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The Pathology of *Trypanosoma vivax* Isolated from Foreign Breeds Outside the Tsetse Belt in Sudanese Kenana calves

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ملخص البحث

تمت دراسة الأثر المرضي لطفيلي المثقبية الفايفكسية (*T. vivax*) في مجموعة من أبقار الكنانة. لقد تم عزل الطفيل المستعمل في الدراسة من عجول أبقار أوربية في منطقة سنار التي تبعد حوالي 500 كلم من اقرب منطقة لذبابة النوم (Tsetse fly) . وجدت العجول ذات قابلية للعدوى علي الرغم من أن عجول أبقار الكنانة لم تصب بالعدوى الطبيعية في مكان عزل الطفيلى.مات أربعة من الخمسه عجول التي أصيبت بالمرض وكانت أهم أعراض المرض هي حمي وفقد للشهية وخمول. كما شملت صور الأعراض المرضية في العجول تواجد طفيلي المثقبية الفايفكسية في الدم، انخفاض في عدد كرويات الدم الحمراء (أقل من $10x2^{12}$ /ل) و تركيز خضاب الدم (6 جم/دل) ومتوسط حجم الخلايا المرصومة – PCV-(20%) وزيادة في عدد كريات الدم البيضاء ($10x11^{9}$//ل) ومتوسط حجم الخلية فلMCV-(60-120/ فل) ومتوسط خضاب الكرية- MCH-(15-30 بجم/ل) بينما كان متوسط تركيز خصاب الكرية الكلي (MCHC) في نطاق الحدود العادية (25-40%).أظهر تشريح جثث العجول الميتة اكتساء كل الجثة باللون الأصفر (يرقان)، رخاوة في القلب وضمور مصلي بينما لم يتأثر حجم كل من الكبد أو الطحال (حجم عادي). كما اتضح من دراسة الأنسجة الدقيقة المريضة أن هناك انتشاراً للخلايا الليمفاوية والبالعة وخلايا البلازما في العضلات الهيكلية والكبد والكلي والخصيتين والعقد الليمفاوية وتضخم وتفكك في ا لخلايا الكبدية ونزف في الكلي.

Summary

The pathology of *Trypanosoma vivax* was studied in a group of Kenana calves. The strain was isolated from a naturally infected foreign breed calves in Sinnar area, 500 km north of the tsetse area. Kenana calves were susceptible to the experimental infection, although animals of local types were not found to be naturally infected in the farm where the parasite was isolated. Four out of the five experimentally infected calves died. The main symptoms were fever, inappetence and dullness. The clinical pathological picture of the disease was characterized by parasitaemia, reduction in RBCs count ($2x10^{12}$/L), Hb concentration (6g/dl) and PCV (20%) and increase in WBCs count ($11x10^{9}$/l), MCV (60-120/fl) and MCH (15-30 pg/l) whereasMCHC was within the normal limits (25%-40%). In gross pathology, jaundice all over the carcass, serous atrophy, flabby heart and, normal spleen and liver were observed.

Histopathologically, infiltration of lymphocytes, macrophages and plasma cells into the skeletal muscles, liver, kidneys, lungs, testis and lymph nodes, swelling and dissociation of hepatocytes, and haemorrhages in the kidneys were the prominent features.

Introduction

Trypanosomosis which occurs across more than a third of Africa, inarguably the most important infectious disease, holds back the development of livestock production in the continent with an estimated annual loss of more than 500 million USA dollars (Anon, 1992). The pathology of the disease had been the subject of so many reports and reviews (Losos and Ikede, 1972, Clarkson, 1976, Anosa and Isoun, 1980; Ikede *et al*., 1988). Structural, functional and immunopathological changes were pictured, but anaemia remained the cardinal sign of the disease (Fiennes, 1970, Moulton, 1988). The anaemia is haemolytic in nature, occurring intravascularly in the acute stages and extravascularly in the subacute and chronic stages (Esieveo and Saror, 1991). The aetiology of the anaemia has not been well documented, but haemodilution, erythophagocytosis, bone marrow hypofunction, haemolytic factors, immunosuppression and immunological disorders are counted as the main factors in the causation of the diseases (Fiennes, 1970, Saror, 1980). Losos and Ikede (1972) believed that the parasite is strictly confined to the plasma, but Losos (1986) later on, confirmed the findings of Hornbey (1952) that the parasite can invade tissues.

In the Sudan the importance of *T. vivax* as a fulminating parasite causing a multitude of outbreaks in cattle residing extremely far north of the tsetse areas is gaining importance in the last ten years. This has initiated the drive to know the role of *T. vivax* as a potential pathogen in the supposed tsetse free areas. The disease has assumed a frequent presence in milk production farms in the central states and even around Khartoum (Mousa *et al.,* 1990).

An outbreak of *T. vivax* in calves in a dairy farm at Sinnar Sugar company, Sinnar State, had initiated this study of the disease.

Material and Methods

Area:

This study was conducted in Sinnar town and Sinnar sugar factory area. It is 300 km South of Khartoum, lying between latitudes 12^{o}-13^{o} North and longitudes 32^{o}-33^{o}.

Experimental animals:

Nine Kenana calves were used. They were two-years-old, kept in animal pens at the premises of Sinnar Veterinary laboratory, fed on roughages of abusabeen, alfa alfa and additives of sorghum grains, cotton seed cakes and salts, and watered *ad-libitum*. Five calves numbered 5628, 5637, 5654, 5680 and 5692 were infected with the trypanosomes. The other four calves were used as controls.

Trypanosomes:

Trypanosoma vivax was isolated from naturally infected calves at Sinnar Sugar factory dairy farm. The trypanosomes were identified as *Trypanosoma vivax* on the basis of morphology. The infected blood was obtained in anticoagulant (EDTA), kept in ice and transported to the Veterinary Laboratory in Sinnar.

Experimental infection:

The experimental calves were infected directly by the intravenous route through the jugular vein.

Haematological examinations:

Blood samples were aseptically taken from the jugular vein in EDTA.The packed cell volume (PCV) was determined by the microhaematocrit technique (MHT). The red blood cells (RBC) and the white blood cells (WBC) counts were determined using the modified Hawksley haemocytometer, while the haemoglobin (Hb) concentration was estimated by the oxyhaemoglobin method (Schalm, *et al*., 1975).The mean cell haemoglobin (MCH), the mean cell haemoglobin concentration (MCHC) and the mean cell volume (MCV) were computed. The parasite was diagnosed by the direct wet smear, the microhaematocrit technique (MHT), and the thin and thick Giemsa stained smears.

Post mortem and Histological examinations:

Post-mortem examination was carried out in all dead animals. Samples from the heart, lungs, liver, kidneys, testis, skeletal muscles and lymph nodes were taken in 10% formalin. They were then, processed for histopathology, embedded in paraffin wax, sectioned and stained with H &E.

Results

Haematology:

All the infected calves developed parasitaemia within seven days p.i. The parasitaemia was high in all the calves and reached

uncountable numbers in some of them. The steady drop in Hb and PCV values after infection coincided with the first peak of parasitaemia,and reached levels of about 6 g/dl and 20%, respectively, within the first two weeks p.i. At the different weekly periods of the experiment, the Hb concentration showed a significant difference from that of the controls ($P<0.05$) and so did the PCV ($P<0.014$) (Fig. 1; 2).

Four of the infected animals died during the study period (4 months). Calves Nos.5680 and calf 5654 showed a degree of endurance untill the end of the experiment when calf no. 5654 died. The total RBCs count declined gradually to levels below $2x10^{12}$/L ($P<0.05$; Fig. 3). The WBCs increased and attained levels of $11x10^{9}$/L in all the calves at about 3 weeks p.i. ($P<0.001$; Fig. 4), when it dropped before showing another peak in two calves and a 3rd peak in calves Nos. 5680 and 5654. The MCV increased above 50fl (mean 40-50) reaching 60-120 fl in almost all the calves, with a significant difference ($P<0.05$), till about 2 months p.i. when 3 calves died. The other two calves showed a MCV of above 60 fl at the end of the experiment (Fig. 5).The MCHC, on the other hand, fluctuated between the levels of 25% and 40%, which was the range of the mean level of the controls (Fig. 6). The MCH increased above the mean controls level attaining up to 15-30pg/L (Fig. 7; $P<0.01$).

Gross pathology:

Prominent p.m. lesions were serous atrophy around the heart and kidneys, oedematous lymph nodes, flabby heart and jaundiced carcasses. The liver and spleen were normal in size.

Histopathology:

The consistent histological finding was infiltration of mononuclear cells, the majority of which were lymphocytes, besides few macrophages and plasma cells. This was seen in the cardiac and skeletal muscles, liver, kidneys, lungs, testis as well as in paracortical, subcapsular, peritrabecular and medullary sinuses of lymph nodes, which had also prominent germinal centres. Further changes had included swelling and dissociation of hepatocytes, particularly in the periportal area and haemorrhages in the kidneys (Figs. 8, 9, 10 and 11).

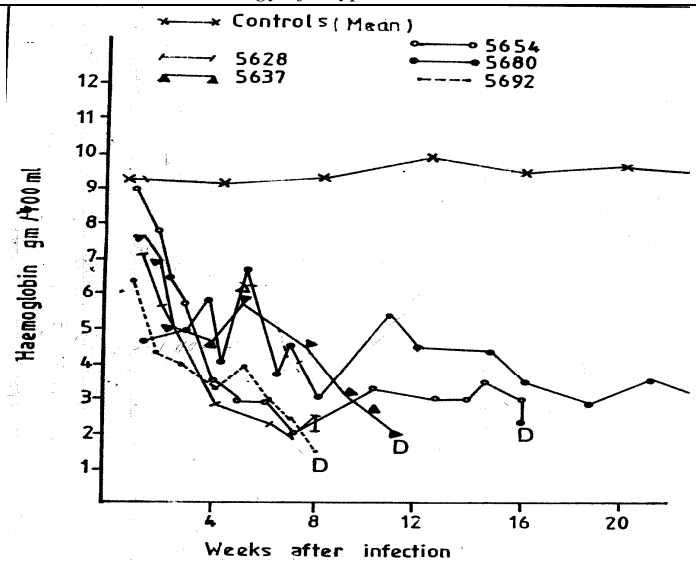

Fig. 1: Determination of haemoglobin concentration (Hb) of infected d control calves.

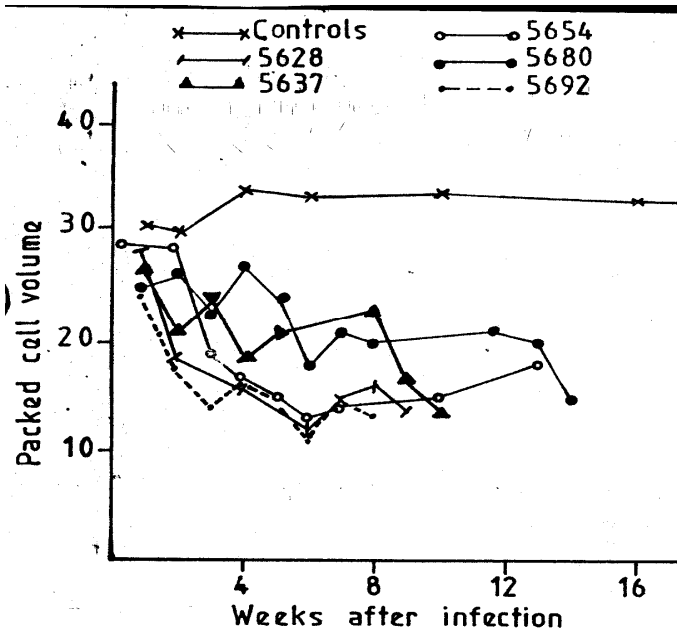

Fig. 2: Determination of packed cell volume (PCV) of infected and control calves.

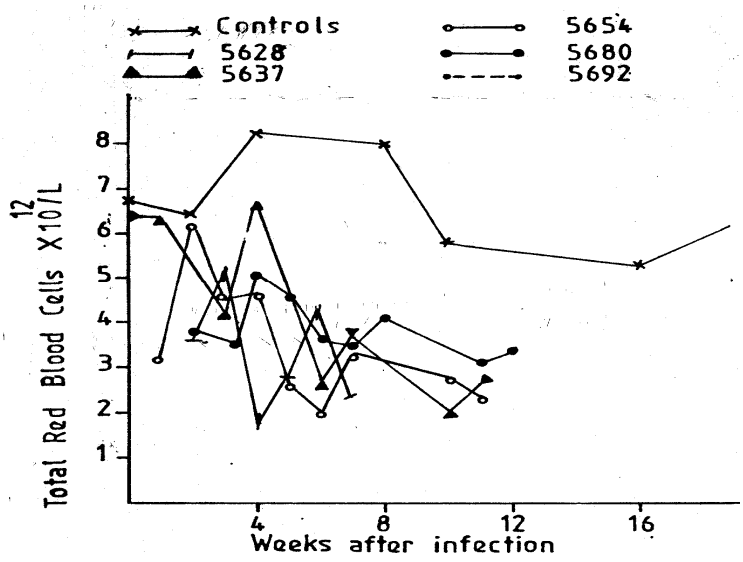

Fig. 3: Determination of total red blood cell (RBC) of infected and control calves.

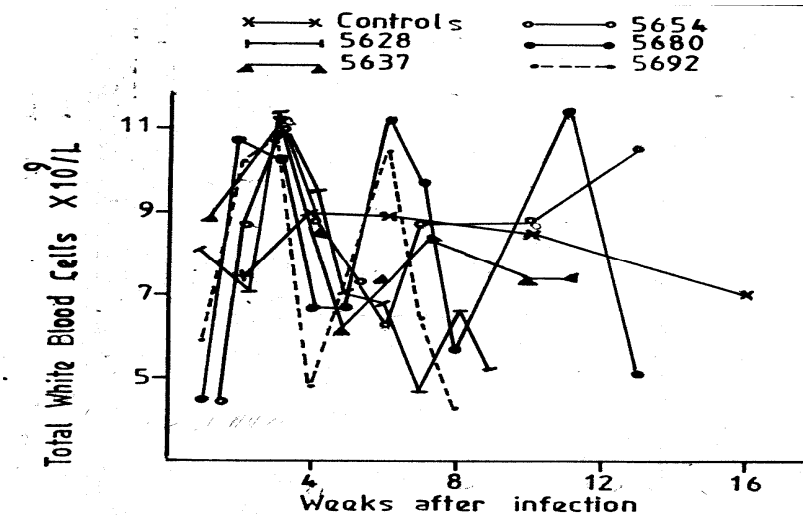

Fig. 4: Determination of total white blood cell (WBC) of infected and control calves.

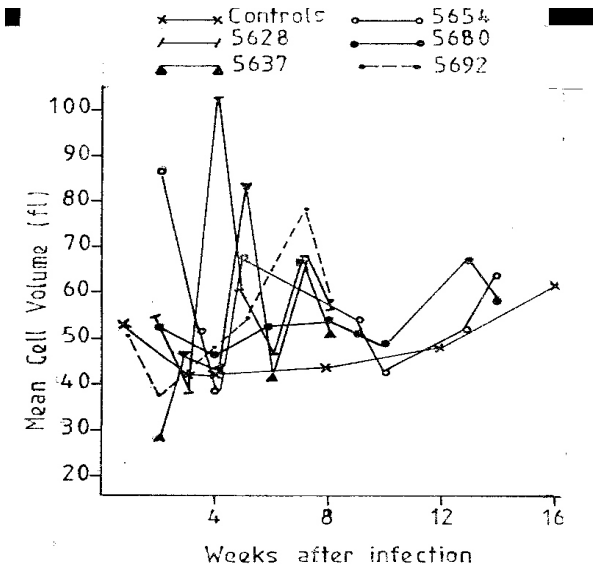

Fig. 5: Determination of mean cell volume (MCV) of infected and control calves.

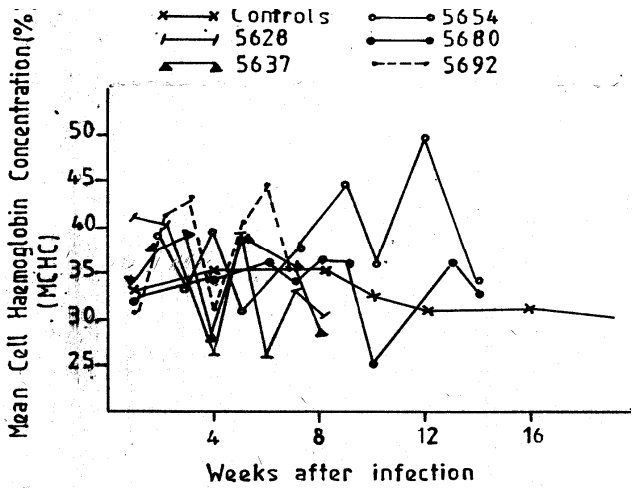

Fig. 6: Determination of mean cell haemoglobin concentration (MCHC) of infected and control calves.

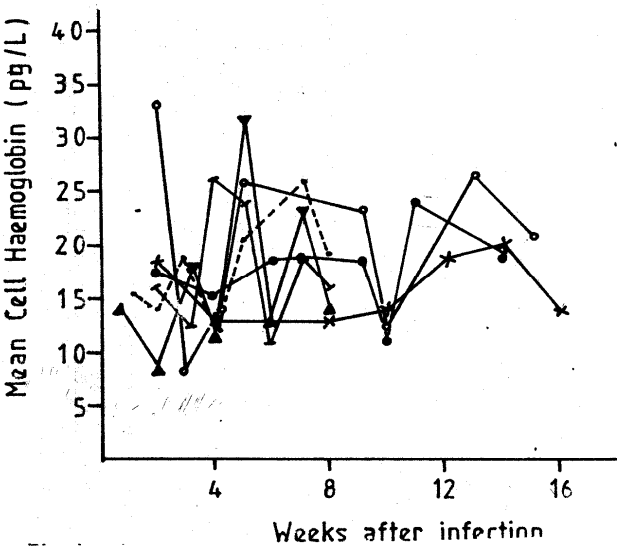

Fig. 7: Determination of mean cell haemoglobin (MCH) of infected and control calves.

Discussion

As indicated from the course of the disease, the death of 4 out of 5 calves reflected a high susceptibility to the *T. vivax* strain isolated from Sinnar sugar factory area, a supposed tsetse free area (Suliman, 1992). The anaemia at the onset of the disease was the cardinal sign of beginning of the infection was coincidental with a drop in the first wave of parasitaemia. Erythrophagocytosis might have taken place at this stage. Murray and Dexter (1988) have attributed the presence of large numbers of macrophages to infection, and held them responsible for phagocytosis of red blood cells. The phenomenon was first observed in *T. brucei* infected rabbits (Boycott and Price-Jones, 1913) and in cattle infected with *T. congolense* infection (Fiennes, 1970; Naylor, 1971; Valli and Foresberg, 1979). This phagocytosis could be due to antigens precipitated on the surface of RBC (Mackenzie *et al*., 1978) or the cleavage produced by sialidase enzyme (Kuster and Silegham involvement in immunosuppression in *T. congolense* infected Boran cattle. This in addition to the haemolytic factors proved to be produced (Fiennes, 1970).

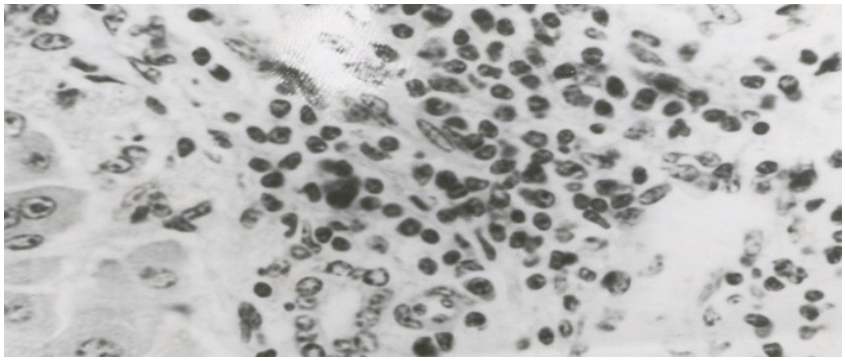

Fig.8 Liver: Infiltration of lymphoid cells in the portal triads H & E x 40 (objective).

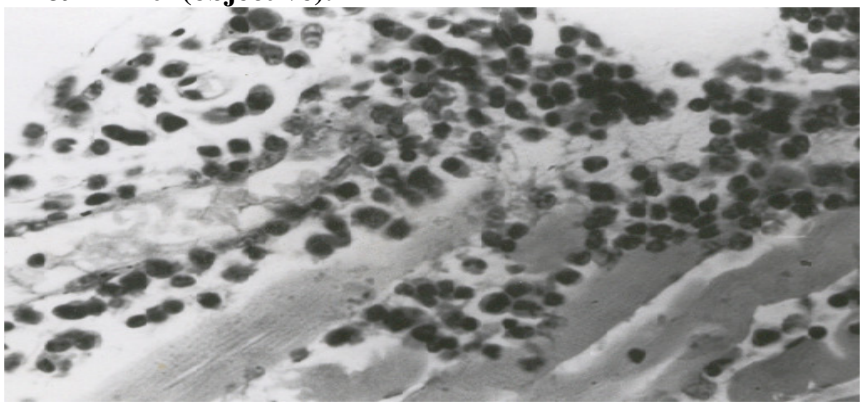

Fig.9 Skeletal Muscles: Effusion of lymphoid cells in the interstitial connective tissue H & E x 40 (objective).

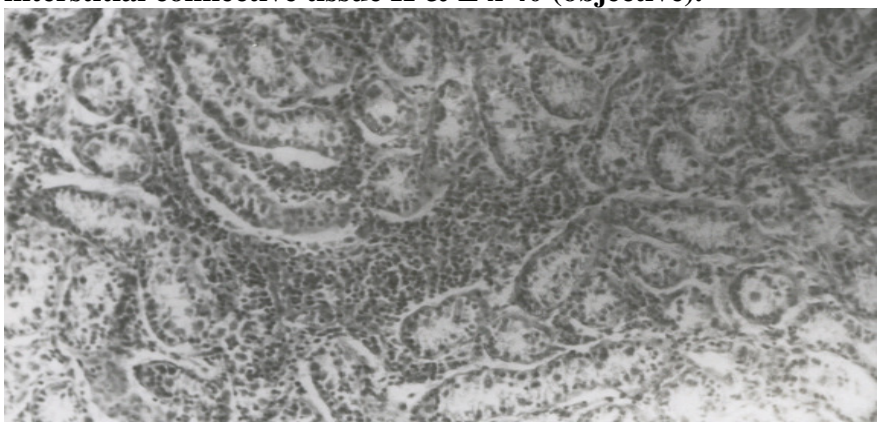

Fig.10Testis:Infiltration of lymphoid cells in the interstitial connective tissue H & E x 40 (objective).

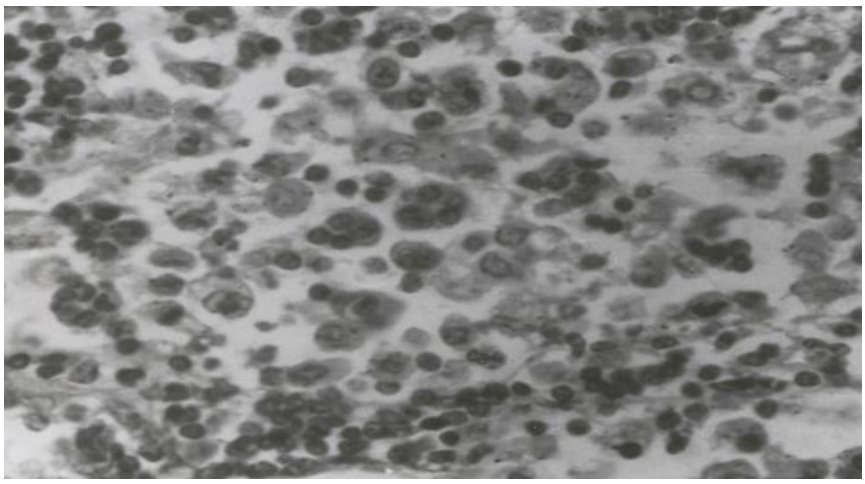

Fig.11 Lymph node: Infiltration of lymphoid cells in the medullary sinuses H & E x 40 (objective).

The MCV elevated,in this experiment, to 80-100 fl in a few calves and continued to be above the normal (Fig. 5). This finding was in agreement with that of Jenkins *et al*. (1980) in *T. brucei* infected rabbits. The MCHC described showed that the calves performed around normal (Fig. 6). On the contrary, Jenkins *et al*. (1980) have found hat MCHC was increased during the infection while the MCH remained constant. Controversially, Anosa (1988) has showed that MCHC was around the normal while MCH increased in calves infected with *T. congolense*.

Murray and Dexter (1988) described two phases of the disease, an acute and a chronic ones. The acute phase is manifested by a drop in Hb, PCV, a decrease in the number of RBC coupled with a hepato-spleenomegaly and an enlargement of the lymph nodes, especially the visible ones. In the chronic phase where anaemia was still a feature, a regression in the size of the spleen, liver, together with changes in the bone marrow, heart, skeletal muscles, kidneys and the connective tissue were observed. In this experiment, the liver and spleen were found to be normal at the time of death probably at the stage of a chronic infection. A proliferative and regressive infection in lymphnodes and spleens of calves and goats, was also described preparations from solid organs. This finding was in agreement with that of Losos and Ikede (1972), although some other (Masake, 1981). The trypanosomes were not

seen in the histological workers had found trypanosomes in solid organs outsides the circulation.No coagulation or thrombosis was encountered in this study despite the fact that the disease had run an acute stage. Intravascular coagulation was reported from *T. vivax* infection by a number of workers (Murray and Dexter, 1988).

The *T. vivax* strain used in this study was isolated from a natural outbreak in the sugar factory dairy farm where calves of the local cattle types (Kenana & others) were not naturally infected. Nevertheless, the experimental inoculations of Kenana calves with the parasite showed that they were susceptible. We think that further research in this area is needed.

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