HAEMATOLOGICAL PROFILE AND PARASITOLOGICAL DIAGNOSIS OF *TRYPANOSOMA VIVAX* INFECTION IN SUDANESE NUBIAN GOATS

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المستخلص

أجريت هذه الدراسة لدراسة تأثير طفيل التربانوسوما فيفاكس الذي تم عزله من خارج منطقة ذبابة التسى تسى على الماعز النوبي و قابليته للإصابة بالمرض. تمت مقارنة الطرق التقليدية لتشخيص المرض باستخدام عينات دم مأخوذة من وريد الرقبة ومن وريد الأذن.

تم استخدام عشرون ماعز نوبي. خمسة عشر تمت عدوتها بالطفيل بينما أستخدمت خمسة كمجموعة غير مصابة. أظهرت الدراسة قابلية الماعز النوبي للإصابة بطفيل التربانوسوما فيفاكس و أدت الإصابة إلى انخفاض في حجم الدم المكداس، العدد الكلى لكريات الدم الحمراء ، تركيز الهيموقلوبين ، ارتفاع ملاحظ في متوسط الحجم الكلى للكريات و متوسط الهيموقلوبين الكلى بينما لم يظهر أي اختلاف في متوسط التركيز الكلى للهيموقلوبين. هذه النتائج أثبتت وجود فقر دم من نوع كبير حجم الخلية و سوية الصبغة. العدد الكلى لكريات الدم البيضاء كان طبيعي. طريقة الطرد المركزي و المسحة الدموية المصبوغة وجدت ملائمة لتشخيص المرض.

Abstract

The present work was carried out to study the haematological profile and infectivity of a stock of *Trypanosoma vivax* which was isolated from an area outside tsetse zone to Nubian goats and their

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susceptibility to the infection. Different conventional parasitological techniques were also compared to diagnose the infection when taking blood samples from jugular and ear veins. Twenty Nubian goats aged 9-12 months were used in the experiment. Fifteen animals were experimentally infected with *T. vivax* stock, while five animals were served as uninfected control group. The study showed that Nubian goats are susceptible to *T. vivax* infection. The infection resulted in significant decrease in packed cell volume (PCV), total red blood cells counts (RBCs) and haemoglobin concentration (Hb) values. Significant increases were encountered in mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values. No significant change was observed in mean corpuscular haemoglobin concentration (MCHC). These results indicated the presence of macrocytic normochromic anaemia. Total white blood cells (WBCs) counts were at normal range. The Haematocrit Centrifugation Technique and thin stained smears were found to be good diagnostic tools for *T. vivax* infection.

Key words: - Trypanosoma vivax, mechanical transmission, Sudanese Nubian goats.

Introduction

Trypanosomiasis are an important group of diseases affecting both man and animals caused by members of the genus *Trypanosoma*. The disease has a major economic importance in Africa, Asia, Latin and South America. In Sudan, trypanosomiasis was firstly reported in 1904 in cattle arrived to Khartoum from Upper Nile state of Southern Sudan (Karib, 1961). Later on, studies and surveys concerning tsetse and trypanosomiasis distribution were done in different parts of Sudan

(A/Rahman, 2002). *Trypanosoma vivax* infections were found in Central Sudan, Abdalla *et al*. (2005) reported that 177 heads of cattle were sampled in Singa area, Sennar State. A total of 89 animals were found to be infected with trypanosomes. All infected animals were infected with *Trypanosoma vivax*. While in AbuHajar 22 out of 500 animals were found to be infected with trypanosomes; the average parasitological prevalence was 4.4%. The infected animals were, also, found harbouring *Trypanosoma vivax* (Abdalla *et al.*, 2008)· In South Kordofan, A/Rahman *et al*. (1991), reported that *T. vivax* infection was predominated in cattle followed by *T. brucei* and *T. congolense*. Experimental *Trypanosoma congolense* infections of goats and calves were conducted by Mahmoud and Elmalik (1977). Goats developed a chronic form of trypanosomiasis, often recovering spontaneously from a strain which caused an acute fatal disease in calves. They concluded that goats may be important in the maintenance of *T. congolense* in nature in the Sudan. A survey was conducted in Kassala Province Using both parasitological tests and enzyme immunoassays, Trypanosomes were found in thick blood films prepared from camels but not in films from sheep and goats. Antibodies to *T. evansi* were found in camels, sheep and goats species suggesting that sheep and goats may be involved in the transmission of *T. evansi* in the Sudan (Boid *et al*., 1981).

A number of parasitological tests are available for detection of trypanosomes infection. Kendrick (1968) reported that the wet smear is a useful method for detection of parasites in drug trials, it is simple and cheap but has low sensitivity and one cannot identify the species of trypanosomes. Thin stained smear is useful in identification of the parasite

species. However, thick stained smear is more sensitive than both wet and thin smears in detection of trypanosomes, but there will be difficulty in recognizing the parasite species (Kendrick, 1968). The Haematocrit centrifugation technique (HCT) as described by Woo (1970) is useful in early detection of trypanosomes infections than other parasitological techniques, and it is an efficient tool in surveys of trypanosomes that are non infective to laboratory animals like *T. vivax* and some strains of *T. congolense*. On the other hand the dark ground phase contrast buffy coat technique (BCT) has proved to be a reliable method than other standard techniques in detecting trypanosomes in the circulation of cattle (Murray, 1977, Paris *et al*. 1982).

The most cardinal sign of animal trypanosomiasis is anaemia associated with decrease in PCV, haemoglobin and RBCs counts as reported by many authors in different animal species (Saror, 1980, Brown *et al*., 1990, Maikaje *et al*., 1991, Bengaly *et al*., 1993, Silva, 1999 and Lukins 1999). The severity of anaemia which follows trypanosomes infection could be related to differences in virulence among trypanosome strains and species and factors associated with the host like age, breed, and nutritional status of infected animals (Murray and Dexter, 1988).

This work is intended to investigate the haematology and parasitological diagnosis of *Trypanosoma vivax* infection in Sudanese Nubian goats and sensitivity of different parasitological diagnostic techniques for detection of infection.

Materials and Methods

Animals:

Twenty male goats aged 9-12 months belonging to the Black Nubian ecotypes, which is a common goat ecotypes in Northern Sudan, were used in this experiment; Animals were kept in fly-proof premises of the Central Veterinary Research Laboratory at Soba for a month as an adaptation period. The goats were ear-tagged, examined for presence of trypanosomes and other blood parasites using conventional parasitological methods. They were also examined for presence of internal parasites and external parasites. The animals were dewormed using albendazole, (Alben, Vety Care pharmaceuticals Ltd., Pakistan), treated with Oxytetracycline Hydrochloride, (Oxigel, Formaceutici Gellini S.P.A, Italy) and sulphonmides as anticoccidial drugs (Sulpha diamidine sodium, Vetwic, Elnasr pharmaceutical chemicals Co, Egypt) and washed with Coumaphos as an acarcide (Asuntol, Bayer, A.G, Germany). All animals were fed dry sorghum hay and concentrates and water throughout the experimental period.

***Trypanosoma vivax* stock:**

The stock was isolated from blood of a confirmed naturally infected cattle found in Suki district (Sennar State). The infected blood was inoculated intravenously in a goat at site of collection, and then transported to Central Veterinary Research Laboratories in Khartoum. Blood samples were collected from the infected goat and preserved in liquid nitrogen (-196c^0). I ml of this blood was used to infect a donor goat.

Fifteen goats were experimentally infected with trypanosome.

Each animal received 1 ml of infected blood IV when parasitaemia scores was ++++ from the donor goat and 5 goats served as uninfected control group.

Parasitaemia and dosing:

The following conventional grades were used to estimate the scores of parasitaemia in wet smears of the infected animals according to Lumsden *et al.* (1973) using 40x magnification..

0 = no trypanosomes seen in minute examination.

\+ = 1 trypanosome per 20 microscopic fields-1 per microscopic field.

++ = 2-6 trypanosomes per microscopic field.

+++ = 7-15 trypanosomes per microscopic field

++++ = 16-25 trypanosomes per microscopic field.

+++++ = more than 25 trypanosomes per microscopic field.

Blood Sampling:

The experimental animals were bled twice weekly for 13 weeks. Blood samples were collected from both jugular and ear veins into heparinised vacutainers and capillary tubes (70mm) for determining the different parasitological and haematological parameters. A total of 253 were collected by the end of experimental period.

Parasitological methods:-

Heparinised blood samples collected from both the Jugular and ear veins were examined for the presence of trypanosomes using the following standard parasitological methods; wet blood smears, thin and thick blood stained smears ,stained with 10% giemsa stain (Kendrick, 1968), Haematocrit centrifugation technique (HCT), (Woo, 1971) and

dark ground /phase contrast buffy coat technique (BCT), (Murray ,1977).

Haematological methods

Different blood parameters were determined according to Schalm *et.al* (1975). Packed cell volume (PCV) was using a microhaematocrit centrifuge (Hawksley and Sons, Ltd., England) for five minutes and the PCV percent was read off on the scaling instrument. Hb was determined by the cyanomethaemoglobin technique using a haemoglobin meter (CIBA Corning, 950 Hb meter, England) at wave length 450 nm. The Hb values were measured in g/dl of blood. White and Red blood cells were counted by the use of an improved Neubauer haemocytometer (Hawksley and Son Ltd., England). Values were expressed in Thousands and million cell/ mm^3 blood respectively.

Blood indices

Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were determined using the following formulas .

$$\text{MCV (fl)} = \frac{\text{PCV (l/l)}}{\text{RBCs } (x10^{12}/l)} \times 1000$$

$$\text{MCH (pg)} = \frac{\text{Hb g/l}}{\text{RBCs } (x10^{12}/l)}$$

$$\text{MCHC (g/l)} = \frac{\text{Hb g /l}}{\text{PCV (l/l)}}$$

Statistical analysis:

Statistical analysis was performed using SPSS program version 9.05. Significant levels were taken at $p < 0.05$.

Results

Parasitaemia:

All infected animals become positive and showed fluctuating parasitaemia throughout the experimental period. Jugular vein parasitaemia had more fluctuations than ear vein parasitaemia. (Fig. 1). Ear veins blood samples revealed higher levels of parasitaemia than jugular vein blood.

Parasitological techniques:

The wet, thin and thick stained blood smears, haematocrit centrifugation techniques (HCT) and dark ground phase contrast buffy coat techniques (BCT) revealed positive results with ratios of 47.15%, 47.15%, 49.05%, 53.61% and 51.33 % respectively for samples taken from the jugular vein, while ratios of 49.05%, 49.05%, 50.57%, 53.61% and 53.23 % respectively were obtained for samples of the ear vein. No significant difference was observed among the results obtained using the different parasitological techniques at the two different sites of sample collection (Table 1).

Haematological findings:

Hematological changes in blood of all experimental goats are summarized in Table 2 and Figures (2-8). A significance decrease was found in PCV, Hb content and RBCs counts, while a significant increase occurred in MCV and MCH values in infected groups. On the other hand no significant difference was found in WBCs count and MCHC values of infected groups in relation to uninfected animals.

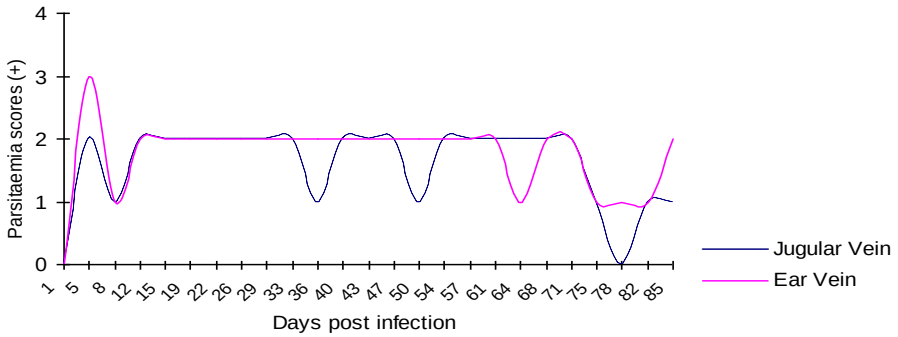


Fig 1. Mean parasitaemia levels in experimentally infected goats with *T. vivax* using blood samples from jugular and ear vein

Table 1: Comparison of the sensitivity of different parasitological techniques in testing jugular and ear veins blood samples in goats experimentally infected with *T. vivax*

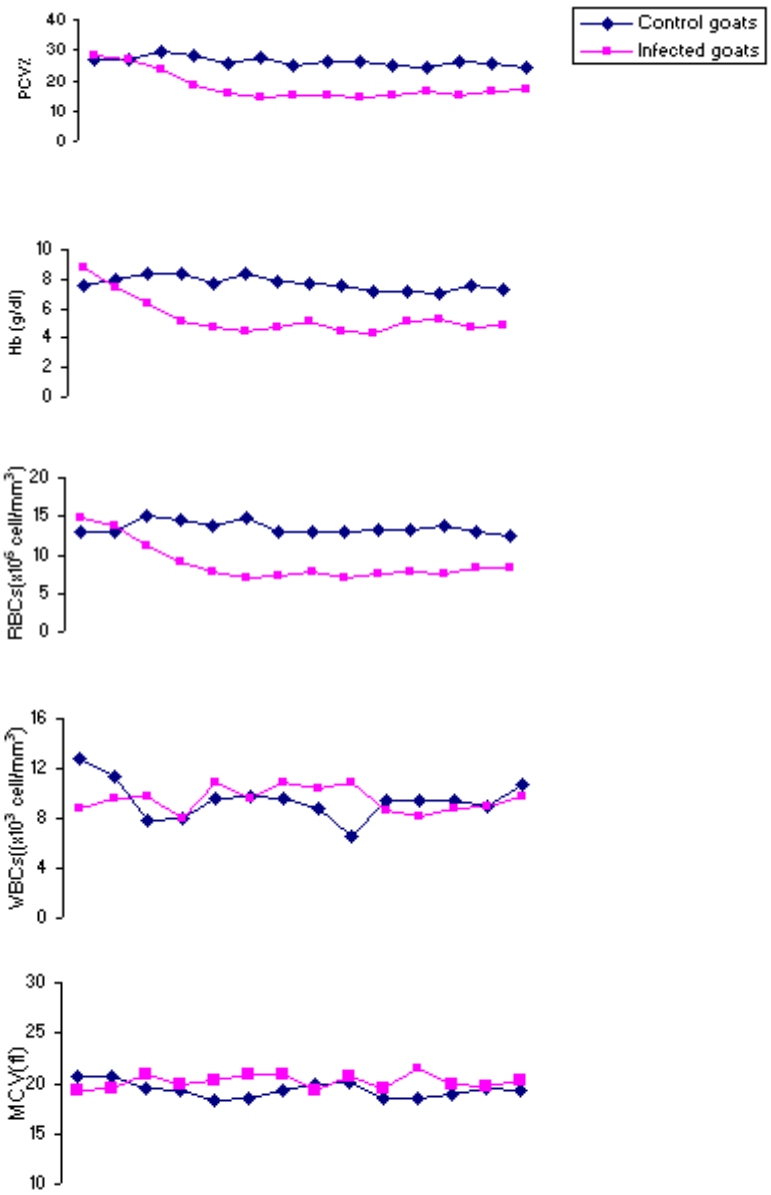
Techniques	Total blood samples examined	Positive jugular veins blood samples	%	Positive ear veins blood samples	%
Wet blood smears	263	124	47.15%	129	49.05%
Thin stained blood smears	263	124	47.15%	129	49.05%
Thick stained blood smears	263	129	49.05%	133	50.57%
HCT	263	141	53.61%	141	53.61%
BCT	263	135	51.33 %	140	53.23 %

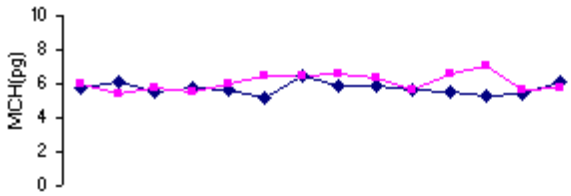
No significant difference is observed

Table 2: Means (±SD) and ranges of values of haematological parameters in experimentally infected with *T. vivax* and uninfected control goats

Parameter	Uninfected group Range	Mean(±SD)	Infected group Range	Mean(±SD)
PCV %	24.0–29.4	26.04±1.64	14.6–23.4	16.37 ±2.48 *
Hb (g/dl)	7.00–8.42	7.69±0.50	4.3 – 6.4	4.93±1.55 *
RBCs ($x10^6/mm^3$)	12.4–15.05	13.60±0.86	7.01–11.18	8.06±1.15 *
WBCs ($x10^3/mm^3$)	6.58–10.69	9.01±1.08	8.84–10.96	9.57±1.06
MCV(fl)	19.85–20.08	19.15±0.57	19.34–21.49	20.29±0.65 *
MCH (pg)	5.09–5.97	5.66±0.25	5.66–7.01	6.15±0.48 *
MCHC (g/l)	26.92–30.95	29.56±1.09	27.35–35.33	30.34±2.42

- *Significant change at $P \leq 0.05$





Discussion

The *Trypanosoma vivax* stock used in this experiment was isolated from infected cattle outside the tsetse zone and is thought to be circulating for along time outside the tsetse zone and adapted to mechanical transmission, yet the results of this study showed that infected animals suffered very high levels of parasitaemia and anemia.

HCT rank first in detecting parasitaemia followed by the BCT, thick stained smears then both the wet smear and thin stained smears in both jugular and ear veins collected samples. However, no significant difference was observed between the two methods of sampling. The superiority of HCT in detecting infection in goats agrees with that of Kalu *et al*. (1986) in *T. vivax* and *T. brucei brucei* infections in goats, and with Kalu and Lawani (1986) who recommended the use of both HCT and BCT in trypanosomiasis epizootiological surveys of goats. Although thin stained smears didn't show high sensitivity in detecting parasitaemia during this study, yet the method has an important role in parasite

identification, a finding which agreed with that of Kendrick (1968).

The slightly higher parasitaemia in the ear vein samples might be explained by the accumulation of parasites in peripheral blood veins as realized by several authors in *T. congolense* infections (Fiennis *et al.*, 1946; Goodwin, 1971 and Banks 1978). This might indicate the suitability of ear vein blood sampling in surveys especially in furious animals.

In this study significant decreases were reported in the PCV levels, Hb concentration and RBCS counts of infected groups, compared to the control group. These findings were supported by the findings of Van den Ingh *et al*. (1976a); Saror, (1980); Sekoni *et al*. (1990b) and Silva *et al*. (1998) in *T. vivax* and *T. congolense* infections in goats and cattle and Sharma *et al*. (2000) in *T. evansi* infection in Barbari goats, the decrease in PCV might be correlated with the decrease in total red blood cell count or due to haemodilution while the decrease in Hb concentration might be due to the decrease in PCV and RBCs count. The decrease in the above mentioned parameters indicate a state of anaemia in the infected groups and ascertained the presence of anaemia reported by several authors in *T. vivax* infections in different hosts (Van den Ingh *et al*., 1976b; Saror, 1980; Igbokwe and Anosa, 1989; Bengaly *et al*., 1993; Okech *et al*., 1996; Silva *et al*., 1998 and Sharma *et al*., 2000). This anaemia might be due to the haemolysins such as proteases, phospholipases and neuraminidases induced by the trypanosomes (Soulsby, 1982), haemodilution which is a state when the fluid content of blood is increased and this results into lowered concentration of the formed elements. For the case of the red cell component, this can result in apparent anaemia (Biryomumaisho and Rwakishaya, 2012), increased erythrophagocytosis, which was found an

important mechanism leading to anaemia in the pathophysiology of *T. congolense* infection in Zambian goats (Witola and Lovelace, 2001), different immunological factors and dyshaemopoiesis on which the bone marrow failed to produce RBCs (Kobayashi *et al*., 1976; Van den Ingh, 1976b; and Murray and Dexter, 1988).

The significant increase in the MCV and MCH values indicates the macrocytic type of anaemia that occurred in infected groups. This finding is in agreement with Saror (1979) and Katunguka (1994) in *T. vivax* and *T. congolense* infection in small ruminants. On the other hand the insignificance change of MCHC percentage indicates that the anaemia occurred was normochromic. This agrees with the finding of Gardiner (1989) who described the anaemia induced by *T. vivax* as normocytic normochromic with a tendency to be macrocytic normochromic.

No significant difference in total white blood cells count (WBCs) was observed though a slight leucocytosis was observed in infected group when compared to the control. These findings disagree with Maxie *et al*. (1979) and Bengaly *et al.* (1993) who realized leucopaenia in experimental trypanosomes infection with both *T. congolense* and *T. vivax* in cattle and small ruminants. The slight increase in WBCs might be correlated to the immunological reactions against the organism.

The results of this revealed that Nubian goats are susceptible to *T. vivax* infection by showing variable haematological changes, those were manifested by the severe responses displayed by the infected animals. The Haematocrit centrifugation technique and thin stained smears especially when taking samples from the ear veins were found to be good diagnostic tools for *T. vivax* infection.

Acknowledgements:-

This work was funded by the Central Veterinary Research Laboratories (CVRL), Khartoum, Sudan. The assistance received from colleagues in tsetse and trypanosomiasis control department at CVRL is greatly appreciated.

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