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Haematological changes in Wistar rats experimentally infected with *Trypanosoma congolense* and *Trypanosoma brucei brucei* obtained from North-west Nigeria

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Abstract

This study determined haematological changes in Wistar rats experimentally infected with local strains of *Trypanosoma congolense* and *Trypanosoma brucei brucei*. Forty-five Wistar rats between 10 – 12 weeks old weighing between 210 – 240 g were used. The Wistar rats were randomly divided into four groups (A, B, C and D), with the infected groups (B, C and D) having 10 rats each, while the uninfected control group (A) had 15 rats. Group A rats were not infected and served as the control, group B were infected with *Trypanosoma congolense*, group C were infected with *Trypanosoma brucei brucei* and group D were co-infected with *Trypanosoma congolense* and *Trypanosoma brucei brucei*. Infection was achieved using 0.1mL of blood containing approximately 1×10^3 trypanosomes intraperitoneally into each Wistar rat in the infected groups. Clinical signs were observed. The changes in the blood cells were assayed in the groups post-infection. Duncan's Least Square Deviation showed significantly ($p < 0.05$) higher parasitaemia in infected groups. However, group D showed a higher significant ($p < 0.05$) difference in parasitaemia when compared to groups B and C. The pattern of mean parasitaemia for the infected groups, revealed a positive correlation with days of post-infection ($p < 0.05$) before the decline. The packed cell volume, total red blood cell count and haemoglobin concentration were significantly ($p < 0.05$) lower in infected groups B, C and D. The total white blood cell count, platelet counts and differential leucocyte count were significantly ($p < 0.05$) lower in infected groups when compared to the uninfected group. These findings suggest that co-infection with *Trypanosoma congolense* and *Trypanosoma brucei brucei* obtained from Wuruno and Ngaski in Sokoto and Kebbi States respectively produced a more damaging effect on haematological parameters.

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Introduction

Trypanosomiasis is one of the most devastating diseases of man and animals in sub-Saharan Africa and has a profound effect on rural development (Silva *et al.*, 1999). Trypanosomiasis occurs between latitudes 14°N and 29°S, approximately one third (1/3) of total land mass in tropical Africa, covering an area of 10 million Km² (Nigerian Institute for Trypanosomiasis Research (NITR), Annual Report, 1989; Molyneux *et al.*, 1996; Steverding, 2008; Sumayin *et al.*, 2010). Animal trypanosomiasis constitutes a major threat to food security in Nigeria and other parts of sub-Saharan Africa (Onyiah, 1997; Swallow, 2000; Abenga *et al.*, 2003; Fajinmi *et al.*, 2006; Samdi *et al.*, 2010). The disease is a major cause of livestock death in Africa each year leading to reduction in livestock populations, calving rates, milk yield, meat supply, work efficiency of draft animal and mixed farming (Swallow, 2000). African trypanosome species are hemoflagellate, extracellular and single cell protozoan parasites and the disease caused is associated with anemia, leucopenia, thrombocytopenia, plasma biochemical changes and lesions in some tissues and organs (Maikai & Auda, 2007). Tsetse flies of the genus *Glossina* species are the principal vector of trypanosomes that are of major threat to livestock industry and include *Trypanosoma vivax* (*T. vivax*), *Trypanosoma congolense* (*T. congolense*) and *Trypanosoma brucei brucei* (*T. b. brucei*). Understanding the manifestations of trypanosome infection in the past has been largely based on single species experimental infection (Losos & Ikede, 1970, 1972; Poltera, 1985; Anere *et al.*, 2009) while there is dearth of investigation on experimental mixed trypanosome infections. Several of the natural occurrences of trypanosomiasis in animals arise from mixed infection (Nantulya, 1990). There is therefore likelihood that the true impact of the disease on the animal might have been under estimated. In West Africa including Nigeria, *T. congolense* and *T. b. brucei* are pathogenic to animals, though they are said to be of less threat to livestock compared to *T. vivax* (Jordan, 1986; Losos, 1986). Haematological parameter determination in relation to trypanosome infection is very important as it normally gives the true health status of the animals as well as indicator for anaemic conditions. In this work, attempt was made to investigate the haematological changes and compare the severity of *T. congolense*, *T. b. brucei* and co-infection on some haematological parameters in Wistar rats experimentally infected.

Materials and Methods

Wistar rats

A total number of 45 Wistar rats of average weight between 210 – 240 g were used for this study. All the Wistar rats were bred at Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna in commercial rat cages. The Wistar rats were also conditioned for a period of two weeks in the laboratory and certified parasite free by blood film microscopy prior to the infection. The Wistar rats were fed daily on vegetables supplemented with potatoes and water was made available *ad libitum*

Experimental design

The Wistar rats were randomly assigned into four groups with the infected groups having 10 rats each, while the uninfected control group had 15 rats. The groupings and their treatments were, uninfected Wistar rats / the control (Group A), Wistar rats infected with *Trypanosoma congolense* (Group B), Wistar rats infected with *Trypanosoma brucei brucei* (Group C) and Wistar rats infected with *Trypanosoma congolense* and *Trypanosoma brucei brucei* (group D).

This study was scrutinized and approved by the Nigerian Institute for Trypanosomiasis Research Committee on Medical and Scientific Research Ethics. General care of the rats was provided in accordance with the Institutional Animal Care and Use Committee, as outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching.

Parasite

Trypanosome strains used were *T. congolense* (Sokoto/Wurno) and *T. b. brucei* (Kebbi/Ngaski). Both strains were isolated from cattle in Wurno Local Government Area Sokoto and Ngaski Local Government Area of Kebbi States respectively and cryo-preserved in liquid nitrogen at the Institute. The parasite strains were inoculated into donor uninfected Wistar rats intraperitoneally and maintained by repeated passaging. Parasitaemia was monitored by preparing a thin film of blood obtained from animal tail according to the method of (Woo, 1970).

Inoculation of Wistar rats with parasite

Blood from the infected donor Wistar rats (at peak parasitaemia) was collected and diluted with phosphate buffered saline (PBS). The number of parasites in the diluted blood was determined as

described (Herbert & Lumsden, 1976). Each rat in groups B, C and D was inoculated intraperitoneally with 0.1ml of blood containing approximately 1×10^3 trypanosomes as described (Abenga *et al.*, 2017). Each rat in group A was inoculated intraperitoneally with 0.1 ml of PBS (uninfected group) as placebo. Wistar rats in all the groups were observed daily for clinical signs, and for any changes in the body temperature and weights using a top-loading balance (ALISTONR AL730) and digital Precision Scale electronic balance (ATOMR-110C (Platinum) (Kelly, 1979) for 21 days post-infection (PI).

Daily rectal temperature ($^{\circ}\text{C}$) and packed cell volume (PCV) were taken every morning. The numbers of trypanosomes in the buffy coat were counted using the haematocrit centrifuge techniques (HCT) and a light microscope at $\times 40$ magnification (Woo, 1969 1970). Trypanosome species were identified by fixing thin blood smears in methyl alcohol and stained with 10% Giemsa solution (Woo, 1970). The slide was examined using $\times 100$ oil immersion objective.

Haematology

Blood obtained from the tail of infected rats was used for the daily estimation of parasitaemia as described (Herbert & Lumsden, 1976) and packed cell volume (PCV) by micro haematocrit method (Cheesbrough, 2000) and Haemoglobin (Hb) concentration by (Jain, 1986). At 21 days PI, blood was collected from the heart of surviving rats using a sterile 2ml syringe and used immediately for determination of total red blood cell (RBC) and total white blood cell (TWBC) counts by the hemocytometer method as described by Dacie & Lewis (1995), and platelets count as described by Kelly (1979). The blood smears were directly prepared and stained by Leishman's stain for differential white blood cell count (DWBC) by Battlement method (Houwen, 2001).

Statistical analysis

Data were analyzed using SPSS and Minitab packages. ANOVA and Duncan's multiple range tests were used to identify the cause of difference among the variables as described by Anere *et al.* (2009). The regression and correlation analyses were performed to determine the trend of the variables with PI period. The coefficient of determining R^2 , was used as a measure of the degree of variance in the variables as explained by Cohen & Cohen (1983).

Results

Clinical signs were observed between days 4 to 5 in groups B and C PI while group D developed clinical

signs between days 2 to 3 PI. There was a significant difference ($p < 0.05$) in the mean temperature of the infected groups (B, C and D) compared to the control group (A). The Duncan's LSD revealed that group D and C recorded higher significant difference in temperature (Table 1). The temperatures of groups B and D increased at the same pace with increase in parasitaemia (group B, $R^2 = 83.59\%$, group D, $R^2 = 83.50\%$, $p < 0.05$) while the temperature of group C had no significant increase with increase in temperature ($R^2 = 0.04\%$, $p > 0.05$) (Figure 1). All the Wistar rats in groups B and C started developing parasitaemia between days 5 to 6 PI, while group D developed parasitaemia between days 2 to 3 PI (Figure 2).

Peak parasitaemia occurred between days 14 to 15 for group B; group C had its peak between days 16 to 17 while group D had its peak between days 10 to 11. The parasitaemia reduced from day 17 till death for group C, group D from day 18 till death while group B reduced from days 14 to 17. Mortalities were recorded earlier in group C than in groups B and D. However, there was no significant difference between the parasitaemia of the infected groups, ($p > 0.05$) as presented in Table 1. There was a significant difference ($p < 0.05$) in the mean PCV of the infected groups (B, C and D) compared to the control group (A). The Duncan's LSD revealed that groups D and C recorded higher significant difference in PCV (Table 1). The PCV of the uninfected group (50.56 ± 02.42) almost doubled those of infected groups (36.88 ± 8.75 , 39.37 ± 7.65 and 38.34 ± 8.97) respectively (Figure 3). Group B exhibited the lowest PCV value, which declined about 15% between day 0 to 21 PI while other groups showed 8.9% and 5.9% respectively. Generally, there was significant change in mean PCV of the infected groups, ($p < 0.05$) compared to the control group. The initial parasitaemia and PCV correlated well from days 1 to 4, (Figure 4). At zero parasitaemia, the Wistar rats had normal PCV of 44.00 ± 7.81 to $54.26 \pm 4.32\%$ while the PCV significantly reduced with increasing parasitaemia from day 7 till death. The R^2 values of groups B, C and D were (87.0, 87.1 and 89.6%) respectively, showing significant ($p < 0.05$) trend with increase PI period of 21 days.

There was a significant difference ($p < 0.05$) in Hb concentration and MCV of the infected groups (B, C and D) compared to the control group (A). The Duncan's LSD revealed that group D and C recorded higher significant difference in MCV (Table 1). The

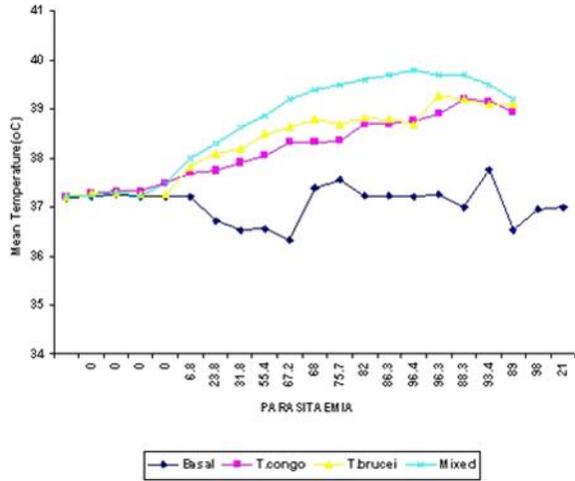


Figure 1: Relationship between mean temperature and parasitaemia of infected Wistar rats

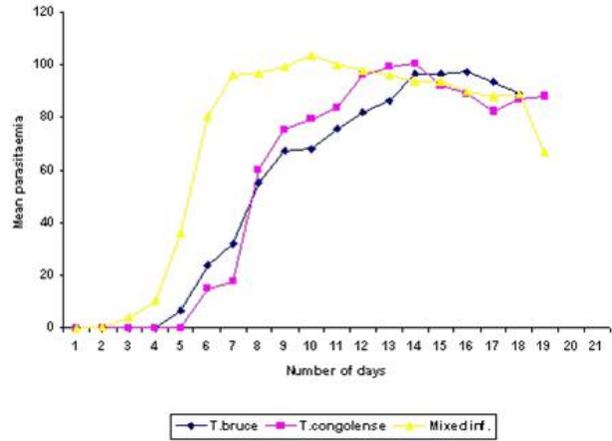


Figure 2: Mean parasitaemia of infected Wistar rats

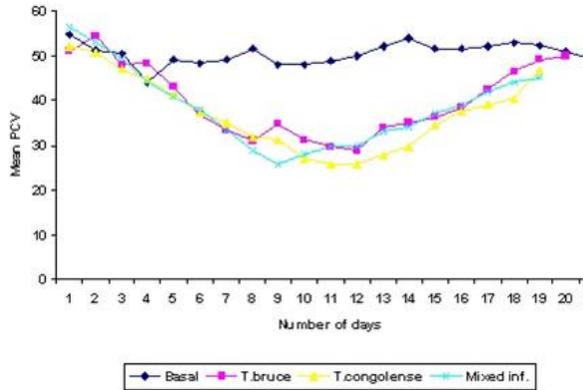


Figure 3: Relationship between PCV of control and infected Wistar rats

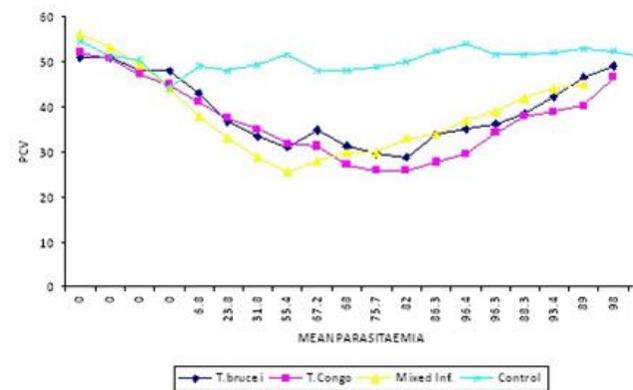


Figure 4: Relationship between parasitaemia (*T. b. brucei*) and PCV in Infected Wistar rats

Table 1: Average changes in weights, mean temperature, PCV, MCV, Hb and parasitaemia of the control and infected Wistar rats

Groups	W (g)	Mean temperature (°C)	Mean PCV (%)	MCV (fl)	Hb (g/dl)	Mean Parasitaemia
A	240	37.18 ± 0.36	50.56 ± 2.42	47.25 ± 0.64	16.85 ± 1.39	-
B	230	38.10 ± 0.76	36.88 ± 8.75	35.12 ± 0.28	12.29 ± 2.43	76.12 ± 27.32
C	220	38.13 ± 0.69	39.37 ± 7.65	52.49 ± 0.63	13.12 ± 1.01	69.24 ± 29.47
D	210	38.71 ± 1.00	38.34 ± 8.97	52.52 ± 0.71	12.78 ± 1.91	74.62 ± 35.71

W: Weight, PCV: Packed Cell Volume, MCV: Mean Corpuscular Volume, Hb: Haemoglobin Concentration

Table 2: Average changes in RBC, total WBC and platelet counts of control and trypanosome infected Wistar rats at 21 days post-infection

Parameters	Group A N = 15	Group B N = 10	Group C N = 10	Group D N = 10
RBC (×10 ⁶ /μL)	10.7 ± 2.0	*(10.9 ± 2.8) 10.5 ± 3.8	(9.8 ± 3.8) 7.5 ± 4.3	(7.9 ± 3.6) 7.3 ± 4.5
WBC (×10 ³ /μL)	16.8 ± 5.6	(16.5 ± 5.3) 13.2 ± 5.8	(16.2 ± 4.6) 12.1 ± 5.3	(14.91 ± 5.5) 6.8 ± 1.3
Platelet Counts (×10 ³ /μL)	461 ± 0.2	(481.5 ± 3.3) 396.5 ± 1.8	(426.5 ± 2.9) 279.2 ± 5.3	(412.3 ± 3.6) 144.4 ± 3.9

*: In parenthesis are pre-infection values

total WBC counts also declined most in group D ($p < 0.05$) while the least was recorded in group B (Table 2). A similar trend of decrease in the mean platelets counts was observed in group D showing highest decrease in platelets counts at the end of 21 days PI (Table 2). The absolute differential WBC counts of the experimentally infected Wistar rats are presented in Table 3. The decrease in lymphocyte and neutrophil counts were also highest in group D than groups B and C. There was higher marked increase in monocytes counts in group D ($p < 0.05$). Likewise, eosinophilia occurred only in group D. A total of six (6) Wistar rats died within the last week of the experiment in group D while three (3) mortalities each were recorded in the other two groups (B and C). There was no significant difference in body weight in all the infected groups (B, C and D) when compared to the control group.

Discussion

Anemia is a major clinical feature of African trypanosomiasis in man and animals (Anosa, 1988a; Ogunsanmi *et al.*, 1994). It has been established that the measurement of anemia gives a reliable indication of the disease status and productive performance of trypanosome infected animals (Ekanem *et al.*, 2005; Ekanem *et al.*, 2006). Anemia, indicated by a significant drop in PCV, Hb and MCV of infected Wistar rats (Table 1) is in agreement with earlier reports (Anosa, 1988b; Igbokwe *et al.*, 1994; Ekanem *et al.*, 2008; Faremi & Ekanem, 2011) in trypanosome infected animals. The low PCV observed in the infected groups may be as a result of acute hemolysis due to growing parasitaemia infection. Previous studies have shown that infection with trypanosomes resulted in increased susceptibility of red blood cell membrane to oxidative damage probably as a result of depletion of glutathione on the surface of the red blood cell (Igbokwe *et al.*, 1994, 1996; Taiwo *et al.*, 2003; Akanji *et al.*, 2009). Severity of anemia usually reflects the intensity and duration of parasitaemia. A number of reports (Ogunsanmi & Taiwo, 2001; Umar *et al.*, 2007; Ekanem & Yusuf, 2008; Saleh *et al.*, 2009) have also ascribed acute anemia in

trypanosomiasis to proliferating parasites. The lower counts of TWBC, lymphocytes and neutrophils observed in the infected groups may be attributed to the immunosuppressive actions of trypanosome infection (Abubakar *et al.*, 2005; Ekanem & Yusuf, 2008). Leucocytosis which may be due to lymphocytosis have been implicated in trypanosomiasis and these conditions are usually as a result of 'wax and wear' syndrome on the animal immune system caused by the ever-changing variable surface glycoprotein of the infecting trypanosomes (Abubakar *et al.*, 2005). This study showed that infected Wistar rats developed anemia as characterized by significant reduction in PCV values especially between days 7 and 19 PI when they had to die due to non-treatment. The co-infected group showed up early parasitaemia from day 2 PI, followed by *T. b. brucei* group.

The mean PCV of the infected groups did not differ significantly ($p > 0.05$). However, the mean PCV of the infected groups were significantly different when compared with the control group ($p < 0.05$). The initial signs of parasitaemia were intermittent pyrexia, lethargy, isolation, reduced feed in-take and rough hair coat later followed by anorexia and recumbency. The relationship between the parasitaemia and PCV (Figure 4) was determined to identify the trend of PCV during the period of infection. From days 1 to 4 PI, the PCV was high when the parasitaemia did not yet occur. There was 81.7% decrease in PCV from days 5 to 6 PI with 60.8% increase in parasitaemia. Throughout the period of infection, there was a general significant decrease ($p < 0.05$) in PCV with increasing parasitaemia. As the parasitaemia progressively increased by 90.9% from days 7 to 8 PI, the PCV significantly reduced. These present findings agree with similar works in which a significant fall in PCV of goats infected with *T. vivax* was reported by (Anosa & Isoun, 1977; Masake, 1980; Murray & Dexter, 1988; Sekoni *et al.*, 1990). The parasitaemia load of *T. b. brucei* (69.24 ± 29.47), *T. congolense* (76.12 ± 27.32) and co-infected group (74.62 ± 35.71) respectively are not significantly different on day 21 PI ($p > 0.05$). The degree of relationship between

Table 3: Average changes in absolute differential WBC ($\times 10^3/\mu\text{L}$ of blood) of control and trypanosome infected Wistar rats at 21 days post-infection

Parameters ($\times 10^3/\mu\text{L}$)	Group A N = 15	Group B N = 10	Group C N = 10	Group D N = 10
Neutrophils	4.2 ± 2.6	*(421 ± 2.7) 3.2 ± 2.5	(4.5 ± 2.9) 3.0 ± 3.5	(2.8 ± 2.0) 2.6 ± 3.2
Lymphocytes	13.2 ± 3.1	(13.2 ± 4.3) 10.9 ± 0.8	(12.9 ± 5.6) 7.8 ± 1.3	(13.3 ± 4.8) 6.1 ± 0.2
Monocytes	0.4 ± 4.1	(0.2 ± 3.5) 0.2 ± 2.5	(0.4 ± 1.0) 0.4 ± 0.9	(0.34 ± 4.6) 0.5 ± 3.4
Eosinophils	0.0 ± 0.0	(0.0 ± 0.0) 0.0 ± 0.0	(0.0 ± 0.0) 0.0 ± 0.0	(0.0 ± 0.0) 0.6 ± 0.5

*: In parenthesis are pre-infection values

mean parasitaemia and *T. b. brucei* infected group was ($R^2 = 0.8707$). This reveals a significant 87.1% rise in parasitaemia with increasing number of days of PI ($p < 0.05$) before the decline. Likewise, the pattern of *T. congolense* ($R^2 = 0.870$) reveals a significant increase in mean parasitaemia with increase in number of days PI ($p < 0.05$) before decline. The co-infection with *T. b. brucei* and *T. congolense* showed much more significant increase in mean parasitaemia in relation to the single infections ($R^2 = 0.896$, $p < 0.05$).

The temperature of the infected groups doubled that of control. However, the co-infection group exhibited higher wave of temperature and maintained their temperature within 39.54°C till death. All the infected groups developed persistent fever within range of 38.05 – 39.80°C till death. It was observed that the mean temperature of the four variables which are (37.18 ± 0.36 , 38.10 ± 0.76 , 38.13 ± 0.69 and 38.71 ± 1.00), respectively, under consideration exhibited a significant increase from the day of post-infection ($p < 0.05$). This increase was generally observed from days 4 and 5 to 17 PI when the temperature began to decline. The Duncan's LSD revealed that co-infection and *T. b. brucei* caused the high significant difference.

This study showed that Wistar rats experimentally infected with *T. b. brucei*, *T. congolense* and co-infection developed acute form of trypanosomiasis which is associated with high rectal temperature, decrease in body weight, anemia, low PCV and finally death. The severity of the disease was more in co-infection and *T. b. brucei* infected Wistar rats.

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Conflicts of Interest

The authors declare no conflict of interest.

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