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HEMATOLOGICAL DERANGEMENT PATTERNS IN NIGERIAN DOGS INFECTED WITH *TRYPANOSOMA BRUCEI*: A SIMPLE PROTOTYPE FOR ASSESSING TOLERANCE TO TRYPANOSOME INFECTIONS IN ANIMALS

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RUNNING TITLE: HEMATOLOGY OF *T. BRUCEI* INFECTED DOGS

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ABSTRACT

The haematology of Nigerian local puppies experimentally infected with the Federe strain of *Trypanosoma brucei* was studied in a total of six 9-weeks old puppies born to two local bitches. Four were randomly selected and inoculated with about 0.8×10^6 of *T. brucei* subcutaneously and the remaining two served as the uninfected control. The parasitaemia was monitored daily using wet mount microscopy. The packed cell volume (PCV), red blood cell (RBC) counts, total and differential white blood cell (WBC) counts and rates of both red blood cell and white blood cell loss per day and per parasitaemia log equivalent value (LEV) were monitored twice in a week. Parasitaemia was detected in the infected group four days after infection which was followed by an acute disease course, though with low fatality rate in the dogs. The anemia was characterized by a fluctuating PCV decrease from the pre-infection value of $29.5 \pm 4.5\%$ and $15.3 \pm 3.3\%$ at two weeks after infection when one of the dogs died. There was a mild decrease in the overall erythrocyte values which was attributable to trypanotolerance in the local breed of dogs. The post infection hematological derangement pattern was characterized by an overall post-infection RBC count drop of $1.92 \pm 0.23 (\times 10^{12}/\mu\text{l})$ (39.0%), mean daily drop of $0.07 \pm 0.05 (\times 10^{12}/\mu\text{l})$ and an overall drop per LEV of $0.69 (\times 10^{12}/\mu\text{l})$. The overall mean post-infection total WBC count drop was $0.61 \pm 0.15 (\times 10^9/\mu\text{l})$ (43.6%) with a mean daily drop of $0.02 \pm 0.14 (\times 10^9/\mu\text{l})$, and an overall drop per LEV of $0.22 \pm 0.44 (\times 10^9/\mu\text{l})$. There was an overall higher post infection leukocyte drop compared to erythrocyte. The result poses fundamental research questions on the likelihood of differential surface sialic acid contents of erythrocytes and leukocytes and the possible roles of trypanosome sialidase in creating this difference as well as enhancing pathogenesis of leucopenia in the dogs. It was concluded that the patterns of hematological derangements demonstrated as erythrocyte and leukocyte drop (loss) rates and drop per parasitaemia Log Equivalent Values could serve as a prototype for comparing susceptibility to animal and human *T. brucei* infections and, other trypanosome species.

Key words: Federe, *Trypanosoma brucei*, haematology, derangement, patterns.

MODELES DE DERANGEMENT HAEMATOLOGIQUES CHEZ LES CHIENS NIGERIANES INFECTES PAR *TRYPANOSOMA BRUCEI* : UN PROTOTYPE SIMPLE POUR L'EVALUATION DE LA TOLERANCE AUX INFECTIONS TRYPANOSOME CHEZ LES ANIMAUX.

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TITRE COURANT : HEMATOLOGIES DES CHIENS INFECTES PAR *T. BRUCEI*

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RÉSUMÉ

L'hématologie de chiots locales nigérianes infectées expérimentalement avec la souche de FEDERE de *Trypanosoma brucei* a été étudiée dans un total de 6 - 9 semaines chiots nées à deux chiennes locales. Quatre ont été choisis au hasard et inoculés avec

environ 0.8×10^6 de *T. brucei* sous-cutanée et les deux autres ont servi de témoin non infecté. La parasitemie a été contrôlée quotidiennement en utilisant la microscopie humide montage. L'hématocrite (PCV), numération globulaires rouges (RBC), numération et différentiel des globules blancs (WBC) et les taux de la perte des globules rouges et globules blancs par jour et par la parasitemie connecter valeurs équivalentes ont été contrôlées deux fois par semaine. La parasitemie a été détectée dans le groupe infecté quatre jours après infection à été suivie par une évolution de la maladie aiguë, mais avec faible taux de mortalité chez les chiens. L'anémie a été caractérisée par une diminution variante de PCV de la valeur pré-infection de $29,5 \pm 4,5\%$ et $15,3 \pm 3,3\%$ à deux semaines après l'infection quand l'un des chiens est mort. Il y avait une légère diminution de l'ensemble des valeurs érythrocytes ce qui est attribuable à la trypanotolérance dans la race locale des chiens. La modèle de dérangement hématologique après l'infection a été caractérisée un ensemble de la baisse des numérations globules rouges de $1,92 \pm 0,23$ ($\times 10^{12}/\mu\text{l}$) (39,0%), la baisse moyenne quotidienne de $0,07 \pm 0,05$ ($\times 10^{12}/\mu\text{l}$) et une baisse globale par LEV de $0,69$ ($\times 10^{12}/\mu\text{l}$). La baisse moyenne globale totale après infection du WBC a été $0,61 \pm 0,15$ ($\times 10^9/\mu\text{l}$) (43,6%) avec une baisse moyenne quotidienne de $0,02 \pm 0,14$ ($\times 10^9/\mu\text{l}$), et une baisse globale par LEV de $0,22 \pm 0,44$ ($\times 10^9/\mu\text{l}$). Il avait une plus baisse leucocytes globales après infection par rapport aux érythrocytes. Le résultat pose des questions de recherche fondamentale sur la probabilité de surface différentielle de rôles possibles de sialidase trypanosomes dans la création de cette différence ainsi que l'amélioration de la pathogenèse de la leucopénie chez les chiens. Il a été conclu que les tendances des dérangements hématologiques démontrées comme le taux de la baisse (perte) d'érythrocyte et la baisse par la parasitemie connecter valeur équivalente pourrait servir de prototype pour comparer la sensibilité aux infections *T. brucei* chez les animaux et les humaines et d'autres espèces de trypanosomes.

Mots Clés : FEDERE, *Trypanosoma brucei*, hématologie, dérangement, tendances

INTRODUCTION

Pancytopenia is one of the consistent pathological features of trypanosomiasis in man and animals whose severity is often dependent on parasite and host factors (1). Host's ability to control parasitaemia and development of anemia had been identified as hallmarks of tolerance to trypanosomiasis (2, 3). The African trypanosomiasis arising from animal infective *Trypanosoma brucei* and the human infective sub species, *T. brucei gambiense* and *T. b. rhodesiense* are recognized as stage dependent pathologies, characterized by the early and late stage syndromes in infected hosts (4, 5) and are characterized by anemia. Its socio-economic impact arises principally from the characteristic wasting nature of the disease as it causes severe losses in production due to poor growth, weight loss, low milk yield, reduced capacity for work, infertility and abortion (6). The disease had been recognized as a major barrier to the development of African continent (6).

The disease in dogs, arising from *T. brucei* is generally acute and fatal (7, 8) and resulting to fever, anemia, myocarditis, corneal opacity, marked body edema, and central nervous system disturbances. The *T. brucei* group of trypanosomes generally localize in solid tissues of various organs causing extensive degenerative disease and anemia (4). The major processes associated with pathogenesis of *T. brucei* infection were described by (7). These include; extravasation of parasites into body tissues leading to severe lesions, vasculitis, increased vascular permeability and thrombosis, direct toxic damage caused by biological active substance produced by dead or living trypanosomes and increased erythrophagocytosis, resulting to excess destruction of erythrocytes.

In the last decade, canine trypanosomiasis arising from tse-tse transmitted *T. brucei* and *T. congolense* had been identified as an important threat to hunting dogs (9) as well as exotic and local city dogs (10, 11), in Nigeria. Due to the absence of sustainable surveillance, the exact prevalence situation and socio-economic impacts of the disease in Nigeria are not well known. Furthermore, the trypanosusceptibility status of most African dog breeds, against the backdrop of emerging new trypanosome strains, is not well known. We reported mild anemia in *Trypanosoma congolense* infected Nigeria puppies characterized by a slight drop in packed cell volume (PCV), hemoglobin and red blood cell counts which did not occur until the last half of 8 weeks post infection period which was attributable to trypanotolerance in the local breed of dogs (12). The Federe strain of *T. brucei* is a newly emerging and highly pathogenic trypanosome, often producing acute disease. The drop in number of new cases of *T. b. gambiense* sleeping sickness in Nigeria which share etiological, structural and host properties with animal infective *T. brucei* coupled with emergence of this strain has generated research interest in the Federe strain of *T. brucei* (13 - 15).

The aim of this study was to investigate the hematological derangement patterns as a basis for assessing the susceptibility of young Nigerian young dogs to the Federe and other strains of *Trypanosoma brucei*. This is with the view to providing additional hematological data needed for the characterization of this strain and assessing of tolerance to *Trypanosoma brucei* infections in animals and man.

MATERIALS AND METHODS

Experimental Animals

A total of six-9 weeks old local puppies of mixed sexes weighing 3.0 to 4.0 kilograms body mass were used. The six puppies were whelped by four different mothers in the same village near the Federal University of Agriculture, Makurdi, Nigeria. The bitches were local dogs and mounted by other local male dogs from within the area. The puppies were acclimatized for one week at the Veterinary Teaching Hospital, Federal University of Agriculture, Makurdi before use. During this period they were dewormed with Albendazole suspension, 25mg/kg against round worms, tapeworms and hook worms. The puppies were fed with pap made from millet (3/4) and fish (1/4), rice, yams, beans, milk, fish, and occasionally biscuit bones, while water was provided *ad libitum*.

Trypanosome specie

The trypanosome specie used was *T. brucei* Federe strain, obtained from the Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria. The parasite was isolated from cattle, cryopreserved in liquid Nitrogen and sub-passaged into the donor albino rats prior to use.

Experimental Design

The six dogs were randomly selected and tagged numbers 01, 02, 03, 04, 05, and 06. The numbers 01, 03, 04 and 06 constituted the infected group and were

Post ID = Mean Pre-infection Value(MPreIV) – Mean Post-infection Value(MPostIV).

In the course of the experiment, terminally sick animals were given painless chemical euthanasia as described by Severidt *et al.*, (19) for necropsy and the remaining infected dogs given curative treatment with diminazene aceturate at the end of the study.

The data collected in this study was statistically analyzed to test significant difference using the student t-test (20) and the P value of less than 0.05 was regarded as significant.

inoculated with 0.8×10^6 of the parasites via the subcutaneous route. The remaining two dogs served as the uninfected control group. Parasites for inoculation were estimated as described by Lumsden *et al.*; (16). Daily parasitaemia was estimated from wet mount preparation made through ear puncture. However, the packed cell volume (PCV) was determined twice in a week.

Blood Collection

Blood for hematology was obtained through venipuncture of the cephalic vein using 23 gauge hypodermic needles and 5ml syringes. The blood was collected into ethylene diamine tetraacetate (EDTA) bottles prior to use. A total of 2ml of blood was collected into each EDTA bottles.

Hematological Techniques

Commercially heparinized capillary tubes were $\frac{3}{4}$ filled with blood, sealed on one end with plasticine, and centrifuged for 5 minutes in a microhaematocrit centrifuge at 12,000 rpm to determine the PCV. The packed cell volume was read off the microhaematocrit reader (17, 18). The erythrocytes and leukocytes were enumerated using the Neubaur haemocytometer (18). Thin blood smears were made and stained with Giemsa stain (18). 100 cells were counted and differentiated per slide. The patterns of hematological derangements were determined arithmetically using the Post Infection Drop (PostID), Daily Drop (DD), Overall Percentage Drop(OPD) and Drop Per Parasitaemia LEV(DPPL) in PCV, RBC and the total WBC count values of the infected group as follows:

$$DD = \frac{\text{PostID}}{\text{Number of Post-infection Days}}$$

$$OPD = \frac{\text{PostID}}{\text{MPreIV}} \times 100$$

$$DPPL = \frac{\text{MPostID}}{\text{Mean Parasitaemia LEV}}$$

RESULTS

The Nigerian local dogs infected with *T. brucei* became parasitaemic 4 days post-infection (PI). The parasitaemic pattern of the infection is shown on Fig. 1. Between the pre-infection (Day 0) and 29 P.I., the parasitaemia attained peak values four times. These were on days 6, 11, 18 and 25 P.I. with mean log equivalent values of 4.30 ± 0.00 , 4.10 ± 0.40 , 3.50 ± 0.70 , and 4.30 ± 0.00 respectively. Following parasitaemia, the dogs exhibited pyrexia, slight weight loss and anemia. The pre-infection mean PCV of the infected

group was 29.5±4.5 (%) while that of the control group was 26±8.5 (%) (Fig.2). After infection, the PCV did not differ significantly until day 8 PI when it

dropped to 18.5±5.8(%) and then to 15.3±3.3(%) on day 11 (P<0.05), followed later by apparent increase to 17.7±5.5(%) when the experiment was terminated.

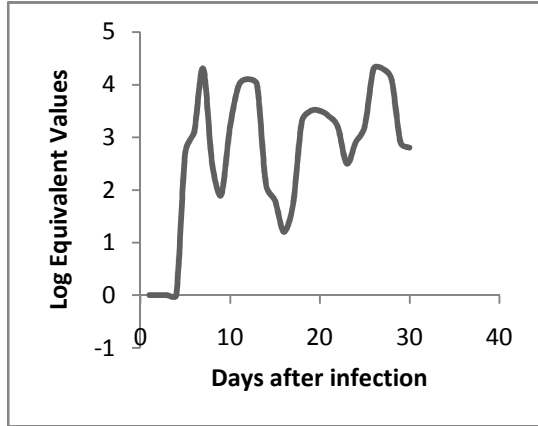


Fig 1. Daily parasitaemia of the *T. brucei*-infected dogs

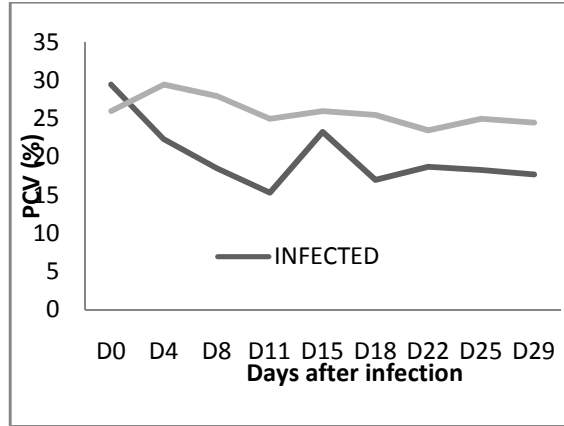


Fig 2. Mean variation in daily PCV of *T. brucei*-infected dogs

This translated to an overall mean post infection drop of 10.6(%), daily drop of 0.37(%) and an overall drop of 3.82% per LEV (Table 1). The mean PCV of control animals fluctuated within normal range. The pre-infection mean RBC count of infected group was 4.92±1.0 (x10¹²/μl) while that of the control group was 4.33±0.21(x10¹²/μl) (Fig.3). After infection, the RBC count of the infected group dropped progressively to 2.55±0.3 (x 10¹²/μl) on day 11PI and thereafter

fluctuated to 2.95±0.47(x10¹²/μl) (P<0.05) on the 29th day before termination of observations. This translated to post-infection value of 3.00±0.13(x 10¹²/μl), post-infection drop of 1.92±0.23(x 10¹²/μl) (39.0%), daily drop of 0.07±0.05 (x 10¹²/μl) and drop per LEV of 0.69 (x 10¹²/μl) (Table 1). The RBC values of the infected group only fluctuated within normal range.

TABLE 1: BLOOD CELL LOSS RATES IN *T.BRUCI*-INFECTED YOUNG DOGS.

Blood cell parameters	Packed Cell Volume(%)	RBC (x10 ¹² /L)	Total WBC (x10 ⁹ /L)
Mean Pre infection Value	29.5±4.5	4.92±0.44	1.40±0.44
Mean Post Infection Value	18.9±4.5	3.00±0.25	0.79±0.15
Post Infection Drop(%)	10.6(36.0)	1.92(39.0)	0.61(43.6)
Daily Drop	0.37	0.07	0.02
Drop per Parasitaemic LEV	3.82	0.69	0.22

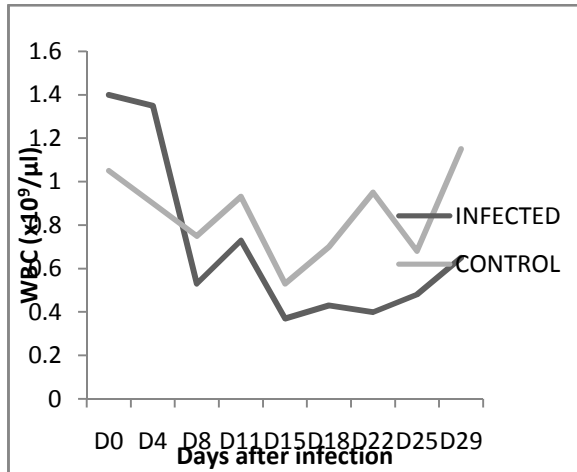
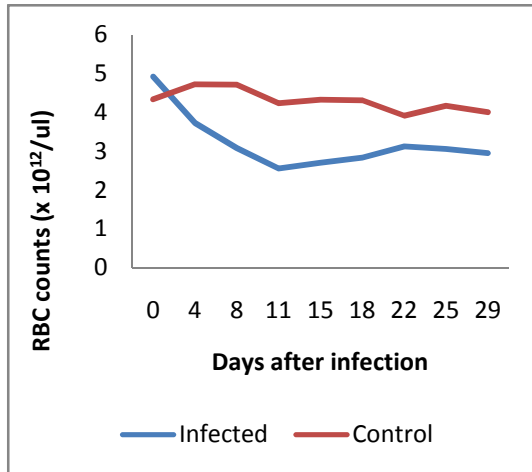


Fig 3: Variation in mean RBC counts in *T. brucei*-infected dogs Fig4: Variation in mean daily total WBC of *T. brucei*-infected dogs

The mean total WBC count of the dogs before infection was $1.4 \pm 0.44 (x10^9/\mu l)$, and $1.05 \pm 0.77 (x10^9/\mu l)$ for the infected and control groups respectively. After infection the total WBC counts dropped to $1.35 \pm 0.44 (x10^9/\mu l)$ on day 4 ($P < 0.05$) and plunged further to $0.53 \pm 0.17 (x10^9/\mu l)$ on day 8 ($P < 0.05$) with subsequent fluctuating values slightly lower than those of the control group ($P > 0.05$) until day 29 (Fig.4). This translated to an overall mean post-infection value of $0.79 \pm 0.25 (x10^9/\mu l)$, post-infection drop of $0.61 \pm 0.15 (x10^9/\mu l)$ (43.6%), daily drop of $0.02 \pm 0.14 (x10^9/\mu l)$, and drop per LEV of $0.22 \pm 0.44 (x10^9/\mu l)$. The Control values fluctuated between $1.15 \pm 0.28 (x10^9/\mu l)$, and $0.53 \pm 0.04 (x10^9/\mu l)$, within this period.

The absolute differential count values of *T. brucei* infected and control dogs is shown on Table 2. The lymphocyte values of infected and control dogs on Day 0 was $0.57 \pm 0.15 (x10^9/\mu l)$ and $0.56 \pm 0.09 (x10^9/\mu l)$ respectively. After infection, there was an apparent increase in the infected group to $0.72 \pm 0.10 (x10^9/\mu l)$ ($P > 0.05$) on Day 4 but thereafter plummeted to

fluctuating values below those of Control group until Day 29 ($P > 0.05$). The neutrophil values of infected and control groups were $0.44 \pm 0.07 (x10^9/\mu l)$ and $0.31 \pm 0.09 (x10^9/\mu l)$ respectively. After infection, the neutrophil values of infected group dropped progressively to $0.21 \pm 0.06 (x10^9/\mu l)$, $0.18 \pm 0.07 (x10^9/\mu l)$, and $0.10 \pm 0.06 (x10^9/\mu l)$ on Days 8, 11 and 15 respectively ($P < 0.05$), but thereafter showed apparent fluctuating improvement to $0.38 \pm 0.09 (x10^9/\mu l)$ on Day 29. After infection, the eosinophil counts in the infected group increased from the pre-infection value of $0.02 \pm 0.00 (x10^9/\mu l)$ to $0.05 \pm 0.01 (x10^9/\mu l)$, on Day 4 and to $0.03 \pm 0.00 (x10^9/\mu l)$ on Days 18 and 25 respectively ($P < 0.05$). The Eosinophil values of Control group ranged from 0.00 ± 0.00 to $0.02 \pm 0.00 (x10^9/\mu l)$ within the period. The post-infection monocyte counts increased from the pre-infection value of $0.02 \pm 0.00 (x10^9/\mu l)$ to $0.04 \pm 0.01 (x10^9/\mu l)$ and $0.05 \pm 0.01 (x10^9/\mu l)$ on Days 11 and 15 respectively which was statistically significant ($P < 0.05$). Values of the Control group ranged from 0.03 ± 0.00 to $0.00 \pm 0.00 (x10^9/\mu l)$ within the period.

TABLE 2: MEAN DIFFERENTIAL ABSOLUTE LEUKOCYTE VALUES OF YOUNG DOGS INFECTED WITH *T.BRUCI*.

Leukocyte Parameter	0	4	8	11	15	18	25	27
	Days of Parasitaemia							
Lymphocytes (x10 ⁹ /L)	0.57±0.15 (0.56±0.09) ^x	0.72±0.10 (0.69±0.15)	0.24±0.07 (0.25±0.15)	0.48±0.11 (0.30±0.09)	0.20±0.05 (0.25±0.06)	0.19±0.07 (0.28±0.09)	0.24±0.07 (0.39±0.10)	0.22±0.09 (0.29±0.07)
Neutrophils (x10 ⁹ /L)	0.44±0.07 (0.31±0.09)	0.27±0.10 (0.34±0.08)	0.21±0.06 (0.49±0.10)	0.18±0.07 (0.59±0.09)	0.10±0.06 (0.39±0.09)	0.20±0.04 (0.39±0.08)	0.19±0.06 (0.34±0.10)	0.38±0.08 (0.36±0.09)
Eosinophils(x10 ⁹ /L)	0.02±0.00 (0.02±0.00)	0.05±0.01 (0.02±0.00)	0.02±0.00 (0.00±0.00)	0.02±0.00 (0.00±0.00)	0.02±0.00 (0.00±0.00)	0.03±0.00 (0.01±0.00)	0.03±0.00 (0.01±0.00)	0.02±0.00 (0.02±0.00)
Monocyte (x10 ⁹ /L)	0.02±0.00 (0.01±0.00)	0.11±0.09 (0.01±0.00)	0.02±0.00 (0.00±0.00)	0.04±0.01 (0.03±0.00)	0.05±0.01 (0.01±0.00)	0.03±0.00 (0.03±0.01)	0.01±0.00 (0.02±0.00)	0.03±0.00 (0.02±0.00)
Besophils (x10 ⁹ /L)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00±0.00)

Note : x- control values in brackets.

DISCUSSION

The Federe strain of *T. brucei* used in this study was pathogenic to the Nigerian local puppies even though they were expected to exhibit tolerance to the infection as a consequence of genetically determined phenomenon(3) and passive maternal antibodies(21,22). This is at variance with our observations in an earlier study on Nigerian local puppies infected with *Trypanosoma congolense* (23) in which the young dogs did not develop anaemia until 4 weeks later. After infection, parasitaemia developed 4days later. Although this is consistent with most experimental infections due to *T. brucei* sub species (4, 24), the parasitaemic peaks attained in *T. brucei*-infected puppies occurred much earlier and with shorter intervals compared to the observations in *T. brucei* infected ewes (25). This points the higher pathogenicity antigenic properties of this strain of trypanosome resulting to severer disease course in the dogs.

The erythrocyte values in *T. brucei* infected dogs varied with the level of parasitaemia as they returned to or near pre-infection values when parasitaemia waves were low and then plumped when parasitaemia was high. The pre-infection PCV was 29.5±4.5(%) but by the 4th day P.I. which coincided with parasitaemia onset, dropped to 22.3±4.5(%) and then further to 15.3±3.3(%) by the 11th day, two days after which puppy No.4 died. The PCV fluctuated with parasitaemia until the 29th day when the study was terminated. In the previous work of Abenga *et al*; (12), *T. congolense* infected Nigerian puppies died at 15% PCV while others were unable to walk. The low PCV values occurring concomitantly with the peak parasitaemia in this study may be attributed to the hemolysis arising from the activities of the parasite (1). Although several factors such as, immunological mechanisms, hemolytic factors, adherence of trypanosomes to red blood cells, RBC fragmentation, high body temperature and hyperactivity of the mononuclear phagocyte system (1, 26) had been associated with events leading to anemia in

trypanosomosis. Advances in research on sialic acids had given credence to the fact that sialidases produced by trypanosomes play leading roles in hemolysis of erythrocytes and pathogenesis of trypanosome induced anemia through cleavage of erythrocyte surface sialic acid thereby leading to RBC senescence and destruction through erythrophagocytosis and development of anemia (27, 28). This was demonstrated by increase in serum sialic acid concentration following cleavage of erythrocyte surface sialic acid and subsequent development of anemia in *T. vivax* infected cattle (27). The slight recovery in erythrocyte values observed between times of high parasitaemia was probably due to compensatory erythropoietic hyperplasia which is often evidenced by reticulocytosis, normoblastaemia, macrocytosis and erythroid hyperplasia of the bone marrow (1).

The hematological derangement patterns are seldom reported in African trypanosomiasis even though they have relevance in assessing sialidase activities of infecting trypanosomes and trypanotolerance. Earlier works (29, 30) had demonstrated that erythrocyte surface sialic acid concentration of trypanotolerant N'dama breed of cattle were five times higher than that of trypanosusceptible Zebu breed. In this study, the overall post infection drop in the PCV of the infected puppies was 36.0% with average daily drop of 0.37% and an overall drop of 3.82% per parasitaemia LEV. The overall post infection percentage drop in RBC counts was 19.8% with an average daily drop of 0.02 ($\times 10^{12}/\mu\text{l}$) and an overall drop of 0.20 ($\times 10^{12}/\mu\text{l}$) per parasitaemia LEV. These drop rates logically represent a measure of sialidase activities of the infecting trypanosome which is specific. Neutropenia is believed to arise from bone marrow granulocyte hypoplasia as a result of significant depression of precursor cells in early part of trypanosome infection (1), as well as phagocytosis of these cells and their precursors in the bone marrow and elsewhere (33), initial lymphocytosis followed later by lymphocytopenia, is believed to result from trypanosome antigenic challenge leading to an increased proliferation of immunocompetent cells into antibody and or lymphokine producing cells(34) but followed later by the depletion of the earlier hyperplastic lymphoid follicles and germinal centers, resulting to ultimate lymphopenia (35). This phenomenon may have played out in the *T. brucei* infected dogs. Eosinophilia and monocytosis are common features of African trypanosomiasis. Monocytosis had been reported to be matched by proliferation of macrophages in several tissues in trypanosome infected animals whose proliferation and activation are believed to be stimulated by

dependent. This is consistent with responsive anemia in African trypanosomiasis (1, 31).

Although decreases in the total WBC counts of infected dogs did not differ significantly from those of the control group ($P>0.05$), they were observable. This differs widely from our observations on the same parasite strain in pigs leading to overwhelming lymphocytic leucocytosis throughout the observation period (13). The percentage overall mean post infection total WBC drop in the dogs was 43.6% with the average mean daily drop of 0.02 ($\times 10^9/\mu\text{l}$) and overall drop per parasitaemic LEV of 0.20 ($\times 10^9/\mu\text{l}$) (Table 1). Although leucopenia occurs in experimental and naturally occurring *T. brucei* infections (Anosa 1988), leucocytosis frequently occurs(13, 32) depending on parasite antigenic properties. Leucopenia observed in the infected puppies may have arisen from massive peripheral utilization, phagocytosis in the bone marrow and other organs such as liver and spleen as well as general depression of granulopoiesis which had been identified as some of the common causes of leucopenia in African trypanosomiasis (8). Although literature on the role trypanosome sialidase activities in the pathophysiology of leucopenia in trypanosomiasis is lacking, it suffices to assume, their roles here may be similar to those earlier described(27, 28), thereby leading to the cleavage of leukocyte surface sialic acids, senescence and phagocytosis by macrophages in tissues. This being the case, rates of leukocyte loss observed in the *T. brucei* infected dogs may be a reflection of sialidase antigenic property of the parasite strain. This was characterized by, a sporadic lymphocytosis on Day 4 and then lymphopenia, neutropenia, eosinophilia and monocytosis. Whereas increased demands to remove particulate matter, including trypanosomes, red blood cells, leukocytes and dead tissue cells (1). *T. brucei* being a tissue invasive parasite is therefore likely to generate monocyte in circulation as demonstrated in this study. We (36, 37) had earlier demonstrated monocytosis in *T. brucei gambiense* infection of rabbits and vervet monkeys respectively. Eosinophilia on the other hand is a response to massive inflammation in tissues (38) in the *T. brucei* infected puppies.

On the whole, the overall post infection percentage drop in leukocyte values was slightly higher than those of erythrocytes. This suggests that leukocyte loss outweighed erythrocyte loss in *T. brucei* infected young dogs, and that, immunosuppression may be one of the vital antigenic properties that has placed the Federe strain above other strains of *T. brucei* in Nigeria in terms of pathogenicity. Although the trypanosome sialidase and sialyltransferase activities

were not investigated here, since it has been speculated that resialylation (putting back of sialic acids) of erythrocyte surface by the enzyme Sialyltransferase occurs in *T. brucei* infected hosts, thereby resulting to self-cure from anemia (27), it suffices to assume this phenomenon was responsible for the relatively lower post infection percentage drop in the erythrocyte values of the puppies. This being the case, this study poses four important research questions; firstly, what is the leukocyte surface sialic acid concentration in comparison to that of erythrocytes?, secondly, do sialidases play any roles in the pathogenesis of leucopenia in African trypanosomiasis?, thirdly is there any sialyl transferase preferential activities that cause self-cure in *T. brucei* induced anaemia but not leucopenia?, and fourthly, are there leukocyte surface molecules that antagonize resialylation in trypanosome infections?

It is concluded that *Trypanosoma brucei* caused anaemia, which began in the first week post infection but fluctuated throughout the observation period,

signifying the susceptibility of the young local dogs to the infection. The overall higher post infection leukocyte drop or loss compared to erythrocyte which raises fundamental questions on the differential surface sialic acid content of erythrocytes and leukocytes and roles played by trypanosome sialidase in creating the difference. The patterns of haematological derangement demonstrated as erythrocyte and leukocyte drop (loss) rates and drop per parasitaemia Log Equivalent Values could serve as simple means of comparing susceptibility to *T. brucei* and other trypanosome species. Trypanotolerance would in this case be expressed as lesser numbers of erythrocyte or leukocyte loss per parasitaemia log Equivalent Value.

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