

EFFECT OF *TRYPANOSOMA CONGOLENSE* AND *TRYPANOSOMA BRUCEI* MIXED INFECTION ON THE PATTERN OF HAEMATOLOGICAL CHANGES IN MURINE TRYPANOSOMOSIS

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The effect of *Trypanosoma congolense* and *T. brucei* mixed infection on the pattern of haematological changes was demonstrated in a rat model. At the end of 21 days post infection (PI), anaemia which was characterised by drop in the packed cell volume (PCV), was found to be significantly ($P < 0.05$) severer in rats with mixed infection than those infected with *T. congolense* or *T. brucei*. Similar pattern of drop in the total white blood cell (WBC), differential WBC, and platelet counts was observed in the group with mixed infection. It was concluded that even though *T. congolense* and *T. brucei* may cause milder haematological changes in animals compared to *T. vivax*, mixed infection by these parasites may cause severer haematological changes in the natural hosts.

Key words: Mixed infections, pattern of haematological changes, rats, *Trypanosoma congolense*, *Trypanosoma brucei*

INTRODUCTION

African animal trypanosomosis still constitutes a major threat to food security in several parts of sub-Saharan Africa (1-3). Although several advances have been made on research into various aspects of the pathogenesis of African trypanosomosis, the exact factors involved in the disease process have not yet been fully known. *Trypanosoma vivax*, *T. congolense* and *T. brucei* are the major causes of disease in ruminants (4, 5). *T. congolense* and *T. brucei* also cause disease in dogs (5).

Anaemia constitutes a major pathological feature of trypanosomoses of man and domestic animals besides other haematological and serum biochemical changes (6, 7). Infecting trypanosome species are known to differ both in their host tissue of primary parasitization and disease pattern in animals (5).

The current understanding of the pathogenesis of African trypanosomes rests largely on the observation of results of experimental single trypanosome specie infections in animals (8-10) while the result of infection arising from mixed

trypanosome species has not been properly investigated.

Several of the natural trypanosomoses in animals arise from mixed infections (11). There is therefore, the likelihood that the true impact of the disease on the animal has been underestimated. In West Africa, *T. congolense* and *T. brucei*, though pathogenic to animals, are less a threat to livestock when compared to *T. vivax* (4, 5). Not much has been known on the effect of mixed infection arising from these trypanosome species on disease course. In this study we attempt to investigate the outcome of *T. congolense* and *T. brucei* mixed infection on the course of haematological changes using rat as a model.

MATERIALS AND METHOD

A total of 80 albino rats were used for the investigation. All the rats were bred at our Institute in Kaduna and Vom. Commercial rat cubes and water were fed *ad libitum* through out the period of investigation. The rats were randomly divided into three groups; A, B and C of 20 rats each while 15 other rats served as control.

Trypanosome species used were *T. congolense* (NITR/BASSA) and *T. brucei* (NITR/LAFIA). Both parasites were isolated from cattle and cryopreserved in liquid nitrogen at the Institute from where they were sub-passaged once into rats before use. Rats in group A were inoculated with *T. congolense*, 1×10^3 parasites through the intraperitoneal (IP) route while group B were inoculated with *T. brucei* using the same number of parasites as in group A IP. Group C rats were infected with a mixture of *T. congolense* and *T. brucei* made up of 0.5×10^3 parasites each. Parasites for inoculation were estimated as described by Lumsden *et al* (12).

Daily parasitaemia was estimated from wet blood preparations made through tail prick while the packed cell volume (PCV) was determined as described by Kelly (13). At the end of 21 days post infection (PI), blood was collected from the heart of surviving rats using a 21 gauge hypodermic needle for the estimation of red blood cells (RBC), total white blood cell (WBC) and Platelet counts as described by Kelly (13). The Thin blood smears stained with Giemsa were used for WBC differentiation. The data collected were analysed using the student's t-test and analysis of variance (ANOVA).

RESULTS

Parasites were first detected in the tail blood of rats in groups B and C infected with *T. brucei* and mixed infection respectively, 3 to 4 days PI while that of *T. congolense* infected group A was

not detected until 5 to 7 days PI. Thin smear made from mixed infection group on the first day of parasitaemia revealed *T. brucei* while *T. congolense* was detected in the smears 3 days later. Control rats showed 1.0% drop in PCV between day 0 and 21 PI while infected rats showed 9.1%, 6.7% and 17.6% drop in PCV in *T. congolense*, *T. brucei* and mixed infection groups respectively.

On the overall, the drop in mean PCV was significantly highest in group C with mixed infection ($P < 0.05$) followed by group B infected with *T. brucei* and least in *T. congolense* infected group B (Fig 1). Drop in the mean RBC count at the end of 21 days followed a similar pattern of change in PCV (Table 1).

The total WBC counts also dropped most in the mixed infection group ($P < 0.05$) but least in the *T. congolense* group. A similar pattern of decrease in the mean platelet counts was observed with the mixed infection group recording highest decrease in platelet counts at the end of 21 days PI (Table 1).

Absolute differential WBC counts of infected rats were as shown on Table 2. The decrease in the mean lymphocyte and neutrophil counts were also highest in the mixed infection group than in *T. congolense* and *T. brucei* infected groups. There was also higher increase in monocyte counts in the mixed infection group ($P < 0.05$). Similarly eosinophilia occurred only in the mixed infection group. A total of 5 rats died within the last week of infection in the mixed infection group while no mortality was recorded in the other groups.

Table 1: Changes in RBC, total WBC and Platelet Counts of control and trypanosome-infected rats at 21 days PI

	Control Rats n=20	Infected A n=20	Infected B N=20	Infected C N=15
RBC ($\times 10^6/\text{ul}$)	9.6 \pm 1.0	*(9.8 \pm 1.7) 9.4 \pm 2.7	(8.7 \pm 2.7) 6.4 \pm 3.2	(6.8 \pm 2.5) 6.2 \pm 3.4
WBC ($\times 10^3/\text{ul}$)	15.9 \pm 4.5	(15.4 \pm 4.2) 12.1 \pm 4.7	(15.0 \pm 3.5) 11.0 \pm 4.2	(13.80 \pm 4.7) 5.9 \pm 0.1
Platelet Counts ($\times 10^3/\text{ul}$)	450.0 \pm 0.1	(470.4 \pm 2.2) 385.1 \pm 0.7	(415.4 \pm 1.9) 268.1 \pm 4.2	(401.1 \pm 2.5) 133.3 \pm 29

*Pre infection values in brackets.

Table II: Summary of changes in absolute differential WBC ($\times 10^2$ /ul of blood) of control and trypanosome-infected rats at 21 days PI

	Control Rats N=20	Infected A n=20	Infected B n=20	Infected C N=15
Neutrophils ($\times 10^3$ /□l)	3.3±1.5	*(3.1±1.6)2.1±2.5	(3.4±2.9)2.0±2.5	(2.8±2.0)1.5±2.1
Lymphocytes ($\times 10^3$ /□l)	12.2±2.1	(13.2±4.3)10.9±0.8	(11.9±4.6)6.8±1.3	(12.3±3.8)5.1±0.1
Monocytes ($\times 10^2$ /□l)	0.3±3.1	(0.2±3.5)0.2±2.5	(0.3±1.0)0.3±0.9	(0.2±3.6)0.4±2.4
Eosinophils ($\times 10^2$ /□l)	0.0±0.0	(0.0±0.0)0.0±0.0	(0.0±0.0)0.0±0.0	(0.0±0.0)0.1±0.5

* Pre-infection values in brackets.

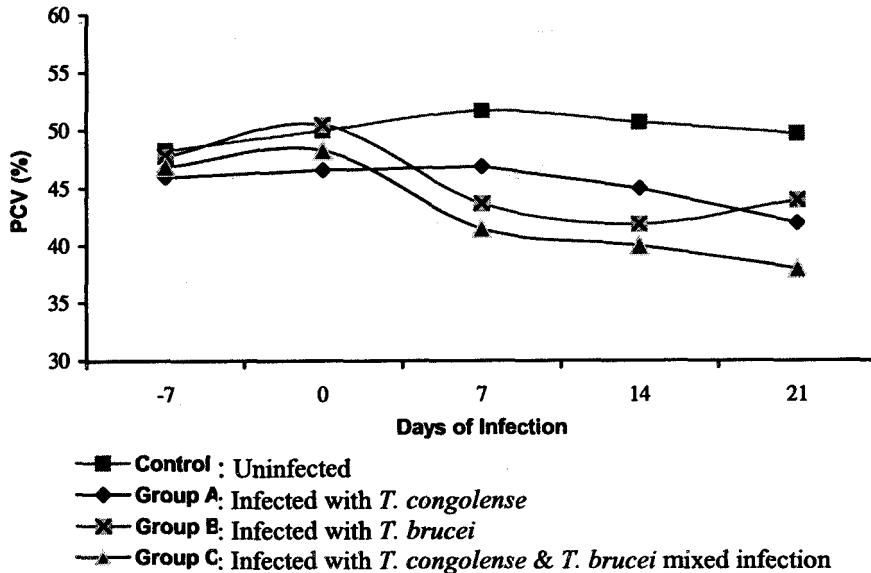


Fig 1: Mean Packed Cell Volume (%) of control and infected rats

DISCUSSION

Mixed infection did not appear to affect pre-patent period of either of the parasites in rats. Rather *T. congolense* and *T. brucei* mixed infection group exhibited more severe haematological changes characterised by marked significant drop in the PCV and RBC. The pattern of anaemia in *T. congolense* and *T. brucei* single infection groups did not differ from the pattern previously observed in *T. congolense* (14, 15) and *T. brucei* (14, 16) infected albino rats. By day 21 PI, anaemia in the *T. brucei* infected group seemed to recover. This has earlier been observed by Anosa (6) and arises from increase in erythropoietic activity in *T. brucei* infection. Similar observation was made in *T.*

gambiense infected monkeys in which the PCV showed apparent recovery in the chronic phase (17).

Both *T. congolense* and *T. brucei* have been shown to differ in their sites of localization in the tissues of infected host. While *T. brucei* is extravascular and localise in solid tissues, *T. congolense* is largely intravascular (5, 18). Losos (5) classified trypanosome lesions into primary and secondary lesions; primary lesions being those changes caused by the direct effect of injurious mechanisms on the infected target organs while secondary lesions are those resulting from the subsequent malfunctioning of organs and tissues affected by the primary lesions. In *T. congolense*, primary lesions occur mainly in the blood, blood

vessels and lymphoid tissue, (5). In *T. brucei* infection, this occurs in the connective tissue of solid organs (2).

A combination of these different mechanisms of pathology may have been responsible for precipitation of an overall severer anaemia in the mixed infection group. Anaemia in trypanosomiasis arises from haemolysis, haemodilution, haemorrhage and dyshaematopoiesis (6, 19). The mechanisms involved in these factors probably become exaggerated in mixed infection. Although *T. brucei* group appeared to recover from anaemia, no such changes occurred in the mixed infection group. This may be due to severity of stem cell injury (19), and marked phagocytosis of erythroid cells (6, 20, 21).

Leucocytopenia and thrombocytopenia was also a general feature in *T. congolense* and *T. brucei* infected rats, but were more marked in the group with mixed infection. This was also characterised by lymphopaenia, neutropaenia, eosinopaenia and monocytosis. Eosinophilia was observed in *T. evansi* infected buffalo calves (10, 22) and *T. brucei* infected deer mice (20). The pattern of fall in lymphocyte numbers suggests that *T. congolense* and *T. brucei* mixed infection also caused more marked antigenic stimulation leading to accelerated transformation of lymphocytes to

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plasma cells and transferred lymphocytes resulting to lymphopaenia (7, 23) in this group. Similarly marked depression of precursor cells and marked phagocytosis of neutrophil precursors in the bone marrow (7, 24) and spleen (6, 25) may have been responsible for the severe fall in neutrophil numbers in the mixed infection group.

Monocytosis may also be due to increased demand for removal of particulate matter (23) arising from severer pathology in rats with mixed infection, which was matched by proliferation of macrophages in several tissues (26). The aetiology of thrombocytopenia on the other hand is multifactorial and it include platelet phagocytosis by splenic and bone marrow macrophages (7, 21, 24), platelet agglutination (28, 29), ineffective thrombopoiesis (30) and splenic pooling (31). These factors were probably exaggerated leading to more severe fall in platelet counts in mixed infection rats.

It is concluded that *T. congolense* and *T. brucei* cause severer pathological changes under mixed infection and that the true impact of African Trypanosomes on animals has indeed been underestimated.

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