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Parasitaemia and clinical manifestations in *Trypanosoma brucei* infected dogs

NWOSU (C.O.), IKEME (M.M.). Parasitémie et manifestations cliniques chez des chiens infectés par *Trypanosoma brucei*. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 273-277

Quatre chiens ont été infectés par *Trypanosoma brucei* (souche Mkar), alors que quatre autres chiens ont servi de témoins. Deux des chiens ont manifesté la maladie de façon aiguë et sont morts au cours de la première vague de parasitémie aux 7e et 8e jours après l'infection, alors que les deux autres sont morts de maladie sub-aiguë aux 24e et 28e jours après l'infection, correspondant à la seconde vague de parasitémie. Au cours de la première vague de parasitémie, l'hématocrite, la numération érythrocytaire, l'hémoglobine, la numération leucocytaire totale, le nombre d'éosinophiles, neutrophiles et lymphocytes étaient diminués ; au cours de la période intermédiaire de faible parasitémie, les valeurs des leucocytes totaux et des lymphocytes remontaient légèrement, bien qu'elles rediminuaient ensuite de façon continue, de même que les autres valeurs, au cours de la deuxième vague de parasitémie. En revanche, il existait une monocytose constante dans les maladies aiguë et sub-aiguë. Globalement, la maladie se manifestait par une altération de l'état général, une anémie, une leucopénie, une monocytose, des troubles oculaires, une élévation de la température et des fréquences cardiaques et respiratoires. La différence entre les manifestations aiguë et sub-aiguë était dans leur degré d'intensité. L'importance de l'anémie et les troubles circulatoires associés à l'infection pourraient être à l'origine de la mort de tous les animaux infectés. *Mots clés* : Chien - *Trypanosoma brucei* - Trypanosomose - Modification hématologique - Symptôme - Nigeria.

INTRODUCTION

Among the pathogenic species of African trypanosomes, the dog is capable of harbouring infection with *Trypanosoma congolense, T. evansi*, but infections with *T. brucei* appear to be the most pathogenic. Unlike in domesticated livestock very little work has been done on canine trypanosomosis whose incidence rate in Nigeria varies between 10 and 16 % (1, 10). However, reported cases suggest that the clinical manifestations in dogs are the same as in other domestic animals (1) and include several waves of parasitaemia with a constant feature of anaemia. The diagnosis of canine trypanosomosis is based on clinical manifestation with the detection of the parasites in the peripheral blood. In this paper we investigated the relationship between parasitaemia and clinical manifestation in canine trypanosomosis with the results expected to throw more light on the laboratory diagnosis of the disease.

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MATERIALS AND METHODS

Eight indigenous Nigerian dogs aged between seven and nine months and negative for haemoparasites were used in the experiment. They were prophylactically treated with pyrantel pamoate* (MSD, USA) against gastro-intestinal parasites and were allowed to acclimatise to the new environment for two weeks. Subsequently, four of the dogs were used as uninfected controls while the other four were each infected with 10^6 Mkar strain (MKAR/84/NITR/6) of *Trypanosoma brucei brucei*. The organism was first isolated in 1984 from a fatal outbreak of porcine trypanosomosis in Mkar in the Benue State of Nigeria (2). It was identified as *T. brucei brucei* based on morphology and negative blood inhibition infectivity test (BIIT) and stabilized by four passages in rats before storage in liquid nitrogen. The stabilate was passaged twice in rats before inoculation into the experimental dogs.

Blood for haematological examinations was collected through the cephalic vein with disodium ethylene diamine tetracetic acid (EDTA) as anticoagulant. The packed cell volume (PCV) was determined by the microhaematocrit method, the erythrocyte (RBC) and total leucocyte (WBC) counts by the haemocytometer method, the haemoglobin concentration (Hb) by Sahli's method and the differential WBC by counting 100 cells (11). All haematological examinations were repeated once every week and on day five post-infection (PI) when parasitaemia was first noted. The initial detection of parasitaemia was done by the buffy coat method while the degree of parasitaemia was determined with the improved Neubauer haemocytometer. Records of the temperature, pulse and respiratory rates as well as the general appearance of each animal was recorded daily.

RESULTS

Parasitaemia

The maximum survival period was 28 days during which the infected dogs manifested two levels of disease. One group of two dogs showed acute disease which terminated in death on days seven and eight PI. The other two

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dogs showed a sub-acute form with death on days 24 and 28 PI. Irrespective of whether the disease was acute or sub-acute, detectable parasitaemia (1.35 x 10^3/ml of blood) appeared on day five PI in all infected dogs (fig. 1). Thereafter, parasitaemia rose sharply with only one phase in the acute but two phases in the sub-acute disease, the second showing a diminishing intensity.

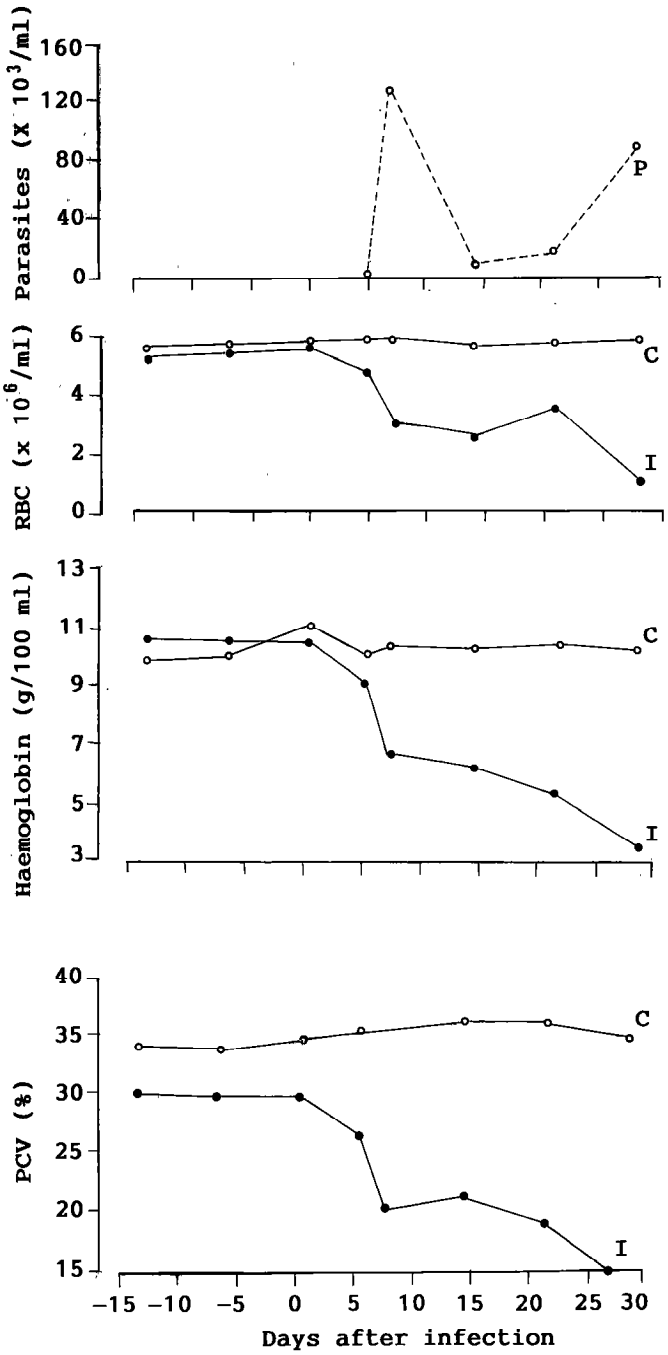

Fig. 1 : Relationship between parasitaemia and RBC, PCV and Hb values (P : parasitaemia ; I : infected dogs ; C : control dogs).

Haematology

Following infection the PCV, RBC and Hb values showed sharp falls in comparison to uninfected (control) dogs corresponding to the first wave of parasitaemia during which the acute cases died (table I). However, in the sub-acute cases the falls became gradual as parasitaemia decreased, but were sharp again at the second wave of parasitaemia with PCV, RBC and Hb values of 15 %, 1.20 x 10^6/ml and 3.90 g/100 ml, respectively (fig. 1). The values of total WBC, neutrophils, eosinophils and lymphocytes showed an inverse relationship with parasitaemia. This was more evident in the sub-acute cases (fig. 2). However, there was consistent monocytosis from 1 % at infection to 4 and 19 %, respectively during the first and second waves of parasitaemia.

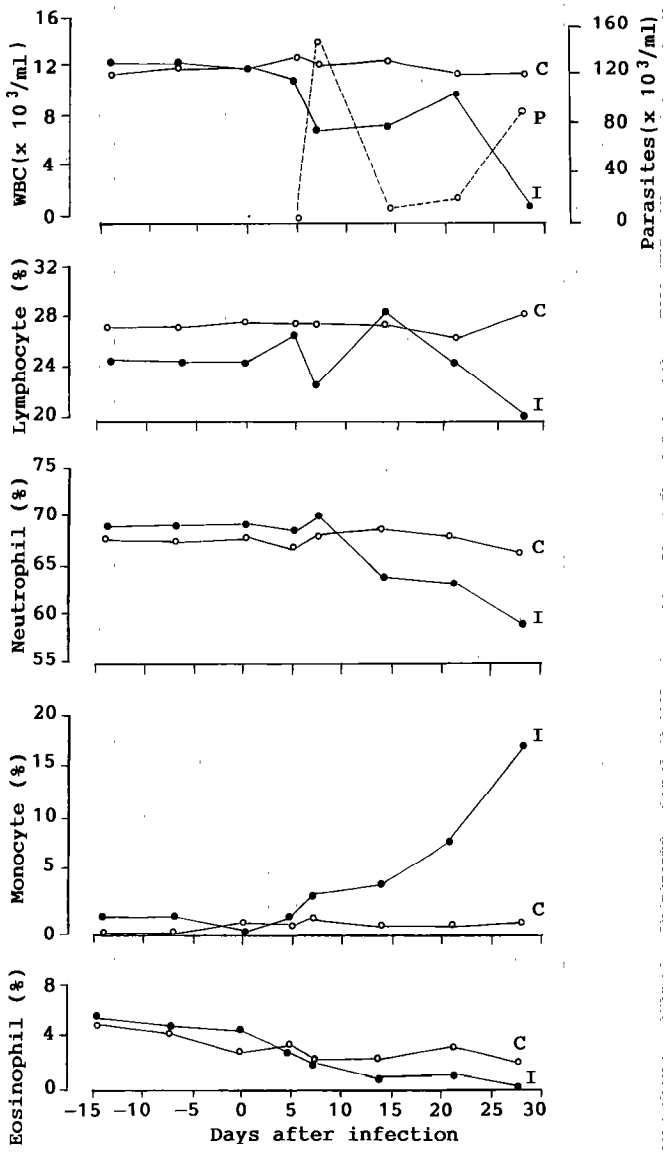

Fig. 2 : Relationship between parasitaemia and WBC, lymphocyte, neutrophil, monocyte and eosinophil values (P : parasitaemia ; I : infected dogs ; C : control dogs).

TABLE I Means of clinical and haematological data of infected and control dogs before and after infection.

Parameter	Before (BI) or After (AI) infection	Infected ± SD				Infected Mean (± SE)	Control Mean ± SE
		1	2	3	4		
Temperature (°C)	BI	38.39 ± 0.17 (38-38.6)	38.44 ± 0.27 (38-38.9)	38.35 ± 0.17 (38-38.7)	38.50 ± 0.20 (38.2-38.9)	38.42 ± 0.04	38.42 ± 0.46 (37.7-38.9)
	AI	38.87 ± 0.63 (38.4-40.0)	39.16 ± 0.90 (38.2-40.4)	39.04 ± 0.75 (38.3-39.9)	38.93 ± 1.01 (36.4-40.8)	39.0 ± 0.06	
Pulse (rate/min)	BI	139.33 ± 3.81 (130-145)	139.73 ± 6.46 (126-149)	135.47 ± 7.28 (120-148)	137.33 ± 8.78 (119-150)	137.97 ± 0.98	175.86 ± 4.36 (160-192)
	AI	158.86 ± 15.88 (139-180)	167.25 ± 23.90 (139-208)	185.54 ± 23.10 (138-214)	186.68 ± 25.78 (130-217)	174.58 ± 6.88	
Respiratory (rate/min)	BI	35.53 ± 2.36 (32-38)	37.13 ± 3.14 (32-42)	32.67 ± 2.23 (32-38)	33.47 ± 3.82 (26-38)	34.70 ± 1.01	30.05 ± 1.75 (24-40)
	AI	41.71 ± 10.42 (30-58)	52.50 ± 20.56 (32-88)	38.92 ± 5.15 (30-48)	39.21 ± 8.37 (26-60)	43.09 ± 3.20	
PCV (%)	BI	28.07 ± 0.12 (28-28.2)	28.57 ± 0.32 (28.2-28.8)	30.67 ± 0.76 (30-31.5)	32.70 ± 0.07 (32.5-33)	30.00 ± 1.06	35.63 ± 0.60 (67.5-69)
	AI	19.00 ± 5.66 (15-23)	21.80 ± 5.37 (18-25.6)	21.00 ± 4.02 (16.5-26)	24.14 ± 5.80 (15-30)	21.49 ± 1.07	
RBC (× 10^6/ml)	BI	5.27 ± 0.12 (5.2-5.4)	5.8 ± 0.2 (5.6-6.0)	5.4 ± 0.00 (5.4-5.4)	5.73 ± 0.31 (5.4-6.0)	5.55 ± 0.13	5.84 ± 0.07 (5.6-6.0)
	AI	3.35 ± 1.63 (2.2-4.5)	4.25 ± 1.49 (3.2-5.3)	3.38 ± 1.03 (3.2-4.7)	3.5 ± 1.45 (1.2-5.1)	3.62 ± 0.21	
Hb (g/100 ml)	BI	10.33 ± 0.12 (10.2-10.4)	10.53 ± 0.12 (10.4-10.6)	10.53 ± 0.12 (10.4-10.6)	11.3 ± 0.12 (11-11.2)	10.67 ± 0.22	10.53 ± 0.21 (9.9-11.92)
	AI	7.3 ± 2.12 (5.8-8.8)	8.3 ± 1.56 (7.2-9.4)	5.48 ± 4.45 (4.6-9.0)	7.18 ± 2.21 (3.9-10)	7.07 ± 0.59	
WBC (× 10^3/ml)	BI	11.27 ± 0.12 (11.2-11.4)	11.8 ± 0.2 (11.6-12.0)	12.93 ± 0.23 (12.8-13.2)	13.47 ± 0.31 (13.2-13.8)	12.37 ± 0.51	12.33 ± 0.25 (11.5-13)
	AI	8.8 ± 1.98 (7.4-10.2)	10 ± 1.70 (8.8-11.2)	8.25 ± 2.80 (5.6-11.4)	8.28 ± 4.18 (1.6-12.2)	8.83 ± 0.41	
Lymphocytes (%)	BI	24.27 ± 0.06 (24.2-24.3)	23.87 ± 0.40 (23.5-24.3)	25.53 ± 0.23 (25.4-25.8)	26.2 ± 0.20 (26-26.4)	24.97 ± 0.54	27.94 ± 0.28 (27-29)
	AI	23.7 ± 1.84 (22.4-25)	23.00 ± 1.41 (22-24)	26.5 ± 2.65 (23.4-28.8)	25.78 ± 2.0 (21-28)	24.75 ± 0.83	
Neutrophil (%)	BI	69.17 ± 0.29 (69-69.5)	69.5 ± 0.5 (69-70)	69.6 ± 0.2 (69.4-69.8)	69.23 ± 0.21 (60-71.1)	69.38 ± 0.11	68.31 ± 0.27 (67.5-69)
	AI	69.5 ± 0.71 (69-70)	70.5 ± 0.71 (70-71)	67.03 ± 3.31 (63.8-70.9)	65.8 ± 4.26 (60-71.1)	68.21 ± 1.09	
Eosinophil (%)	BI	5.53 ± 0.23 (5.4-5.8)	5.47 ± 0.50 (5-6)	4.0 ± 0.00 (4-4)	3.93 ± 0.12 (3.8-4.0)	4.73 ± 0.45	2.5 ± 0.90 (2-4.5)
	AI	2.3 ± 0.11 (1.8-2.8)	2.3 ± 0.71 (1.8-2.8)	1.38 ± 0.79 (0.6-2.2)	1.26 ± 0.89 (0-2.4)	1.81 ± 0.29	
Monocyte (%)	BI	1.3 ± 0.25 (0.8-1.3)	1.17 ± 0.06 (1.1-1.2)	0.8 ± 0.12 (0.8-1.0)	0.6 ± 0.15 (0.5-0.8)	0.9 ± 0.13	0.88 ± 0.29 (0.0-1.5)
	AI	4.5 ± 1.84 (3.2-5.8)	4.2 ± 1.41 (3.2-5.2)	5.1 ± 4.02 (0.6-10.2)	7.16 ± 7.32 (1-19)	5.24 ± 0.07	

Clinical observations

The daily variations between temperature, pulse and respiratory rates are shown in figure 3. Following the onset of parasitaemia the values of these parameters were all elevated and remained high, thereafter with minor fluctuations in both the acute and sub-acute cases. There were varying degrees of lethargy, pale mucous membranes, oculo-nasal discharges, anorexia, rough haircoats, oedema of the head and dependent parts and enlargement of the superficial lymphnodes which progressed as the disease developed into the second wave of parasitaemia. Ocular involvement in the form of photophobia, cloudiness of the cornea and corneal opacity was initially unilateral, but later bilateral before progressing into total blindness in the last of the sub-acute cases. One dog with both acute and sub-acute disease developed nervous signs with mild salivation towards the terminal stages of the disease.

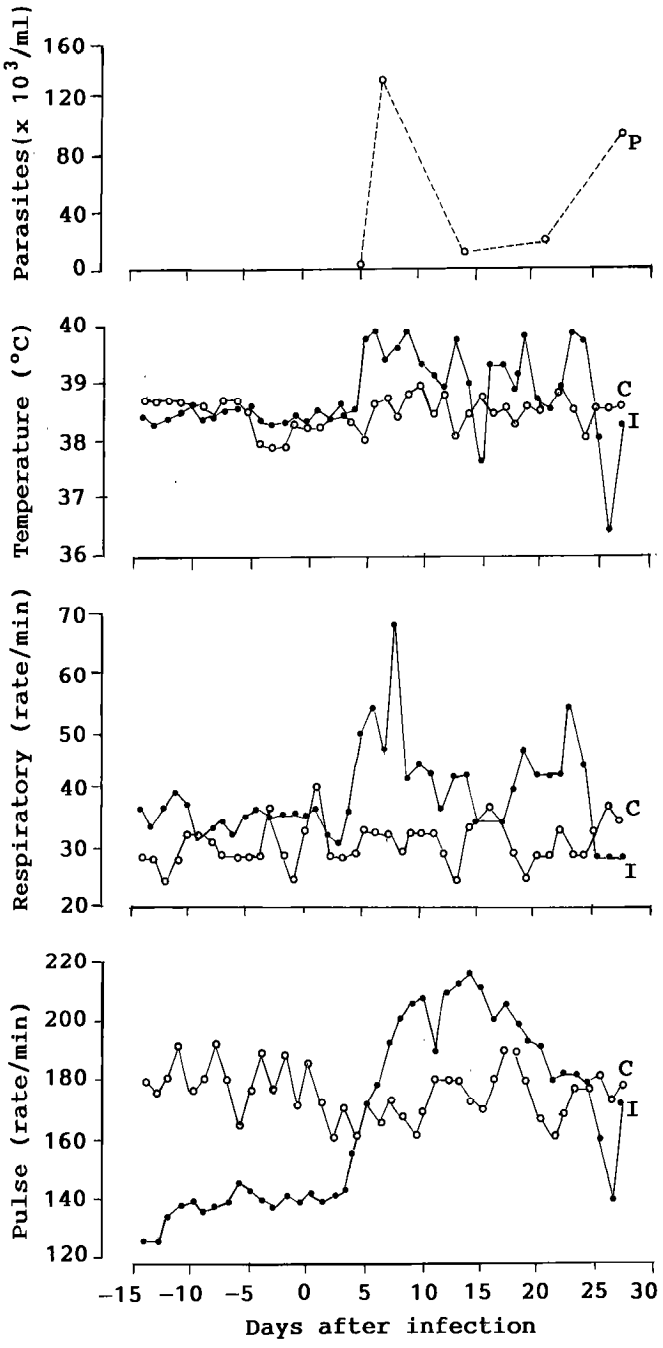

Fig. 3 : Relationship between parasitaemia and temperature, pulse and respiratory rates (P : parasitaemia ; I : infected dogs ; C : control dogs).

DISCUSSION

It is a known characteristic of the salivarian trypanosomes to show an antigenic variation which is responsible for the successive waves of parasitaemia. The ability of the host to limit the peak and number of each wave of parasitaemia and even to eliminate the infection, determines when it would be acute, sub-acute or chronic and thus the outcome of the disease. In the present study, where a standard infection was administered and similar prepatent periods observed with either acute or sub-acute disease, the initial parasite replication rates were similar, irrespective of the susceptibility of the host.

The anaemia of trypanosomosis usually starts during the first wave of parasitaemia (4). In this experiment, its development was the same for both the acute and sub-acute forms, the difference being only in the intensity.

According to ANOSA (5) several factors, especially haemolysis, contribute to anaemia. The exact incidence of each of these factors is not known, but the fact that the PCV, RBC and Hb values decreased sharply in periods of high parasitaemia but maintained a gradual decrease during the period of low parasitaemia show a direct relationship with parasitaemia. Similar fluctuations in erythrocyte values have been demonstrated in *T. brucei* infection of dogs (7) and *T. brucei* and other trypanosome infections of various other animals (3). The haemolytic nature of the anaemia will therefore depend on the trypanosomes and according to ANOSA (5) this has a multifactorial aetiology including the expanded and active mononuclear phagocytic system (MPS). Erythrophagocytosis was mainly caused by MPS which developed soon after infection and continued therafter in both the acute and sub-acute diseases. Their presence must have been necessitated by the increased demands on the system to remove dead blood and tissues cells, trypanosomes, antigen-antibody complexes and to participate in immune responses. This must have been responsible for the consistent monocytosis recorded in both the acute and sub-acute diseases in this experiment since macrophages are formed from blood monocytes. Consistent monocytosis has been observed in other trypanosome infections of animals (6).

CONCLUSION

Organisms of the *T. brucei* sub-group are known to invade extravascular tissues (8) with considerable variation in the degree of invasion and extent of tissue damage from one host to another. However, the most severe tissue lesions occur in dogs with the heart, central nervous system and eyes always being the most severely affected (9). This may have been responsible for nervous and ocular signs observed in this experiment. The degree of anaemia and the circulatory disturbances associated with the infection could have caused the death of the infected dogs.

NWOSU (C.O.), IKEME (M.M.). Parasitaemia and clinical manifestations in *Trypanosoma brucei* infected dogs. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 273-277

Four dogs were infected with *Trypanosoma brucei* (Mkar strain) while another four were used as uninfected controls. Two of the dogs showed acute disease and died in the first wave of parasitaemia on days 7 and 8 post infection (PI) while the other two died from the sub-acute disease on days 24 and 28 PI corresponding to the second wave of parasitaemia. In the first wave of parasitaemia there was a sharp decrease in the packed cell volume, red blood cell, haemoglobin, total leucocytes, eosinophil, neutrophil and lymphocyte values, but during the period of low parasitaemia there was a slight recovery of the values of total leucocytes and lymphocytes although these and the other values showed a continuous decrease during the second wave of parasitaemia. In contrast, there was a consistent monocytosis in both acute and sub-acute diseases. The general picture was that of loss of condition, anaemia, leucopenia, monocytosis, ocular impairment, elevated temperature, pulse and respiratory rates, the difference between the acute and sub-acute diseases being in the degree of intensity. The degree of anaemia noted and the circulatory disturbances associated with the infection could have caused the death of all the infected dogs. *Key words* : Dog - *Trypanosoma brucei* - Trypanosomosis - Haematological changes - Symptom - Nigeria.

NWOSU (C.O.), IKEME (M.M.). Parasitemia y manifestaciones clínicas en perros infestados con *Trypanosoma brucei*. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 273-277

Se infectaron cuatro perros con *Trypanosoma brucei* (cepa Mkar) y se utilizaron otros cuatro como controles sanos. Dos de los animales presentaron una forma aguda de la enfermedad y murieron al sétimo y octavo día post infeción (PI), durante el primer pico de parasitemia ; los otros dos perros mostraron cuadros subagudos y murieron a los 24 y 28 días PI, correspondiendo al segundo pico de parasitemia. Durante el primer pico de parasitemia, se observó una caida drástica del volumen celular total, de los eritrocitos, de la hemoglobina, de los leucocitos totales, así como de los niveles de eosinófilos, neutrófilos y linfocitos. Durante el período de baja parasitaemia, se observó una ligera recuperación de los valores de linfocitos y de leucocitos totales, a pesar de que tanto éstos como los otros valores sanguíneos disminuyeron durante el segundo pico de parasitemia. Tanto en la forma aguda como en la subaguda, se observó una monocitosis consistente. El cuadro general fue de pérdida de condición general, anemia, leucopenia, monocitosis, problemas oculares, fiebre, pulso y tasa respiratoria elevados, con una diferencia en la intensidad de los síntomas entre los cuadros agudos y subagudos. La muerte de todos los perros podría haber sido ocasionada por el grado de anemia observado y los problemas circulatorios asociados a la infección. *Palabras claves* : Perro - *Trypanosoma brucei* - Tripanosomosis - Modificación hematologica - Síntoma - Nigeria.

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