A.O. Ogunsanmi[1]
S.O. Akpavie[1]
V.O. Anosa[1]

Haematological changes in ewes experimentally infected with *Trypanosoma brucei*

OGUNSANMI (A.O.), AKPAVIE (S.O.), ANOSA (V.O.). Modifications hématologiques observées chez des brebis infectées expérimentalement par *Trypanosoma brucei*. *Revue Élev. Méd. vét. Pays trop.*, 1994, **47** (1) : 53-57

Des modifications des valeurs hématologiques ont été observées chez des brebis de la race Naine de l'Afrique de l'Ouest infectées par *Trypanosoma brucei*. La maladie avait pour caractéristique une anémie normochrome normocytique en phase aiguë, et une macrocytose sévère en phase chronique. D'après les modifications hématologiques observées, l'érythropoïèse était insuffisante en phase aiguë, alors qu'en phase chronique elle était plus élevée, mais néanmoins insuffisante, conduisant à une anémie persistante. La numération leucocytaire était normale durant la phase aiguë, tandis que la leucocytose était une caractéristique constante de la phase chronique.

Mots-clés : Ovin - Brebis - Mouton Djallonké - Sang - Hématopoïèse - *Trypanosoma brucei* -Nigeria.

INTRODUCTION

Haematological changes have been reported in human and animal African trypanosomosis (3, 4, 5, 10, 15, 16). Anaemia has long been established as one of the most consistent haematological features (3, 4, 5), but in spite of in-depth investigation, the mechanism involved in its development has not yet been determined.

The present study was conducted to investigate the etiology of the disease and the haematological changes in female sheep experimentally infected with *Trypanosoma brucei*, during the acute and chronic phases.

MATERIALS AND METHODS

Animals

Eight West African Dwarf (WAD) ewes about 2 years old and weighing between 14 and 18 kg, were purchased from a local market in Ibadan, Nigeria. The animals were housed in fly-proof pens, provided with grass, water and salt lick *ad libitum* and sheep concentrate at 0.5 kg per head and per day.

All were treated intramuscularly with diminazene aceturate at 7 mg.kg^{-1} body weight, oxytetracycline hydrochloride per os at 50 mg.kg^{-1} and thiophanate at 50 mg.kg^{-1}. Other treatments were given as appropriate and ticks were controlled using a coumaphos bath.

The animals were conditioned for 4 weeks during which they were examined for trypanosomes and other blood parasites as well as clinically with rectal temperatures taken daily between 7 and 8.00 a.m. and body weight was recorded weekly.

After this period, five of the eight sheep were infected intraperitoneally with 2.3 x 10^6 trypanosomes of *T. brucei* stock MKAR/84/NITR/6 isolated during an outbreak of fatal *T. brucei* infection of pigs in Mkar, Benue State, Nigeria. Three animals were kept as controls.

Parasitology and Haematology

Jugular blood was collected from each animal twice weekly. Animals were examined post-infection (PI) for the presence of trypanosomes by the buffy coat method (29). Packed cell volume (PCV) was determined by haematocrit method, the red blood cell (RBC) and white blood cell (WBC) counts by the haemocytometer method, and the haemoglobin concentration (Hb) by the cyanomethaemoglobin method. Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated according to SCHALM *et al.* (32).

Data analysis

Data were subjected to Student's "t" test using the Statistical Analysis System (33) computer programme. Tests were carried out at 95 % level of confidence ($p < 0.05$) or 99 % ($p < 0.01$ and $p < 0.001$).

RESULTS

Clinical signs

Following infection, trypanosomes were detected in the blood by microscopic examination of the buffy coat within 7 to 10 days. The clinical disease was characterized by marked pyrexia at an average of 40.4 °C. The temperature fluctuated daily (fig. 1A) during the period of infection. Infected sheep had intermittent, irregular parasitaemia and only a few parasites were seen per 100 microscopic

1. Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria.

Reçu le 16.2.1993, accepté le 15.2.1994

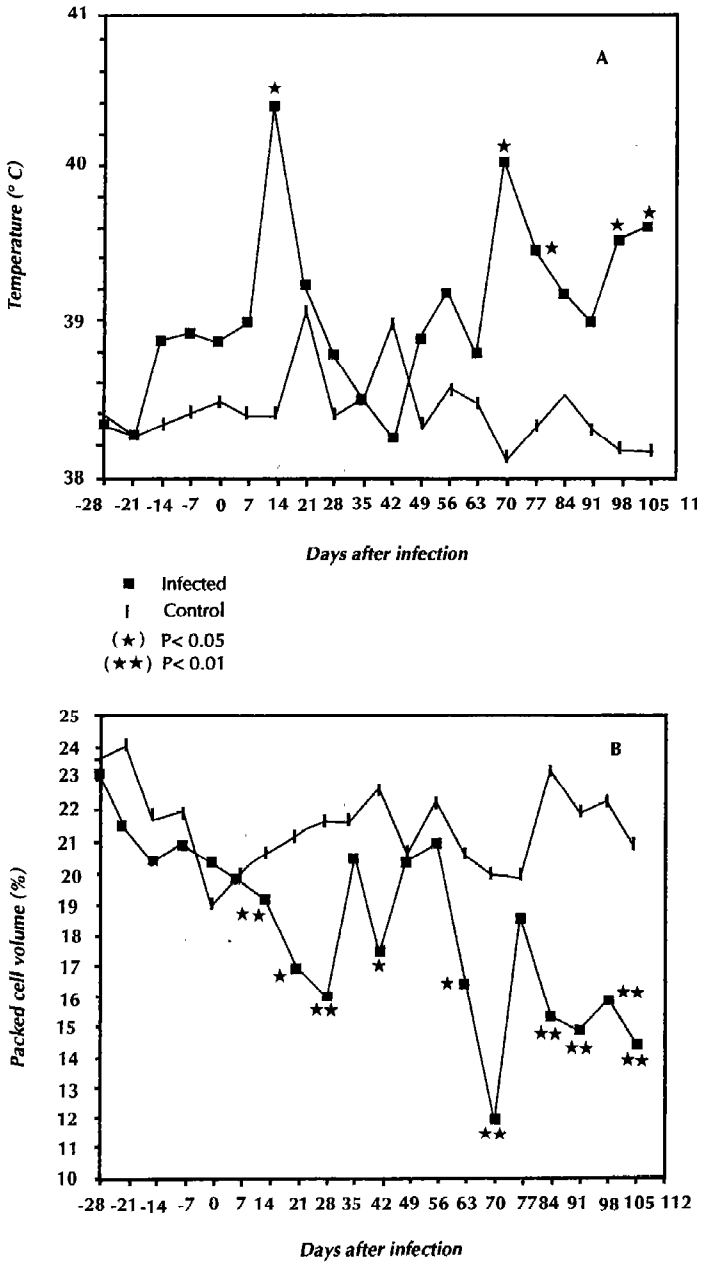

Figure 1 : Mean temperature (°C) and PCV (%) levels in T. brucei *infected and control sheep.*

fields. Sheep were anaemic with mean PCV of 11.8 ± 1.7 % 70 days PI. Infected sheep were emaciated with very pale mucous membranes, anorexic with facial and submandibular oedema, enlarged lymph nodes, occular discharges, and they showed signs of central nervous system disturbances. All animals, with the exception of the controls, showed a decrease in total body weight. Infected ewes lose as much as 7.9 kg within 56 days post infection which corresponds to 45.3 % of their body weight at the beginning of the study.

Haematological changes

The changes in the erythrocyte (PCV, HB, RBC count, MCV, MCH and MCHC) values of sheep infected with *T. brucei* and controls are shown in figures 1 to 4. There was a negative correlation between temperature and PCV in the infected animals (fig. 1B). With the onset of parasitaemia, all the infected sheep developed anaemia with a drop in erythrocyte (PCV, RBC and HB) values (fig. 1A,B and fig. 2A,B). These reflected a 19.6, 19.6 and 20.6 % drop in HB, PCV and RBC values, respectively. The anaemia developed progressively during the experiment. There were no appreciable variations in the erythrocyte values of the controls. However, the mean MCV values of infected sheep fluctuated but did not vary significantly from the normal values for the first 35 days PI. Thereafter, from day 42 of infection, the MCV values increased and remained elevated until the end of the experiment (fig. 3A). MCH values during infection relatively followed the pattern of MCV changes (fig. 3B). There was no significant variation in the MCHC values during the experiment (fig. 4A).

The mean total WBC counts during the infection fluctuated but increased on day 35 PI and also from day 70 PI until the end of the experiment (fig. 4B).

DISCUSSION

The occurrence of a negative correlation between temperature and erythrocyte values (i.e. PCV, RBC and HB) in the infected sheep confirmed previous observations in goats infected with *T. vivax* (20).

An inverse relationship was also observed between pyrexia and anaemia. Previous reports (11, 15, 20) partly attributed pyrexia to the effects of toxic metabolites produced by the trypanosomes and this might be the case in the present study. The anaemia, a major clinical feature, has also been partly attributed to the effects of *T. brucei* on host erythrocytes (3, 5, 25). It contributed to anorexia, weakness, facial and submandibular oedema and death (21, 22).

Severe anaemia and leucocytosis observed during the infection were the major haematological changes in *T. brucei* infected sheep. Anaemia, as a major consequence of the disease, contributed more to the outcome of the infection than any other pathological entity (17). It was characterized by depressed erythrocyte values (i.e. PCV, RBC and HB) and this result is in agreement with observations of LOSOS and IKEDE (25) and IKEDE and LOSOS (21) in sheep infected with *T. brucei*, ANOSA (2) in *T. vivax* infection of sheep and goats, IKEDE *et al.* (23) in *T. congolense* or *T. brucei* infection of mice and IGBOKWE and ANOSA (18) in *T. vivax* infection of sheep (2). In the present study, the anaemia was normocytic in the acute phase while the chronic phase was characterised by macrocytosis. This observation differs from results

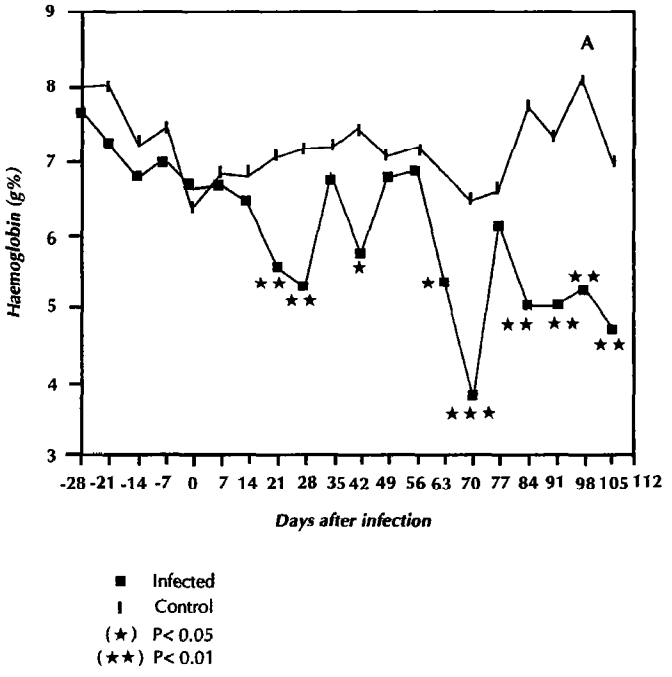

Figure 2 : Mean Hb (g %) and RBC (x 10⁶/µl) counts levels in T. brucei *infected and control sheep.*

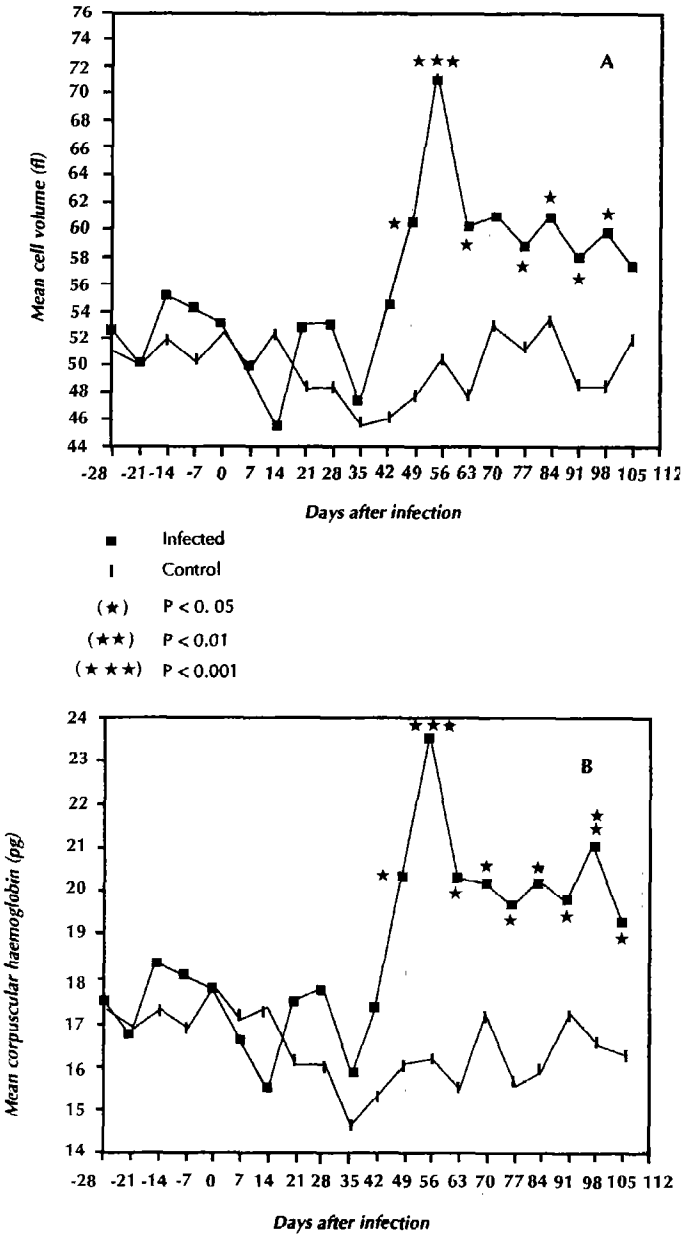

Figure 3 : Mean MCV (fl) and MCH (pg) in T. brucei *infected and control sheep.*

of previous workers (6, 15, 18, 34) who reported macrocytosis in the early acute phase and normocytic or microcytic changes in the chronic phase of trypanosomosis.

There was an increase in the MCH values of infected sheep during the chronic phase and this correlated with an increase in MCV values. It is noteworthy that the rise in MCH was observed at the onset of anaemia in the chronic phase. Similar observations were made by FIENNES *et al* (16) in *T. congolense* infected cattle. The increase in MCH and MCV values were obviously due to increased erythropoiesis indicating that erythroid response peaks as the anaemia enrages. The results indicate that although dyserythropoiesis occurs in *T. brucei* infection, it is not absolute. Bone marrow erythroid hyperplasia has been observed in *T. congolense* and *T. vivax* infections (26, 30, 35), in addition to an increase in the uptake of ^{59}Fe (10, 27), leading to an increase in young erythrocytes as indicated by macrocytosis. The failure of the bone marrow to generate sufficient erythrocytes was part-

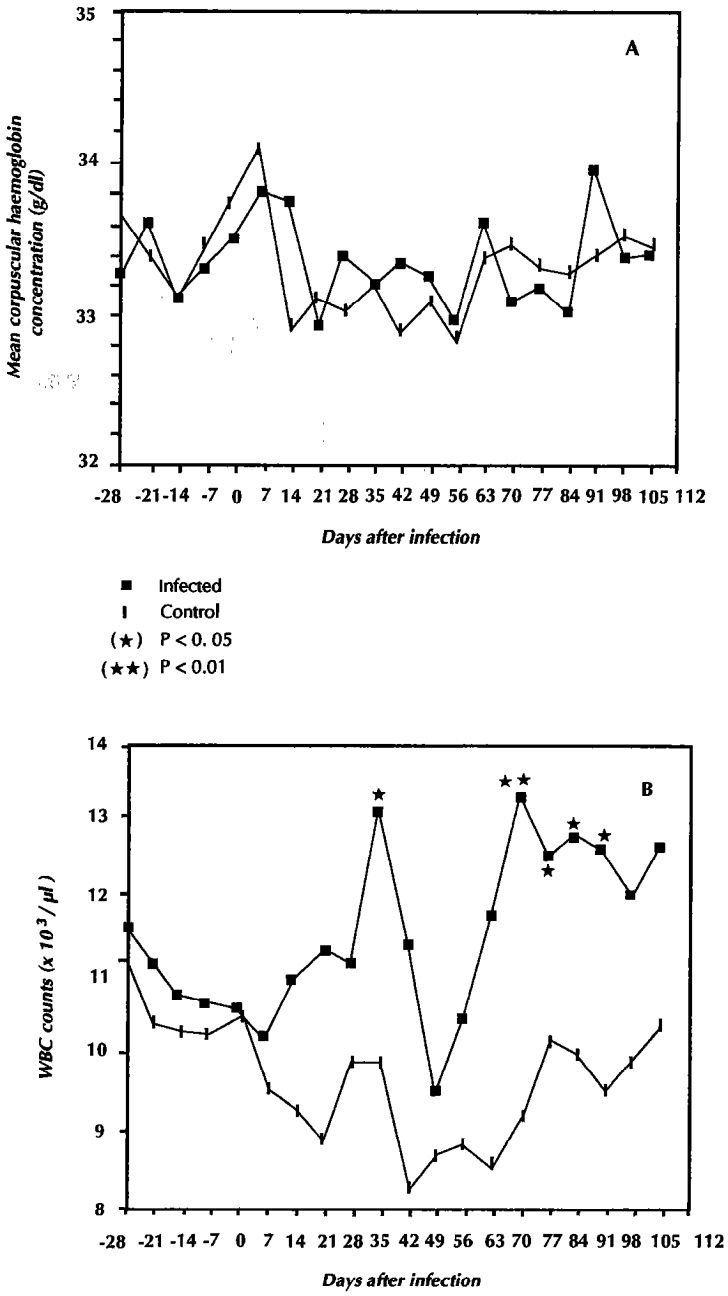

Figure 4 : Mean MCHC (g/dl) and WBC counts (x 10^3/µl) in T. brucei *infected and control sheep.*

ly responsible for the persistent anaemia. MCHC values of the infected sheep were within the normal range during both phases of the infection, hence the anaemia was normochromic.

In this study, the chronic phase of *T. brucei* infection was characterized by leucocytosis. Similar observations were reported by ANOSA and KANEKO (8) in *T. brucei* infected deer mice and KAGGWA *et al.* (24) in *T. brucei* infected dogs but differs from those of ANOSA and ISOUN (7) in *T. vivax* infected sheep and goats. It has been suggested that the change in the number of leucocytes in animal trypanosomosis may be due to the effects of trypanosomes on the host (5, 13). The finding of leucocytosis, concurrent with a relatively stable reduction in PCV, HB and RBC during the chronic phase of infection is in keeping with this observation. That the development of anaemia and macrocytosis were most pronounced during this period are also presumptive evidence of possible damage to the host cells (9, 12, 13) and tissues (5, 22) by the circulating and invading trypanosomes.

CONCLUSION

These findings indicate that changes in blood haematological values for sheep during the course of infection have significant effects on the pathology. It seems that the erythroid cell line of the bone marrow was partially damaged and incapacitated as its responsive efforts to reduce the advancement of anaemia in both acute and chronic phases were indequate.

REFERENCES

1. AKPAVIE (S.O.), IKEDE (B.O.), EGBUNIKE (G.N.). Ejaculate characteristics of sheep infected with *Trypanosoma brucei* and *T. vivax* : changes caused by treatment with diminazene aceturate. *Res. vet. Sci.*, 1987, **42**: 1-6.

2. ANOSA (V.O.). Studies on the mechanism of anaemia and the pathology of *Trypanosoma vivax* (Ziemann 1905) infection in sheep and goats. Ph.D. thesis. Nigeria, University of Ibadan, 1977.

3. ANOSA (V.O.). Diseased produced by *Trypanosoma vivax* in ruminants, horses and rodents. *Zentbl. VetMed.*, 1983a, **30**: 717-741.

4. ANOSA (V.O.). Mammalian blood cells in health and in trypanosomiasis. *Trop. Veterinarian*, 1983a, **1**: 177-199.

5. ANOSA (V.O.). Haematological and biochemical changes in human and animal trypanosomiasis. Part I. *Revue Élev. Méd. vét. Pays trop.*, 1988, **41** (1): 65-78.

6. ANOSA (V.O.), JENNINGS (F.W.), URQUHART (G.M.). The effect of splenectomy on the anaemia of *Trypanosoma brucei* infection of mice. *J. Comp. Path.*, 1977, **87**: 569-580.

7. 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.

8. ANOSA (V.O.), KANEKO (J.J.). Pathogenesis of *Trypanosoma brucei* infection in deer mice (*Peromyscus maniculatus*). Haematologic, eryhtrocyte biochemical and iron metabolic aspects. *Am. J. vet. Res.*, 1983, **44** (4): 639-644.

9. BANKS (K.L.). Injury induced by *Trypanosoma congolense* adhesion to cell membranes. *J. Protozool.*, 1980, **66**: 34-37.

10. DARGIE (J.D.), MURRAY (P.K.), MURRAY (M.), GRIMSHAW (W.R.T.), McINTYRE (W.I.M.). Bovine trypanosomiasis : the red cell kinetics of N'Dama and Zebu cattle infected with *Trypanosoma congolense*. *Parasitology*, 1979, **78**: 271-286.

11. DE GRUCHY (G.C.). Clinical haematology. 3rd. ed. London, Oxford Blackwell Scientific Publication, 1970. p. 333-358.

12. ESIEVO (K.A.N.), SAROR (D.I.), ILEMOBADE (A.A.), HALLAWAY (M.H.). Variation in erythrocyte surface and free serum sialic acid concentrations during experimental *Trypanosoma vivax* infection in cattle. *Res. vet. Sci.* , 1982, **32**: 1-5.

13. ESIEVO (K.A.N.), SAROR (D.I.). Leucocyte response in experimental *Trypanosoma vivax* infection in cattle. *J. Comp. Path.*, 1983, **93**: 165-170.

14. FACER (C.A.), CROSSKEY (J.M.), CLARKSON (M.J.), JENKINS (G.C.). Immune haemolytic anaemia in bovine trypanosomiasis. *J. Comp. Path.*, 1982, **92**: 393-401.

15. FIENNES (R.N.T.W.). Haematological studies in trypanosomiasis of cattle. *Vet. Rec.*, 1954, **66**: 423-434.

16. FIENNES (R.N.T.W.), JONES (R.E.), LAWS (S.G.). The course and pathology of *Trypanosoma congolense* (Broden) disease of cattle. *J. Comp. Path.*, 1946, **56**: 1-27.

17. GOODWIN (G.). The pathology of African trypanosomiasis. *Trans. R. Soc. trop. Med. Hyg.*, 1970, **64**: 797-812.

18. IGBOKWE (I.O.), ANOSA (V.O.). Response to anaemia in experimental *Trypanosoma vivax* infection of sheep. *J. Comp. Path.*, 1989a, **100**: 111-118.

19. IGBOKWE (I.O.), ANOSA (V.O.). Leucopenia in *Trypanosoma vivax* infection of sheep. *Revue Élev. Méd. vét. Pays trop.*, 1989, **42** (2): 219-221.

20. IKEDE (B.O.). African trypanosomes. *Insect sci. appl.*, 1986, **7**: 368-378.

21. IKEDE (B.O.), LOSOS (G.J.). Pathological changes in cattle infected with *Trypanosoma brucei. Vet. Path.*, 1972a, **9**: 272-277.

22. IKEDE (B.O.), LOSOS (G.J.). Pathology of the disease in sheep produced experimentally by *Trypanosoma brucei. Vet. Path.*, 1972b, **9**: 278-289.

23. IKEDE (B.O.), LULE (M.), TERRY (R.J.). Anaemia in trypanosomiasis. Mechanisms of erythrocyte destruction in mice infected with *Trypanosoma congolense* or *T. brucei. Acta trop.*, 1977, **34**: 53-60.

24. KAGGWA (E.), MUNGUA (W.K.), MUGERA (G.M.). Pathogenecity of *Trypanosoma brucei* in dogs. *Bull. Anim. Hlth Prod. Afr.*, 1984, **32**: 360-368.

25. LOSOS (G.J.), IKEDE (B.O.). Review of pathology of diseases of domestic and laboratory animals caused by *Trypanosoma congolense, T. vivax, T. brucei, T. rhodesiense* and *T. gambiense*. *Vet. Path.*, 1972, **9** suppl. : 1-71.

26. MACKENZIE (P.K.I.), CRUICKSHANK (I.G.). Phagocytosis of erythrocytes and leucocytes in sheep infected with *Trypanosoma congolensis* (Broden 1904). *Res. vet. Sci.*, 1973, **15**: 256-262.

27. MAMO (E.), HOLMES (P.H.). The erythrokinetics of Zebu cattle chronically infected with *Trypanosoma congolense. Res. vet. Sci.*, 1975, **18**: 105-106.

28. MAXIE (M.G.), LOSOS (G.J.), TABEL (H.). *In* : SOULSBY (E.J.L.) Ed. Pathophysiology of parasitic infection. New York, Academic Press, 1976. p. 1183-1198.

29. MURRAY (M.), MURRAY (P.K.), McINTYRE (W.I.M.). An improved parasitological technique for the diagnosis of African trypanosomiasis. *Trans. R. Soc. trop. Med. Hyg.*, 1977, **71**: 325-326.

30. NAYLOR (D.C.). The haematology and histopathology of *Trypanosoma congolense* infection in cattle. Part. I. Introduction and histopathology. *Trop. Anim. Hlth Prod.*, 1971, **3**: 159-168.

31. ROBINS-BROWNE (R.W.), SCHNEIDER (J.), METZ (J.). Thrombocytopenia in trypanosomiasis. *Am. J. trop. Med. Hyg.*, 1975, **24**: 226-231.

32. SCHALM (O.W.), JAIN (N.C.), CARROL (E.J.). Veterinary haematology. 3rd ed. Philadelphia, Lea and Febiger, 1975. p. 15-81.

33. Statistical Analysis Systems (SAS). Version 6.03, SAS User's Guide : Statistics. Cary., North Carolina, USA, SAS Institute Inc., 1987.

34. VALLI (V.E.O.), FORSBERG (C.M.), McSHERRY (B.J.). The pathogenesis of *Trypanosoma congolense* infection in calves. II. Anaemia and erythroid response. *Vet. Path.*, 1978a, **15**: 732-745.

35. VALLI (V.E.O.), FORSBERG (C.M.), ROBINSON (G.A.). The pathogenesis of *Trypanosoma congolense* infection in calves. I. Clinical observations and gross pathological changes. *Vet. Path.*, 1978b, **15**: 608-620.

OGUNSANMI (A.O.), AKPAVIE (S.O.), ANOSA (V.O.). Haematological changes in ewes experimentally infected with *Trypanosoma brucei*. *Revue Élev. Méd. vét. Pays trop.*, 1994, **47** (1) : 53-57

Changes in the haematological values were studied in West African Dwarf ewes infected with *Trypanosoma brucei*. This disease was characterized by a normocytic normochromic anaemia in the acute phase and a severe macrocytosis during the chronicity. The observed changes suggest inadequate erythropoiesis in the acute phase while the chronic phase had a superior but still inadequate erythropoietic response with persistent anaemia. Normal total leucocyte values were observed during the acute phase while leucocytosis was a permanent feature in the chronic phase.

Key words : Ewe - Djallonké sheep - Blood - Hematopoiesis - *Trypanosoma brucei* - Nigeria.

OGUNSANMI (A.O.), AKPAVIE (S.O.), ANOSA (V.O.). Modificaciones hematológicas observadas en ovejas infectadas experimentalmente con *Trypanosoma brucei*. *Revue Élev. Méd. vét. Pays trop.*, 1994, **47** (1) : 53-57

Se observaron modificaciones de los valores hematológicos en ovejas de la raza oeste africana infectadas con *Trypanosoma brucei*. La enfermedad se caracterizaba por una anemia normocroma normocitica de fase aguda y por una macrocitosis grave de fase crónica. Según las modificaciones hematológicas observadas, la eritropoiesis estaba insuficiente durante la fase aguda mientras que durante la fase crónica, estaba más elevada, pero sin embargo insuficiente, causando una anemia persistente. Estaba normal el recuento de leucocitos durante la fase aguda, mientras que la leucocitosis era una característica constante de la fase crónica.

Palabras clave : Oveja - Ovino Djallonké - Sangre - Hematopoiesis - *Trypanosoma brucei* - Nigeria.