

## Haematological Studies in Sheep Experimentally Infected with *Trypanosoma vivax* in Sudan

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### Abstract

This study was carried out on twelve sheep aged 5-7 months. They were divided into 2 groups, infected group and control group. The infected group was composed 8 sheep while the control group was composed of 4 sheep. The infected group was inoculated with *T.vivax* (DRSS06) strain which isolated from Southern Sudan. Blood samples were taken every day from all animals for parasite detection and haematological examination. The strain of *T.vivax* used resulted in an acute infection with incubation period of 4-6 days. The first peak of parasitaemia appeared on day 7 post infection (p.i) and increased gradually till the end of the experiment. The highest peak reached ( $\log_{10} 5 \pm 0.8$ ). Body temperature also increased, with the course of the disease. It is noteworthy that, parasitaemia is correlated well with the temperature rising. Haematological values were decreased steadily till the end of study. Hb was reached  $5.3 \pm 0.2$  at week 5 p. i. with some animals showed level as low as 2.7 mg/dl (2 animals). PCV also decreased to  $13.2 \pm 1.1$  at week 5 p.i. RBCs counts were decreased with a count of  $5 \pm 2.8 \text{ mm}^6/\text{ml}$  at week 5, with some animals showed level as low as  $1.9 \text{ mm}^6/\text{ml}$  (2 animals). WBCs were reaching  $5 \pm 1.2 \text{ mm}^3/\text{ml}$  at week5, some animals showed level as low as  $1.8 \text{ mm}^3/\text{ml}$  (3 animals). The main clinical signs exhibited by diseased animals were dullness, coughing, slight diarrhoea, weakness, nervous signs, very good appetite, abdominal pain, ear oedema, conjunctivitis with plugged eye, loss weight, lethargy, emaciation, collapse and death.

### Introduction

Sudan is the largest country in Africa and Arabic area that extends over 2.5 million  $\text{Km}^2$ . Livestock population of the Sudan is estimated to be 134.3 million heads (20). Trypanosomosis of tropical African comprises a group of diseases of great social and economic importance that infect man and his livestock. It is caused by parasite belonged to the genus *Trypanosoma*,

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transmitted by haematophages flies, cyclically by *Glossina* (tsetse flies) and mechanically by biting flies, the most important of which are *tabanus* and *stomoxys*. In animals, the disease is called Nagana which caused by *T. vivax*, *T. congolense* and *T. brucei*, while in human, called sleeping sickness caused by *T. hodesiense* and *T. gambiense*. It has been responsible for serious mortality and much ill-health, besides, its contribution to the widespread malnutrition in Africa by decreasing the amount of protein available to man it also considered as a hindrance to the development of animal production with an estimated annual loss of more than 500 million USA dollars (Anon, 1992). The most important species of trypanosome in Sudan are *T. congolense*, *T. vivax*, *T. brucei* and *T. evansi*. Because of their presence in the blood of animals they caused numerous changes in its cellular and biochemical constituents. It can lead to most important clinical signs as anaemia, weakness, and nervous signs, in coordination, poor condition, and increase of body temperature, emaciation, collapse and death. (4). This paper reports the haematological findings of sheep experimentally infected with *T. vivax*.

### **Materials and Methods**

#### **Experimental animals:**

Twelve healthy local breeds male sheep, aged 5 to 7 month weighed 17-19 Kg were purchased from the local market and housed in fly-proof barn in the College of Veterinary Medicine and Animal Production, Sudan University of Science and Technology (CVMAP/SUST). They were supplied with limited concentrates (Kenana concentrate), grain Sorghum stalks (Abo-Sab' een) from the farm of the (CVMAP/SUST), twice a day. Water and salt lick were provided *ad libitum*. All animals were examined for blood parasites, internal parasites. Supportive treatment was induced.

#### **Parasite:**

The stock of *Trypanosoma vivax* which was identified as DRSS06 strain (Deleba Raga South Sudan 2006) was isolated from natural infected cattle in village Deleba near Raga city; a known tsetse flies zone. Tow ml of the

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natural infected cow's blood was used to infect a kid subcutaneously. Thereafter, the infected kid was transported to Khartoum. At the laboratory of Professor Ahmed Ali Ismail at CVMAP/SUST. Then the parasite was harvested from the kid and preserved in Liquid Nitrogen (-196C°) and kept till needed.

Blood for parasitological and haematological investigations was collected from the jugular vein into clean vacutainers with ethylenediamine tetra acetic acid (EDTA) as anticoagulant.

**The experimental design:**

The animals were divided randomly into two groups, eight as experimentally group and four as a control group. Tow ml of *T. vivax* isolate  $5 \times 10^5$  parasites contained 1 ml of phosphate buffered saline with glucose (PSG, pH 8.0), was inoculated intravenously (I/v) into a donor Nubian goat.

The experimentally group of sheep was inoculated (I/v) with 1 ml  $5 \times 10^5$  parasites of the infected blood initially denied from the donor goat while the control group was left without inoculation.

**Sample analysis:**

All animals of the two groups were blood sampled daily till the day 35 p. i., the end of experiment.

**a. Clinical signs:**

Each morning, blood was sampled for haematological assays as well as body temperature was taken. Body weight was weekly taken until the end of the experiment. Every abnormal clinical signs were recorded throughout a day.

**b. Levels of parasitaemia:**

For parasitaemia examination, wet blood film, Buffy coat method of (21) and scoring method of (13) modified by (18) were used.

**c. Haematological examinations:**

Packed Cell Volume (PCV %) was determined by microhaematocrit technique (MHT). Red blood cells (RBCs) and white blood cells (WBCs) counts were carried out by using the conventional methods of (22) modified by (18). Haemoglobin concentration (Hb) was estimated by oxyhaemoglobin method (22) and the erythrocytes indices; Mean

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corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were calculated by following the method of (22).

## Results

### Body Temperature:

The temperature records in infected sheep revealed slight but steady increase with the course of the disease (Fig. 1).

### Parasitaemia:

The prepatent period in experimentally infected sheep ranged from 4-6 days p.i., then the parasitaemia increased gradually till week 3 when it was recorded as  $\log_{10} 4.2 \pm 0.4$  parasites/mm<sup>3</sup>. Thereafter it elevated gradually and by week 5 of infection it was recorded almost a mean of  $\log_{10} 5 \pm 0.1$  parasites/mm<sup>3</sup>. (Fig. 2)

### Haematological changes:

In general, the changes of the host blood picture have been associated with the level of parasites in the blood especially during the early stage of the disease.

#### a. PCV:

The sheep started with a mean of  $30.6 \pm 4.1$  then dropped steadily until the end of study with a mean of  $13.2 \pm 1.1$  (Figure 4). One animal showed levels as low as 6 %. The PCV values in infected animals was significantly lower ( $p > 0.001$ ) than that of control.

#### b. Haemoglobin concentration (Hb):

The animals were started with a mean of Hb  $10.6 \pm 0.9$  mg/ dl then dropped gradually post infection till reached  $5.3 \pm 0.2$  mg/ dl at week 5. (Figure 5). Some animals showed levels as low as 2.7mg/dl (2 animals). The control animals kept significantly higher Hb value ( $p > 0.005$ ) compared to infected group.

#### c. RBCs counts:

Animals in both groups were started with a mean of  $11.8 \pm 1.9$  mm<sup>6</sup>/ml. Following infection, there was a significant drop in the infected sheep ( $p > 0.005$ ), thereafter the RBCs counts decreased sharply till week 5 p.i.,

when the levels approached  $5 \pm 2.8$  mm<sup>6</sup>/ml. (Figure 6). Some infected animals revealed levels as low as 1.9, mm<sup>6</sup>/ml (2 animals).

**d. WBCs counts:**

WBCs counts were started with a mean of  $11.8 \pm 3.2$  mm<sup>3</sup>/ml, and then decreased almost steadily throughout the course of the disease until week 5 p.i when it recorded  $5 \pm 1.2$  mm<sup>3</sup>/ml. (Figure7). Some infected animals showed levels as low as 1.8 mm<sup>3</sup>/ml (3 animals).

**e. Erythrocyte indices:**

In general, the RBCs indices followed the Hb, PCV and RBCs counts. The MCV started with a mean of  $26.2 \pm 4.8$  f/l then showed a fluctuating decrease till the end of study with a mean of  $18.3 \pm 9.7$  f/l. The MCH values were started with a mean of  $9.3 \pm 2.2$  p.q then showed a fluctuating decrease till the end of study with a mean of  $6.3 \pm 3.2$  p.q. The MCHC started with a mean of  $36.3 \pm 7.2$  g/dl. Thereafter it fluctuated within a narrow range. Some infected animals recorded values as low as 23 g/dl.

**f. Body weight:**

Infected sheep started with a mean body weight of  $18 \pm 1.8$  Kg, then animals began to lose weight after infection gradually till reached  $13.5 \pm 1$  Kg at the end of experiment. (Figure7). However, one of them reached 10 Kg.

**g. Clinical signs and general condition:**

In general, the infected animals showed dullness, coughing, slight diarrhoea, weakness, convulsions, recumbency, abdominal pain, grunting teeth, ear oedema, conjunctivitis with plugged eye, lethargy, emaciation, animals always lay down and walked very slowly to eat or drink with very good appetite, as the disease progressed weakness, hind limb and neck paralysis, progressive loss of condition were observed and white anaemic mucus membrane. The disease was terminated in death of the animals. (Fig. 8, 9, 10 and 11).

## Discussion

In this study, the experimental infection with *T.vivax* caused an acute disease with incubation period of 4 to 6 days. This agreed with (15) and (1). The high parasitaemia observed coincided with high level of body temperature was in accordance with (27) and (1) but in contradiction with

(17) and (9). However, (29) noticed that there's no good correlation between the changes in the temperature during fever and levels of parasitaemia in goat infected with *T. vivax*.

Though the haematological changes observed in sheep in this study are higher than those reported by (24) and (23), however; they are in accordance with (25) and (7).

The severe anaemia reported in this study was evident by the decrease in PCV, Hb and RBCs counts. Moreover, the coinciding reduction of RBCs with the increased number of the parasites in the blood, this agreed with (5) and (3) emphasizing the opinion that haemolysis is the cause of anaemia. Similarly, Taiwovo and (4) reported erythrophagocytosis mainly caused reduction of RBCs in cattle infected with *T. congolense* and *T. vivax*.

In this study, significant decreases in WBCs counts were recorded. This is in agreement with (28), (3), and (16). However, (11) did not observe any consistent changes in the leukocyte values of sheep and goats infected with *T. vivax*. However, the MCHC values reported agreed with (25) and (12), nevertheless, the decreased MCH and MCV values opposing the findings of (25) and (12). As it indicated from the erythrocyte indices the type of anaemia recorded in this study was characterized as microcytic hypochromic.

In the present study, the severe body weight loss reported agreed with (8) who noticed that goats infected with *T. evansi* had substantial loss in body weight. This is also in agreement with (10). The main clinical signs shown in this study are fever, emaciation, anaemia, coughing, diarrhoea collapse and death. This is in agreement with (4) and (9). The nervous signs observed in this study agreed with (6). Keratitis and conjunctivitis reflected by infected animals in the present work were in accordance with (14) and (19).

In conclusion, it has been shown that sheep trypanosomosis due to *T. vivax* affects greatly the blood picture of the animal as well as its weight.

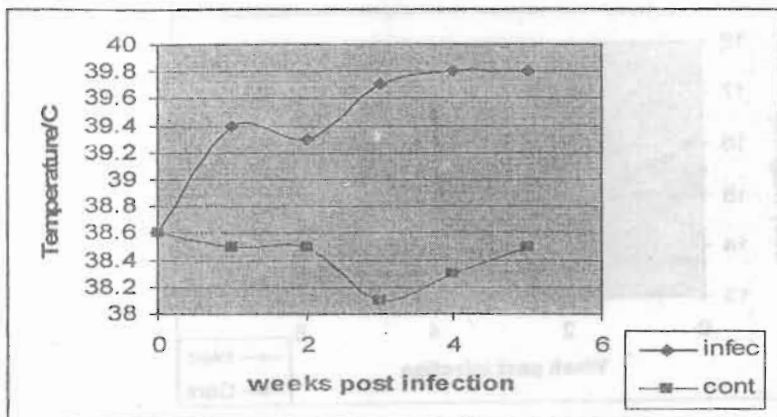


Fig.1: Mean of body temperature in sheep infected with *T. vivax*

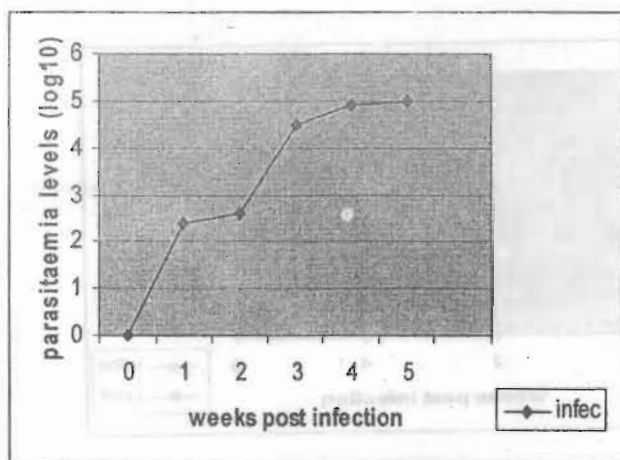


Fig.2: Mean of Parasitaemia levels (log<sub>10</sub>) in sheep infected with *T. vivax*

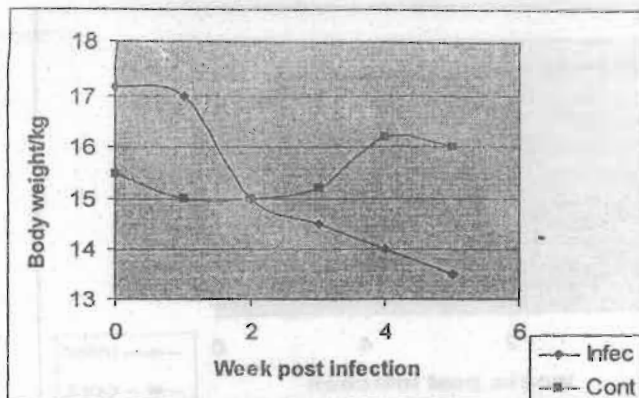


Fig.3: Mean of body weight (/kg) in sheep infected with *T.vivax*

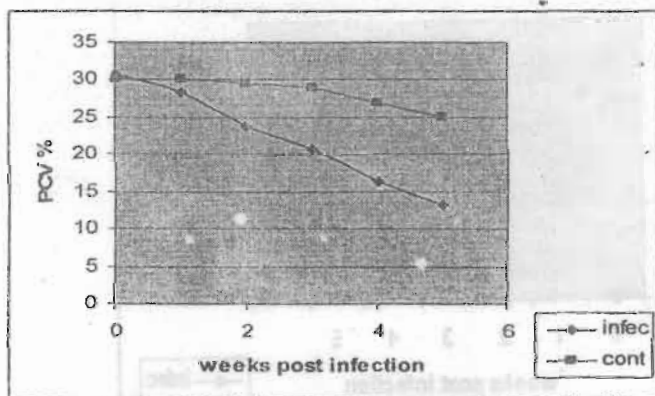


Fig.4: Mean of PCV % in sheep infected with *T.vivax*



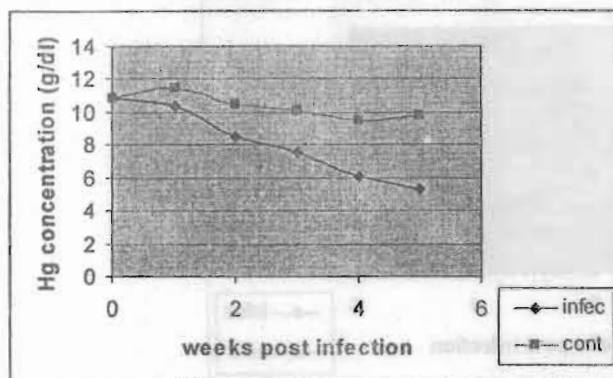


Fig.5: Mean of Hb concentration (g/dl) in sheep infected with *T. vivax*

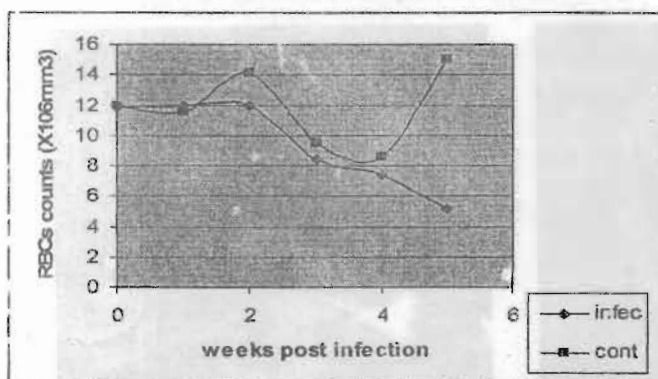


Fig.6: Mean of RBCs counts ( $\times 10^6/\text{mm}^3$ ) in sheep infected with *T. vivax*

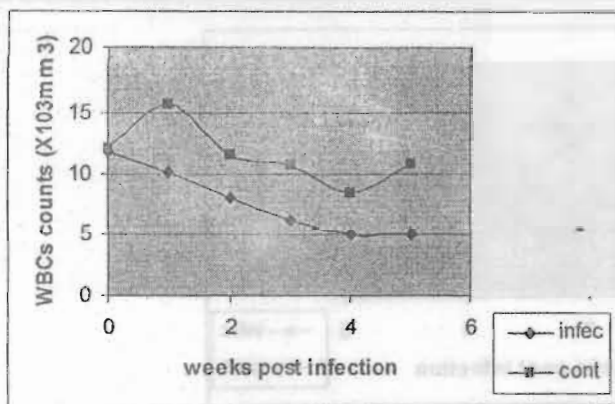


Fig7: Mean of WBCs counts ( $\times 10^3/\text{mm}^3$ ) in sheep infected with *T. vivax*



(Fig.8) Neck paralysis



(Fig.9).Ear oedema



(Fig.10). Recumbency



(Fig.11). Conjunctivitis

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دراسات على صورة الدم في الاغنام المعدية اصطناعيا بطفيل التريبانوزوما فيفاكس في السودان

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الملخص العربي

اجريت هذه الدراسة على ١٢ من الغنم تتراوح اعمارها من ٥ - ٧ شهور قسمت الى مجموعتين، مجموعة للعدوى الاصطناعية ومجموعة لم تتعرض للعدوى للقياس. المجموعة الاولى ضمت ٨ حيوانات ومجموعة القياس على ٤. وتم حقن المجموعة الاولى بواسطة طفيل التريبانوزوما فيفاكس المعزولة من جنوب السودان. وتم تجميع عينات دم يوميا من جميع الحيوانات لتشخيص وجود الطفيل وعمل فحوص معملية. ولقد لوحظ أن الطفيل المستعمل قد أدى الى ظهور عدوى حادة بعد فترة حضانة تتراوح بين ٤ - ٦ أيام وصاحب ذلك ارتفاع في درجة الحرارة مع استمرار تقدم المرض ومن الجدير بالذكر أن ارتفاع درجات الحرارة يتناسب تناسباً طردياً مع ظهور الطفيل في الدم. وأظهرت صورة الدم نقصاً مستمراً في القياسات حتى انتهاء التجربة. وكانت الاعراض الاكلينيكية الاساسية في الحيوانات المريضة هي حمول وسعال واسهال وضعف عام وبعض الاعراض العصبية وبالرغم من وجود شهية جيدة للطعام عانت الحيوانات من الام في البطن والتهابات باللعينين ونقص في الاوزان انتهى بزيادة في النحافة ثم الوفاة.