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Haematology of experimental *Trypanosoma brucei gambiense* infection. II. Erythrocyte and leucocyte changes

EMERIBE (A.O.), ANOSA (V.O.). Hématologie de l'infection expérimentale à *Trypanosoma brucei gambiense* II. Changements érythrocytaires et leucocytaires. *Revue Élev. Méd. vét. Pays trop.*, 1991, **44** (1) : 53-57

L'infection chronique à *Trypanosoma brucei gambiense* chez le lapin engendre une anémie légère initialement macrocytique et normochrome mais qui évolue, ultérieurement, vers une forme microcytique et hypochrome. Une anisocytose moyenne et une poïkilocytose sont apparues dès le 14e jour après l'infection (*p.i.*). Les cellules rouges à noyau - normales chez le lapin -, que l'on pouvait observer avant l'infection, ont vu leur nombre diminuer au fur et à mesure que la maladie progressait. Ont également été observées : une leucocytose à neutrophiles et éosinophiles, une monocytose et une lymphopénie en phase terminale. Le changement essentiel dans la morphologie des lymphocytes était la présence de lymphocytes atypiques liée à des niveaux accrus de jeunes neutrophiles dans la circulation périphérique. En conclusion, les changements majeurs des lignées érythrocytaire et leucocytaire, lors de l'infection expérimentale à *T.b. gambiense* chez le lapin, sont ceux d'une anémie légère devenant, en phase terminale, microcytique et hypochrome, et une leucocytose transitoire due à une neutrophilie et à une monocytose *Mots clés* : Lapin - Trypanosomose - Erythrocyte - Leucocyte - Lymphocyte atypique - Monocytose - *Trypanosoma brucei gambiense* - Nigeria.

INTRODUCTION

In West and Central Africa, *Trypanosoma brucei gambiense* is an important cause of human sleeping sickness. Despite its low prevalence in some territories, sleeping sickness is still a major health problem in many African countries (28). African trypanosome infections are generally characterized by haematological and serum biochemical aberrations the severity of which is often determined by the level of parasitaemia attained in the host (7). *T. b. gambiense* usually, produces low parasitaemia and a chronic disease (18).

Anaemia is a consistent finding in various trypanosome infections. *T. b. gambiense* produced anaemia in rabbits (13) and man (21) while *T. b. rhodesiense* produced anaemia in chimpanzees and man (8, 9). There is usually a negative correlation between the degree of anaemia and each outbreak of parasitaemia (4). Various mechanisms have been reported to contribute to anaemia in relation to trypanosomosis. Inconsistent pattern of total leucocyte changes have been reported in African trypanosomosis. Leucocytosis was reported in *T. brucei* infection of highly tolerant deer mice (7) whereas SAVOR (23) described an initial leucopaenia over the first three weeks of *T. vivax* infection in cattle with values subsequently rising above the pre-infection levels. Investigations on the sequential red and white blood cell changes induced by *T. b. gambiense* parasites have not been reported in man or experimental animals. This study is therefore an attempt to evaluate some of the haematological alterations induced by *T. b. gambiense*, using the rabbit as a model as well as a review of the mechanisms of red and white cell changes in gambiense trypanosomosis.

MATERIALS AND METHODS

Adult male rabbits initialy weighing 1.7-2.7 kg were used. The *T.b. gambiense* parasite (TRIK I) used was isolated from a human patient's gland juice in Gboko, Benue State in June, 1990 (Gboko/80/Hom/NITR/Kad). It was passaged in rats and subsequently cryopreserved at the Nigeria Institute for Trypanosomosis Research, Vom, from where it was brought in for these experiments. The stabilate was passaged through 5 Whistar rats before 4 x 10^7 parasites were inoculated subcutaneously into each of the experimental rabbits. Seven rabbits were infected while 3 served as controls.

The procedure for blood collection into EDTA has earlier been described (14). Haemoglobin (Hb) estimation was made by the cyanmethaemoglobin method, packed cell volume (PCV), visual red blood cell and reticulocyte counting using formal citrate and new methylene blue solutions, respectively as well as total white cell counting using 1 % acetic acid solution, as described by DACIE and LEWIS (11). Absolute differential white cell counts were calculated from the blood film differential percentages using total white cell counts. Varying levels of parasitaemia were estimated as earlier described (14). Pre-infection base line data were obtained from all animals.

On the basis of the severity of the clinical, haematological and parasitological changes, the disease in rabbits was roughly classified into (a) a sub-acute group (A) as shown by 4 of the infected rabbits, and (b) a chronic group (B), as shown by 3 of the rabbits.

Statistical analysis for significance was made by Student's t-test.

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RESULTS

Rabbits appeared to tolerate *T.b. gambiense* infection fairly well. Control animals showed 8 % drop in PCV between day 0 and 42. The same effect should therefore operate in the infected groups in addition to the trypanosome effect. Group A animals showed a 23.7 % drop in PCV while group B (chronically infected) showed a 12.5 % drop in PCV. Group B animals which were more mildly affected by the infection had higher PCV results at the onset of the experiments. On day 42 post-infection (p.i.) group A rabbits manifested significant anaemia ($P < 0.05$, table I). Control rabbits showed persistent macrocytosis while group A rabbits showed initial increase and subsequent decrease in the mean cell volume (fig. 1). Group B infected rabbits also showed a significant decrease in MCV on day 42 p.i. ($P < 0.05$). Reduced mean cell haemoglobin concentration and mean cell haemoglobin values were recorded from group A infected rabbits on day 42 p.i. (fig. 2). There was no statistically significant difference between the reticulocyte counts of both infected and control animals throughout the experiments. However, figure 2 shows that the infected animals generally had lower reticulocyte counts. From day 14 p.i., moderate anisocytosis and poikilocytosis became evident in the blood smear of infected rabbits (A and B). The initially observed macrocytosis and polychromia gradually gave way for microcytosis and hypochromia. Nucleated red cells which were observed pre-infection (normal feature of rabbits) declined in number as the infection progressed.

The total white cell counts of infected rabbits showed an apparent increase which was statistically significant on day 28 p.i. ($P < 0.05$, table II). On day 42 p.i. the total WBC counts of infected rabbits dropped to comparable values with those of controls. Infected rabbits showed a statistically significant increase in absolute neutrophil counts at week 6 p.i. ($P < 0.05$, table III) even though the total counts were not significantly higher than those of the control rabbits (table II). Conversely, lymphocytes dropped from 3 355 ± 616 x 10^6/l on day 0 to 2 665 ± 709 x 10^6/l on 42 p.i. ($P < 0.05$) after a transient increase. Monocyte counts increased significantly from a pre-infection level of 280 ± 116 x 10^6/l to 501 ± 228 x 10^6/l at week 6 p.i. There was a marked eosinopaenia from day 21 p.i. Eosinophil counts fell from the pre-infection level of 128 ± 98 x 10^6/l to 80 ± 159 x 10^6/l on day 21 p.i. No eosinophils were seen in the blood of infected rabbits on days 28 and 35 p.i., while a mean count of 26 ± 72 x 10^6/l was recorded on day 42 p.i. (table III).

The main changes in the morphology of leucocytes were the presence of atypical lymphocytes (403 + 148 x 10^6/l on day 28 p.i.) as well as increased levels of band neutrophils (515 + 10^6/l on day 42 p.i.) in the peripheral circulation.

TABLE I** Sequential changes in PCV, MCV, MCHC and reticulocyte counts in control and* **T. b. gambiense-infected rabbits.*

	PCV (%)	MCV (f1)	MCHC (gm/dl)	Retics (%)
Day 0				
Controls	37.0 ± 4.0	75 ± 1.5	32.7 ± 0.6	2.6 ± 0.7
Inf. A	38.1 ± 1.3	76 ± 1.7	32.8 ± 0.5	1.9 ± 0.4
Inf. B	42.0 ± 4.4	74 ± 0.6	32.0 ± 0.0	2.6 ± 0.8
Day 14 p.i.				
Controls	34.0 ± 1.5	78 ± 2.5	34.7 ± 1.2	5.4 ± 2.5
Inf. A	43.1 ± 1.7	77 ± 2.4	33.5 ± 0.6	5.8 ± 5.4
Inf. B	38.0 ± 5.7	72 ± 5.6	34.7 ± 1.5	4.5 ± 3.6
Day 28 p.i.				
Controls	33.0 ± 4.0	79 ± 4.4	34.3 ± 0.6	3.8 ± 0.6
Inf. A	31.0 ± 1.7	80 ± 5.0	33.0 ± 2.2	3.3 ± 1.5
Inf. A	36.0 ± 4.7	71 ± 2.0**	34.6 ± 2.5	2.5 ± 2.1
Day 42 p.i.				
Controls	34.0 ± 3.5	81 ± 2.1	33.7 ± 0.6	4.2 ± 0.9
Inf. A	29.0 ± 1.3*	68 ± 6.1*	30.0 ± 1.6***	3.4 ± 2.0
Inf. B	36.0 ± 3.2	70 ± 7.1*	33.0 ± 1.7	2.3 ± 1.3

Inf. A = Subacutely infected rabbits (3). Inf. B = Chronically infected rabbits (5).
** P < 0.05. ** P < 0.025. *** P < 0.005*

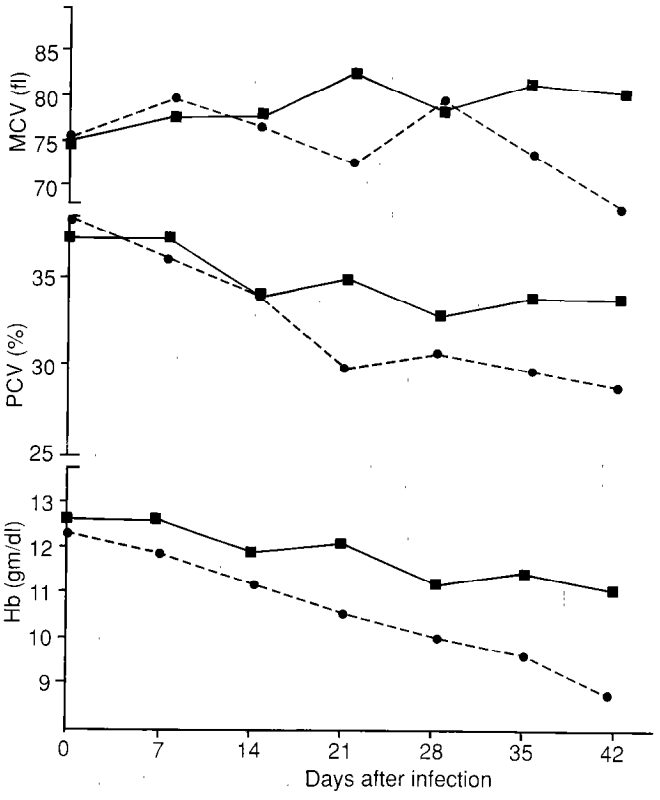

Fig. 1 : Seqential changes in Hb, PCV and MCV values in control ( ) and subacutely infected rabbits (—).

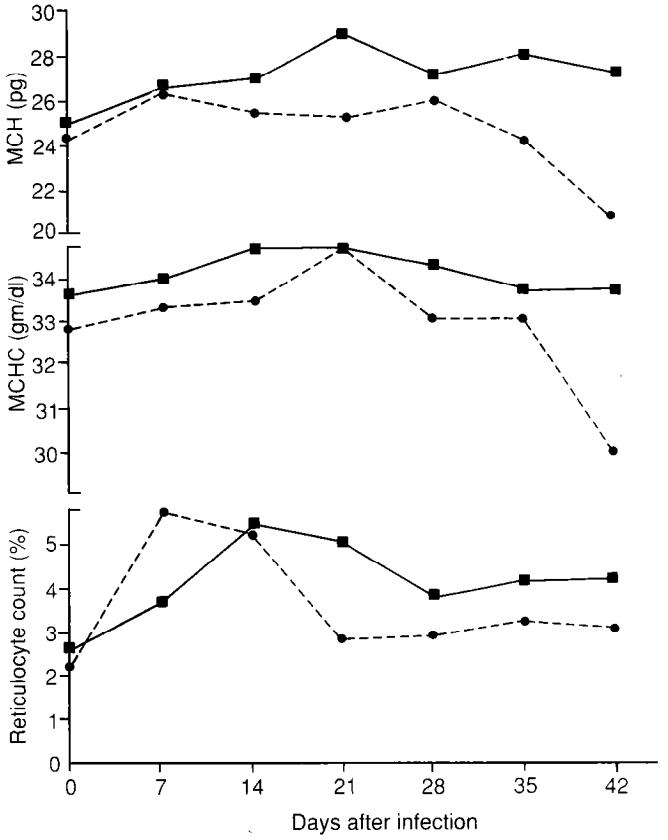

Fig. 2 : Sequential changes in reticulocyte count? MCHC and MCH in control ( ) and subacutely infected (—) rabbits.

TABLE II Changes in total WBC counts in control and* T. b. gambiense-*infected rabbits.

	Controls (n = 3)	Inf. A (n = 4)	Inf. B (n = 3)
Pre-infection	6.5 ± 2.2	6.4 ± 1.2	5.6 ± 0.3
Day 14 p.i.	6.0 ± 1.2	6.8 ± 0.8	7.0 ± 0.8
Day 28 p.i.	6.7 ± 2.0	8.6 ± 3.3	10.6 ± 2.0*
Day 12 p.i.	6.3 ± 1.1	6.8 ± 0.9	6.2 ± 0.9

Inf. A = Subacutely infected rabbits. Inf. B = Chronically infected rabbits.
** P < 0.05*

TABLE III Summary of changes in absolute differential WBC counts (cells per mm³ of blood) of seven* T. b. gambiense-*infected rabbits at week 6 p.i.

Cell type	Pre-infection	42 days p.i.
Neutrophils	2 318 ± 437	3 315 ± 722*
Lymphocytes	3 355 ± 616	2 665 ± 709**
Monocytes	280 ± 116	501 ± 228**
Eosinophils	128 ± 98	26 ± 72**

** P < 0.025. ** P < 0.05.*

DISCUSSION

The drop in PCV of control rabbits might be due to the constant sampling of blood especially between day 1 to 14. Reduction in the red cell parameters of infected rabbits began from day 7 in some animals. The anaemia was generally very mild compared to findings in other trypanosome infections such as *T. vivax* and *T. congolense* infections in ruminants (16, 5) and *T.b. rhodesiense* infection in man and rodents (8, 9). Changes in MCV values during the first 12 weeks were similar to those of control animals, *i.e.* MCV of controls also increased. This may be the possible effect of daily bleeding for concurrent platelet studies. After the daily bleeding was discontinued in favour of a weekly schedule, MCV of controls stabilised at higher normal levels. Conversely those of infected animals together with their MCHC and MCH dropped to below pre-infection levels resulting in a microcytic hypochromic anaemia in contrast to the earlier macrocytic picture. These changes correspond to the increase and later drop in the number of circulating reticulocytes despite the persistence of anaemia. Microcytic hypochromic anaemia after initial macrocytosis have been reported in trypanosomosis (12, 26). Microcytic hypochromic anaemia could be caused via either of the two following main ways : firstly, because of a general deficiency of iron needed for haemoglobin synthesis : secondly, due to the failure in iron incorporation into red cell precursors in the presence of adequate or even excessive iron storage in the bone marrow. Studies by DARGIE *et al.* (17) in cattle infected with *T. congolense* have shown that failure in iron incorporation rather than iron deficiency is the cause of the microcytic hypochromic anaemia seen in some types of trypanosomosis. Higher reticulocyte counts would have been expected if the mechanisms of anaemia were mainly haemolytic as in *T.b. rhodesiense* infection (22). The poor erythrocyte response obtained in the present study may indicate that factors such as shunting off of iron from the erythroid cell like in anaemia of chronic disorder (12), stem cell injury (24), phagocytosis of erythroid cells (7) may play major roles in the anaemia of *T.b. gambiense* infection. Moderate poikilocytosis with few schistocytes observed could be the result of an RBC damage of the spleen or could be due to an increased consumption coagulopathy. Generally, erythrocyte changes observed in this study agree with those earlier recorded in *T.b. gambiense* infection of man and rodents (13, 21).

Transient recurrent leucocytosis was a feature of *T.b. gambiense* infection in this study. This result agrees with the leucocytosis reported in *T.b. gambiense* infection of man and *T.b. brucei* infection in highly tolerant deer mice (7). More commonly, however, leucopaenia has been a feature of early phases of trypanosomosis (2, 8, 15). The leucocytosis was accompanied by mild neutrophilia, lymphopaenia, eosinopaenia and monocytosis. Monocytosis, lymphopaenia and eosinopaenia have all been reported

as consistent findings in trypanosomosis (5). Neutrophilia with a mild left shift observed in this experiment has been reported in *T. evansi* infections of camels, dogs and rats (25). The aetiology of these leucocyte aberrations have not been adequately investigated, but several deductions can be made. The neutrophilia could be due to an irritation of the bone marrow by *T. b. gambiense* probably too weak to cause marrow granulocyte hypoplasia (21) which contributes to the neutropaenia more commonly reported in *T. vivax* (5), *T. brucei* (8) and *T. congolense* (20) infections. Eosinopaenia which has been reported more consistently appears to be a pathognomonic haematological feature of trypanosomosis (4). The aetiology of eosinopaenia may include marrow granulocyte hypoplasia (1), splenic sequestration considering that hypersplenism is common in trypanosomosis (19) and coating of granulocyte progenitor cells with trypanosome antigen/antibody complexes thus predisposing them to phagocytosis (17). However, in this study, since eosinopaenia occurred together with neutrophilia and yet eosinophils share same progenitor cells with neutrophils, it would thus be worthwhile investigating the level of ACTH and adrenal cortex hormones which have been reported to have eosinopaenic and neutrophilic effects (27) during *T.b. gambiense* infection. The cachetic nature of the disease may induce eosinopeania via the stress pathway. The atypical lymphocytes seen in some of the blood smears could result from trypanosoma antigenic challenge leading to an increased proliferation of immunocompetent cells into antibody and/or lymphokine producing cells with a characteristic deeply basophilic cytoplasm. The intense antigenic stimulation by the trypanosomes may result in a depletion of the earlier hyperplastic lymphoid follicles and germinal centres resulting in the ultimately observed lymphopaenia. Loss of germinal centres and thinning of the cortex have been reported in *T.b. brucei* and *T. congolense* infections in rats (10) and *T. vivax* infection of sheep and goats (6). Monocytosis which may be due to increased demands to remove particulate matter has been reported in several infections (2). This was matched by proliferation of macrophages in several tissues in trypanosomosis (3).

It is concluded that the main erythrocytic and leucocytic changes during *T. b. gambiense* infection were mild anaemia which was ultimately microcytic, hypochromic and transient leucocytosis due to neutrophilia and monocytosis.

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Chronic *Trypanosoma b. gambiense* infection of rabbits induced mild anaemia which was initially macrocytic normochromic, but became later microcytic hypochromic. Moderate anisocytosis and poikilocytosis were evident from 14 days post infection (p.i.). Nucleated red cells which were observed prior to the infection (normal feature of rabbits) declined in number as the infection progressed. Leucocytosis with neutrophilia, eosinophilia, monocytosis and terminal lymphopaenia were also observed. The main changes in the morphology of leucocytes were the presence of atypical lymphocytes as well as increased levels of band neutrophils in the peripheral circulation. It is concluded that the main erythrocytic and leucocytic changes in the *T.b. gambiense* infection were mild anaemia which was terminally microcytic hypochromic and transient leucocytosis due to neutrophilia and monocytosis. *Key words* : Rabbit - Trypanosomosis - Erythrocyte - Leucocyte - Anaemia - Transient leucocytosis - Atypical lymphocyte - Monocytosis - *Trypanosoma brucei gambiense* - Nigeria.

EMERIBE (A.O.), ANOSA (V.O.). Hematologia de la tripanosomosis experimental a *Trypanosoma brucei gambiense* II. Modificaciones eritrocitarias y leucocitarias. *Revue Élev. Méd. vét. Pays trop.*, 1991, **44** (1) : 53-57

La tripanosomosis crónica a *Trypanosoma brucei gambiense* en el conejo provoca una anemia reducida, inicialmente macrocitica y normocroma pero que vuelve, ulteriormente, microcitica e hipocroma. A partir dle día 14 después de la infección (*p.i.*) una anisocitosis media y una poikilocitosis se aparecieron. El número de las células rojas con núcleo - normales en el conejo - que se podían observar antes de la infección aumentaba a medida que la enfermedad progresaba. Se observaron también : una leucocitosis con neutrofilos y eosinofilos, una monocitosis y una linfopenia en fase terminal. La modificación esencial en la morfologia de los linfocitos era la presencia de linfocitos atipicos ligada con niveles de jovenes neutrofilos en la circulación periférica. En conclusión, las modificaciones importantes de las lineas eritrocitaria y leucocitaria, durante la infección experimental a *T. b. gambiense* en el conejo, son las de una anemia reducida volviendo, en fase terminal, microcitica e hipocroma, y una leucocitosis transitoria causada por una neutrofilia y una monocitosis. *Palabras claves* : Conejo - Tripanosomosis - Eritrocito - Leucocito atipico - Monocitosis - *Trypanosoma brucei gambiense* - Nigeria.

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