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Experimental infections of domestic rabbits (*Oryctolagus cuniculus*) with *Trypanosoma brucei* and *Trypanosoma congolense*: A comparative study

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Abstract

Comparative study of single infections of domestic rabbits (Oryctolagus cuniculus) with Nigerian isolates of Trypanosoma brucei (Gboko strain), and Trypanosoma congolense (Binchi strain) was carried out in the laboratory for clinical and haematological effects. Eighteen rabbits of 10-14 weeks old weighing between 600- 1200 grams were used for the study. The rabbits of both sexes were randomly selected and divided into groups. The level of infection was studied by determining total red blood cell (RBC) count, haemoglobin estimation, total and differential white blood cell(WBC) count, changes in body weight, mortality, rectal temperature changes and other clinical signs of trypanosomiasis. There was significant reduction ($P< 0.001$) in the total red blood cell counts and haemoglobin level in the infected rabbits when compared to the control rabbits with the effect being more pronounced in rabbits infected with T. congolense. The white blood cell count was also highest in those rabbits infected with T. congolense. Both parasites produced similar clinical symptoms which included weight loss, unthriftiness, anorexia, fever, paleness of mucous membrane, and oedema of the facial region. One death was recorded in each of the infected group. Possible reasons for the significant differences in the total red blood cell count, haemoglobin level, and total white blood cell count are discussed.

Key words: Single infections, *T. brucei, T. congolense,* rabbits, comparative.

Introduction:

African trypanosomes are haemoprotozoan parasites which are normally transmitted by tse tse flies and affect man and economically important domestic animals throughout 10 million km^2 of the African continent (Finelle, 1983). The area affected includes large expanses of sub-saharan Africa between latitudes 14° N and 29° S covering about 37 African countries (Hursey, and Slingenbergh, 1995). The economic impact of African trypanosomosis is enormous resulting in an estimated 3 million cattle deaths annualy translating into about US $6000 m to US $12000 m yearly in monetary terms (Hursey and Slingenbergh, 1995).

The various methods of prevention and control of trypanosomiasis which include chemotherapy, breeding resistant breeds of cattle, measures directed against the vector and immunological control by way of vaccination using the immunodominant variant surface glycoprotein (VSG) have not been successful. Also current efforts to harness the potentials of the invariant parasite antigens in the flagellar pocket and the surface of the trypanosomes is yet to yield any significant result. (Powel, 1993; Mkunza *et al.*, 1995; Nolan *et al.*, 1999).

The ever increasing acute shortage of both calories and protein for human consumption in

the tropics have given rise to the commercial production of highly prolific small animals like rabbits for meat purposes and rabbit skin for shoe and bag making. (Fielding, 1991). It has been shown that rodents including rabbits can be infected both naturally and experimentally by protozoan parasites including the pathogenic African trypanosomes like *T. brucei*, and *T. congolense* leading to either acute or chronic infections terminating in loss of productivity and death (Anosa, 1983; Albright and Albright, 1991).

There is at present paucity of information on diseases produced in rabbit by different *Trypanosoma* species. This work was therefore undertaken to investigate the clinical and haematological effects of *Trypanosoma* infections in rabbits with *T. brucei* and *T.congolense* in domestic rabbits with a view to establishing the possible role of *Trypanosoma* species in the numerous undiagnosed causes of death of rabbits in our rural communities.

Materials and methods:

Experimental animals:

Eighteen domestic rabbits (9 males and 9 females) aged between 10- 14 weeks and weighing between 600 and 1200 grams were used for the study. They were kept in fly proof housing, fed *ad libitum* with commercial pelleted feed (Guinea feed) and grasses. They were given access to unlimited supply of clean water. Prior to the study, a conditioning period of two weeks was allowed during which time the rabbits were given anticoccidial drug (Duocoxin, Merck Sharp, and Dohme B.V. Harlem, Holland) at a dose of 1 mg per litre of drinking water for six days (3 : 2: 3). All rabbits were negative for trypanosomes by wet blood film. Giemsa stained thin blood smears and the hematocrit buffy coat method (Murray *et al.*, 1977; Paris *et al., 1982*)

T. brucei(Gboko stain) and *T.congolense* (Binchi strain) used in this study were obtained from Nigeria Institute for Trypanosomiasis Research, Vom, Nigeria. The parasites were maintained in mice.

Experimental design:

Eighteen rabbits were divided into 3 groups of 6 rabbits each (Groups 1, 2, and 3). Each group had 3 females, and 3 males. Group 1 animals were infected with *T. brucei*; Group 2 animals were infected with *T. congolense*, while group 3 animals were not infected at all and served as controls.

Infected mice blood was used to infect the rabbits. Their levels of parasitaemia were determined using the rapid match/counting method (Herbert and Lumsden, 1976). The rabbits in groups 1 and 2 were each infected intraperitoneally (i/p) with 4 x 10^5 *T. brucei* and *T. congolense* per milliliter of phosphate buffered saline diluted infected blood respectively. This concentration of trypanosomes used was obtained through limiting dilutions. After infection, the onset of prasitaemia was determined by a daily examination of wet blood film, Giemsa stained blood smear and the hematocrit buffy coat method (Murray *et al.*, 1977).

The parameters monitored include RBC count, total and differential WBC count, haemoglobin estimation, rectal temperature, weight changes, general body conditions, and mortality. Readings were taken on weekly basis and the experiment ran for 6 weeks post infection. The standard techniques of Coles (1980) were used for the RBC count, total and differential WBC counts. The haemoglobin level was estimated using spectrophotometer (model 6/20) at absorbance maximum 540 nm, filter: Hg. 546 and light path 1 cm.

Statistical analysis:

Data was analyzed by analysis of variance (ANOVA) and significant mean tested using Duncan's multiple range test (Steel and Torrie, 1980).

Results:

Clinical signs observed:

Infection with *T.brucei:*

Parasitaemia occurred 14 days post infection and was relatively low. The clinical signs include dullness, fever, ruffled hair coat, emaciation, anorexia, paleness of the mucous membrane, oedema of the facial region including the ears, eyelids, and the external nares; oedema of the inguinal region in males, erosive exudative dermatitis of the ear, lips, nostril, and scrotum; Mild conjunctivitis with occular discharges.

Infection with *T. congolense:*

Parasitaemia occurred 7 days post infection and was relatively low. The clinical signs include dullness, fever, ruffled hair coat, anorexia, emaciation, paleness of the mucous membrane of the eyes, and mild conjunctivitis with slight nasal and occular discharges, oedema of the abdominal region, and scrotum in the male. There were scabby necrotic lesions in the ears.

Mortality:

One rabbit died in each of the groups 1 and 2 on days 28 and 21 post infection respectively representing a mortality rate of 16.67% in each group. There was no death recorded in the group 3 which was the control group.

Rectal temperature:

The mean rectal temperature was 39.4°C in group 3 (control) whereas the rectal temperature significantly increased ($P < 0.001$) in infected groups (1 and 2) as the experiment progressed. However no significant differences were recorded between groups 1 and 2 ($P > 0.05$) (Fig.1).

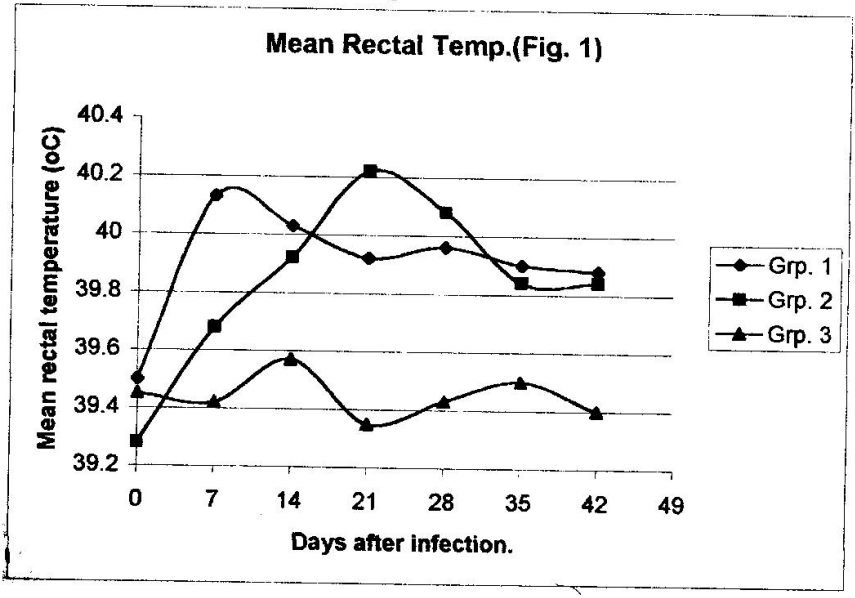

Fig 1: Mean rectal temperature (°C) of rabbits experimentally infected with *T. brucei* (Grp.1), *T. congolense* (Grp.2) and uninfected control (Grp.3).

Body weight:
The absolute body weight of rabbits in group 3 (control) increased as the experiment progressed. The reverse was the case with the infected groups (1and 2). The reduction in weight of the infected rabbits was significant ($P< 0.001$). However, the difference in weight loss in groups 1 and 2 was not significant ($P< 0.05$) (Fig. 2).

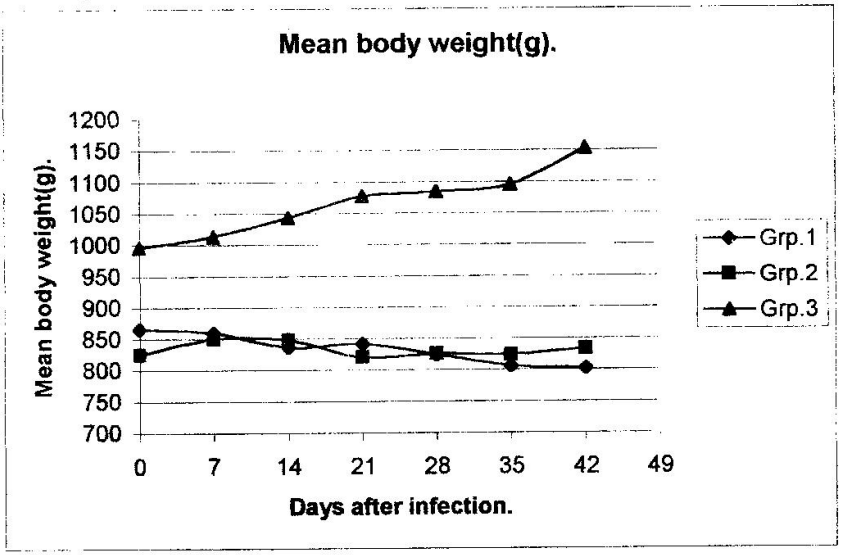

Fig. 2: Mean body weight (g) of rabbits experimentally infected with *T. brucei* (Grp.1), *T. congolense* (Grp.2), and uninfected control (Grp.3)

Hematology:
Total RBC counts:
The mean RBC counts of rabbits ranged between 4.6 – 5.1 x 10^6 ery/ul in the control. There was a significant reduction ($P < 0.001$) in the mean RBC counts in groups 1 and 2. However, the reduction in the mean RBC count as a result of *T. congolense* infection was significantly lower ($P< 0.001$) than the RBC count from rabbits infected with *T. brucei*. (Fig. 3)

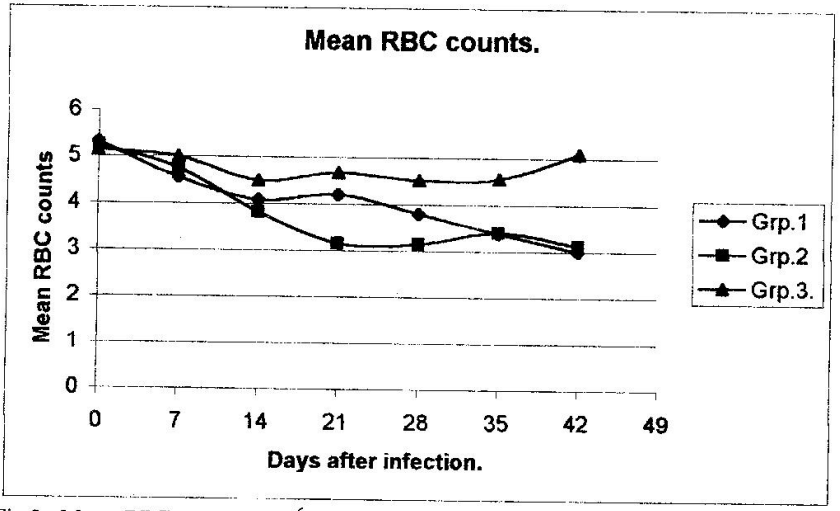

Fig.3: Mean RBC counts ($x10^6$) of rabbits experimentally infected with *T. brucei* (Grp.1), *T. congolense* (Grp.2) and uninfected control (Grp.3).

Haemoglobin values:

The mean haemoglobulin values of the rabbits remained stable at about 11.7% in the uninfected group 3 (control) whereas there was significant reduction in the mean haemoglobin values in the infected groups (1 and 2). Also, the changes in the mean proportion of the haemoglobin values from the initial values at day 0 of the experiment remained constant as the experiment progressed in the control where as there was significant reduction ($P< 0.001$) in the mean proportion of haemoglobin values from the initial value at the pre-infection (day 0) in the infected groups (1 and 2). The reduction in haemoglobin in the rabbits infected with *T. congolense* was more ($P< 0.001$) than those infected with *T. brucei*.(Fig. 4).

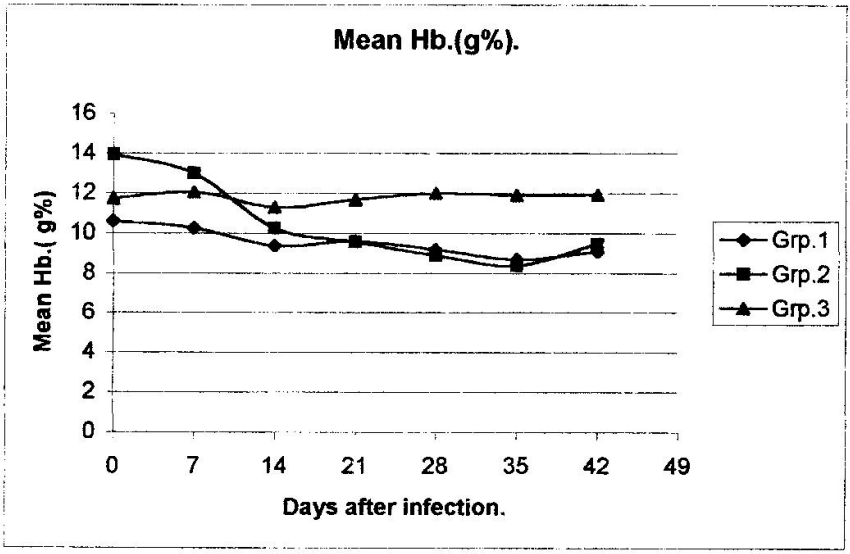

Fig.4: Mean Hb (g%) of rabbits experimentally infected with *T. brucei* (Grp.1), *T. congolense* (Grp.2) and uninfected control (Grp.3)

Total WBC counts:

The mean WBC count in the group 3 (control) ranged between 3.9 and 4.8 x 10^3 leu/ul. There was significant increase ($P < 0.001$) in the mean WBC count to as much as 11.78 x 10^3 leu/ul at day 21 post infection in group 2. However, there was a greater increase ($P< 0.001$) in mean WBC count in rabbits infected with *T. congolense* than those infected with *T. brucei*.(Fig. 5).

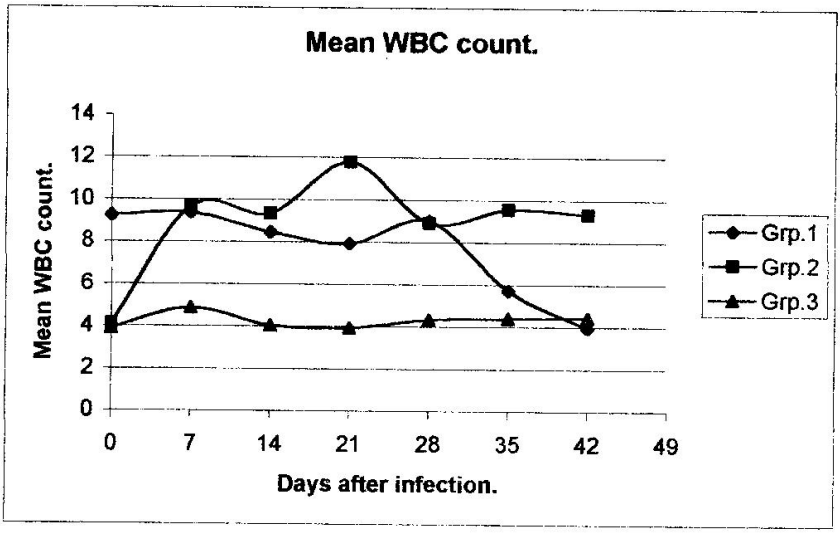

Fig. 5: Mean WBC ($x10^3$) of rabbits experimentally infected with *T. brucei* (Grp.1), *T. congolense*(Grp.2) and uninfected control (Grp. 3).

Differential Leucocyte count:

Lymphocytes:

The mean differential lymphocyte percentage ranged between 69% and 77.8% in group 3 (control). There was a significant increase ($P< 0.001$) in the mean differential lymphocyte count to as much as 87.5% at day 14 post infection in group 2. However, there was no significant difference ($P> 0.05$) in the increase in the lymphocyte percentage between groups 1 and 2 (Fig.6).

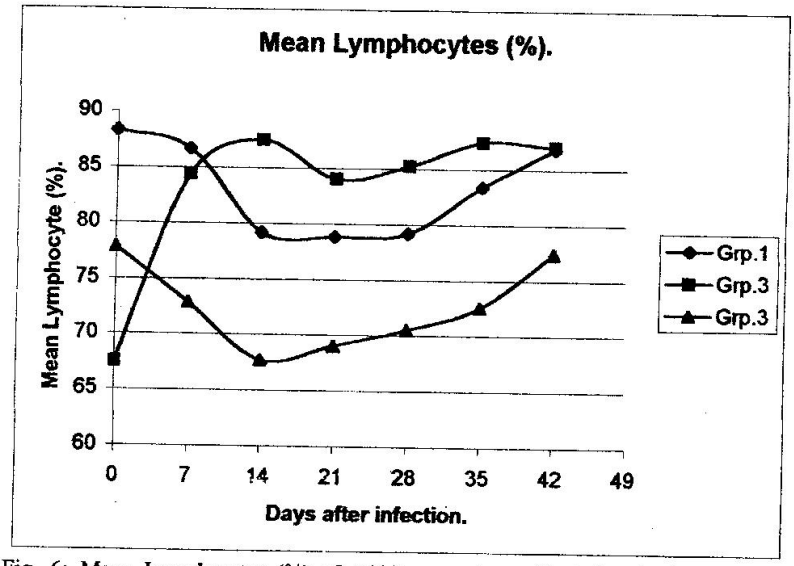

Fig. 6: Mean Lymphocytes (%) of rabbits experimentally infected with *T. brucei* (Grp.1), *T. congolense* (Grp.2) and uninfected control (Grp.3).

Neutrophils:

The mean differential neutrophil percentage ranged between 21 and 32% in group 3 (control). There was a significant decrease ($P < 0.001$) in the mean differential neutrophil in groups 1 and 2. This decrease however was not significant ($p > 0.05$) when groups 1 and 2 were compared (Fig. 7).

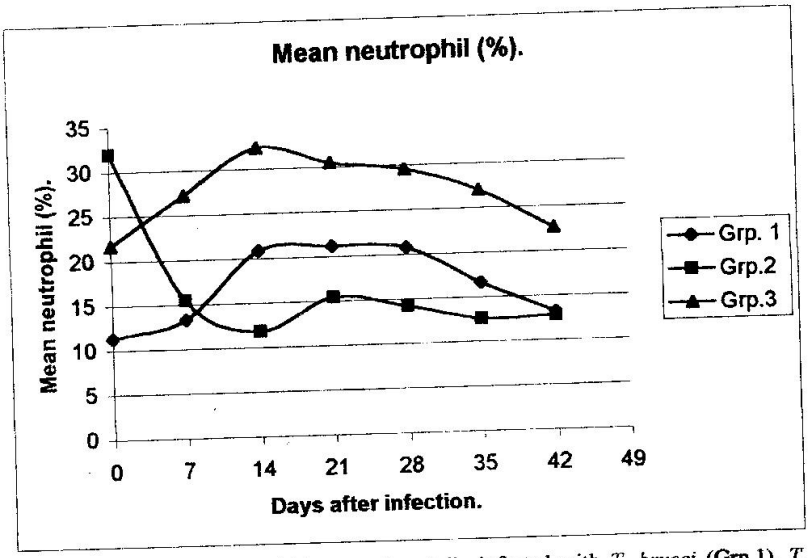

Fig. 7:Mean neutrophil (%) of rabbits experimentally infected with *T. brucei* (Grp.1), *T. congolense* (Grp.2) and uninfected control (Grp.3).

Discussion:

The results of this study showed that Nigerian Outbred domestic rabbits (Oryctolagus cuniculus) are susceptible to both *Trypanosoma congolense* (Binchi strain) and *T. brucei* (Gboko strain) producing parasitaemia at day 7 and day 14 of syringe inoculation respectively. The difference in the onset of parasitaemia between the two *Trypanosoma* species may be due to the fact that *T. congolense* is a strict plasma parasite and remained confined to the blood and *T. brucei* being a humoral parasite invades the plasma, connective tissue, and body cavity fluids (Losos, and Ikede, 1972; Grundler, 1988).

The clinical signs exhibited by the infected rabbits were typical of trypanosomosis, (Losos, and Ikede,1972; Anene, 1989; Onyeyili, and Anika 1989). These clinical signs did not differ significantly in terms of severity between the infections due to *T. brucei* and *T. congolense* and hence the clinical signs are not pathognomonic for any of the parasites in rabbits.

The reduction in the absolute mean body weight of the infected rabbits is in agreement with the earlier findings of (Fiennes, 1970; Anosa and Isoun, 1976 ; Anene *et al.*, 1991). This reduction in body weight in the infected rabbits may be due to the anorexia and lethargy which normally characterize *Trypanosoma* infections in livestock.

The resultant anaemia characterized by gradual reduction in RBC count and haemoglobin concentration in the rabbits with *T. congolense* and *T. brucei* in the study is in conformity with the findings of other workers (Agu and Bajeh, 1986; Anene *et al.*, 1989, 1991, and 1999; Dwinger *et al.*, 1994). The significant reduction in the total RBC counts and haemoglobin concentration by *T. congolense* in this study may be due to the fact that *T. congolense* being a strict plasma parasite remains restricted to the blood causing its primary pathogenic effect in the blood compared to the *T. brucei* which extravasates into the tissues, and the body

cavities leading to anaemia which is of secondary importance to the extensive degenerative necrotic and inflammatory changes that characterize the infection. (Losos and Ikede, 1972).

The leucocytosis observed in the infected rabbits in this study is in agreement with the findings of Kagwa *et al.,* (1984); Anene, (1989); Onyeyili and Anika, (1989) and Onah, *et al.,* (1998). This however contrasted with the findings of Ikede *et al.,* (1977), Anosa, (1983); Omamegbe and Uche, (1985) who reported leucopenia in Trypanosoma infected mouse, and dogs respectively. This leucocytosis may be due mainly to lymphocytosis since high relative percentage of lymphocyte counts were observred in the infected rabbits. Lymphocytosis have been reported in *T. brucei* infected dogs, (Anene, 1989; Omamegbe and Uche, 1985), in *T. brucei* infected calves (Moulton and Sollod, 1976), and in *T. theileri* infected cattle (Cross et al, 1968) while Anosa (1983) and Kagwa *et al.,* (1984, 1988) reported low lymphocyte count in *T. brucei* infected mouse and dog respectively. Lymphocytosis may reflect the immunological competence of the host and hence the ability to withstand acute *Trypanosoma* infections in animals.

The neutropenia recorded in the infected rabbits in this study may be attributed to a depression of bone marrow granulocyte precursors by *Trypanosoma* toxins and or massive elimination of neutrophils when they engulf trypanosomes. This finding was in agreement with that of Anosa, (1983) and Onyeyili, (1983) who reported neutropenia in mouse and dogs respectively.

This study has shown that trypanosomiasis poses a serious danger to intensive and commercial rabbit production in Nigeria since it was possible to infect these rabbits experimentally. Also the findings that there was actually no significant difference between the infections due to *T. congolense*, and *T. brucei* in terms of changes in the body weight, pyrexia, and mortality which are the major clinical indices of trypanosomiasis in livestock goes further to suggest that none of these clinical signs is pathognomonic for trypanosomiasis in rabbits. It is thus concluded that trypanosomiasis may be one of the causes of the so many undiagnosed cases of death and low productivity of rabbits in our rural communities in Nigeria.

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