Communication

Leucopenia in *Trypanosoma vivax* infection of sheep

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IGBOKWE (I. O.), ANOSA (V. O.). Leucopénie chez les moutons infectés par *Trypanosoma vivax*. *Revue Élev. Méd. vét. Pays trop.*, 1989, **42** (2) : 219-221.

L'infection expérimentale par *Trypanosoma vivax* a provoqué chez les moutons une leucopénie modérée associée à une lymphopénie et une éosinopénie. Les numérations totales des leucocytes des souris adultes n'ont pas significativement diminué lors de l'inoculation avec le plasma de mouton infecté par *T. vivax*. Ces observations suggèrent que le plasma des moutons infectés ne possède pas un facteur capable de réduire la leucopoïèse *in vivo*. *Mots clés* : Mouton - Trypanosomose - *Trypanosoma vivax* - Leucopénie - Facteur plasmatique - Nigeria.

Trypanosomosis, a haemoprotozoan disease of animals and man, constitutes a limiting factor to livestock production in most parts of Africa (1, 10, 11). The pathogens, which are transmitted biologically by *Glossina sp* and/or mechanically, include *Trypanosoma congolense, T. vivax, T. brucei, T. simiae, T. evansi* and *T. equiperdum* (8). The haematological changes associated with the disease are anaemia, leucopenia and thrombocytopenia (3, 7). The pathogenesis of the leucopenia has been reviewed by ANOSA (3). The serum of cattle infected with *T. vivax* has been observed to depress the granulocyte/macrophage colony formation *in vitro* and it is speculated that the serum factor may have toxic effect on circulating leucocytes (9). There appears to be no information on the role of the plasma factor *in vivo* in the pathogenesis of the leucopenia.

The present study assesses the possible role of the plasma factor in the pathogenesis of the leucopenia in experimental *T. vivax* infection of sheep.

Out of 6 West African Dwarf sheep used for this study, 4 were experimentally infected intravenously with 5×10^6 of *Trypanosoma vivax* strain V8 while 2 served as controls. Blood was collected from each sheep by jugular venipuncture at 0, 2, 3 and 4 weeks post-infection (PI). Blood for haematology was anticoagulated in ethylene diamine tetracetate (EDTA). Plasma was harvested from heparinized blood by centrifuging the blood at 3,000 g for 10 minutes. The plasma was stored in sterile plastic tubes at -20 °C for between a few days and one week, after which the plasma from each sheep was thawed and inoculated subcutaneously into 4 mice at the rate of 1.5 ml of plasma per mouse in 3 divided doses of 0.5 ml per day for 3 consecutive days. At 72 hours after the last dose, the mice were anaesthetized with ether, the chest opened and the heart blood anticoagulated in EDTA, was collected for total white blood cell counts.

Experimental animals

Sheep : They were 1-2 years of age, weighing 10-20 kg and were bought at the local markets in Ibadan. They were blindly treated for bacterial and protozoan infections endemic in the area and were stabilized for more than one month in fly-proof pens before infection.

Mice : They were in-bred Swiss albino mice above 8 weeks of age.

The aim of the study was to evaluate the presence or absence of plasma factors in infected sheep which may be responsible for induction of leucopenia. It was therefore necessary to use mice which were not susceptible to *T. vivax* infection so that changes in the mice could be attributable to the effects of infected sheep plasma and not to actual infection of mice by the organism. Furthermore, the small size of the mouse was suitable since the amount of plasma injected would produce a greater effect than it would have been the case if a larger animal was used.

Haematological techniques

Packed cell volume (PCV) estimation was by microhaematocrit method while haemoglobin (Hb) estimation was by cyanmethaemoglobin method. Total white blood cell count was carried out by haemocytometry. White blood cell differential count was done on thin blood smears stained with Wright's stain.

The *T. vivax*-infected sheep developed a parasitaemia at about 1 week PI. The appearance of the parasites in the blood was associated with a drop in PCV (Fig. 1). Changes in the leucogram of the infected sheep is presented in table I. There was a depression of the total WBC counts at 2, 3, and 4 weeks PI. The total WBC counts at 2 and 3 weeks PI did not significantly differ from the pre-infection and control values. At 4 weeks PI, the total WBC count was significantly lower ($P < 0.05$) than the pre-infection value.

There was no significant change in the neutrophil counts. The lymphocyte count was significantly decreased at 3 and 4 weeks PI ($P < 0.05$). The monocyte count decreased progressively from its pre-infec-

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Reçu le 29.07.88, accepté le 27.09.88.

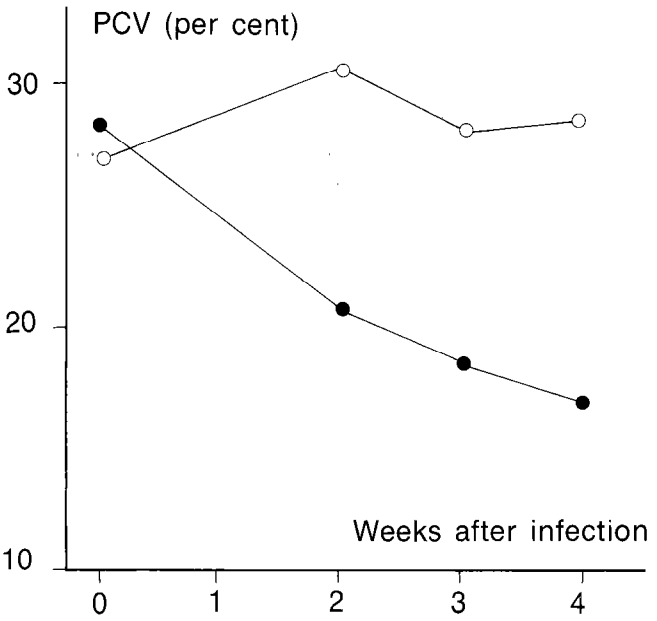

Fig. 1 : Changes in the mean PCV of sheep infected with T. vivax.

tion value but the value attained was not significantly different from that of the control. The eosinophil count dropped at 2 weeks PI, rose above the pre-infection value at 3 weeks PI and dropped below the pre-infection value at 4 weeks PI. The drop at 2 weeks PI and the rise at 3 weeks PI were significant ($P < 0.05$).

Inoculation of mice with plasma : subcutaneous injection of the plasma into the mice produced an elevation of the skin at the site of injection. The elevation disappeared after a few hours without an obvious inflammatory reaction. The mice were active and none died during the period of inoculation. The mean total WBC counts of the heart blood of the mice at 72 hours after the last dose of plasma are presented in table II. There were no significant difference between the mean total WBC counts of the mice that received no plasma inoculation (NP) and the mice inoculated with

TABLE II The mean white blood cell counts of the heart blood of mice inoculated with plasma.

Mice (*) group	Number of mice	WBC ($\times 10^3/\mu l$)
NP	6	4.0 ± 0.8**
CP	10	4.5 ± 2.2
TP-2	8	4.5 ± 2.1
TP-3	8	5.1 ± 1.2
TP-4	16	4.8 ± 1.6

* NP = no plasma inoculation.
CP = mice received plasma from control sheep.
TP-2, 3, 4 = mice received plasma from *T. vivax* infected sheep at 2, 3, 4 weeks PI.
** mean ± standard deviation.

plasma from the control sheep (CP), on one hand, and between the mice inoculated with plasma from the control sheep and mice inoculated with plasma from *T. vivax*-infected sheep (TP), on the other.

The *T. vivax*-infected sheep developed a progressive anaemia which indicated a progression in the severity of the disease. The leucopenia observed in the infected sheep was not very severe. Earlier workers who reported leucopenia in *T. vivax* infection of sheep observed the lowest values of the total WBC counts between 2 and 4 weeks PI (1, 4). While ANOSA and ISOUN (5) reported that the leucopenia was associated with lymphopenia, neutropenia, eosinopenia and monocytosis, in this study, only lymphopenia and eosinopenia were marked. The lymphopenia may have arisen because of the depletion of lymphocytes from the lymphoid nodules and the sequestration of many lymphocytes in the inflammatory reactions in *T. vivax* infection of ruminants (5). The eosinopenia was presumed to be due to depression of the granulocyte precursors in the bone marrow by trypanosome toxins (2). Neutropenia in *T. vivax* infection had been postulated to be caused by the depression of granulocyte precursors in the bone marrow coupled with trapping of neutrophils in the spleen due to hypersplenism syndrome (2). Since the eosinopenia observed in this study did not occur with neutropenia, the pathogene-

TABLE I Changes in the mean white blood cell counts (total and differential) of sheep infected with **T. vivax.**

	Weeks post-infection				Control/ non-infected
	0	2	3	4	
Total WBC ($\times 10^3/\mu l$) :	11.3 ± 0.8*	10.3 ± 1.7	10.1 ± 1.0	8.3 ± 1.3	10.9 ± 2.7
Neutrophils ($\times 10^3/\mu l$)	5.9 ± 1.0	5.6 ± 1.2	5.5 ± 1.8	4.1 ± 1.5	5.5 ± 2.4
Lymphocytes ($\times 10^3/\mu l$)	4.1 ± 0.1	3.7 ± 0.3	3.1 ± 0.2	3.1 ± 1.0	4.2 ± 1.2
Monocytes ($\times 10^2/\mu l$) :	8.4 ± 0.2	8.2 ± 1.4	6.8 ± 5.6	6.6 ± 3.2	6.7 ± 2.9
Eosinophils ($\times 10^2/\mu l$)	4.4 ± 2.9	2.1 ± 0.3	8.5 ± 0.1	3.9 ± 3.5	5.0 ± 2.1
Basophils ($\times 10^2/\mu l$) :	0	0	0	0	0

* Mean ± Standard deviation.

sis of the eosinopenia may not be associated with the depression of granulocyte precursors. Monocytosis is consistently reported in trypanosomosis (3, 4, 6) in which it co-exists with marked proliferation of macrophages in the tissues (5). In this study, it is not clear why monocytosis was not apparent.

KAAYA *et al.* (9) reported that serum from *T. vivax*-infected cattle depressed granulocyte/macrophage colony formation *in vitro*. The effect of the serum factor *in vivo* was assessed in the mice subcutaneously inoculated with plasma from *T. vivax*-infected sheep. The total WBC count of the mice inoculated with plasma from the control sheep did not differ significantly from that of the mice inoculated with plasma from the *T. vivax*-infected sheep. This may be an indication that the plasma of the infected sheep does not have a factor which could depress leucopoiesis *in vivo*. It may, however, be that the titre of this factor was not high in the plasma and/or that it did not act for long enough time to produce an observable effect on the leucocyte counts of the mice.

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Experimental *Trypanosoma vivax* infection of sheep produced a moderate leucopenia associated with a lymphopenia and eosinopenia. The total white blood cell counts of adult mice were not significantly depressed when inoculated with plasma from *T. vivax*-infected sheep. These observations suggested that the plasma of the infected sheep did not have a factor which could depress leucopoiesis *in vivo*. *Key words* : Sheep - Trypanosomosis - *Trypanosoma vivax* -Leucopenia - Plasma factor - Nigeria.

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