Leucocyte and thrombocyte responses in dogs experimentally infected with *Trypanosoma brucei*

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Three dogs were subcutaneously infected with *Trypanosoma brucei* strain ILRAD 1797. Artificial haemolytic anaemia was induced in 2 other dogs by phlebotomy, heat treatment and re-infusion of the blood, while 2 dogs were kept as control animals. The infected animals developed pan-leucopenia and thrombocytopenia, while the dogs with artificial haemolytic anaemia developed leucocytosis and thrombocytosis. These findings suggest that there was a bone marrow depressing factor in the plasma of *T. brucei*-infected dogs especially as it affected leucocyte production.

Key words : Dog - *Trypanosoma brucei* - Experimental infection - Anaemia - Blood - Nigeria.

INTRODUCTION

Although natural canine trypanosomosis has already been reported (11, 18), and experimental canine trypanosomosis described (13, 14, 17, 19), the leucocyte and thrombocyte changes have not yet been described.

Pan-leucopenia is generally reported in acute trypanosomosis (1, 4), but leucocytosis is a feature of *Trypanosoma brucei*-infected deer mice (5). The decrease in the total number of white blood cells (WBC) is reflected in all the WBC components, but monocytosis has consistently been reported in *T. brucei* infection of rodents (5) and in *T. vivax* infection of ruminants (3, 8). However, no significant change in monocyte counts was reported in *T. congolense* infection of cattle (15).

Thrombocytopenia is a common characteristic of both human and animal trypanosomosis (2, 9, 22). Detailed leucocyte and thrombocyte responses have not been reported in the literature on canine trypanosomosis. This paper reports the leucocyte and thrombocyte responses in *T. brucei* infected dogs.

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MATERIALS and METHODS

Experimental design

The design of this work was described in (19). Seven local dogs (mongrels) of 7 to 18 months old were used in this experiment. They were kept in standard kennels at the Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

The dogs were kept for about 4 months to acclimatize before the experiment, when they were dewormed, deticked, and screened and treated against babesiosis. Three dogs were subcutaneously infected with the *T. brucei* strain ILRAD 1797. Artificial haemolytic anaemia was induced in 2 other dogs by phlebotomy, heat treatment and re-infusion of the blood, while 2 dogs were kept as controls.

Animal infection and plasma inoculation into mouse

The infection and induction of artificial haemolytic anaemia (AHA) were carried out as described by Omotainse and Anosa (19), as was the plasma inoculation into mice.

Haematological techniques

The WBC counts were determined in a Neubauer haemocytometer using a blood dilution of 1 : 20 in 2 % aqueous solution of acetic acid to which gentian violet was added. For platelet counts, the blood was diluted 1 : 20 with 1 % ammonium oxalate and counted in a haemocytometer. WBC differential counts were made on thin blood smears stained with Wright's stain (20).

The plasma samples harvested from each dog were inoculated into 4 mice and the mice were heart-bled 6 days later for haematology. This was done to evaluate the leucopoietic potential of the sera in the mice.

RESULTS

T. brucei infection produced a hyperacute disease in dogs. There was a sudden development of anaemia. This anaemia, already described by Omotainse and Anosa (19) led to an unequal and unsustained increased production of new red blood cells (reticulocytes).

Leucocyte counts in *T. brucei* infected dogs

On average, there was a sharp decrease in the total leucocyte counts, especially in the first 4 weeks of infection when there was a significant decrease ($p < 0.001$) (table I, fig. 1). Thus the total WBC dropped from a pre-infection value of $12.72 \pm 3.23 \times 10^3/\mu l$ to $5.175 \pm 3.01 \times 10^3/\mu l$ by the fourth week of infection. The animal that survived for 8 weeks after infection had a terminal total WBC count of $5.50 \times 10^3/\mu l$.

Leucocyte counts of dogs with artificially induced haemolytic anaemia (AHA)

In dogs with AHA, the total leucocyte counts rose sharply from $11.61 \pm 2.43 \times 10^3/\mu l$ before onset of bleeding to $19.5 \pm 7.78 \times 10^3/\mu l$ by the third week. Terminally, a dog with AHA had a total WBC count of 23,600/µl of blood compared to 5,500/µl recorded for an infected dog (tables I and II, fig. 1). While the leucopenia recorded in *T. brucei* infected dogs was characterized by neutropenia, lymphopenia, eosinopenia and mild monocytopenia, the dogs with AHA had leucocytosis with neutrophilia, eosinophilia, slight early lymphocytosis and early monocytosis.

Leucocyte counts in mice with plasma inoculation

The WBC counts of mice that received anaemic dog plasma showed maximum values in those mice that received plasma of *T. brucei*-infected dogs after 10 days of infection. Mice that received plasma from terminal parasitaemic dogs or AHA plasma yielded similar WBC counts, slightly higher than those of the recipients of control plasma (table III).

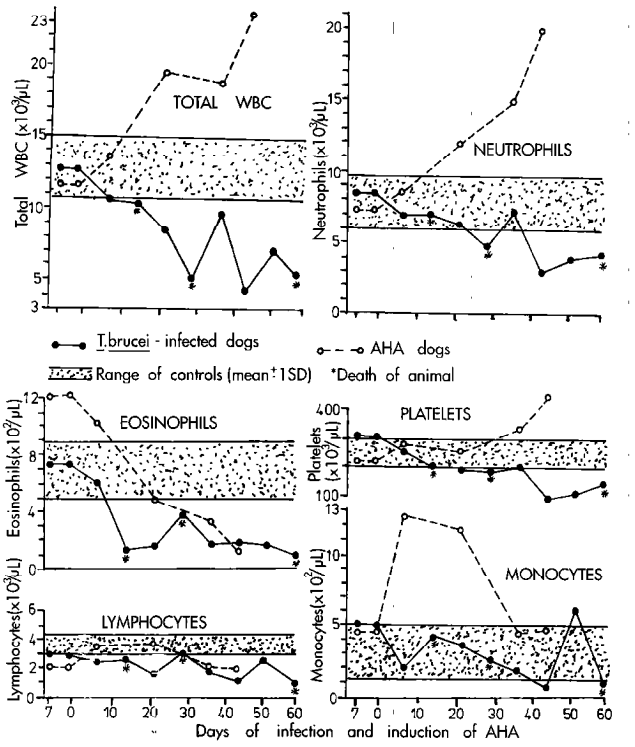

Figure 1 : Total and absolute leucocyte and platelet counts of T. brucei-*infected dogs and dogs subjected to artificial haemolytic anaemia (AHA).*

TABLE I
Leucocyte counts and their changes in *T. brucei* infected dogs

Leucocytes	Control Dogs*	Weeks post infection								
		0**	1	2	3	4	5***	6***	7***	8***
Total WBC ($\times 10^3/\mu l$)	12.82 ± 2.11	12. 72 ± 3.23	10.69 ± 0.81^e	10.30 ± 3.13^e	8.57 ± 1.55^d	5.17 ± 3.01^a	9.30	4.50	7.33	5.50
Neutrophils ($\times 10^3/\mu l$)	7.81 ± 1.35	8.58 ± 2.3	6.95 ± 1.59^e	7.12 ± 3.38^e	6.45 ± 1.83^e	4.77 ± 1.76^b	7.07	3.15	3.98	4.22
Lymphocytes ($\times 10^3/\mu l$)	3.71 ± 0.92	2.86 ± 0.65	2.57 ± 0.59^e	2.62 ± 0.70^e	1.60 ± 0.21^c	3.12 ± 1.70^e	1.86	1.08	2.64	1.11
Monocytes ($\times 10^2/\mu l$)	3.17 ± 2.01	4.95 ± 1.36	2.09 ± 1.16^e	4.08 ± 0.65^e	3.51 ± 1.30^c	2.63 ± 2.06^e	1.86	0.90	6.11	1.11
Eosinophils ($\times 10^2/\mu l$)	6.89 ± 2.02	7.45 ± 5.63	6.06 ± 6.54^e	1.25 ± 1.47^e	1.60 ± 1.44^e	3.73 ± 5.21^e	1.86	1.80	1.80	1.11
Basophils ($\times 10^2/\mu l$)	0.42 ± 0.95	0.35 ± 0.43	0.00	0.24 ± 0.48^e	0.00	0.17 ± 0.29^e	0.00	0.00	0.00	0.00
Platelets ($\times 10^3/\mu l$)	246.5 ± 47.4	306.0 ± 44.1	259.75 ± 63.63^e	239.0 ± 70.9^e	178.3 ± 33.4^c	137.67 ± 48.0^d	204	98	102	142

* Mean of 10 counts from 2 control dogs taken during the experiment. ** Pre-infection values. *** Values for dogs number 04 alone. a to e indicated levels of significance relative to corresponding values of controls for each parameter (a = $p < 0.001$; b = $p < 0.005$; c = $p < 0.05$; e = not significant).

Except for mice inoculated with 10th day infection plasma and showing platelet counts of 135,000/µl, all mice inoculated with infected plasma had slightly lower platelet counts than the 118,000/µl found in mice inoculated with AHA plasma.

Whereas there was a gradual decrease in the platelet counts of dogs with *T. brucei* infection, there was a gradual increase in those with AHA (tables I and II, fig. 1). Platelet counts in dogs with AHA were also slightly higher than those of control dogs.

DISCUSSION

There was pan-leucopenia in *T. brucei*-infected dogs. This is consistent with earlier results in *T. vivax* and *T. congolense* infections of ruminants (4, 16, 21) and in *T. brucei* infection of mice (1), although monocytosis has been reported in *T. brucei*-infected mice (1) and *T. vivax*-infected sheep and goats (4).

Thrombocytopenia which occurred in *T. brucei*-infected dogs has also been reported in *T. gambiense* infection of

TABLE II
Mean leucocyte values of dogs subjected to artificial haemolysis

		Weeks after onset of haemolysis				
Leucocytes	**Control dogs***	**0****	**1**	**2**	**5+**	**6+**
Total WBC (× 10^3/µl)	12.82 ± 2.11	11.61 ± 2.43	13.65 ± 1.49	19.5 ± 7.78	18.88 ± 7.6	23.6
Neutrophils (× 10^3/µl)	7.81 ± 1.35	7.41 ± 2.3	8.74 ± 2.8	12.13 ± 0.52	14.89 ± 8.6	20.06
Eosinophils (× 10^2/µl)	6.89 ± 2.02	12.33 ± 5.86	10.30 ± 9.2	4.93 ± 0.96	3.41 ± 2.8	1.42
Lymphocytes (× 10^3/µl)	3.71 ± 0.92	2.13 ± 0.88	3.46 ± 3.6	3.83 ± 3.43	2.15 ± 1.7	1.89
Monocytes (× 10^2/µl)	3.17 ± 2.01	4.61 ± 4.40	12.71 ± 9.1	11.70 ± 4.40	4.44 ± 5.6	4.72
Basophils (× 10^2/µl)	0.42 ± 0.95	0.34 ± 0.68	0.0	6.70 ± 2.40	0.0	0.0
Platelets (× 10^3/µl)	246.5 ± 47.37	221.5 ± 52.35	277.0 ± 52.0	253.0 ± 35.36	333.5 ± 2.4	472.0

* Mean of 10 counts from 2 control dogs taken during the experiment. ** Values before induction of haemolysis. + Death of one dog.

TABLE III
Mean white blood cell counts of heart blood of mice six days after inoculation with plasma from control dogs, dogs with *T. brucei* infection and dogs with artificial haemolytic anaemia

Leucocytes	**Dogs No. 8 with 5 days of parasitaemia**	**Dogs with 10 days of parasitaemia**	**Dogs with 17 days of parasitaemia**	**Dog No. 4 with 24 days of parasitaemia**	**Terminal parasitaemia of dogs Nos. 3 & 4**	**AHA**	**Control dogs**
Total WBC (× 10^3/µl)	3.875	6.671	4.463	4.750	5.876	5.788	5.469
Neutrophils (× 10^3/µl)	2.149	3.647	2.326	2.055	3.247	2.525	2.798
Lymphocytes (× 10^3/µl)	1.525	2.563	1.980	2.395	2.448	2.434	2.386
Eosinophils (× 10^2/µl)	0.37	1.27	0.68	0.71	0.94	1.59	0.79
Monocytes (× 10^2/µl)	0.76	1.44	1.75	2.04	0.87	6.49	1.99
Basophils (× 10^2/µl)	0.0	0.0	0.0	0.0	0.0	0.0	0.08
Platelets (× 10^3/µl)	77.25	135.0	104.0	105.0	117.63	118.0	112.38

man and rats (10), *T. brucei* infection of rats and rabbits (12), and in *T. vivax* infection of cattle (15), and goats (6). Important mechanisms that precipitate thrombocytopenia in trypanosomosis include thrombocyte aggregation, production of anti-platelet auto-antibodies, and phagocytosis of thrombocytes in the spleen and haemolymph nodes of infected animals (2), and these may be major factors involved in the *T. brucei*-infected dogs. The changes recorded in thrombocyte counts as a result of mouse inoculation with plasma from *T. brucei*-infected dogs, were essentially similar to those inoculated with plasma from AHA and control dogs. They all resulted in marginal increases in thrombocyte counts. This shows that in general, the production of thrombocytes from the bone marrow was not depressed by factors in the plasma.

The response of dogs to AHA is characterized by leucocytosis with neutrophilia, lymphocytosis and monocytosis, as well as by thrombocytosis, thus differing from that of dogs with *T. brucei* infection. The failure of the dogs infected with *T. brucei* to develop similar responses despite the haemolytic nature of the accompanying anaemia suggests that fundamental changes might be taking place in the bone marrow of trypanosome-infected animals, depressing its responsiveness to the haematological events occurring in trypanosomosis. It is pertinent that the marrow composed of metamyelocyte, band and segmented cells were markedly depressed in *T. vivax* infection of sheep (4) and in *T. congolense* infection of cattle (21). Recent studies in calves infected with *T. vivax* has shown that the marrow granulocyte reserve was depressed and that this was due to massive phagocytosis of these cells by macrophages in the bone marrow (7).

In view of the drop in myeloid : erythroid ratio usually occurring in the marrow of trypanosome-infected animals (2), it is also possible that the cytokines that control various haemopoietic lines are modified to enhance the production of some and depress others such as granulocytes, though there was an initial but unsustained induced leucocytosis in mice which received plasma from dogs recently infected with *T. brucei.*

CONCLUSION

There was leucopenia in *T. brucei* infection of dogs, characterized by neutropenia, lymphopenia, eosinopenia, monocytosis and thrombocytopenia. In contrast, dogs with AHA developed leucocytosis with neutrophilia, lymphocytosis, monocytosis and eosinopenia. The plasma of *T. brucei* infected dogs induced increased leucocytosis in mice in the early part of infection, but this potential was lost with time. Similarly, lower platelet counts were recorded generally in mice injected with infected plasma than those injected with plasma from AHA dogs.

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References

1. ANOSA V.O., 1980. Studies on the parasitaemia, plasma volumes, leucocyte and bone marrow cell counts and the moribund state in *T. brucei* infection of splenectomized and intact mice. *Zentbl. vet. Med.,* **27 :** 168-180.

2. ANOSA V.O., 1988. Haematological and biochemical changes in human and animal trypanosomiasis. Part II. *Revue Élev. Méd. vét. Pays trop.,* **41 :** 151-164.

3. ANOSA V.O., ISOUN T.T., 1974. Experimental *T. vivax* infection in sheep and goats : the relationship between the parasitaemia, the growth rate, and the anaemia. *J. Niger. vet. Med. Assoc.,* **3 :** 102-108.

4. ANOSA V.O., ISOUN T.T., 1980. Haematological studies of *T. vivax* infection of goats and intact and splenectomized sheep. *J. comp. Path.,* **90 :** 155-168.

5. ANOSA V.O., KANEKO J.J., 1983. Pathogenesis of *T. brucei* infection in deer mice *(Peromyscus maniculatus) :* Haematologic, erythrocyte biochemical, and iron metabolic aspects. *Am. J. vet. Res.,* **44 :** 639-644.

6. ANOSA V.O., KANEKO T.T., 1989. Ultrastructural pathology of haemopoietic organs in *Trypanosoma vivax* infection of goats. *Vet. Path.,* **26 :** 78-83.

7. ANOSA V.O., LOGAN-HENFREY L.L., SHAW M.K., 1992. Acute haemorrhagic *T. vivax* infection in calves : A light and electron microscopic study of changes in blood bone marrow. *Vet. Path.,* **29** (1) : 33-45.

8. ESIEVO K.A.N., SAROR I., 1983. Leucocyte response in experimental *T. vivax* infection cattle. *J. comp. Path.,* **93 :** 165-170.

9. FORSBERG C.M., VALLI V.E., GENTRY P.W., DUAWORTH R.M., 1979. The pathogenesis of *T. congolense* infection in calves. IV. The kinetics of blood coagulation. *Vet. Path.,* **16 :** 229-242.

10. GREENWOOD B.M., WHITTLE H.C., 1976. Coagulation studies in Gambian trypanosomiasis. *Am. J. trop. Med. Hyg.,* **25 :** 390-394.

11. IKEDE B.O., LOSOS G.J., 1972. Spontaneous canine trypanosomiasis caused by *T. brucei :* Meningoencephalomyelitis with extravascular localization of trypanosomes in the brain. *Bull. Epizoot. Dis. Afr.,* **20 :** 221-228.

12. JENKINS G.C., FORSBERG C.M.. BROWN J.L., PARR C.W., 1974. Some haematological observation on experimental *T. brucei* infections in rabbits. *Trans. R. Soc. trop. Med. Hyg.,* **68 :** 154.

13. KAGGWA E., MUNGUA W.K., MUGERA G.M., 1984. Pathogenicity of *T. brucei* in dogs. *Bull. Anim. Hlth Prod. Afr.,* **32 :** 360-368.

14. KAGGWA E., MUNGUA W.K., MUGERA G.M., 1988. Relapse in dog experimentally infected with *T. brucei* and treated with Diminazene aceturate of isometamidium Chloride. *Vet. Parasitol.,* **27 :** 199-208.

15. MAXIE M.G., LOSOS G.J., TABEL H., 1979. Experimental bovine trypanosomiasis *(T. vivax* and *T. congolense) :* I. Symptomatology and clinical pathology. *Tropenmed. Parasitol.,* **30 :** 274-282.

16. MAYLOR D.C., 1971. The haematology and histopathology of *T. congolense* infections in cattle. Part II. Haematology (including symptoms). *Trop. Anim. Hlth Prod.,* **3 :** 159-168.

17. MORRISON W.I., MURRAY M., SAYER P.D., PRESTON J.M., 1981. The pathogenesis of experimentally induced *T. brucei* infection in the dog. I. Tissue and organ damage. *Am. J. Path.*, **102 :** 168-181.

18. MULLIGAN H.W., 1970. The African trypanosomiasis. London, U.K., Allen and Unwin, p. 763-782.

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

20. SCHALM O.W., JAIN N.C., CARROL E.J., 1975. Veterinary haematology. 3rd ed. Philadelphia, U.S.A., Lea and Febiger.

21. VALLI V.E.O., FORSBERG C.M., LUMSDEN J.H., 1979. The pathogenesis of *T. congolense* infection of calves. III. Neutropenia and myeloid response. *Vet. Path.*, **16 :** 96-107.

22. WELLDE B.T., KOVATCH R., CHUMO D., WYKOFF D., 1978. *T. congolense.* Thrombocytopenia in experimental infected cattle. *Exp. Parasitol.*, **45 :** 26-33.

OMOTAINSE (S.O.), ANOSA (V.O.). Réponses leucocytaires et thrombocytaires à l'infection expérimentale à *Trypanosoma brucei* chez les chiens. *Revue Élev. Méd. vét. Pays trop.*, 1995, **48** (3) : 254-258.

Trois chiens ont été infectés par une souche ILRAD 1797 de *Trypanosoma brucei* par voie sous-cutanée. Une anémie hémolytique a été provoquée artificiellement chez deux autres chiens par phlébotomie, traitement thermique et réinjection de sang, alors que deux autres chiens ont servi de témoin. Les animaux infectés ont manifesté une panleucopénie et une thrombocytopénie, alors que les chiens atteints d'une anémie hémolytique provoqué artificiellement ont présenté une leucocytose et une thrombocytose. Ces résultats laissent supposer l'existence d'un facteur d'aplasie médullaire dans le plasma des chiens infectés à *T. brucei,* d'autant plus que la production leucocytaire a été affectée.

Mots-clés : Chien - *Trypanosoma brucei* - Infection expérimentale - Anémie - Sang - Nigeria.

OMOTAINSE (S.O.), ANOSA (V.O.). Respuesta leucocitaria y trombocitaria en perros infectados experimentalmente con *Trypanosoma brucei.* *Revue Élev. Méd. vét. Pays trop.*, 1995, **48** (3) : 254-258.

Se infectaron via subcutánea 3 perros con *Trypanosoma brucei* cepa ILRAD 1797. En otros 2 perros se indujo artificialmente une anemia hémolítica, por flebotomía, tratamiento de calor y re infusión sanguínea, mientras que 2 perros se mantuvieron como animales control. Los animales infectados desarrollaron pan leucopenia y trombocitopenia, mientras que los perros con anemia hemolítica artificial desarrollaron leucocitosis y trombocitosis. Estos hallazgos sugieren la presencia de un factor de depresión de la médula ósea en el plasma de los perros infectados con *T. brucei,* especialmente debido a la afección de la producción leucocitaria.

Palabras clave : Perro - *Trypanosoma brucei* - Infección experimental - Anemia - Sangre - Nigeria.