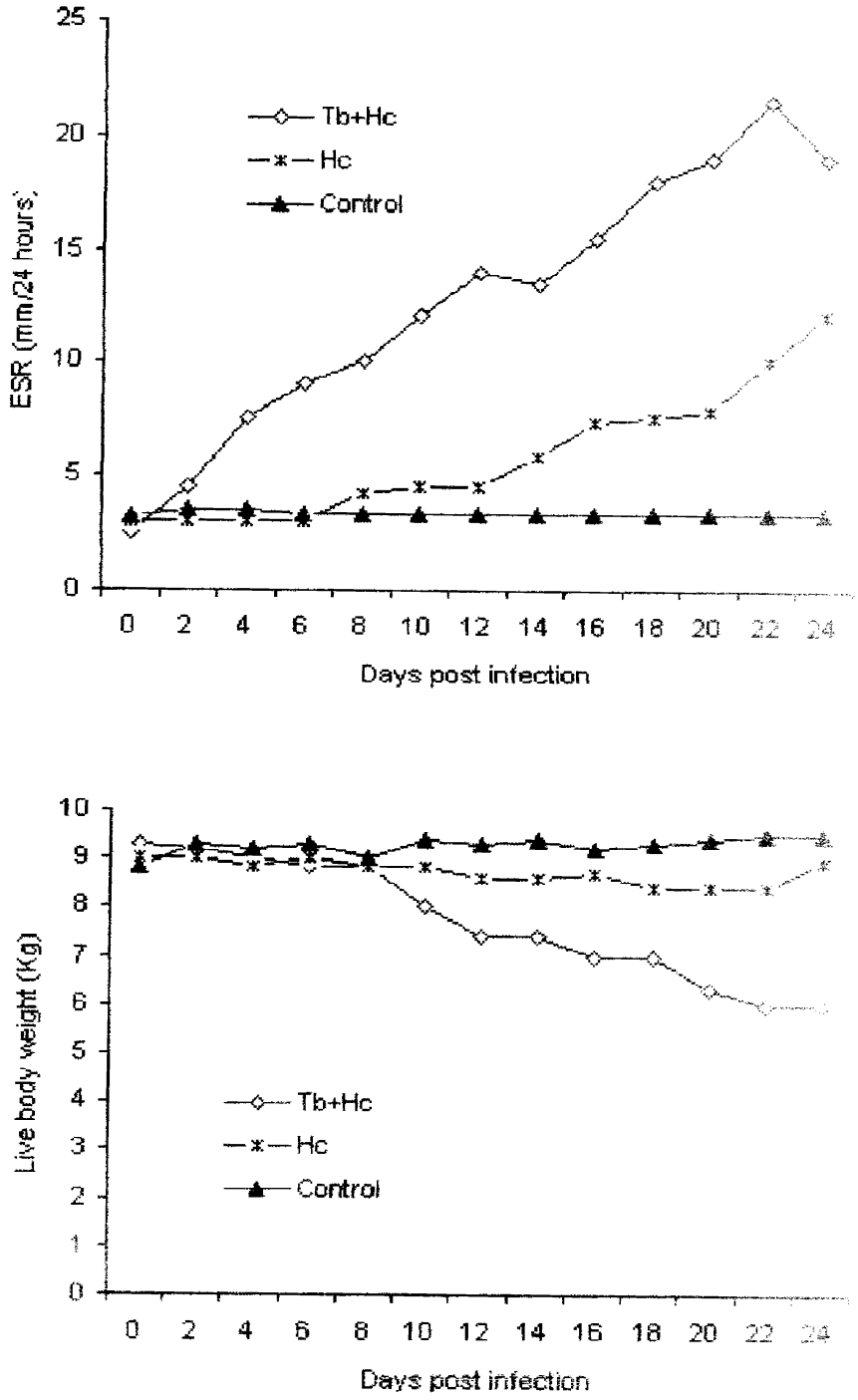

Fig. 1. Live body weight and erythrocyte sedimentation rate of Red Sokoto (Maradi) goats infected with *Haemonchus contortus* alone (Hc) or concurrently with *Trypanosoma brucei* (Hc + Tb) and their control (Control)

Eosinophil counts remained within their normal pre-infection value throughout the study in the concurrently infected goats while persistent eosinophilia was observed in the group infected with *H. contortus* alone (Fig. 5). In contrast, persistent monocytosis was recorded in the concurrently infected group whereas no significant changes were observed in the monocyte counts in the goats infected with *Haemonchus* species alone. In general, no significant changes were observed in the differential leucocyte counts of the uninfected control goats throughout the study period (Fig. 5).

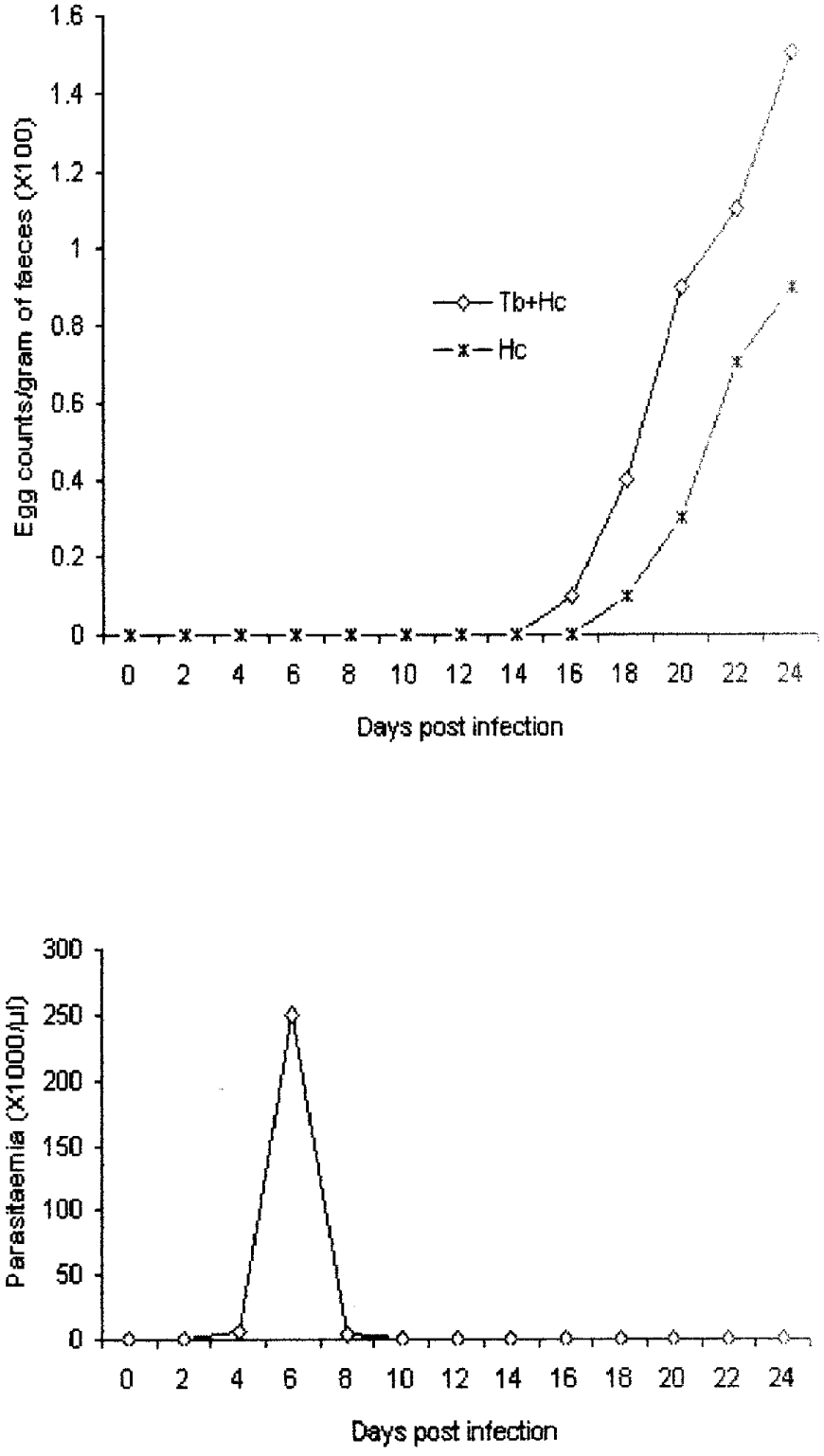

Fig. 2. Parasitaemia and faecal egg counts of Red Sokoto (Maradi) goats infected with *Haemonchus contortus* alone (Hc) or concurrently with *Trypanosoma brucei* (Hc + Tb)

DISCUSSION

Concurrent infections of the host have been recognized to affect the outcome of parasitic diseases with the potentiation of the effects of one parasite by another reported in some cases (Christensen *et al*., 1987). In this study, the Red Sokoto goats infected with *H. contortus* alone showed only a mild disease whereas concurrent infection with *T. brucei* was associated with a severe clinical disease that culminated in the death of all the affected animals

within 24 days. The greater clinical severity of the concurrent infection became manifest from the first appearance of *T. brucei* parasitaemia and was associated with slightly earlier maturation of *Haemonchus* species, higher faecal worm egg output and significantly ($P<0.05$) higher worm burden than that recorded in the goats suffering from haemonchosis alone. The mild nature of the *H. contortus* infection alone and the absence of marked anaemia in the infected goats further suggested that the trypanosome species may have contributed to the greater clinical severity and marked anaemia recorded in the concurrently infected group.

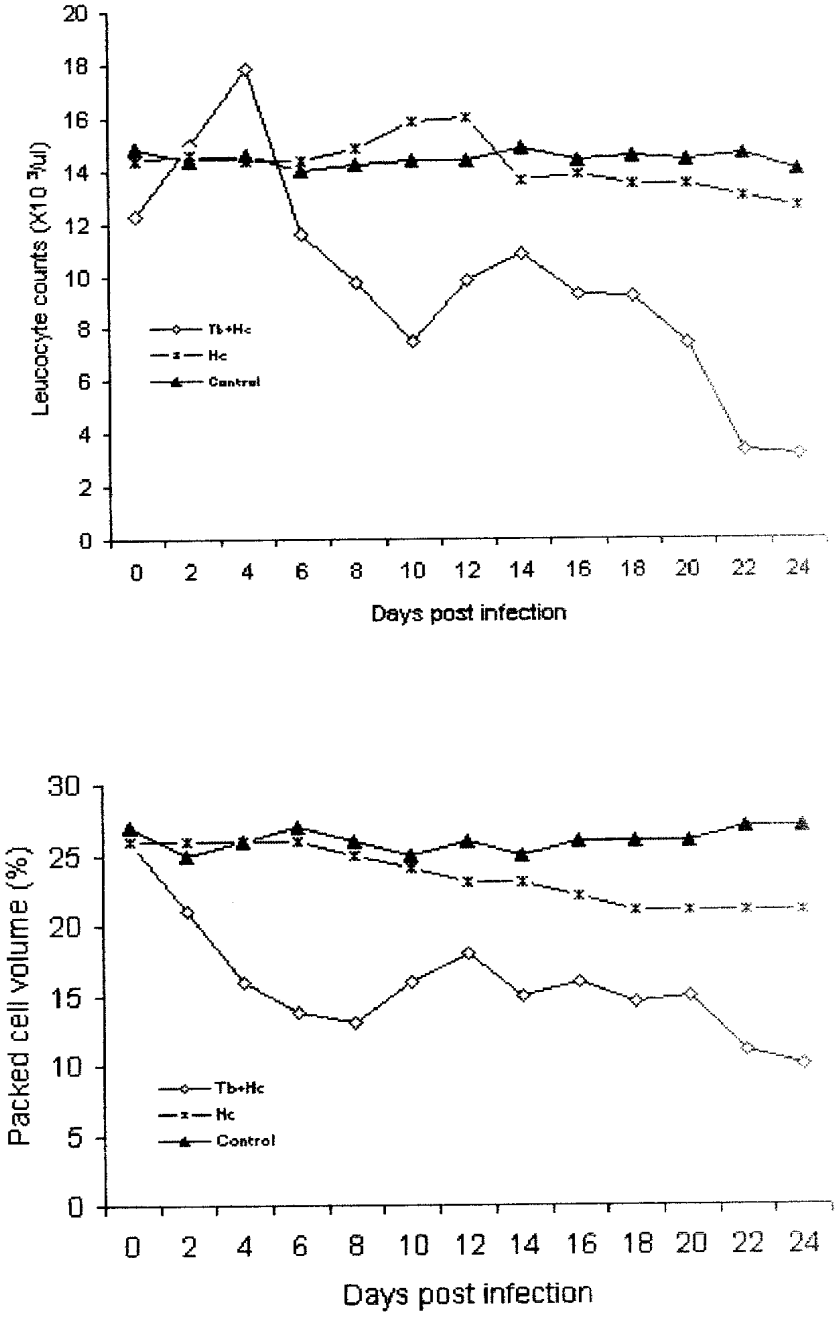

Fig. 3. Packed cell volume and total leucocyte counts of Red Sokoto (Maradi) goats infected with *Haemonchus contortus* alone (Hc) or concurrently with *Trypanosoma brucei* (Hc + Tb) and their control (Control)

The findings of this study with respect to the *H. contortus* infection alone further confirm previous observations that under natural conditions, apparently healthy small ruminants in Nigeria frequently harbour burdens of over 1,000 adult *H. contortus* (Fabiyi, 1970; Nwosu *et a*l., 1996a, b). Based on the results of this study, such animals may, however, succumb to clinical disease when subjected to the additional stress of concurrent trypanosome infection. Both trypanosomes and *Haemonchus* or mixed strongyline species are prevalent in small ruminants in

most parts of Nigeria (Schillhorn van Veen, 1973; Joshua and Ige, 1982; Chiejina, 1986). According to Fakae and Chiejina (1993), about 12% of the domesticated small ruminants in endemic areas of Nigeria harbour concurrent infections with trypanosomes and *Haemonchus* or mixed nematode species.

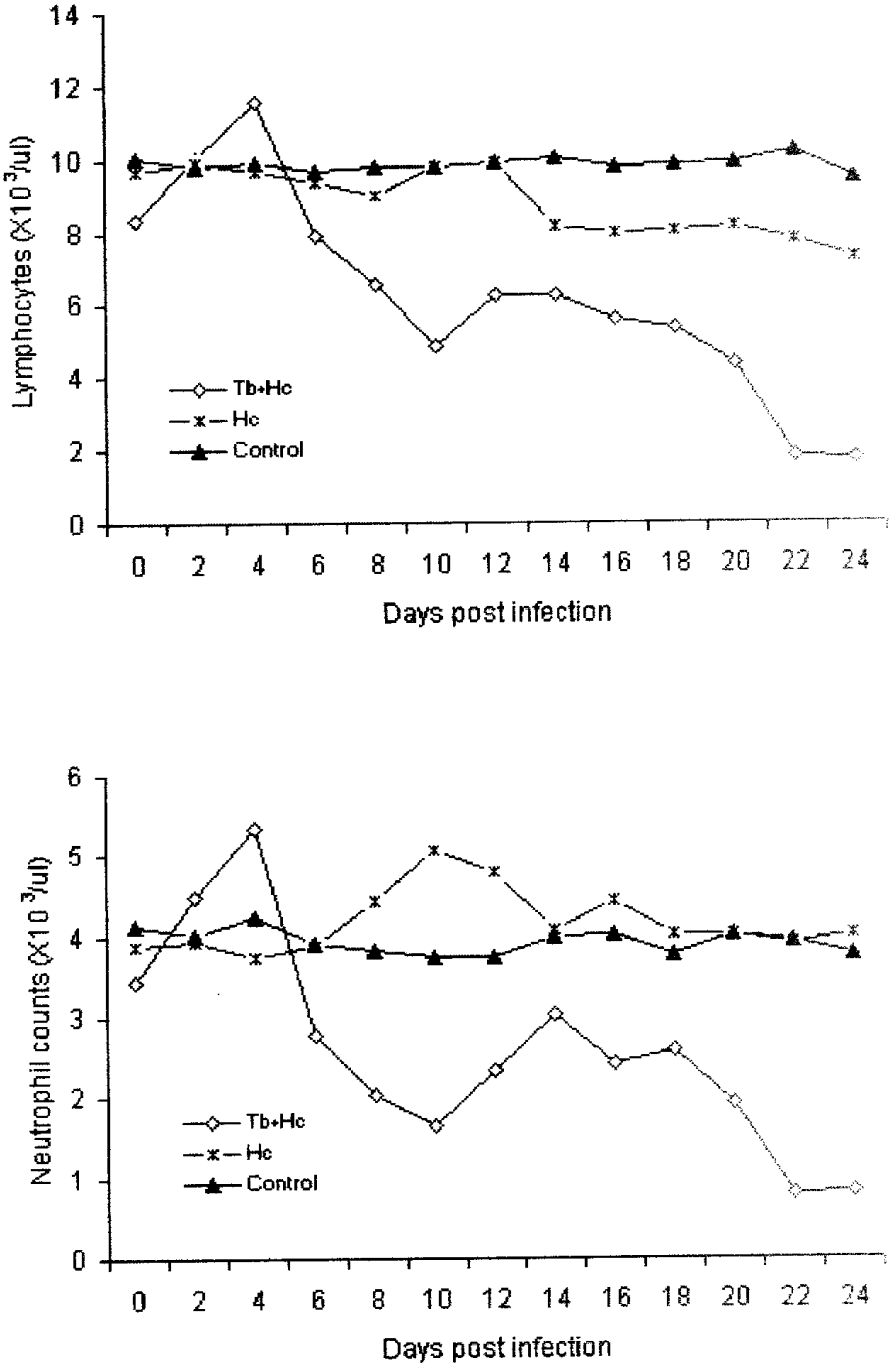

Fig. 4. Mean lymphocyte and neutrophil counts of Red Sokoto (Maradi) goats infected with *Haemonchus contortus* alone (Hc) or concurrently with *Trypanosoma brucei* (Hc + Tb) and their control (Control)

Anaemia is the commonest feature of both haemonchosis (Anosa, 1977; Chiejina, 1987) and trypanosomosis (Anosa, 1988). In the present study, anaemia associated with significant reductions in the PCV was noted in the concurrently infected goats but not in those infected with *H. contortus* alone. This, therefore, suggests that in addition to the haematophagia by the abomasal worms, the destruction of erythrocytes in the concurrently infected goats was closely related to the intercurrent trypanosome infection in the animals. In addition to anaemia, varying degrees of tissue damage and necrosis have been reported to occur in animals suffering from either haemonchosis

(Chiejina, 1987; Nwosu *et al*., 2001) or trypanosomosis (Anosa, 1983; Igbokwe, 1994). Such conditions are known to result in alterations in the suspension stability of the erythrocytes with an elevation of the ESR (Coles, 1980; Schalm *et al*., 1995). It is therefore likely that the synergistic pathological effect of the dual parasite infection was responsible for the significantly higher ESR recorded in the concurrently infected goats than in those suffering from haemonchosis alone.

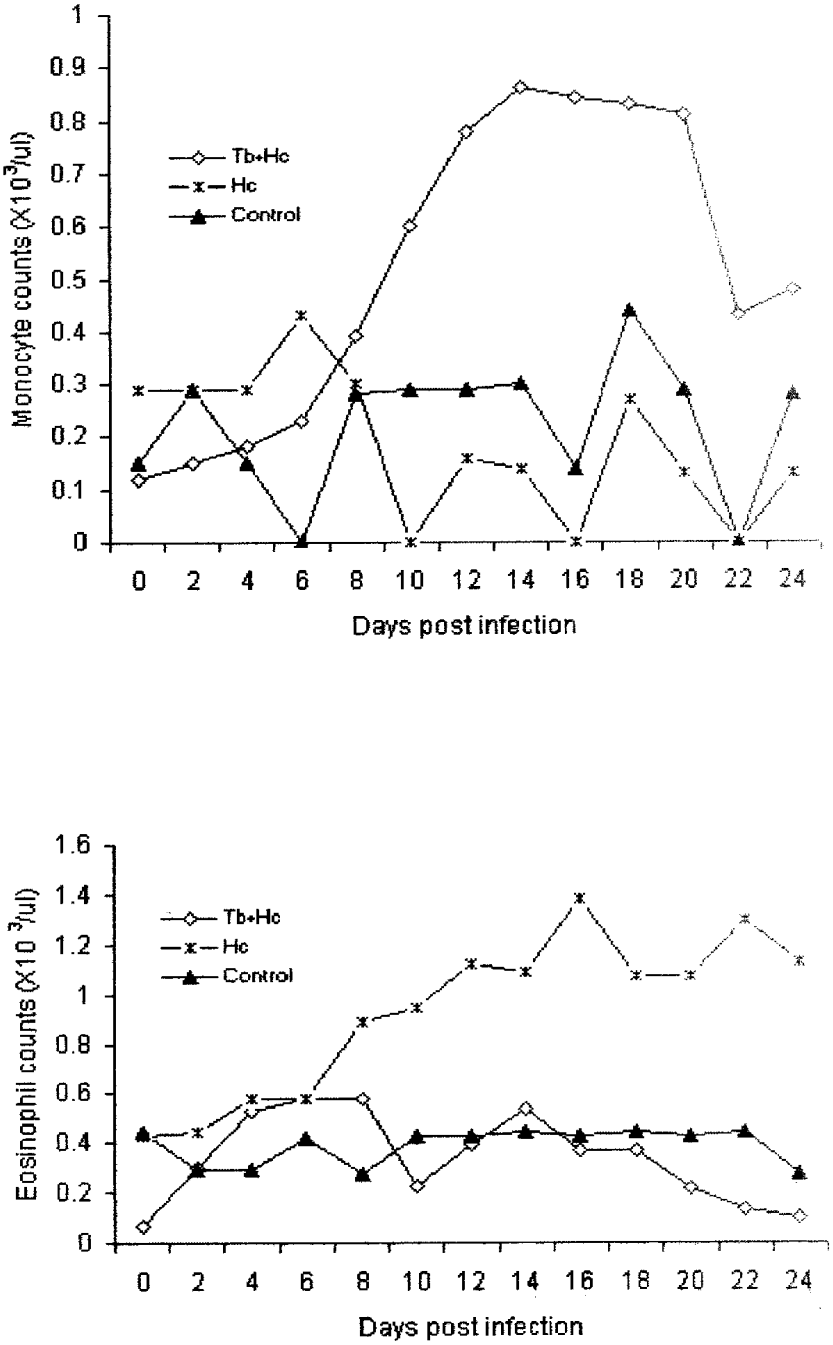

Fig.5. Mean eosinophil and monocyte counts of Red Sokoto (Maradi) goats infected with *Haemonchus contortus* alone (Hc) or concurrently with *Trypanosoma brucei* (Hc + Tb) and their control (Control)

In the present study, significant differences were recorded in the lymphocyte counts of the concurrently infected goats but not in those infected with *Haemonchus* species alone. The intense antigenic stimulation which usually occur during both trypanosomosis (Anosa, 1988) and haemonchosis (Chiejina, 1987) may have resulted in increased demands on the system for lymphocytes to be involved in both immune and inflammatory responses in the concurrently infected goats. These may have caused the lymphopaenia and thus the leucopaenia observed in the

concurrently infected goats since cellular infiltrations involving lymphocytes were noted in several tissues of the goats during this study (data not shown). On the other hand, the reduced neutrophil counts recorded in the concurrently infected group from day 6 post infection might have been due to the splenic sequestration of leucocytes reported to occur during trypanosomosis (Anosa, 1983).

Monocytosis is a consistent finding in trypanosomosis and has been reported in *T. brucei* infections of mice, rabbits and dogs (Jenkins *et al*., 1974; Anosa, 1980; Nwosu and Ikeme, 1992) and in *T.vivax* infections of sheep and goats (Anosa and Isoun, 1983). Monocytosis due to trypanosomiasis have also been associated with a proliferation of tissue macrophages probably necessitated by the increased demands on the system to remove dead blood and tissue cells, trypanosomes, antigen-antibody complexes and to participate in immune responses. Since macrophages are derived from blood monocytes, this increased need for macrophages may have been responsible for the persistent monocytosis observed in the concurrently infected goats but not in the uninfected controls nor in those suffering from haemonchosis alone.

Resistance to most nematode diseases, including haemonchosis, is usually mediated through immunoglobulin E (IgE) response. Since IgE antibodies are elaborated by eosinophils, most nematode diseases have consequently been associated with eosinophilia (Sahaia, 1966; Chiejina, 1987) as was observed during this study in the goats infected with *Haemonchus* species alone. On the other hand, eosinophil numbers have been suppressed in animals infected with *Trypanosoma* species alone or concurrently with *H. contortus* (Griffin *et al*., 1981a,b; Wakelin, 1984). Observations in the present study are consistent with the above reports.

Based on the results of this study, sub-clinical trypanosome infections could potentiate the effects of inapparent nematode infections with resultant high mortalities. This may be especially important during the dry season when grazing animals in Nigeria are frequently malnourished as a result of reduced herbage availability and are forced to migrate into tsetse-trypanosome endemic areas of the country. Therefore, in addition to the regular anthelmintic treatment of grazing animals at strategically determined periods of the year (Nwosu *et al*., 1996b), animals in tsetse/trypanosomiasis endemic regions of Nigeria should be prophylactically treated against sub-clinical trypanosomiasis. Improved general hygiene and supplementary feeding will further complement the control of helminthiasis in grazing animals during this period of the year.

In conclusion, therefore, the results of this study show that a concurrent trypanosome infection may enhance the clinical severity of an otherwise sub-clinical infection with *H. contortus* in Red Sokoto goats through reduced prepatent period, increased worm burden and faecal egg counts coupled with anaemia and significant reduction in live body weight and haematological parameters of such animals.

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Table 1. Prepatency, survival period and worm burden of red Sokoto (Maradi) goats infected with *Haemonchus contortus* alone or concurrently with *Trypanosoma brucei*.

Treatment Groups	No. of Animals	Prepatent period (days)	Survival period (*H. contortus*) (days)	Worm recovery	
				Mean ± S. D.	Range
Group I (*H.contortus* + *T.brucei*)	6	16[a] ± 0.5	18 - 24	137[a] ± 24	99 - 162
Group II (*H.contortus* alone)	4	18[a] ± 0	> 24	98[b] ± 26	67 - 128
Group III (Uninfected control)	4	0	> 24	0	0

[ab] Figures in the same column with different superscripts are significantly different (P<0.05)