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Erythrocyte response to *Trypanosoma brucei* in experimentally infected dogs

OMOTAINSE (S.O.), ANOSA (V.O.). Réponse érythrocytaire à l'infection expérimentale à *Trypanosoma brucei* chez les chiens. *Revue Elev. Méd. vét. Pays trop.*, 1992, 45 (3-4) : 279-283

L'infection à *Trypanosoma brucei* a conduit à un processus aigu et fatal chez les chiens communs nigériens en raison d'une anémie à évolution rapide. Les chiens infectés ont répondu par une réticulocytose accrue qui ne s'est pas prolongée avec la chronicité. En comparaison, la réponse à une anémie hémolytique artificiellement induite a été progressive, bien marquée et prolongée. L'anémie consécutive à l'infection à *T. brucei* chez le chien était soit normocytaire et normochromique dans le cas d'une infection aiguë, soit microcytaire et normochromique dans l'infection chronique. Quant à l'anémie induite artificiellement, elle était soit macrocytaire et normochromique, soit normocytaire et normochromique. Le potentiel érythropoïétique du plasma *in vivo* chez la souris était plus élevé chez les chiens infectés, sauf à la fin de la parasitémie. Cette anémie à *T. brucei* chez les chiens infectés est donc une réaction initiale mais devient un facteur de peu d'importance avec l'établissement de la chronicité. *Mots clés* : Chien - Trypanosomose - *Trypanosoma brucei* - Infection expérimentale - Anémie - Modification hématologique - Erythrocyte - Nigeria.

INTRODUCTION

Like in other animals, *T. brucei* infection of dogs is characterized by anaemia (6, 8, 11). There is no available information on bone-marrow responses to anaemia generated in *T. brucei* infection of dogs.

The aim of this work was to study the erythrocytic response of dogs to anaemia generated by *T. brucei* infection and the ability of their plasma to stimulate reticulocytosis in mice. This study contributes to understanding the function of bone-marrow in trypanosome-infected animals.

MATERIALS AND METHODS

Experimental design

Three dogs were infected with *T. brucei*. Their red blood cell (RBC) parameters were determined weekly pre-and post-infection. Ten ml of blood was collected weekly from each dog with heparin as anticoagulant and used to separate plasma. Similarly, blood for plasma was collected

from 2 control dogs. All the plasma samples were stored at - 20 °C. Artificial haemolytic anaemia (AHA) was induced in two other adult dogs, and the erythrocytic parameters measured during this period. The plasma was also collected when the packed cell volume (PCV) values were comparable to those attained in *T. brucei*-infected dogs.

The stored plasma from the 3 groups of dogs were thawed and inoculated into mice at a dose of 1.5 ml per mouse in three divided doses of 0.5 ml daily for three consecutive days designated as days 0, 1 and 2. The mice were tail-bled 7 days before (day-7) the first plasma inoculation into 2 heparinized capillary tubes. One tube was used for PCV determination while the other was stained for reticulocyte counts. On day 6 (4 days after the last plasma inoculation) each mouse was tail-bled; heart blood was also collected for determination of red cell counts, while the absolute reticulocyte counts were calculated from the RBC and percentage reticulocyte counts.

Experimental animals

Seven mongrel dogs aged between 7 to 18 months were purchased from the Ibadan region and acclimatized in kennels at the Department of Veterinary Medicine of the University of Ibadan for an average of 4 months before experiment. During this period, they were regularly treated with piperazine citrate, dicestal, dinithrophenol and diaminaquine acetate against worms and protozoan diseases. They were also regularly deticked with AsuntoI[®]* at 1-2 week intervals. Ferrous sulphate was also given to supplement the iron supply. The diet was made up of rice, beans, gari (*Manihot manihot*), vegetables, meat and bones.

Adult Swiss albino mice aged above 10 weeks were supplied from the breeding unit of the Department of Virology, College of Medicine, University of Ibadan, and kept in conventional cages. The mice were fed with mouse cubes** *ad libitum*. They were treated with terramycin soluble powder fortified with multi-vitamins for five days and with Berenil[®]*** to eliminate possible bacterial and protozoan infections. They were allowed to acclimatize for 2 weeks before the experiment.

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Infections with trypanosomes

The dogs were all infected subcutaneously with 2×10^6 of the *T. brucei* strain ILRAD 1797, obtained from the International Laboratory for Research on Animal Diseases, ILRAD, Kenya.

Induction of artificial haemolytic anaemia (AHA)

Twenty to 60 ml of blood from each dog was collected through the jugular vein into 3.8 % disodium citrate sterile solution and incubated in a water bath at 50 °C for 30 min.

The blood was mixed every 10 min in the bath and was thereafter allowed to cool down to room temperature before it was re-infused into the peritoneal cavity through the inguinal region. This heat-treatment of blood denatures the RBC and renders them susceptible to phagocytosis (10).

Plasma inoculation into mice

The plasma harvested from the dog blood and stored at -20 °C was thawed and each sample was inoculated subcutaneously into four mice at a dose of 0.5 ml for 3 days.

Haematological techniques

The PCV was determined by the microhaematocrit method, while the RBC count and haemoglobin concentration were determined by haemocytometer and cyanomethaemoglobin methods, respectively (13). Thin films of 1 % new methylene blue-stained blood were made on clean glass slides, and the numbers of reticulocytes in every 500 red cells enumerated.

RESULTS

The anaemia of *T. brucei*-infected dogs

Table I shows the changes in the mean values of erythrocyte parameters of the *T. brucei*-infected dogs. Anaemia developed rapidly in infected dogs with the PCV values dropping from an average value of 0.392 ± 0.11 (l/l) to values between 0.165 ± 0.0 (l/l) and 0.24 ± 0.05 (l/l) (fig. 1, table I) before death. Similarly there was a decrease in haemoglobin concentration of the *T. brucei* infected dogs with the onset of parasitaemia. There were increases in the reticulocyte counts of the *T. brucei*-infected dogs (table I, fig. 2) as the PCV decreased. Except for the first week of infection when there was an increase in the mean corpuscular volume (MCV) of the infected dogs, there was a normocytic normochromic anaemia.

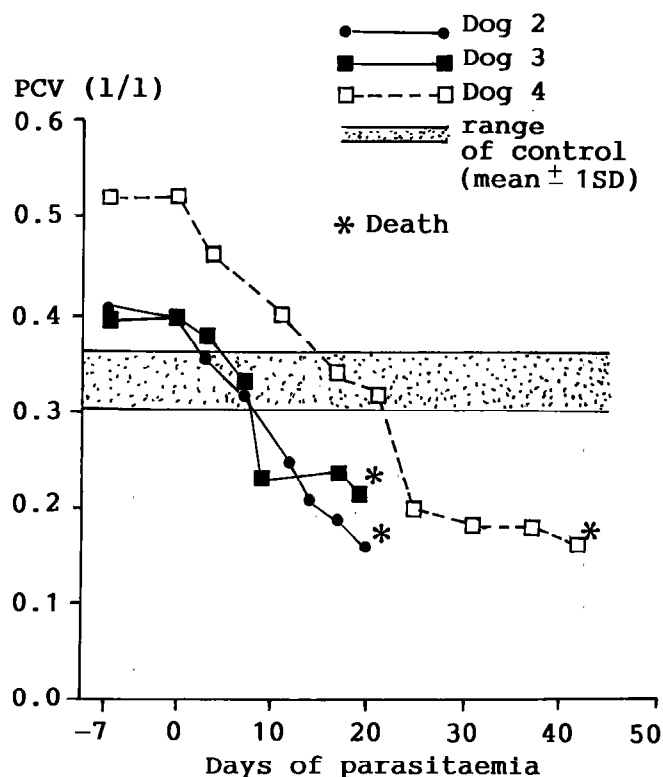


Fig. 1: Changes in the PCV of dogs infected with *T. brucei*.

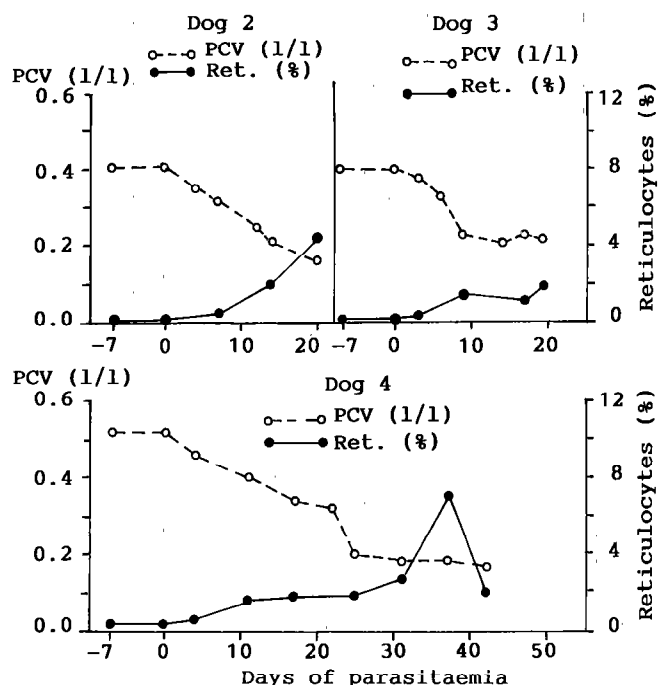


Fig. 2: Changes in the PCV and reticulocyte counts of dogs infected with *T. brucei*.

TABLE I Changes in the mean values of erythrocyte parameters of 3 dogs infected with *T. brucei*.

Erythrocyte parameters	Pre-infection	Weeks after infection							
		1	2	3	4	5*	6*	7*	8*
PCV (l/l)	0.393 ± 0.11	0.384 ± 0.12 ^d	0.332 ^c ± 0.13	0.296 ^c ± 0.13	0.272 ^c ± 0.13	0.337 ± 0.02	0.243 ± 0.05	0.183 ± 0.01	0.165 ± 0.0
Hb (g/dl)	12.2 ± 3.9	12.87 ± 3.7 ^d	11.53 ± 1.83 ^d	11.23 ± 1.35 ^a	10.57 ± 1.42 ^b	8.2 ± 0	8.0 ± 0	6.25 ± 0	6.25 ± 0
RBC (× 10 ⁶ /μl)	6.08 ± 1.77	5.85 ± 2.14 ^d	5.33 ± 1.83 ^d	5.09 ± 1.35 ^a	4.42 ± 1.42 ^b	4.96 ± 0	4.45 ± 0	2.71 ± 0	2.71 ± 0
Reticulocyte (%)	0.33 ± 0.15	0.6 ± 2.14 ^d	1.38 ± 0.59 ^c	1.53 ± 0.42 ^c	2.4 ± 1.04 ^d	3.25 ± 0	7.0 ± 0	3.8 ± 0	2.0 ± 0
MCV (fl)	60.51 ± 2.73	64.88 ± 5.99 ^e	59.45 ± 3.56 ^e	53.19 ± 10.97 ^d	57.59 ± 9.76 ^d	58.05 ± 0	56.48 ± 0	67.65 ± 0	65.73 ± 0
MCHC (g/dl)	31.33 ± 3.91	33.41 ± 1.34 ^d	35.67 ± 3.08 ^d	39.50 ± 7.14 ^d	40.93 ± 4.77 ^d	24.39 ± 0	31.54 ± 0	34.24 ± 0	36.61 ± 0
MCH (pg)	18.67 ± 2.69	22.67 ± 0.78 ^d	21.78 ± 1.37 ^d	22.24 ± 2.75 ^d	23.45 ± 2.84 ^d	16.53 ± 0	17.26 ± 0.98	23.06 ± 0	23.06 ± 0

* Values for dog number 4 with chronic infection.

a to d indicate levels of significance relative to corresponding values of controls for each parameter (* = $P < 0.01$; * = $P < 0.025$; * = $P < 0.05$; * = not significant).

MCV = mean corpuscular volume; MCHC = mean corpuscular haemoglobin concentration; MCH = mean corpuscular haemoglobin.

Anaemia in dogs with artificially-induced haemolysis

Changes in the erythrocyte parameter of dogs with artificially-induced anaemia (AHA) are presented in table II. There was a decrease in the PCV values comparable to that of *T. brucei*-infected dogs. The MCV and mean corpuscular haemoglobin concentration values did not markedly change. Therefore, the anaemia in dogs with AHA was either macrocytic normochromic (week 3) or normocytic normochromic during other periods. Dogs with AHA showed higher reticulocyte responses than those with *T. brucei* infection (table II). The increase in reticulocyte counts with low PCV was progressive and sustained.

Mouse inoculation with plasma of normal and anaemia dogs

The changes in tail-blood that these types of plasma produced when injected subcutaneously into mice are presented in table III.

There were slight non significant decreases in post inoculation PCV of mice that were injected with all types of anaemic plasma and slight non significant increases in those injected with the control plasma (table III). Plasma

from all groups of dogs, except those with terminal parasitaemia, induced increases in reticulocyte counts in recipient mice (fig. 3, table III). The best erythropoietic response to anaemic dog plasma was recorded from dogs on day 17 of *T. brucei*-parasitaemia, while the response induced by plasma from dogs with AHA was superior to those of all other groups of *T. brucei*-infected dogs. Of particular significance were the responses of mice to plasma from dogs with very severe anaemia after 24 days of parasitaemia and at terminal parasitaemia.

DISCUSSION

There was a rapid development of anaemia in *T. brucei* infected-dogs with the PCV dropping as low as 0.16 to 0.18 (l/l) terminally. This was a more serious anaemia than that previously recorded where there was a ratio of PCV values of 0.25 (l/l) to 0.30 (l/l) in *T. brucei* infection (11), but less severe than PCV value of 0.11 (l/l) recorded in naturally *T. brucei*-infected dogs (6).

In this study, the reticulocyte count increased from the pre-infection levels of 0.3 ± 0.2 % to 1.45 ± 0.92 % and 1.93 ± 1.45 % in the second and fourth weeks of infection respectively, with some individual dogs exhibiting counts

TABLE II Changes in the mean erythrocyte values of dogs during the course of artificial haemolytic anaemia (AHA).

Erythrocyte parameter	Pre-induction of AHA	Weeks after onset of haemolysis				
		1	2	3	4*	5*
PCV (l/l)	0.354 ± 0.04	0.318 ± 0.09	0.318 ± 0.09	0.26 ± 0.03	0.195 ± 0.07	0.19 ± 0
Hb (g/dl)	11.6 ± 1.70	10.25 ± 2.2	10.08 ± 2.4	7.75 ± 1.2	6.25 ± 0.4	6.0 ± 0
RBC (× 10 ⁶ /μl)	5.59 ± 1.5	5.73 ± 1.5	5.21 ± 0.9	3.83 ± 0.1	3.18 ± 0.3	3.09 ± 0
Reticulocyte (%)	0.9 ± 0.42	0.05 ± 1.1	1.45 ± 0.9	2.9 ± 1.3	5.0 ± 0	7.9 ± 0
MCV (fl)	63.17 ± 5.35	59.84 ± 0.5	61.42 ± 2.2	67.25 ± 6.4	61.35 ± 2.3	61.49 ± 0
MCHC (g/dl)	33.05 ± 0.83	30.61 ± 1.5	31.30 ± 1.3	30.17 ± 0.8	31.04 ± 0.7	31.57 ± 0
MCH (pg)	20.88 ± 1.24	18.05 ± 0.9	19.18 ± 1.5	20.28 ± 2.5	19.7 ± 0.4	19.42 ± 0.1

* Value for one dog.

MCV : mean corpuscular volume; MCHC : mean corpuscular haemoglobin concentration; MCH : mean corpuscular haemoglobin.

TABLE III Changes in the PCV values and reticulocyte counts of the tail blood of mice inoculated with normal and anaemic plasma.

Types of plasma inoculated	Packed cell volume (l/l)			Reticulocyte counts/ μ l	
	Pre-inoculation	Post-inoculation	% change	change**	% change
Normal plasma from control dogs (mean PCV of 0.36 ± 0.1 l/l), n = 8*	0.49 ± 0.04	0.498 ± 0.02^a	+ 1.5	$+ 8,850 \pm 14,440^a$	+ 2.6
<i>T. brucei</i> dog plasma at 10 days of parasitaemia (3 dogs with PCV = 33.0 ± 10 %), n = 12	0.552 ± 0.07	0.539 ± 0.07^a	- 2.3	$+ 132,529 + 87,429^b$	+ 49.4
<i>T. brucei</i> dog plasma at 17 days of parasitaemia (3 dogs, mean PCV = 26.0 ± 6.8 %), n = 12	0.548 ± 0.08	0.543 ± 0.03^a	- 1.0	$+ 350,791 \pm 72,788^b$	+ 108.9
<i>T. brucei</i> dog plasma at 24 days of parasitaemia (1 dog, PCV = 27 %), n = 4	0.528 ± 0.05	0.470 ± 0.05^a	- 10.9	$+ 142,920 \pm 74,691^b$	+ 43.4
<i>T. brucei</i> dog plasma at terminal parasitaemia (2 dogs, mean PCV = 19.0 ± 4.2), n = 8	0.491 ± 0.02	0.483 ± 0.03^a	- 1.8	$- 13,335 \pm 83,350^c$	- 1.5
AHA dog plasma (2 dogs, mean PCV = 19.0 ± 0), n = 8	0.488 ± 0.06	0.464 ± 0.05^a	- 4.9	$+ 221,313 \pm 96,349^b$	+ 74.5

^a: not significant ; ^b: $P < 0.001$; ^c: $P < 0.005$.

*: number (n) of mice that received the plasma ; **: difference between pre- and post-inoculation values.

+ : increase in value ; - : decrease in value.

\pm : standard deviation.

from 3.6 to 75 % as the anaemia progressed. The increase in percentage counts observed in *T. brucei* infection was however lower than that observed in rodents infected with *T. brucei* (3, 7) in *T. rhodesiense* infection of monkeys (12) and in *T. evansi* infection of rats (4). The reticulocyte response of dogs to AHA was higher and more sustained than that in *T. brucei* infection in contrast to the moderate increases in reticulocyte counts recorded in *T. brucei* infection of dogs. Sheep infected with *T. vivax* did

not show any reticulocytes in circulation (5). Similarly, *T. congolense* and *T. vivax* infections in ruminants generated only mild or no reticulocyte responses (1, 9, 14).

Although in acute trypanosomosis there is usually macrocytosis which may become normocytic or microcytic anaemia with chronicity, the anaemia in these *T. brucei* infected dogs was mainly normocytic or microcytic.

In mouse inoculation, the maximal reticulocyte response to infection was induced by plasma on day 17 of parasitaemia, which increased absolute reticulocyte counts by 350, 790/ μ l. This response was superior to that induced by AHA plasma which increased the absolute reticulocyte count of mice by 221, 313/ μ l. However, the response to AHA plasma was superior to that recorded at other periods of *T. brucei* infection, with plasma from terminally infected dogs actually depressing reticulocyte counts (table III). The reticulocyte responses of mice to inoculation with plasma from *T. brucei*-infected anaemic dogs peaked on day 17 of parasitaemia when PCV was 0.26 ± 0.07 l/l and when the response was superior to that induced by AHA plasma, and was followed by a significant decrease with subnormal values being recorded terminally. This indicates that erythropoiesis in *T. brucei*-infected dogs was accelerated but subsequently became depressed. The erythropoietic response, measured by reticulocyte counts, to plasma from sheep infected with *T. vivax* was consistently inferior to that of plasma from sheep with AHA, although a similar peaking of erythropoietic activity was recorded on day 21 post-infection. This suggests that the erythropoietic response to *T. brucei* infection of dogs is superior to that of ruminants infected with *T. vivax*, and presumably *T. congolense*.

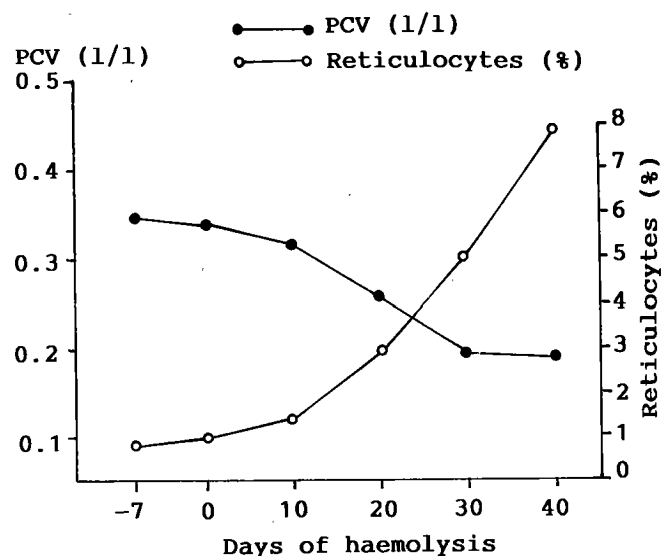


Fig. 3 : Changes in the PCV and reticulocyte counts of dogs with artificially-induced haemolytic anaemia.

CONCLUSION

The direct quantitative analysis of erythropoietic factors was not undertaken in this work. However, the lower reticulocyte counts in the *T. brucei* infected dogs, and the lower capacity of their plasma to stimulate increased reticulocyte responses in mice as compared to AHA in dogs, indicate an initial increase followed by the presence of inadequate erythropoiesis stimulatory factors like erythropoietin, in trypanosomiasis, especially in the case of chronicity.

OMOTAINSE (S.O.), ANOSA (V.O.). Erythrocyte response to *Trypanosoma brucei* in experimentally infected dogs. *Revue Elev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 279-283

Trypanosoma brucei infection produced an acute and fatal disease in Nigerian mongrel dogs due to a rapidly developing anaemia. Infected dogs responded with increased reticulocytosis, which was not sustained with chronicity. In comparison the response to artificially-induced haemolytic anaemia was progressive, marked and sustained. The anaemia of *T. brucei* infection of dogs was either normocytic normochromic in acute infection or microcytic normochromic in chronic infection. Artificially-induced haemolytic anaemia was either macrocytic normochromic or normocytic normochromic. The erythropoietic potential of plasma *in vivo* in mice increased in *T. brucei*-infected dogs except at the terminal parasitaemia. The anaemia in *Trypanosoma brucei*-infected dogs is therefore initially responsive but becomes poorly involved with chronicity. **Key words** : Dog - Trypanosomiasis - *Trypanosoma brucei* - Experimental infection - Anaemia - Haematological change - Erythrocytes - Nigeria.

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OMOTAINSE (S.O.), ANOSA (V.O.). Respuesta eritrocitaria a *Trypanosoma brucei* en perros infectados experimentalmente. *Revue Elev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 279-283

La infección por *Trypanosoma brucei* en perros nigerianos, produjo una enfermedad aguda y fatal, debida al desarrollo rápido de una anemia. Los perros infectados respondieron con un aumento de la reticulocitosis, la cual no se mantuvo en forma crónica. La respuesta a una anemia hemolítica inducida en forma artificial fue, por el contrario, progresiva, marcada y se mantuvo en el tiempo. La anemia producida por *T. brucei* en perros fue normocítica y normocrómica en las infecciones agudas o microcítica y normocrómica en las infecciones crónicas. La anemia hemolítica inducida en forma artificial fue macrocítica y normocrómica o normocítica y normocrómica. El potencial eritropoyético en plasma *in vivo* en ratones, aumento en los perros infectados con *T. brucei*, excepto en el estadio terminal de la parasitemia. La anemia en perros infectados con *T. brucei* se presenta, pues, como una respuesta inicial, cuya importancia disminuye con la cronicidad. **Palabras claves** : Perro - Trypanosomiasis - *Trypanosoma brucei* - Infección experimental - Anemia - Modificación hematológica - Eritrocitos - Nigeria.

REFERENCES

1. ANOSA (V.O.), ISOUN (T.T.). Haematological studies on *Trypanosoma vivax* infection of goats and intact and splenectomized sheep. *J. Comp. Path.*, 1980, **90** : 155-168.
2. ANOSA (V.O.), ISOUN (T.T.), OLADOSU (L.A.). Splenectomy in sheep. Technique, haematological changes and the haematology of the precipitated anaplasmosis and babesiosis. *Zentbl. Vet.Med.*, 1979, **26A** (4) : 327-336.
3. ANOSA (V.O.), KANEKO (J.J.). Pathogenesis of *T. brucei* infection in deer mice (*Peromyscus maniculatus*) : haematologic, erythrocyte biochemical, and iron metabolic aspects. *Am. J. vet. Res.*, 1983, **44** : 639-644.
4. ASSOKU (R.K.G.). Immunological studies of the mechanism of anaemia in experimental *T. evansi* infection in rats. *Int. J. parasitol.*, 1975, **5** : 137-147.
5. IGBOKWE (I.O.), ANOSA (V.O.). Response of anaemia in experimental *T. vivax* infection of sheep. *J. comp. Path.*, 1988, **1100** : 111-118.
6. IKEDE (B.O.), LOSOS (G.T.). Spontaneous canine trypanosomiasis caused by *T. brucei*. Meningoencephalomyelitis with extravascular localization of trypanosomes in the brain. *Bull. Epizoot. Dis. Afr.*, 1972, **20** : 221-228.
7. JENKING (G.C.C.M.), FORSBERG (J.L.), BROWN (B.L.), PARR (C.W.). Some haematological observation on experimental *T. brucei* infections in rabbits. *Trans. R. Soc. trop. Med. Hyg.*, 1974, **68** : 154.
8. KAGGWA (E.), MUNYUA (W.K.), MUGERA (G.M.). The pathology of *Trypanosoma brucei brucei* in the dog. *Bull. anim. Hlth Prod.*, 1983, **33** : 69-75.
9. MACKENZIE (P.K.J.), CRUTCKSHANK (J.G.). Phagocytosis of erythrocytes and leucocytes in sheep infected with *T. congolense* (Broden, 1904). *Res. Vet. Sci.*, 1973, **15** : 256-262.
10. MARTON (P.F.). Erythrophagocytosis in the rat bone-marrow following transfusion of heat-denatured erythrocytes. *Scand. J. Haematol.*, 1971, **8** : 328-335.
11. MORRISON (W.L.), MAXMURRAY (P.D.), SAYER (P.D.), PRESTON (J.M.). The pathogenesis of experimentally induced *T. brucei* infection in the dog. I. Tissue and organ damage. *Am. J. Pathol.*, 1981, **102** : 168-181.
12. SADUN (E.), JOHNSON (A.), NAGLE (R.), DUXBURY (R.I.). Experimental infections with African trypanosomiasis. V. Preliminary parasitological, clinical, haematological, serological and pathological observations in rhesus monkeys infected with *T. rhodesiense*. *Am. J. trop. Med. Hyg.*, 1973, **22** : 323-330.
13. SCHALM (O.W.), JAIN (N.C.), CARROL (E.J.). Veterinary haematology. 3rd ed. Philadelphia, Lea and Febiger, 1975. Pp. 340-470.
14. VALLI (V.E.O.), MILLS (J.N.). The quantitation of *T. congolense* in calves. I. Haematological changes. *Tropenmed. Parasitol.*, 1980, **31** : 215-231.