THE PATHOGENCITY AND DRUG RESISTANCE OF *TRYPANOSOMA VIVAX* IN CALVES AND GOATS

By

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A thesis submitted in fulfillment of the requirement for the degree of doctor of philosophy (Ph.D.)

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December 2005
DEDICATION

To the soul of my father whom I missed.
To my mother, brothers and sisters
In recognition of kindness and support
To my husband and sons

I dedicate this work
ACKNOWLEDGEMENT

I am grateful to Sudan government for granting the scholarship which enabled me to carry out this work. I am very much indebted to my supervisor Prof. Hamid Suliman for his guidance, encouragement, and assistance throughout the execution and writing of this study.

I would like to express my appreciation and gratitude to Dr. Ahmed Hussein A/Rahman Head Department of Tsetse and Trypanosomosis control for his generous advice, encouragement, assistance and kindness during the survey and throughout this study.

My thanks and gratitude to Prof. Mohammed Mohammed Salih the Director of Central Veterinary Research Laboratories for his encouragement and support. I am gratefully indebted to Dr. Zakia Abass for the histopathology and assistance, my thanks extended to Dr. El Gundi Suliman and Dr. Halima Mohamed Osman.

I am grateful to Dr. ElZain Bashir for helping with statistical analysis, my unlimited gratitude to Dr. Ibtisam Goraish for unlimited help and support and my sincere thanks to Dr. Nadia Mohamed Osman for her help during the survey for her cooperation.

I am also grateful to the technical staff in particular Mr. Nasir Ibrahim and Mr. Ahmed El Shikh, Ali Elgamri for their unlimited help and care.
My thanks to the tsetse and trypanosomosis department technical staff. Mr. Ismail, Ahmed Eltayib, Wisal ElNur, Faiza, Umsalma and Safia.

My special sincere thanks to all the staff at Umbenain Dairy farm Station for their guest and hospitality during the survey. My special sincere thanks to my colleague Dr. Enaam ElSanousi for generous moral support, and my family for their patience and understand, I am also grateful to Mrs. Samira Amin for typiing the manuscript.
A survey was conducted at the Blue Nile area to determine the prevalence of *Trypanosoma vivax* in cattle, using haematocrit centrifugation technique, wet blood film, thin and thick stained smears. The results showed that the prevalence rate during the dry season varied between 6.5 to 2.2% and 6.35 to 1.43% during rainy season, and the tabanids catches were 25±5.1 fly/trap/day in early dry season. This indicated that cattle suffer due to *T. vivax* is quite prevalence in the area, the rate is high with seasonality.

The trypanocidal drug resistance studies of *T. vivax* stock isolated from non-tsetse area were conducted in 32 goats. Trypanocidal drug tested were Homidium bromide (16 goats). The doses tested for homidium bromide were the recommended treatment dose of 0.5 /kg body weight and double that dose. The doses tested for diminazine aceturate were the recommended treatment dose (7mg/kg).

The *T. vivax* stocks tested against both drugs relapsed indicating the development of drug resistance against both drugs at the tested doses. Both groups of goat treated showed significant reduction in PCV Hb. WBC and RBCs count.

The pathological effect of *T. vivax* stocks isolated from non tsetse area was conducted by experimental infection of 10 Kenana calves. The infected animals showed obvious, emaciation anaemia, enlargement of lymph node, high, temperature, increase in heart and respiratory rates, which were positively correlate with...
peak of parasiteaemia. The blood showed significant haematological changes. The PCV values dropped to 15%, Hb 8%, WBCs 5001.696 ±162.725, RBCs 3554018 ±172889.0. The gross findings showed petechial and ecchymotic haemorrhages in all the visceral organs spleen, liver, heart and lymph nodes were considerably enlarged, oedema and pulmonary exudate were observed in the lungs, the heart showed myocardial degeneration. Also proliferative lymphoid changes and infiltration of lymphatic cells were observed in all tissues. The kidneys showed interstitial glomerulonephritis, and inflammatory changes and the testis showed prominent orchitis, epididymitis, atrophic seminiferous tubules and degenerative changes. The brain showed infiltration of lymphoid cells, histocytes, mononuclear and glial cells and necrotic foci. Generally wide tissue damage, and haematic changes were observed in all tissues of the infected animals died of and the congestive heart failure.
ملخص الأطروحة

أجريت المسوحات في منطقة النيل الأزرق لتحديد نسبة الإصابة لطفيل Trypanosoma vivax في الأبقار في فترة الجفاف والخريف. وباستعمال عدة طرق وطريقة تركز الدم في الأنابيب الشعرية بواسطة الطرد المركزي والمسحة الرطبة، المسحة المصبوغة الرقيقة، تثبت الدراسة أن نسبة الإصابة بطفيل T. vivax في فترة الجفاف تتراوح بين 6.5 إلى 2.2% وفي فترة الخريف 6.35 إلى 1.43% مع وجود نسبة 25±1% من الذبابة المصاب. هذه الدراسة تثبت أن طفيل T. vivax الخريف والجاف وتدفاد نسب الإصابة بتكاثر الذبابة العامل مما يؤدي إلى حدوث نسبة إصابة عالية خارج حزام الذبابة.

تمت دراسة مقاومة الطفيل لعقار Ethidium bromide بعد حقن بعض الأغذية النووية بالطفيل T. vivax وظهوره على نسبة من الطفيل في الدم. تمت المعالجة بجرعة 0.5 مل/كيلو جرام و1 مل/كيلو جرام مما نتج عنه مقاومة الطفيل للعقار وظهوره في فترة الخريف، مما يؤكد مقاومة الطفيل لجرعة المعالجة وعليها هذه الإصابة أيضاً أدت إلى انخفاض.

كما أثبتت هذه الدراسة هذه الدراسة مقاومة T. vivax لعقار Berenil بعد حقنة بجرعتين 3.5 مل/كيلو جرام و7 ملليم/كلم ماعز. وبعد علاج الطفيل المصاب به الكويكزي 0.10 مل من الدم، وبعد ظهور الطفيل بنسبة عالية بالعقار نتج عنه تكيف للعقار بعد المعالجة في فترة متفاوتة مما يؤكد مقاومة الطفيل لجرعة المعالجة وعليها هذه الإصابة أيضاً أدت إلى انخفاض.

ملحوظ في مكونات الدم المؤدية إلى فقر الدم.

تمت دراسة الأثر المرضي لطفيل T. vivax في عشرة عقول. كتبة بعد حقنها ستة منها بتركيز 0.10 مل من الدم المصاب. وناتجة الأثر الإكلينيكي والباثولوجي أثبتت الداسة حدوث هزات، فقد تم فقدان شعر الذيل تضخم الغدد المفاصلة. أيضاً ارتفاع درجة حرارة الجسم وزيادة في ضربات القلب زيادة وسرعة في التنفس بدرجة
متجاوبة مع ازدياد عدد وسرعة في التنفس بدرجة متجاوزة مع ازدياد عدد الطفيلة في الدم.

أما تغييرات التشريحيّة العيانية شروب في الأغشية المخاطية والمصلية، لدق الدم الباهت، ضمور العضلات مده النامور، نزيف الكلي واحتقان الدماغ وظهور ارتشاح الخلايا وحيدة النواة في معظم خلايا الأعضاء التهاب واضح في الخصي.
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INTRODUCTION

Trypanosomiosis is a parasitic disease caused by species of flagellate protozoa belonging to the genus *Trypanosoma* which inhibit the blood and various body tissues and fluids. These parasites are found in many animals and were mostly pathogenic to mammals including man (Finnelle, 1983). The disease has direct impacts on livestock productivity, livestock management and human settlement (Swallow, 2000). In Africa most of the species that are pathogenic to man and animal are transmitted cyclically by tsetse flies and or mechanically by other haematophagus insects mainly Tabanidae. Other transmitters incriminated include Muscidae and Stomoxys (Ford, 1970). The most important *Trypanosomes* species that cause bovine *Trypanosomosis* include *T. vivax*, *T. congolense* and *T. brucei*. They are transmitted cyclically by several species of *Glossina* which are adapted to different climatic and ecological conditions. (Ford, 1964). Tsetse transmitted trypanosomosis occurs in about 10 million km$^2$ in 37 sub-Saharan African countries corresponding to almost one third of Africa’s total land area (FAO, 2003). Non-tsetse transmitted trypanosomosis caused by *T. evansi* and *T. vivax*, occurs in various forms in South America,
Africa and Asia including China, and is potential risk for 500 million cattle, 100 million buffaloes and 12 million camels (Peregrine, 1994).

Control of African bovine trypanosomosis continues to rely in most endemic areas on chemotherapy and chemoprophylaxis using the salt of three trypanocidal compounds, isometamidium, homidium and diminazine. All of these drugs have been in widespread use for over 50 years and resistance has been reported in at least 13 African countries (Peregrine, 1994; Geert and Holmes, 1998). Vaccination is not yet possible option due to the antigenic variation of trypanosomes (Nantulya, 1986).

Surveys in Eastern and Southern Africa (Ndung’u et al., 1999) and in west Africa (McDermott et al., 1999, 2000) have shown that the prevalence of trypanocidal drug resistance might even be higher than suspected. However, for most trypanosomosis areas, the extent and impact of drug resistance is not known (Geerts and Holmes, 1998).

Objective

The present study is intended to

Update the prevalence of *T. vivax* in an area of previous history of bovine trypanosomosis outside the tsetse belts of Sudan.

Determine and assess the degree of resistance of *T. vivax* to the Homidium bromide (Ethidium) drug commonly used in Sudan.

Determine and assess the degree of *T. vivax* resistance to Diminazine aceturate (Berenil).

Study the pathology and pathogenicity of *T. vivax* in cattle and goats.
CHAPTER ONE
LITERATURE REVIEW

Classification:

The pathogenic trypanosomes in the genus Trypanosoma are divided into two sections, stercoraria and salivaria, according to the site of development of the organisms, primarily in the insect vector and hence their transmission via saliva or faeces of the vector to the mammalian host. Most species of pathogenic importance in mammals belong to the section salivaria and are mainly transmitted cyclically by haematophagous flies of the genus *Glossina* (Hoare, 1972). Kreier and Baker (1993) proposed the following classification.

<table>
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Animal trypanosomosis is named nagana in cattle and is caused by *T. vivax, T. congolense, T. brucei*. The parasites that affect ruminants, pigs and equines, and are transmitted cyclically by different species of tsetse flies. The disease is distributed in large areas within the tsetse fly belt of Africa. Camels are affected by
*T. evansi* in north Africa, Asia and South America and it was mechanically transmitted by biting flies such as tabanids and *Stomoxys*. Another form of the diseases caused by *T. equiperdum* and termed dourine. This is a venereal disease of equines, transmitted by coitus and is distributed in North Africa and the Middle East (Hunter, 1986).

**Morphology and motility:**
In Giemsa-stained blood smears, the subgenera are distinguished by their size, shape, location and size of kinetoplast, position of the nucleus and the attachment and length of flagellum. In wet mounts, the type of motility and locomotion are also useful in differentiating subgenera. *Trypanosoma vivax* has a fluttering movement resulting in a rapid darting linear locomotion (Losos, 1986). *T. vivax* is a monomorphic species, the posterior part is distinctly broader and bulbous, the kinetoplast is large and terminal and the free flagellum is short. The organism is very motile in fresh blood, moving, rapidly across the field, pushing red cells as it goes (Soulsby, 1982).

**Distribution:**
Non-tsetse transmitted trypanosomosis caused by *T. vivax* and *T. evansi* occurs in various forms in South America, Africa and Asia including China and is a potential risk for 500 million buffaloes and 12 million camels (Woo, 1977, Pregrine, 1994, Tourateir, 2000) *T. vivax* was reported in the New World for the first time in the French Guyana. (Leger and Vienne, 1919) and Latter in other
parts of South and Central America and some Caribbean islands (Melendez, 1995).

The natural home of *T. vivax* is tropical Africa, where it is prevalent outside the tsetse belt. This trypanosome occurs in most parts of west, Central, East and South Africa. It represents a remarkable instance of tsetse borne trypanosome that had spread far beyond its original area to distinct countries (Hoare, 1972).

**Epidemiology:**

Trypanosomiasis is quite prevalent or endemic in many African countries. In Nigeria, the prevalence of ruminant trypanosomosis in Kano State, showed that the infection rate was double during rainy season in comparison with average rate during dry season (Kalu and Lawani, 1996). Griffin and Allonby (1979) studied the epidemiology of trypanosomosis in sheep and goats in Kenya, in tsetse infested areas, and noted that, there was a significant correlation between tsetse numbers and rainfall followed by an increase in prevalence of trypanosomosis in animals. Kalu et al. (2001) investigated the prevalence of trypanosomosis in small ruminants, in Nigeria, the vivax is the predominant parasite. Jones and Davila (2001) were reviewed current problems of *T. vivax* which spread to 10 of 13 countries of South American continent. The epidemiology of *T. vivax* in the Republic of Central Africa was studied and the hypothesis is that the stable flies could be good vectors as they are very much abundant in cattle resting sites, particularly during rainy season. There was also a good
correlation between stable fly densities at resting sites and *T. vivax* in cattle (D’Amico *et al.*, 1996). In Colombia *T. vivax* infection was widely distributed (Otte *et al.*, 1994).

**Transmission and life cycle:**

Transmission of trypanosomes depends on their subgenera and species, and mainly by cyclic and mechanical transmission by insect vectors. Most species are spreaded by more than one method but when insect vectors are involved they usually play the most important role (Losos, 1986). Trypanosomes ingested by the tsetse fly from parasitic host undergo life cycle of up to 20 days during which they undergo morphological changes, multiplication, tissue migration and a final localization in the mouth parts (Losos, 1986). The mechanical transmission of *T. vivax* in Africa has been thought to be responsible for its spread from the tsetse fly belts. Wells (1972) reviewed the evidence for mechanical transmission. Leeflang (1975) proposed that on the periphery of the tsetse fly belts low tsetse flies densities still transmit *T. vivax* with the other salivarian species. *T. brucei* had been transmitted experimentally by flies of the genus *Tabanus* and *Stomoxys* (Dixon *et al.*, 1971). Chaudhuri *et al.* (1965) showed that the species of these flies (*Tabanus* and *Stomoxys*) were more efficient than others in the transfer of *T. evansi*. The classification of the oriental horsefly vectors of *T. evansi* has been revised by (Burger and Thompson, 1981). The role of the vampire bats in
transmission of this species in South America has been reviewed by Hoare (1965) and Uilenberg (1998).

The ability of certain trypanosomes to penetrate the mucous membrane and cause systemic infection is best shown by *T. equiperdum*. It has been claimed that *T. evansi* and *T. brucei* are also infective through ingestion of infected meat which depends on temperature (de Jesus, 1951, 1962). Meat may be infective for periods of up to 66 hours (Moloo *et al*., 1973; Soltys *et al*., 1973; Uilenberg, 1998). Congenital infection have been demonstrated in infected man with the salivarian species (Buyst, 1972). There are few reports on to transplacental infection with *T. vivax* and *T. evansi* in domestic animals (Ikede and Losos, 1972; Abdel-Latif, 1963). In man 2-4% of infected mothers transmit *T. cruzi* infections to children (Bitten Court, 1976). Transplacental transmission of trypanosomes in natural and experimental infection in various species of animals and man, infected with different species of trypanosomes (Ogwa and Nuru, 1981; Uilenberg, 1998). The relatively simple developmental cycles which *T. vivax* undergoes in its vector leads to high infection rates in tsetse (Moloo, 1982a). The proportion of bovine blood meals taken by the flies are positively correlated with the prevalence of *T. vivax* in tsetse (Moloo *et al*., 1980; Tarimo *et al*., 1984; Ryan *et al*., 1986). Further, the ratio of infection rates of *T. vivax* and *T. congolense*, detected in flies in a particular locality may not necessarily correlated with the ratio of the infections with these
two species detected in the livestock population (Bourn and Scott, 1978). Latrogenic transmission can occur by using the same needle or surgical instrument on more than one animal, at sufficiently short intervals that the blood on the needle or instrument does not dry. This type of trypanosomosis transmission occurs when animals are vaccinated or subjected to surgical intervention e.g. dehorning, castration etc. without proper disinfection (Uilenberg, 1998). The use of one needle for several animals during foot and mouth disease campaigns in Brazil and Bolivia might have contributed to the spread of *T. vivax* (Davila and Silva, 2000).

**Parastaemia:**

After inoculation of the trypanosome they, enter the blood either directly or through the lymphatics, during the first wave of parasitaemia, some species leave the blood and localize in various tissues and organs (Losos, 1986). *T. vivax*, is also capable of leaving the circulation and invading solid tissue, particularly the heart, *T. cruzie* differ from other pathogenic trypanosomes as it does not only divide in the blood or tissue fluids but also in various cells (Losos, 1986). The skin reactions caused by *T. congoense*, *T. vivax* and *T. brucei* were different in their histological appearance, severity, and parasitaemia levels (Emery *et al.*, 1980; Emery and Moloo, 1981). After infection with the trypanosomes, the onset of parasitaemia varies according to the species (Losos *et al.*, 1982). High levels of parastaemia have
often been associated with acute severe syndromes of trypanosomosis both in domestic and laboratory animals (Godfrey, 1960; 1961). The levels of *T. vivax* infection in east Africa are lower than in west Africa, and are also related to milder forms of the disease (Murray and Morrison, 1979). It has also been shown that infectivity and virulence of *T. brucei* for mice was associated with high levels of parastaemia, and was dependent on a particular antigenic type (McNeillage and Herbert, 1968; Clayton, 1978). Fluctuations of the numbers of trypanosomes observed in the blood are characteristic of most forms of trypanosomosis, and the periodic peaks of parastaemia suggest rapid changes in the circulating populations. Obvious cyclic waves of parastaemia were observed to occur about every 6 days in *T. vivax* but not in congolense infection (Maxie *et al.*, 1979). During the first peak, the generation time was approximately 8 hours which is equal to the optimal growth rate reported for *T. brucei* culture (Hirumi *et al.*, 1977). Cicardiac rhythm has been reported with *T. congolense* infection in cattle and rats, and in the first species it was associated with morning and after noon temperature changes (Hornby and Baitey, 1931; Hawking, 1978). It has now been clearly established that *T. brucei, T. rhodesiense, T. gambiense* and *T. evansi* invade and localize in solid tissue (Godwin, 1970; Losos and Ikede, 1980; Morales and Carreno, 1976). It was also noted that their penetrating ability has been related to their various twisting
forward motility (Evans and Ellis, 1975). This was found to be in contrast to the findings of Losos and Ikede (1972) who stated that the parasites do not invade tissues. Ormerod (1979) reviewed the presence of trypanozoan species in the blood and tissue forms. *T. congoense* is known to attach themselves to the endothelial cells by their flagellates (Banks, 1978; Bungener and Muller, 1976). The binding to the endothelium wall and erythrocytes of infected hosts, was proposed due to the presence of surface of *T. congoense* which binds to sialic acid of the host cell (Banks, 1979). Comparative study of *T. brucei* and *T. congoense* in tissue of mice and rats, showed that *T. brucei* was found in the connective tissues and body fluids, while *T. congoense* was found in the capillaries (Ssenyonga, 1980). This attachment is disrupted by the action of the trypanocidal drug diminadzine aceturate and the trypanosomes are liberated into the circulating blood (Maxie *et al.*, 1976). Berenil cleared the capillaries of the trypanosomes within 30 minutes (Mills *et al.*, 1980).

**Clinical signs:**

*T. vivax* infections occur in cattle, sheep, goats, horses and camels but not in dogs and pigs (Losos, 1986). In west Africa, the severity of the disease and time of death varied between individual animals (Maxie *et al.*, 1979). The fulminating syndrome has been reported in east Africa (Machenzie, 1970). Clinical signs discribed by Losos and Ikede (1972) in acute form of *T. vivax* infection indicated that the
animal dies within five weeks after having high temperature, lethargy, weakness, anaemia and loss of condition. The chronic form is characterized by anaemia and progressive emaciation. Acute fatal haemorrhagic disease in exotic dairy cattle was reported from Kenya caused by *T. vivax*, characterized by abortion, bloody diarrhoea and death (Mwongela *et al.*, 1981). The fever was well correlated to elevation of parastaemia with respiratory and heart rates increase, and lymph nodes obviously enlarged (Maxie *et al.*, 1979; Van den Ingh *et al.*, 1976). In South America the disease in cattle was predominantly chronic with death only occasionally observed (Show and Lainson, 1972). Pathogenic clinical signs observed in cattle infected with *T. vivax*, others with *T. congolense* were fever increased heart and respiratory rates, anorexia, and emaciation. Rise of parastaemia and body temperature were positively correlated with *T. vivax* infection, the generation time 7.9±2.5 hours (Maxie *et al.*, 1979).

The clinical signs of bovine trypanosomosis, the first occurrence by *T. vivax* in Bolivia, were fever, anaemia, abortion, progressive weakness, loss of appetite, lethargy, weight loss in relatively short time, progressive emaciation (Silva *et al.*, 1998).

Uilenberg (1998) described the disease caused by *T. vivax*, seven to ten days post infection. The temperature raised and the heart and respiratory rates increased and the animal showed continuous loss of condition. In acute forms, lacrimation and corneal opacity were observed.
The anaemia:

Anaemia in trypanosomosis has been attributed to inhibition of haemopoiesis (Losos and Ikede, 1972) and/or to haemolysis (Mamo and Holmes, 1975). Anaemia is the principal sign of trypanosomosis in livestock and persistent anaemia and congestive heart failure due to myocardial damage may cause death (Sannusi, 1979; Murray et al., 1979). Saror (1979) and Sarror et al. (1979) described the T. vivax infections in cattle, and pattern of haematological changes accompanying the first appearance of parasites. The anaemia was described as normocytic normochromic with tendency to being macrocytic normochromic. The anaemia may be chronic (Saror, 1979; Murray et al., 1979). The initial wave of parastaemia and the onset of anaemia are accompanied by leucopaenia and persistent thrombocytopaenia (Maxie et al., 1976; Maxie and Valli, 1979; Esievo and Saror, 1983). The cellular changes in the caprine or ovine host is essentially similar. The haemolytic anaemia associated with acute phase of the disease was accompanied by high parastaemia (Anosa and Isoun, 1980). The white blood cells with control of parastaemia or leucocytosis occurs due to increased lymphocytes number (Saror, 1979; Esievo and Saror, 1983). Spleen was the major site of the red cell destruction in mildly anaemic animals and the liver being the major site in severely anaemic animals (Anosa and Isoun, 1980).
The first peak of parastaemia in *T. vivax* and *T. congolense* was closely associated with development of anaemia (Maxie *et al.*, 1979). In west African dwarf and red sokoto goats experimentally infected with *T. congolense*. The course of the disease showed rapid progressive anaemia, severe drop in PCV 9-11 within 11 to 13 days post infection, leucocytosis and death (Adah *et al.*, 1993). West African Dwarf goats experimentally infected with *T. congolense* developed chronic anaemia and persistent parastaemia (Goossens *et al.*, 1997). Masake (1980) noted the high parastaemia and severe anaemia resulted in 25% deaths in *T. vivax* infected east african goats and cattle. West African dwarf goats infected with *T. congolense* developed acute and chronic disease associated with persistent parastaemia together with chronic anaemia, significant drop in PCV, Hb and RBCs (Goossens *et al.*, 1998).

The anaemia caused by *T. congolense* in Holstein calves was of moderate severity. It was normocytic normochromic, macrocytic in acute phase changing to normocytic with chronicity. The haemolytic anaemia resulted in increased plasma volume and cell destruction (Valli *et al.*, 1978). Also *T. vivax* experimentally infected sheep produced progressive macrocytic normochronic anaemia (Igbokwe and Anosa, 1989). *T. congolense* infected sheep developed fluctuating parastaemia, macrocytic normachromic anaemia and leucocytosis (Katunguka *et al.*, 1992). Trypanosomosis caused anaemia in both sheep and goats and
animals whose PCV fell below 15% rarely recovered (Masiga et al., 2002).

**The pathogenesis of *T. vivax***:

The chancre is the first sign in human and sleeping sickness but it is not marked in animal trypanosomosis. It is a raised skin reaction localized at the site of tsetse fly bite (Dwinger, 1985; Akol and Murray, 1985). However, Losos (1979) expressed doubts about chancres, as they are rarely observed in the skin of wild animals, which were bitten by large numbers of infected tsetse. Others (Emery et al., 1980; Akol and Murray, 1983) noted some *T. vivax* shown chancres 5-8 days post infection, but these lesions were smaller than that caused by *T. congolense*. The parastaemia occurred 12-24 hours after appearance of chancre (Emery and Moloo, 1981). Chancres are not produced by the bite of all flies infected with *T. vivax* (Akol and Murray, 1983).

Primary lesions caused by *T. vivax* occur in the blood, blood vessels, lymphoid system and some of the solid tissue particularly the heart (Losos, 1986). These observations changed the earlier conclusion that *T. vivax* was strict plasma parasite (Losos and Ikede, 1972). During the initial stages of infection with *T. vivax* in cattle the trypanosomes were detected in lymph nodes which showed proliferative response (Masake and Morrison, 1981). *T. vivax* cause a rapidly fatal disease which is associated with high persistent parasitaemia, and the blood was described as swarming with parasites (Losos and Ikede, 1972). The acute syndrome, with
extensive haemorrhages and thrombosis, has been produced experimentally in cattle, sheep and goats with both the west and east African strains of *T. vivax* (Boyt and McKenzie, 1970; Van den Ingh et al., 1976). The organs affected were kidneys, lungs, liver, lymph nodes and adrenal glands (Masake, 1980). The thrombosis causing tissue infection was observed to occur early and late in relatively mild form of the disease associated with peaks of parastaemia (Isoun, 1975). Pericarditis and myocarditis, associated with the invasion of trypanosomes to solid tissue, was seen in particular lesions in *T. brucei* infections (Van den Ingh et al. and DeNeijs-Baker, 1979). Infection in goats and cattle caused marked lymph nodes enlargement associated with increase in number and size of the lymphatic follicles accompanied by germinal center. Lesions were also observed in the heart with myocardial fiber degeneration and necrosis, and mononuclear cell infiltration, extravascular trypanosomes, inflammatory lesions seen in testes and pituitary (Masake, 1980).

With the first wave of parastaemia the packed cell volume (PCV) the white blood cell volumes and thrombocytes decrease in level. PCV may continue to drop gradually until the death of the animal or development of chronic disease (Losos, 1986). Ugochukwu (1986), noted that *T. vivax* and *T. congolense* infections in cattle, caused significant drop in haemoglobin concentrations, packed cell volume and numbers of erythrocytes.
*T. vivax* infection affect the male genital organ and invasion of the featus by trypanosomes was indirectly responsible for reproductive disturbances. (Isoun and Anosa, 1974; Ikede and Losos, 1972a). Testicular degeneration in *T. vivax* infection of sheep and goats was associated with pyrexia fibrin thrombi in testicular vessels which may have resulted in infarction (Anosa and Isoun, 1980b).

The largest numbers of trypanosomes were present towards the end of the first week of elevated temperature, while at the time of death the parastaemia was often low or to undetectable (Losos and Ikede, 1972).

The pathogenesis of acute *T. vivax* adapted to mice showed acute infection with histological lesions such as generalized fibrin, thrombus formation in the blood vessels of heart, lung, spleen and brain (Isoun, 1975).

Trypanosomosis assumes chronic course of infection leading to considerable morbidity. Besides lowering the productivity it was found to interfere with immune status of the host (Freeman *et al.*, 1973). The reproductive system is frequently affected and abortion and infertility are common in herds in areas of high trypanosome challenge (Yagil, 1982).

**The haemorrhagic syndrome:**

Animals infected with *T. vivax* show greater tendency to control the parastaemia and overcome their anaemic condition than do those with *T. congolense* (Gardiner, 1989). In East Africa *T.*
*T. vivax* which cause acute disease in cattle is accompanied by haemorrhagic syndrome (Anonymous, 1966; Mwongela *et al.*, 1981; Wellde *et al.*, 1983; Roeder *et al.*, 1984; Olubaya and Mugera, 1985; Connor and Mukangi, 1986; Schonefeld *et al.*, 1987). The infections are characterized by high and persistent initial parasitaemia, fever, profound anaemia and generalized haemorrhages of the vicera and mucosal surfaces resulting in mortalities and abortion of cattle (Mwongela *et al.*, 1981). *T. vivax* causes acute haemorrhagic infection in calves, peripheral blood changes including anaemia, thrombocytopenia, and initial leukopenia, later in the course of infection, leukocytosis associated with lymphocytosis and neutropenia developed (Anosa *et al.*, 1992). Haemorrhagic pancarditis, haemorrhage, oedema, mononuclear cell infiltration, degeneration atrophy and lysis of myofibres and extra vascular localization of the parasite was observed in *T. vivax* infected cattle (Kimeto *et al.*, 1990).

The main difference between the acute and chronic form seems to be the presence of microthrombi in acute stage of infection, the platelets, trypanosome, monocyteid cells and fibrin, with thrombus formation, which directly related to high parasitaemia, Ischemia, which could explain the haemorrhage (Van den Ingh *et al.*, 1976). *T. vivax* isolated from dairy cattle caused haemorrhagic disease to Ayrshire steers, gastrointestinal bleeding, reduced the thrombocytes and high level of parasitaemia (Welde *et al.*, 1983).
N Dama cattle, which are resistant to *T. congolense* and *T. vivax* from West Africa, were highly susceptible to *T. vivax* which causes acute haemorrhagic disease (Williams et al., 1992).

**Diagnosis:**
The diagnostic methods in salivarian trypanosome infections have been reviewed by Kendrick (1968) and Molyneux, (1975). Various parasitological techniques, microscopic examination and animal subinoculations, have been evaluated in the laboratory infections and under natural conditions. It has been shown that any techniques by itself was unsatisfactory and a combination of either the haematocrit centrifugation or thick smear techniques, together with thin smears was recommended. Examination of lymph node aspirates did not increase the sensitivity (Leeflang et al., 1978). Infections in cattle were more often detected in the peripheral than in jugular blood, and lymph node smears were essential in diagnosis of *T. vivax* (Robenson and Ashkar, 1972a, Rickman and Robenson, 1972). Other workers did not observe an increased efficiency in detecting infections by examination of lymph nodes of sheep and goats (Zwart et al., 1973). With trypanosomes infective for rodents, the subinculation technique is particularly useful in diagnosing in very low parastaemia, as *T. evansi* in camels (Godfrey and Kendrick, 1962; Pegram and Scott, 1976). The other common methods used to detect low numbers of trypanosomes in the blood involve either concentration or separation of trypanosomes from relatively large volume of blood,
(the microhaematocrit techniques), based on the method of Devignat and Dresse (1955), which was modified and adopted for routine use in the field (Woo, 1969a, 1970; Murray et al., 1977).

Several parasite detection techniques can be used, including the microscopic examination of the wet and stained thick or thin blood films. However, diagnostic sensitivity is increased significantly by concentrating the parasites prior to examination in combination with a phase contrast or dark-ground microscope. The parasite concentration techniques have the added advantage that the packed cell volume, and hence the level of the anaemia, can be determined at the individual animal and/or herd level. A highly sensitive test used on a more experimental basis, is the polymerase chain reaction (OIE, manual 2004). This is a highly sensitive and specific chain reaction (PCR) based assay for the detection of *T. evansi* present in the blood of different animals and vectors (Chonsiri et al., 2002). The PCR assay provides a very sensitive tool in diagnosis of active infections of dourine in endemic areas where trypanocidal drug are in common use (Clausen et al., 1998).

DNA probe and PCR based assay, are technically complex. They have high sensitivity, directly detect parasite independent of immuno-competence or previous clinical history (Weiss, 1995). Two trypanosomal antibody detection tests, namely the indirect fluorescent antibody test, and antibody enzyme linked immunosorbent assay (ELISA), have high sensitivity and specificity but
can only be used for presumptive diagnosis of trypanosomosis. They lend themselves to automation and will allow a high degree of standardization when recombinant antigens have been developed and validated (OIE, manual, 2004). Two (FAO/OIE) ELISA, which use microplates precoated with denatured crude *T. congoense* or *T. vivax* antigen for detecting antitrypanosoma antibodies in bovine sera, were evaluated for their sensitivity, specificity and positive and negative predicative value (Magona et al., 2002).

The buffy coat dark ground phase contrast technique (BCT) and (ELISA) were employed to assess the trypanosomal status of N Dama cattle and it was concluded that serological screening could provide useful information complementary to that obtained by the use of BCT (Mattioli et al., 1996; Mattioli et al., 2001.

A monococal antibody-based latex agglutination test for detection of circulating trypanosoma antigen in animal serum was evaluated to be used for testing small numbers of animals under field conditions (Kayang et al., 1997).

Nadia (2005) showed that the haematocrit centrifugation technique for detecting *T. vivax* infection, is superior of the five conventional techniques commonly used, and the higher parasitaemia were detected from the ear vein blood compared to jugular vein blood.

**Culture technique:**
Basic studies on structure, function, metabolism and nutrition of trypanosomes require techniques for growing the organisms in large numbers e.g. *in vitro* culture systems. In order to study the various stages observed in the vector and mammalian host, a number of cultures are used ranging from simple media to culture containing invertebrate and vertebrate organs. Species of pathogenic and non-pathogenic trypanosomes vary in their ability to grow in artificial media. The salivarian pathogens are relatively more difficult to cultivate than the stercorarian trypanosomes, and growth is usually limited to the procyclic forms (Losos, 1986). Investigations have led to the development of methods for the cultivation of *T. vivax* through all its life cycle stages *in vitro*, blood stream forms (Burn and Jenni, 1987) and insect forms (Gray *et al*., 1987). Trager (1959, 1975) made initial attempts to establish insect-form cultures of *T. vivax* in association with tsetse organs. In his first visit to Nigeria Trager (1959) was able to establish cultures at 31°C with blood stream forms of *T. vivax* from the blood of infected sheep. These cultures subsequently showed all the vector stage of *T. vivax* and the trypanosomes were readily sub-cultured to fresh tissue cultures. Two of these sub-cultured trypanosome populations were infective for sheep after maintenance for several weeks *in vitro*. In a later attempt to repeat this work using different stocks of nigerian *T. vivax*, a slightly lower culture temperature was adopted (27-29°C) and successful initiation of cultures was
reported (Trager, 1975). Hirumi et al. (1983, 1984, 1985a,b) developed a technique in which agarose beads treated with a dye derivative (matrix gel green A-beads) served as a substrate for the attachment of *T. vivax*. The trypanosomes are maintained in RPMI-1640 (Rosewell Park Memorial Institute 1640) medium supplemented with 20% foetal bovine serum over a layer of fibroblast cells. Blood stream forms attach to the beads in a manner similar to the proboscis of the tsetse fly (Vickerman, 1973, 1974; Fish et al., 1987).

**Chemotherapy:**

Finnelle (1983) described the trypanocides which have been used extensively in veterinary medicine as follows

**Dimidine** (Diminazene aceturate-Berenil) is widely used as a curative drug against *T. vivax*, *T. congolense* and *T. brucei* in cattle, sheep goats and equines. This drug is rapidly excreted from the circulation.

**Phenanthridine** (Homidium -Ethedium) curative to *T. vivax* and *T. congolense* in cattle, sheep and goats. There is a wide spreading phenanthridine resistance against this drug.

**Isometamedium** Samorin (Trypamidium) is widely used as curative and prophylactic drug to *T. vivax*, *T. congolense* and *T. brucei* in cattle, sheep, goats and equines.

**Pyrithidium** (Prothidium) is used as curative and prophylactic against *T. vivax* and *T. congolense* in cattle, sheep and goats and its resistance generally develops rapidly.
Quinolines (quinapyramine-Antrycide-Trypacide) were used as curative and prophylactic drugs against T. evansi, T. vivax, T. congolense and T. brucei in cattle, sheep, goats, camels, equines and canines. There is widespread resistance against this compound.

Naphthalidine (Suramin, Antrypol-Naganol) curative and prophylactic drug against T. brucei and T. evansi of camel equines, canines and humans, only prior to nervous system involvement.

Arsenical Melaminyl (cymelarasan) curative to T. evansi in camels.

Trypanocides used in treatment and protection are the most common single method employed for the control of animal trypanosomosis (Mbwambo et al., 2003). Effective application of chemotherapy and chemoprophylaxis in the field depends on several factors which include the species of trypanosome causing infection, severity of challenge, species of animals, and occurrence of resistant strains (Leach & Roberts, 1981; Losos, 1986). The oldest drug used in animals, but now with decreasing frequency, was suramin for the brucei-like infections in horses, donkeys and camels (Losos, 1986). Antrycide has replaced this drug (Fair lamb and Bowman, 1980). The phenanthridine compounds were widely used and ethidium was used in East and West Africa (Losos, 1986). Although it was primarily a curative compound (Whiteside, 1960; Losos, 1986) its prophylactic effect
was observed in cattle up to 10 month (Mwambu, 1971). The curative and prophylactic drug isometamidium had been used extensively as a curative forms of tsetse transmitted trypanosomosis as well as against *T. evansi* and *T. equiperdum* (Leach, 1961). Berenil has in many parts of Africa replaced Ethidium and Antrycide as the curative trypanocide of choice (Maxie and Losos, 1977).

**Drug Resistance:**

Resistance to the drugs used for control african animal trypanosomosis is increasingly recognized as constraint to livestock production in sub-Saharan Africa (Eisler *et al*., 2000). The degree of resistance to a drug which is of importance in the field is the resistance to the standard curative dose (Hawking, 1963). Natural resistance (drug tolerance) does not necessarily developed due to previous exposure of trypanosome to drug concentration (Leach and Roberts, 1981). Cross-resistance is a further complication and usually occurs between those compounds, which are related in their chemical structure (Losos, 1986; Zweygarth and Röttcher, 1988).

reported resistance of *T. congolense* to Homidium west Africa. Resistance was also reported in Ethiopia (Scott and Pegram, 1974) and Kenya (Gitatha, 1979; Schillinger, 1985). Homidium has been the drug of choice in southern Sudan where *T. vivax* infections are prevalent (Anonymous, 1975).

Abdel Gadir *et al.* (1981) studied Sudanese isolates of the three major trypanosome species and found them to express resistance to 1mg/kg homidium bromide, the relapse infections were susceptible to 3.5 mg/kg diminazine aceturate. Later, (Mohammed Ahmed *et al.*, 1992) reported *T. vivax*, *T. brucei* and *T. congolense* to be resistant to treatment with the recommended doses of Ethidium, Berenil and Isometamedium.

Iosmetamedium chloride is considered as prophylactic drug among the three trypanocides commonly used (Conner, 1984; Niougu *et al.*, 1985). When used in an area of high tsetse challenge its protection ranged from 3 to 6 months (Williamson, 1976). Resistance of various strains of *T. vivax* and *T. congolense* to isometamedium have been reported in several countries in west Africa (Kupper and Wolters, 1983; Pinder and Authie, 1984; Moloo and Kutuza, 1990) *T. congolense* resistance to isolmetamedium was reported in Ethiopia (Scott and Pegram, 1974), Kenya (Röttcher and Schillinger, 1985; Peregrine *et al.*, 1991) and Somalia (Ainanshe *et al.*, 1992).

Resistance of *T. vivax* to isometamedium was reported in Nigeria (Ilemobade and Na’ Isa 1981), Kenya (Röttcher and Schillinger,
1985; Peregrine et al., 1991) and Somalia (Schönefied et al., 1987). Nega et al. (2004) demonstrated the presence of isometamedium resistance to trypanosome infecting cattle in western Ethiopia.

Diminazine resistance was reported for *T. vivax* in west Nigeria (Jones-Davis, 1967a; 1968b; Maclellan and Na’Isa, 1970), in Uganda (Mwamba and Mayenda, 1971) and Kenya (Schillinger and Röttcher, 1984; 1985).

Diminazene aceturate 3.5 mg/kg has been shown to transiently increase the parastaemia of cattle infected with *T. congolense* 8 minutes after administration (Maxie et al. and losos, 1979). This effect was not noted in cattle infected with *T. vivax* and may reflect the dislodgment of *T. congolense* parasites from sites of capillary adherence (Gardiner, 1988). The relapses following treatment of *T. vivax* infected Zebu cattle with 1mg/kg homidium chloride or 3.5 mg/kg diminazene aceturate was between 37 and 47 days (Leeflang et al., 1976a) and 13 and 42 days in studies of other *T. vivax* from Nigeria (Leeflang et al., 1979).

In northern Ivory coast, cattle infected with *T. vivax* showed resistance to 0.5 and 1mg/kg isometamedium chloride and homidium chloride and were effectively treated with diminazene aceturate at 7mg/kg (Kupper and Walters, 1983). Logan et al. (1984) pointed out relapse infections under fly- free conditions can occur 10 to 25 days after diminazene aceturate treatment to *T. vivax* infection or mixed infection of *T. vivax* and *congolense*. 


Goats infected with west African stocks of *T. vivax* showed severe illness which was fatal if untreated. When they were treated with diminazene aceturate the parastaemia was eliminated but six weeks later the animals became parasitmic. It was concluded that reemergence of trypanosomes might have come from the central nervous system and or the eye, where sequestered parasites may have been inaccessible to the trypanocide (Whitelaw *et al.*, 1988). Rodent-adapted strain of *T. vivax* (Leeflang strain Y58), infected mice were treated with 10mg/kg Berenil, and 4mg/kg novidium, 0.2 gm/kg samorin, show complete cure. Berenil and novidium at lower doses rendered the mice parasitaemic (relapse). Lower doses of samorin were curative for some mice without relapses (Arowolo *et al.*, 1977). It is not possible under field conditions to differentiate between the two mechanisms governing the reappearance of trypanosomes (Logan *et al.*, 1984), and relapse infection may not necessarily be associated with drug resistance (White law *et al.*, 1988).

Röttcher and Schillinger (1985) noted *T. vivax* isolates which caused haemorrhagic disease were resistant to 2 mg/kg isometamedium chloride, 3.5mg/kg diminazene aceturate homidium chloride and quinapyramine sulphate at 5mg/kg. Njogu and Heath (1986) confirmed this isometamedium resistance to *T. vivax* but successfully treated the haemorrhagic infection with 7mg/kg diminazine aceturate. Schonefeld *et al.* (1987) have described other multiple drug resistance *T.vivax* isolates. Agu
(1984, 1985); Moloo and Kamunya (1987) have shown that tsetse harbouring *T. vivax* can be cured of the infection following ingestion of blood meal containing isometamidium, *in vivo* feeding for 5 days on animals treated with 1mg/kg isometamidium chloride. In Tanzania, trypanocidal drug-resistance occurred in isolates resistant to either of the sanative pair, diminazene and isometamidium, or resistant to both (Mbwanabo *et al.*, 1988, 2001).

Recent surveys in Eastern and southern Africa (Ndung’u *et al.*, 1999) and in West Africa (McDermott *et al.* 1999, 2000) have shown that the prevalence of trypanocidal drug resistance might even be higher than suspected. However, for most trypanosomosis areas, the extent and impact of drug resistance is not known (Geerts and Holmes, 1998).

Several tests have been described for the detection of drug resistance in trypanosomes pathogenic for domestic ruminants (Geerts and Holmes, 1998). The most commonly used tests for detection of trypanocidal drug resistance are tests using mice or ruminants (Wellde *et al.*, 1989; Eisler *et al.*, 2000). New, effective trypanocidal drugs are unlikely to become available in the near future due to high costs of development and production (Jordan, 1986).

**Control of trypanosomosis:**

The eradication of trypanosomosis from the entire African continent is an unrealistic goal. Considerable efforts have been
investigated in control of this disease through the use of trypanocidal drugs, management of the vector and exploitation of the genetic resistance exhibited by indigenous breeds. There is little hope that conventional, anti-infection vaccine will be produced sometime in future. However, drug resistance developing faster than generally thought. The control of the tsetse fly has been attempted over many decades. The decreasing efficiency of available trypanocidal drug and difficulties of sustaining tsetse control increase the imperative need to enhance trypanotolerance through selective breeding, either within breeds or cross breeding (Leteren et al., 1988, OIE, 2004).

Vector control, can be applied by simultaneous or consecutive use of a combination of chemical, biological, autocidal, mechanical and other procedures (FAO/WHO report on African trypanosomosis, 1976). Bush clearance would be effective in reducing the tsetse flies numbers in infested areas (Walker, 1986). Game animals resembles, an important reservoir for cattle trypanosomosis, so fencing of these animals would have great adverse effect on tsetse population (Mohammed, 2000). The use of traps targets and hand nets resemble simple, cheap and ecologically acceptable (FAO, 1992; Mohammed et al., 2000). Also biological control methods have been developed, the sterile insect technique is a species specific, and environmentally safe-non polluting method of insect control. It relies on the mass rearing, sterilizing and realizing of
large numbers of sterile males and consequently reduced their reproductive potential (Uilenberg, 1998).

**Trypanosomosis in Sudan:**

Trypanosomosis was reported in Sudan early as 1904 (Karib, 1961). It was noted that *T. vivax* was predominant outside tsetse belt, while *T. brucei* and *T. congolense* were confined to tsetse area. The predominance of *T. vivax* in cattle had been reported by Hall *et al.* (1984) in southern Darfur, A/Rahman *et al.* (1991) in South Kordofan, Suliman (1992); Homeida (1993); Abdelsalam (1996); Fadl *et al.* (2000) and A/Rahman (2002) in different sites of central Sudan and blue Nile state. The predominance of *T. brucei* and *T. congolense* infection in cattle herds in south eastern Sudan was reported by Kheir *et al.* (1993). Karib (1961) stated that camel trypanosomosis locally known as “Guffar” was officially reported in 1908, in Bahar El Gazal province. Mahmoud and Gray (1980) reported that *T. evansi* is manifested by elevation in temperature associated with parastaemia and anaemia which is often fatal. The distribution of *T. evansi* in central and eastern Sudan has changed slightly due to improvement of socioeconomic status of camel owners (ElRayh, 1997). The prevalence of camel trypanosomosis in western Sudan is higher than eastern and central Sudan (Elrayah, 1997). Boied *et al.* (1981) was able to detect *T. evansi* in western Sudan by thick smears from camels.
Tsetse and Tabanids in Sudan:

Only seven species and sub species of tsetse flies (*Glossina*) have been recognized in the Sudan, these are *Glossina morsitans* submorsitans and *G. pallidipes* of Savannah group, *G. fuscipes fuscipies* and *G. tachinoides* of the reverine group, *G. fusca, G. fuscipleuris* and *G. Logipennis* of the forest group (Lewis, 1949; Yagi and Abdel Razig, 1971). *G. morsitance, G. pallidipes* and *G. Tachinoides* in Blue Nile were reported by Lewis, 1949).


Also the presence of *G. fuscipes* in Kurmuk and Khor Yabous was reported by (Yagi and Abdel Razig, 1971; Mohamoud Ahmed *et al*., 1989; and Kheir *et al*., 1992).

Tabanid flies and stomoxys play an important role in the transmission of animal trypanosomosis outside the tsetse belts. Lewis (1953) reported seven species of tabanids. Then four species were added by Yagi (1968). In southern Darfur (AbdelKarim, 1980; Hall *et al*., 1983; Abdel Karim and Banjamin, 1989) and in South Kordofan (A/Rahman *et al*., 1991) in central Sudan (Suliman, 1992; Homeida, 1993) Abdelsalam (1996) in Abunaama and Um benin. Sawsan (1997) in Khartoum
State. Tabandids were distributed over different ecological zones and the dominant species were *T. supis*, *T. taeniola*, *A. agrestis*, *A. fuscipes* and *T. beguttatus*. *Stomoxys calcitrans*, *S. niger* and *haematobota & T. biggutatus*; *H. exigna* and *H. minuta*, were reported by Lewis (1954) in Sudan. Sawsan (1997) also reported the presence of stomoxys species in Khartoum State.

ElRayah and ElMalik (1990) noted that drug resistance was found to develop when trypanosomes were exposed to subcurative doses of trypanosicides. Today chemoresistance was reported against most trypanocidal drugs used (Williamson, 1982).

In the Sudan tsetse control at large scale has ceased for lack of funds and facilities (Hall *et al.*, 1983). However chemotherapy and/or chemoprophylaxis were successfully used for control trypanosomosis in the past. The high efficacy and availability of prophylactic drugs, protected the animals and improved their production and encouraged the nomads to encroach deeper on and stay longer in tsetse belt, but due to the abuse of trypanosicides and unprogrammed prophylactic applications, resistant trypanosomes strains began to emerge very frequently in these areas (A/Gadir *et al.*, 1972; Mohamed Ahmed *et al.*, 1992). Further more, the rise in drug prices with subsequent high cost of treatment impose an additional negative impact on a sound control of the disease.

Efforts aimed at controlling the disease mainly depend on chemotherapy, but this approach has not been without problems.
The most important one is widespread of drug resistant strains reported in the Sudan and as yet no efforts were made to control the biting flies (Mohamed Ahmed et al., 1992; ElRyah, et al., 1999).
CHAPTER TWO

Materials and Methods

1- Survey:

The Study area:
The survey were conducted in some areas Blue Nile State, which included Umbenain, Singa, Wad ElNaeal, Abu Neama, Abuhugar, Eldamazine, Elrosaris and the surrounding villages. Cross-sectional surveys were conducted in such areas, which have previous history of bovine trypanosomosis. Inspite of the absence of tsetse flies, the areas hold many resident herds, and foster of large irrigated agricultural schemes that act as breeding habitats for biting flies through out the year. There are also large mechanized irrigated land agricultural projects where thousands of fedans of woodland and pastures had been transferred to irrigated land were established south of Damazine. The farmers cultivate these lands only during the rainy season (July-October) which is the main source of irrigation. The main crops include sorghum, sesame groundnuts and sunflower. The animals were allowed to graze the post harvest products and many nomadic herds that belong to Ruffaa, Fulani and Ingassna tribes enter those plots for post harvest grazing. The area between Damazine town and Singa may be described as vast plains free from trees except for small patches of cultivated forests of Camphor trees.
Animals movements in the Blue Nile area:
Most of the farmers in the irrigated agricultural projects and the villagers of the region raise small numbers of cattle, sheep and goats. They keep them on the post harvest products and on green fodder grown in small farms. The migratory (nomadic) of the animal wealth in the region is owned by migratory animal owners. These tribes spend the dry season in Sudan, Ethiopian border including Khur Yabus tsetse area where grass and water are available. During the rainy season they come back to their home land around the Blue Nile villages mixing with resident animals of those areas.
Kenana and Rufa'a tribes who inhabit the Blue Nile areas. In the dry season camel owners from Butana and Kawahla come to these areas, whereas, Fallata (Fulani) come with their cattle from the south to this area during rainy season.
Three types of husbandry are practiced in the area namely the sedentary, the semi-nomadic and nomadic types. The sedentary animals graze over limited area around the villages. Nomadic and semi-nomadic animal owners move their herds in search of good grazing. The migrating cattle movement occurs in the dry season southwards to Upper Nile State, where the water and grass are available. At the beginning of the rainy season (Rushash), they return home.
Animals
Cattle:
The cattle examined in this survey were found mostly at the watering sites. Information was taken from the owners regarding, the place of sample collection, sex age of the animals, and the previous treatment with trypanocides used. The samples were immediately examined in the field by the wet smears, thin and thick blood films and buffy coats methods and packed cell volume (PCV) was determined.

Blood collection:
Blood samples from jugular veins were obtained using a vacutainer containing EDTA as anticoagulant. Diagnosis was made by microscopic examinations of wet smear, thin and thick films in addition to buffy coat were carried out on all samples during examination. The samples collected were placed

Ice-boxes and drops of blood from positives animals were sub-inoculated (intravenously in the jugular vein of kid goats, or cryopresrved in liquid Nitrogen. The viability of the trypanosome were confirmed before inoculation into the goats under objective x40 lens. The parastaemia was checked every day. When the animals became parastaemic the parastaemia was then followed until it was 25-30 /field (10^5/ml or 10^6/ml). At this time the isolates were cryopreserved using 10% glycerol as
cryopreservant. The stabilates kept in liquid Nitrogen were retrieved later for subsequent studies.

**Trapping methods:**
Three types of traps were used during these surveys:

**The Blue biconical trap:**
(autoradiographic products, scientific equipment and supplies U.K.; Challier and Laveissere, 1973).

**Collapsible canopy trap:**
Canopy trap of Axtell et al (1975) was modified into collapsible pyramidal frame of four metal pipes, a pyramidal piece and a removable collection non return cage which was fitted in away, similar to that of the biconical trap. Also a black cloth was attached to the netting on the upper edge of the cloth to improve the efficiency of the trap.

**Modified epsilon trap:**
This trap is a locally made design of epsilon trap developed in Zimbabwe. The design differs from the F₂ trap by its three-walled triangular shape (Flint, 1985). The cone, the central pole and the non return collecting cages were similar to those of the biconical trap.

**Experimental Animals:**

**Goats:**
Thirty two male Nubian goats, 6-12 months old were purchased from the local livestock market. All the goats had similar age and weights. They were transferred to the fly-proof premises of the
Central Veterinary Research Laboratory at Soba. They were ear-tagged before examination for presence of trypanosomes and other blood parasites using wet blood smears, thin and thick stained blood films Haematocrit and concentration methods. They were also examined for the presence of internal and external parasites, and were dewarmed by using Ivermectin (Avemec, Aveco, Jordan). The animals were also given anticoccidial drugs and were washed monthly with acarcide (Asuntol, coumaphos, Bayer, A.G., Germany). The cages where the animals were kept were sterilized by flame and antiseptics. The animals were fed dry sorghum and concentrate ad-libdium with free access to clean water.

Cattle:

Ten kenana calves were purchased from the local market. Their ages were less than two years old. All the calves were ear-tagged and blood samples were taken from their ears and jugular vein for examination for the presence of the blood parasites by wet, thin and thick smears, stained blood films and concentration methods. PCV and Hb values were also determined. All the calves were examined for internal and external parasites, and they all were treated with anthelmintics (Alben, albendazole vetycare pharmaceuticals Ltd., Pakistan), antibiotics (Oxigel gellini S.P.A., Italy) and anticoccidial drugs (Sulpha diamidima sodium, vetwic, Enasr pharmaceutical chemical Co. Egypt) and all the calves were sprayed using acarcide (Asuntol, comaphos, bayer, A.G.,
Germany) All the calves were kept in fly-proof pens at the central Veterinary Research Laboratory at Soba, and Fed dry sorghum, hay and concentrates and have free access to clean water.

**Trypanosoma vivax stock:**

Blood infected with *T. vivax* which was previously isolated from a naturally infected cattle in Suki district (Sennar state), was incubated intravenously in goats at site of collection, and then transferred to the Central Veterinary Research Laboratories in Khartoum. The goats were examined daily until they became parasitimic. During the peak of the first parastaemia, the stocks were cryopreserved as 10% stabillates in liquid Nitrogen.

**Sampling:**

Prior to infection the experimental animals were bled from the jugular veins and the blood was collected into clear vials containing EDTA. It was used for determination of the haematological picture. Following infection with *T. vivax* the blood sampling continued daily until the appearance of the parasite 4-5 days after inoculation. Blood was collected once/week for 16 weeks into heparinized vacutainers and heparinized capillary tubes from ear vein every two days for detecting of parastaemia of infected groups. For serum separation blood was collected into sterile plain vacutainers tubes. The whole blood were used for parasitological, haematological investigations.
Trypanocides:

Solution of Ethidium at the rate of 1ml/kg, and Berenil at the rate of 30mg then were prepared in sterile deionized water in accordance with the directions of the manufacturers and used within 30 minutes of preparations.

Treatments:

Homidium bromide:

Sixteen Nubian goats were divided into 4 groups of 4 animals each. Group I was infection with *T. vivax* (10^6 tryp./ml). When the animals became parasitaemic, 5 days post infections, they were treated with the standard recommended dose of Homidium bromide (1mg/kg body weight). Group 2 animals were infected with *T. vivax* as in group I and when the animals became parastaemic 5 days later, they were treated with homidium bromide at a dose rate double the standard dose (2mg/kg body weight). Animals in Group 3 were infected with *T. vivax* stock and served as infected control. Group 4 animals were non-infected non treated control.

Ethidium®:

The active ingredient of Ethidium®, Homidium bromide, belong to a group of compounds known as Phenanthridines.

Chemistry:

Pure Homidium bromide (3.8 diamino-5ethyl-6 phenylphenanthridium bromide) is a dark red, crystalline or amorphons
powder with solubility in water of 3.5% at 20°C (W/v). The product Ethidium® is presented as soluble tablets containing 250 mg of Homidium bromide the recommended dose at the rate of 1mg/kg, is sufficient to treat an average 250 kg animal.

**Preparation:**

A single tablet is prepared for administration as a 2.5% w/v injectable solution by dissolving in 10 ml of sterile, distilled or boiled then cooled water. Tablets are allowed to dissolve completely within two minutes, the resultant solution is clear dark, red and free from insoluble matter.

**Administration:**

Homidium bromide is administered by deep intramuscular injection. The preferred site of injection for adult cattle is the middle third of the neck. (Pro net 2004). The compound tested was produced by (Laprovet, BP 7562) company (France) under trade name Ethidium®.

**Diminazene Aceturate**

Sixteen Nubian goats were divided into 4 groups of 4 animals each. Group I was infected with *T. vivax* (10^6 tryp./ml). When the animals became parastaemic 5 days post infection, they were treated with the standard recommended dose of diminazene aceturate (3.5mg/kg body weight). Group 2 was infected with *T. vivax* (1000 tryp./ml) as in group 1 and when the animals became parastaemic 5 days later, they were treated with double the standard recommended dose of Diminazene aceturate (7mg/kg).
body weight). Group 3 infected with *T. vivax* (10⁶ Tryp/ml) served as infected control. Group 4 was non-infected non treated control.

Diminazene aceturate (Diminasan® compound consists of two amidinophenyl moieties joined a trizene bridge. (P, P-diamidinodiazooaminobenzene diceturate tetrahydrate; N-1-3-diamidino-phenyltriazene diaceturate tetrahydrate; (C₂₂ H₂₄ N₄ O₆-4H₂O). Molecular weight 587.6)

**Indication:**
Berenil is an odourless yellow powder and a dose of 7mg/kg was sufficient to cure infections with *T. vivax*.

The compound tested was produced by (Afasan) company (Woerden, Holland) under trade name Diminasan.

**Clinical parameters:**
The body temperatures of the animals were taken by digital thermometer from the animal rectum once a week for 16 weeks. Heart rates were determined by a stethoscope once a week for 16 week. Respiratory rates were observed using a stethoscope one a week for 16 week of the experiment.

**Haematological methods:**
Blood samples were collected from all the experimental goats and calves by jugular vein puncture into clean dry vials containing the disodium salt of EDTA as an anti-coagulant.
**Haemoglobin concentration: (Hb)**

Hb concentration was determined by the method described by Scham, Jain and Carrol (1975). The cyanmethaemoglobin technique using haemoglobin meter (IBA forming, 950 Hb meter England) at wave length 450nm. The method is based on the conversion of the haemoglobin by means of drabkins solution consisted of 0.2g potassium cyanide, 198mg potassium ferricyanide and 1g sodium bicarbonate per liter of distilled water to a cyanmethaemoglobin. The haemoglobin concentration was measured in gm/ml of blood.

**Haemoglobin determination:**

0.02 ml of blood was added in 4ml of drabkin solution, being allowed to stand for 10 minutes. Samples were then and using a haemoglobin meter. The haemoglobin meter and drew a standard curve haemoglobin solution was used to adjust. Values were obtained out of the haemoglobin meter standard curve and expressed as g/dl.

**Packed Cell Volume (PCV):**

Fresh blood samples were collected from the ear vein into microhaematocrit capillary tubes (70mm) which were centrifuged in a microhaematocrit centrifuge (Hawksley and sons Ltd., England) at 150 rpm for five minutes. The percentages of PCV were read on the scale reader.
Red Blood Cells Count (RBCs):
Red blood cells were counted by an improved Neubauer haemocytometer (Hawksley and Sons Ltd., England). Formal citrate was used as a diluting fluid. It was prepared by dissolving 30gm sodium citrate in addition to 10ml formalin in one liter of distilled water.

Procedure:
Using RBCs pipette, blood was withdrawn till 0.5 mark then completed with RBCs diluent till 101, mixed well and left for 3 minutes. Mixture in the diluent part was discarded and a drop was placed in the a haemocytometer, 5 squares out of 25 in RBCs specified area were calculated under 40x objective. Total number of RBCs were counted and multiplied by the dilution factor 10000. Values were expressed in million/mm blood.

White Blood Cells Count (WBCs):
White blood cells were counted with an improved Neubauer haemocytometer (Hawksley and Sons Ltd., England) Turks solution was used as diluting fluid (prepared by mixing 10ml glacial acetic acid, tinged with crystal violet in I liter of distilled water.

Procedure:
Using WBCs pipette, blood was withdrawn till 0.5 mark then completed with WBCs diluent till 11 mark, mixed well and left for three minutes. The mixture in the diluent part was discarded and a drop of the solution was placed in the haemocytometer.
Four squares in WBCs were counted and multiplied by the dilution factor 50. Values were expressed in thousands/mm$^3$ blood.

**The pathogenicity of *T. vivax***:
Ten kenana calves were divided into two groups. Six animals in group (I) were infected with *T. vivax* ($10^6$ Tryp./ml). Group 2 consist of 4 animals served as non infected control. Several haematological and clinical parameters were recorded every week as follows:

**Histopathological methods**:
At necropsy, all goats and calves were examined for gross pathological changes and specimens from, heart, lung, spleen liver, kidney, lymph nodes testicles and brain were collected fixed in 10% formal saline and processed for histopathology. They were transferred to automatic tissue processor (Elliotts liver pool Ltd., England) for the processes of dehydration and embedding in paraffin sections before staining with haematoxylin and eosin. Processing and staining were done according to the methods described by Clayden (1971).

**Parasitological methods**:
Heparinized blood samples collected from both the jugular and ear veins were examined for presence of trypanosome using the following standard parasiteological methods.
**Wet blood smear:**
A drop of blood was placed on a clean slide and covered with a 22x22 mm cover slip, about 100 microscopic fields were searched under x40 objective (Kendrick, 1968).

**Thin blood film:**
It was done by placing drop of blood on a clean slide, another slide (Spreader) was placed at angle of approximately 30° to the first slides, and drawn back to make contact with the blood droplet. The blood was allowed to run along the edge of the spreader, which was then pushed to the other end of the slide, drowning the blood out into a thin film. The slide was dried quickly by waving in the air, fixed for three minutes in methanol, and stained for 30 minutes with 10% diluted Giemsa stain in buffered water. After staining, the slide was washed gently under tab water x100 oil – immersion objective lens (Kendrick, 1968; OIE, 2004).

**Thick blood films:**
It was done by placing a drop of blood on a clean slide and spreading it with the edge of another slide over an area of approximately 2cm in diameter, dried rapidly by waving in the air, dehaemoglobinized in distilled water for five minutes, then stained in 10% Giemsa stain for 30 minutes, washed and examined under x100 objectives lens (Kendrick, 1968; OIE, 2004).
**Haematocrit Centrifugation technique (CT):**
A capillary tube was filled with blood then sealed from one side using crystaseal, the sealed capillary tube was centrifuged in the microhaematocrit centrifuge (Hawksley and sons Ltd., England) for four minutes at 12,000 rpm. After centrifugation the capillary tube was placed in a McMaster chamber flooded with water, and the junction of the buffy coat layer and the plasma was examined under a microscope using x10 objective. The capillary tube was rotated form time to time during the examination to ensure that all sides of the tube have been examined (Woo, 1971).

**Dark ground/phase contrast buffy coat technique (BCT):**
This was done according to Murray (1977). After examining the capillary tube the tube was cut 1 mm below the buffy coat to include the upper most layers of red blood cells and 3 cm above to include the plasma, the contents of the capillary tube were gently expressed onto a slide, mixed and covered with a cover slip (22x22mm). The preparation was then examined using a phase contrast or dark-ground microscopy or ordinary bright microscope system, with the condenser top out and the diagram closed to achieve critical illumination. About 200 microscopic fields were examined using x10 and x40 objectives.

**The estimation of Parastaemia:**
This method was described by Paris, *et al.* (1982) to estimate the levels of parastaemia. The presence of 1 to 10 parasites in a whole preparation of 22x22 mm cover slip was scored as 2+ and had an equivalent level of 1x10^2 – 1x10^4 parasites /ml. The presence of 1 to 10 parasites in each microscopic field was scored as 4+ and had a level of parastaemia equivalent to 1x10^4 – 5x10^5 parasites /ml. For more than 10 parasites/ml a haemocytometer was used for an accurate count of the number of parasites.
Meteorological data:
The meteorological data were obtained from Damazine Meteorological stations.

Statistical Analysis:
The repeated measurement Anova was used for statistical analysis. (Axill).
CHAPTER THREE

RESULTS
CHAPTER THREE
RESULTS

Survey of Trypanosomosis at the Blue Nile Area:
Examination of a total of 1096 resident and migratory cattle during the dry season (March to June) showed that *T. vivax* was the only species present in the area during the survey period. The number of infected animal were 13 giving a total infection rate of 1.2%. The infection rates was highest at Abohagar (6.5%) follows by Aldamazine (4.1%) Roseris (2.8%) and Singa (2.2%). There was no positive cases at the other localities (Table 1).

During the rainy season (July-December), a total number of examination of 694 showed that 12 were infected giving and overall percentage of 1.7%. The rates at Singa, Abonama and Aldamazine were 6.4%, 6.3% and 1.4% (Table 2).

Positive cases were not encountered at Alsuki and Abohagar.

Tabanid catches during the dry season were highest at Aldamazine 25±7.1, Singa 25±5.1, Wadel Nayal (24) and AlSuki (21), ElRoseris (19). Moderate catches occurred at Abohogar (10) while low numbers were encountered at Wad taktook (5±2.2), ElSuker (4.3±2.1), Wad Abass (2±1.4) (Table 3).
The infection rate of *T. vivax* in cattle during the dry season

Table (1).

<table>
<thead>
<tr>
<th>Locality</th>
<th>Animal examined</th>
<th><em>T. vivax.</em></th>
<th>infection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abohijar</td>
<td>62</td>
<td>4</td>
<td>6.5</td>
</tr>
<tr>
<td>Aldamazin</td>
<td>122</td>
<td>5</td>
<td>4.1</td>
</tr>
<tr>
<td>Alroseris</td>
<td>72</td>
<td>2</td>
<td>2.8</td>
</tr>
<tr>
<td>Singa</td>
<td>313</td>
<td>7</td>
<td>2.2</td>
</tr>
<tr>
<td>Alsuri</td>
<td>154</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Al sukar</td>
<td>155</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Wad al Nyal</td>
<td>41</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Wad taktuk</td>
<td>136</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Wad al Abass</td>
<td>41</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1096</strong></td>
<td><strong>0</strong></td>
<td><strong>1.2</strong></td>
</tr>
</tbody>
</table>
Trypanosomosis infection rates during the rainy season Survey at The Blue Nile Area.

Table (2)

<table>
<thead>
<tr>
<th>Locality</th>
<th>Animals examined</th>
<th>T. vivax</th>
<th>Infection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldamazin</td>
<td>48</td>
<td>3</td>
<td>6.3</td>
</tr>
<tr>
<td>Abo Neamma</td>
<td>63</td>
<td>4</td>
<td>6.4</td>
</tr>
<tr>
<td>Singa</td>
<td>491</td>
<td>5</td>
<td>1.4</td>
</tr>
<tr>
<td>Wadelnyal</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Alsuki</td>
<td>34</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Abo Hajar</td>
<td>16</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>694</td>
<td>12</td>
<td>1.7</td>
</tr>
</tbody>
</table>
Tabanids catches during dry season survey at the Blue Nile area.

**Table (3)**

<table>
<thead>
<tr>
<th>Locality</th>
<th>Tabanid catches means fly/trap/day during dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldamazin</td>
<td>25±7.1</td>
</tr>
<tr>
<td>Singa</td>
<td>25 ±5.1</td>
</tr>
<tr>
<td>Wad Elnayal</td>
<td>24</td>
</tr>
<tr>
<td>Alsuki</td>
<td>21</td>
</tr>
<tr>
<td>Alroseris</td>
<td>19</td>
</tr>
<tr>
<td>Abohijar</td>
<td>10</td>
</tr>
<tr>
<td>WAD taktok</td>
<td>5 ±2.2</td>
</tr>
<tr>
<td>Alsukar</td>
<td>4.3±2.1</td>
</tr>
<tr>
<td>Wad Alabass</td>
<td>2 ±1.4</td>
</tr>
</tbody>
</table>
Treatment of *T. vivax* infected goats with Ethidium bromide

The onset of parasitaemia in the infected animals of the various groups occurred on day 4 or 5 post infection, then the peak was reached with the same week (10^6 parasite/ml). In the Ethidium bromide treated animals the parasites disappeared from the blood to relapse at time intervals started with less than one month up to more than 4 months. Group 3 (infected control) showed different parasitaemia pattern started with low level at the first 3 weeks post infection then it peaked (10^6 parasite /ml) at different intervals to drop up to the death of the animals. (Table 5 and 6).

**Table 5: The Relapse of goats treated with Ethidium bromide**

**Group I (animals treated with standard dose)**

<table>
<thead>
<tr>
<th>Group I Animal No.</th>
<th>Parasite/ml</th>
<th>Onset of parasitaemia</th>
<th>Ethidium dose</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>2865</td>
<td>1x10^6</td>
<td>4 days</td>
<td>0.5ml/kg</td>
<td>Died</td>
</tr>
<tr>
<td>2869</td>
<td>1x10^6</td>
<td>4 days</td>
<td>0.5ml/kg</td>
<td>26 days</td>
</tr>
<tr>
<td>2870</td>
<td>1x10^6</td>
<td>4 days</td>
<td>0.5ml/kg</td>
<td>126 days</td>
</tr>
<tr>
<td>76</td>
<td>1x10^6</td>
<td>5 days</td>
<td>0.5ml/kg</td>
<td>53 day</td>
</tr>
</tbody>
</table>
Table 6: The Relapse of the goats treated with Ethidium Bromide Group II (animals treated with double dose)

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal No.</th>
<th>Parasite/ml</th>
<th>Onset of parasitaemia</th>
<th>Ethidium dose</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2866</td>
<td>1x10⁶</td>
<td>4 days</td>
<td>1ml/kg.</td>
<td>99 days</td>
</tr>
<tr>
<td></td>
<td>2863</td>
<td>1x10⁶</td>
<td>4 days</td>
<td>1ml/kg.</td>
<td>130 days</td>
</tr>
<tr>
<td></td>
<td>2864</td>
<td>1x10⁶</td>
<td>4 days</td>
<td>1ml/kg.</td>
<td>76 days</td>
</tr>
<tr>
<td></td>
<td>2867</td>
<td>1x10⁶</td>
<td>4 days</td>
<td>1ml/kg.</td>
<td>27 day</td>
</tr>
</tbody>
</table>

Haematological Parameters in Ethidium bromide treated animals:

**Packed Cell Volume (PCV):**

The mean PCV values in groups 1, 2 and 3 were found to be $20.482 \pm 0.299$, $21.411\pm0.299$ and $19.464 \pm 0.423$ respectively while that of the uninfected control group was $27.196 \pm 0.299$ (Table 7). Statistically there is a significant difference between the infected groups and the non infected control ($P<.05$).

The PCV in group (1) showed significant decline after the second week post infection reached its minimum values at week 13 and 14. In Group (2) animals showed similar significant drop in PCV value to reach the minimum at week 14. Group 3 showed rapid fall in value to reach the minimum level at week 13 before the
value exhibit slight increase followed by a decrease in week 14. The control group showed normal values during the course of infection (Fig. 1).

**Table 7: Ethidium bromide treatment**

<table>
<thead>
<tr>
<th>PCV</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment groups</td>
</tr>
<tr>
<td></td>
<td>Animal treated with double dose</td>
</tr>
<tr>
<td></td>
<td>Untreated infected control</td>
</tr>
<tr>
<td></td>
<td>Untreated uninfected control</td>
</tr>
</tbody>
</table>

(P<.05)

**Haemoglobin (HB) in the Ethidium bromide treatment:**

The mean haemoglobin values in groups 1, 2 and 3 were 5.038 ± 0.080, 5.282 ± 0.080, and 5.011 ± 0.113 respectively, (Table 8) control animals in group (4) had HB of 8.027 ± 0.080. Statistically there is significant difference between the infected groups and the non-infected control (P<.05).

The Hb in group 1 showed rapid fall and reached its minimum value during week 13 and 14. Group 2 animals showed decline of Hb that reached its minimum value at week 14. Group 3 also witnessed decline to reach the minimum at week 12 and 14.
Animals in group (4) showed normal values during the course of the experiment (Fig. 2).
### Table 8: Ethidium bromide treatment

Hb

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Mean</th>
<th>Std. error</th>
<th>95% confidence interval</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal treated with standard dose</td>
<td>5.038</td>
<td>.080</td>
<td>4.859</td>
<td>5.216</td>
<td></td>
</tr>
<tr>
<td>Animal treated with double dose</td>
<td>5.282</td>
<td>.080</td>
<td>5.104</td>
<td>5.460</td>
<td></td>
</tr>
<tr>
<td>Untreated infected control</td>
<td>5.011</td>
<td>.113</td>
<td>4.759</td>
<td>5.262</td>
<td></td>
</tr>
<tr>
<td>Untreated uninfected control</td>
<td>8.027</td>
<td>.080</td>
<td>7.849</td>
<td>8.205</td>
<td></td>
</tr>
</tbody>
</table>

(P<.05)
Figure (1)

PCV in Homidium Bromide treated goats infected with T. vivax

Treatment groups

- Animal treated with standard dose
- Animal treated with double dose
- Untreated infected control
- Untreated uninfected control

PCV %

Tim e

PCV in Homidium bromide treated goats infected with T. vivax

PCV %

Tim e
Animal treated with standard dose
Animal treated with double dose
Untreated infected control
Untreated uninfected control

Mean haemoglobin in different treatment groups

The level of HB in different groups in 14 weeks
White Blood Cells (WBCs) Ethidium bromide treatment:
The mean white blood cells counts in groups 1, 2 and 3 were 4339.436 ± 1044.408, 7299.607 ± 1381.622, and 9511.857 ± 1953.908 respectively while the noninfected control group (4) was 12443.643 ± 1381.622 (Table 9). Statistically there is significant difference between the groups 1, 2, 3 and group 4 the control group (P<.05). Group 1 also showed rapid fall as WBC reached the minimum level at week 14. Similarly, group (2) goats showed rapid decrease in WBC count to reach the minimum values at week 13 and 14. In group (3) similar drop was observed the values exhibited slight increase at week 8 and 9 before they drop to minimum values during week 13 and 14 (Fig. 3).

Table 9: Ethidium bromide treatment
WBCs

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Mean</th>
<th>Std. error</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
</tr>
<tr>
<td>Animal treated with standard dose</td>
<td>4339.436</td>
<td>1044.408</td>
<td>2083.129</td>
</tr>
<tr>
<td>Animal treated with double dose</td>
<td>7299.607</td>
<td>1381.622</td>
<td>4314.794</td>
</tr>
<tr>
<td>Untreated infected control</td>
<td>9511.857</td>
<td>1953.908</td>
<td>5290.695</td>
</tr>
<tr>
<td>Untreated uninfected control</td>
<td>12443.643</td>
<td>1381.622</td>
<td>9458.830</td>
</tr>
</tbody>
</table>
Red Blood Cells (RBCs):
The mean value of red blood cells in groups 1, 2 and 3 were 6879232 ± 211488.5, 7382954 ± 211488.5, 6742321 ± 299089.9 respectively. The control group (4) showed a count of 12209125 ± 211488.5 (Table 10). Statistically there is significant difference between groups 1, 2, 3 and group 4 (P<.05). Group 1 showed rapid decline of RBC until week 6 post infection before they increase to decline down until week 14 to reach the minimum at week 11 and 12. Group 2 also decline to reach the minimum at week 9 and 10, group 3 were slow down along the course of infection while group 4 the control showed no change in the normal RBCs value along the course of the experiment (Fig. 4).

Table 10. Ethidium bromide treatment

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Mean</th>
<th>Std. error</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
</tr>
<tr>
<td>Animal treated with standard dose</td>
<td>6879232</td>
<td>211488.5</td>
<td>6408006.375</td>
</tr>
<tr>
<td>Animal treated with double dose</td>
<td>7382954</td>
<td>211488.5</td>
<td>6911727.786</td>
</tr>
<tr>
<td>Untreated infected control</td>
<td>6742321</td>
<td>299089.9</td>
<td>6075907.531</td>
</tr>
<tr>
<td>Untreated uninfected control</td>
<td>12209125.0</td>
<td>211488.5</td>
<td>11737899.21</td>
</tr>
</tbody>
</table>
(P<0.5)

The mean WBCs in different groups

- Animal treated with standard dose
- Animal treated with double dose
- Untreated infected control
- Untreated uninfected control

The level of WBCs in different groups

- Animal treated with standard dose
- Animal treated with double dose
- Untreated infected control
- Untreated uninfected control
Figure 4

Mean RBCs count in different groups

The level of RBCs count in different groups
Treatment of *T. vivax* with Dimimazine aceturate (Dimimazine)

Parastaemia:

Generally the pattern in different animals in group 1 and 2 showed low level of parasitamia $10^3$/ml then it increase to $10^6$ in both of the group the animals were treated with the different dose of diminazine aceturate.

The parasites disappeared from the blood to reappear at different periods between month to more than 3 months (relapse) as shown in the (Table 11 and 12.). While the pattern at group III remains at low level with exception of animal number 548 showed $10^6$ parasite /ml at different intervals dropping to lower level.

**Table 11. Relapse after Diminazine Aceturate treated with standard dose**

<table>
<thead>
<tr>
<th>Group (1) Animal No.</th>
<th>Parasite/field</th>
<th>On set of infection</th>
<th>Diminazene aceturate</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>542</td>
<td>$10^6$</td>
<td>4</td>
<td>3.5/mg/kg</td>
<td>30 days</td>
</tr>
<tr>
<td>2929</td>
<td>$10^6$</td>
<td>5</td>
<td>3.5/mg/kg</td>
<td>37 days</td>
</tr>
<tr>
<td>2930</td>
<td>$10^6$</td>
<td>4</td>
<td>3.5/mg/kg</td>
<td>93 days</td>
</tr>
<tr>
<td>2932</td>
<td>$10^6$</td>
<td>4</td>
<td>3.5/mg/kg</td>
<td>26 days</td>
</tr>
</tbody>
</table>
Relapse after Diminazine aceturate treatment with double dose

Table 12.

<table>
<thead>
<tr>
<th>Group (1) Animal No.</th>
<th>Parasite/field</th>
<th>On set of infection</th>
<th>Diminazene aceturate</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>2928</td>
<td>$10^6$</td>
<td>4</td>
<td>7.0mg/kg</td>
<td>53 days</td>
</tr>
<tr>
<td>2941</td>
<td>$10^6$</td>
<td>5</td>
<td>7.0mg/kg</td>
<td>83 days</td>
</tr>
<tr>
<td>2945</td>
<td>$10^6$</td>
<td>4</td>
<td>7.0mg/kg</td>
<td>66 days</td>
</tr>
<tr>
<td>2948</td>
<td>$10^6$</td>
<td>5</td>
<td>7.0mg/kg</td>
<td>56 days</td>
</tr>
</tbody>
</table>

Packed Cell Volume (PCV):

The mean value of packed cell volume in group 1, 2, 3 and 4 were 18.119 ± 0.325, 19.0 ± 0.281, 17.321 ± 0.398, and 27.178 ± 0.281 the control group. (Table 13). Statistically there is significant difference between group 1,3 and 4 (control) (P<0.5).

Group I and II shows similar decline in PCV that reached its minimum value at week 13 and 14, group III showed rapid decline to reach the minimum value at week 3, before they show steady rise in PCV level, group 4 (the controls showed normal values during the course of experiment (Fig. 5).

Table 13: Diminazine aceturate treatment PCV

<table>
<thead>
<tr>
<th></th>
<th>Mean g/l</th>
<th>Standard error</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal treated with standard dose</td>
<td>18.11905</td>
<td>0.325348</td>
<td>17.38306</td>
<td>18.85504</td>
</tr>
<tr>
<td>Animal treated with double dose</td>
<td>19</td>
<td>0.28176</td>
<td>18.36261</td>
<td>19.63739</td>
</tr>
<tr>
<td>Untreated infected control</td>
<td>17.32143</td>
<td>0.398469</td>
<td>16.42003</td>
<td>18.22283</td>
</tr>
<tr>
<td>Untreated uninfected control</td>
<td>27.17857</td>
<td>0.28176</td>
<td>26.54119</td>
<td>27.81596</td>
</tr>
</tbody>
</table>

(P<.05)
Haemoglobin (HB):

The mean value of haemoglobin was 4.309 ± .138, in group I, 4.677 ± .138, in group II, 5.196 ± .138 group III and in group IV the control it was 9.482 ± .138. Statistically there is significant difference between the 3 groups and the control (Table 14).

Group I showed rapid fall down post infection to reach the minimum value at week 13 and 14, group 2 also decline to reach the minimum at week 14, group 3 rapidly fall down to reach the minimum values at week 10 and 13, while group 4 showed normal course and no change was observed during the course of the experiment (Fig. 6).

**Table 14: Diminazine aceturate Treatment Hb**

<table>
<thead>
<tr>
<th></th>
<th>Mean G/d</th>
<th>Standard error</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal treated with standard dose</td>
<td>4.309</td>
<td>.138</td>
<td>4.001</td>
<td>4.617</td>
</tr>
<tr>
<td>Animal treated with double dose</td>
<td>4.677</td>
<td>.138</td>
<td>4.368</td>
<td>4.985</td>
</tr>
<tr>
<td>Untreated infected control</td>
<td>5.196</td>
<td>.138</td>
<td>4.760</td>
<td>5.633</td>
</tr>
</tbody>
</table>

(P<.05)
Figure 5

Mean PCV of Diminazene Aceturate Treatment Groups

- Animal treated with standard dose
- Animal treated with double dose
- Untreated infected control
- Untreated uninfected control

Mean PCV Diminazene Aceturate Treatment Groups

- PCV %
- Time

- Animal treated with standard dose
- Animal treated with double dose
- Untreated infected control
- Untreated uninfected control
Mean Haemoglobin of Diminazene Aceturate Treatment

- Animal treated with standard dose: 4.34 g/ml
- Animal treated with double dose: 4.68 g/ml
- Untreated infected control: 3.67 g/ml
- Untreated uninfected control: 9.48 g/ml

Mean Haemoglobin of Diminazene Aceturate in Treatment

- Animal treated with standard dose
- Animal treated with double dose
- Untreated infected control
- Untreated uninfected control
White Blood Cells (WBCs):
The mean value of White Blood Cells was 7708.810 ± 182.369 in group I, 6570.286 ± 157.936 in group II, 7264.321 ± 223.358 in group III and 12141.929 ± 157.936 in the control group IV. Statistically there is significant difference between the groups 1, 2, 3 and 4 the (control) (P<.05) (Table 15).
Group I showed rapid decline immediately after infection then it increased for a week and it dropped again to reach the minimum value at week 11. Group 2 and 3 declined to reach the minimum at week 14, value at week 14, while group 4 showed normal values during the course of experiment (Fig. 7).

<table>
<thead>
<tr>
<th>WBCs</th>
<th>Animal treated with standard dose</th>
<th>Mean</th>
<th>Standard error</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7708.810</td>
<td>182.369</td>
<td>7296.262</td>
<td>8121.357</td>
<td></td>
</tr>
<tr>
<td>Animal treated with double dose</td>
<td>6570.286</td>
<td>157.936</td>
<td>6213.009</td>
<td>6927.563</td>
<td></td>
</tr>
<tr>
<td>Untreated infected control</td>
<td>7264.321</td>
<td>223.356</td>
<td>6759.056</td>
<td>7769.587</td>
<td></td>
</tr>
<tr>
<td>Untreated uninfected control</td>
<td>12141.929</td>
<td>157.936</td>
<td>11784.652</td>
<td>12499.205</td>
<td></td>
</tr>
</tbody>
</table>

(P<.05)

Red Blood Cells (RBCs):
The mean value of Red Blood Cells in group I was 7034000 ± 289847.6, in group 2 was 7198404 ± 251015.4, in group 3 was
7524643 ± 354989.3, and in group 4 was 9647500 ± 251015.4. Statistically there is significant difference between group 1, 2, 3 and 4 the (control) (Table 16).

Group 1 showed decreased to reached the minimum value at week 14, group 2 rapidly declined to reach the minimum value at week 12 and 14. Group 3 also rapidly declined to reach the minimum value at week 4 then slightly increased then decreased again until week 14. Group 4 showed normal value during the course of infection (Fig. 8).

**Table 16: Diminazine aceturate treatment RBCs**

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard error</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal treated with</td>
<td>7034000</td>
<td>289847.6</td>
<td>6378319.247</td>
<td>7689680.753</td>
</tr>
<tr>
<td>standard dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal treated with</td>
<td>7198404</td>
<td>251015.4</td>
<td>6630567.383</td>
<td>7766239.760</td>
</tr>
<tr>
<td>double dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated infected control</td>
<td>7524643</td>
<td>354989.3</td>
<td>6721601.218</td>
<td>8327684.496</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated uninfected</td>
<td>9647500</td>
<td>251015.4</td>
<td>9079663.812</td>
<td>10215336.19</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(P<.05)
Figure 7

Mean WBCs of Diminazene Aceturate Treatment Groups

![Graph showing WBCs over time for different treatments](image)

Mean WBCs of Diminazene Aceturate Treatment Groups

![Graph showing WBCs over time for different treatments](image)
Figure 8

**Mean RBCs of Diminazene Aceturate Treatment Groups**

- Animal treated with standard dose
- Animal treated with double dose
- Untreated infected control
- Untreated uninfected control

**Mean RBCs of Diminazene Aceturate Treatment Groups**

- Animal treated with standard dose
- Animal treated with double dose
- Untreated infected control
- Untreated uninfected control
The pathogenicity of *T. vivax* infected calves

**Pattern of Parastaemia:**
Generally the parastaemia in different animals followed similar course starting by low number of parasite $10^4$ after 4 to 5 days post-infection then it reached a peak 3-4 days later ($10^6$ parasite/ml), the animals in the infected group showed different patterns. Animal number 1453 showed high level of parasitaemia during the first two weeks then it became aparasitaemic until it died. Animals number 1446 and 1487 showed high level fluctuating parasitaemia through out the course of the experiment. The rest of the animals showed fluctuating low level of parasitaemia that continued until the death of the animals (Fig. 9).

**Haematological parameters:**

**Packed Cell Volume (PCV):**
The analysis of the packed cell volumes of group one was found to be $15.679 \pm 1.457$ and that of group II (non infected control) was $27.589 \pm 1.785$. (Table 17). Statistically there is a significant difference between the two groups ($P<.05$). Group one showed a significant, fall in PCV during the second and third week post infection, then it declined slowly until week 9 after which it remained at low level until week 14. The PCV values of the control group showed no significant change during the course of infection (Fig. 10).
Table 17: PCV of infected and non infected groups of calves

<table>
<thead>
<tr>
<th>Animals group</th>
<th>Mean PCV %</th>
<th>Std. error</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
</tr>
<tr>
<td>Infected</td>
<td>15.679</td>
<td>1.457</td>
<td>12.319</td>
</tr>
<tr>
<td>control</td>
<td>27.589</td>
<td>1.785</td>
<td>23.474</td>
</tr>
</tbody>
</table>

(P<.05)

**Haemoglobin concentration (HB):**

The mean haemoglobin values of group I was 5.775 ± 0.220 and for group 2 (non infected control) was 8.023 ± 0.220. Statistically there is significant difference between the two groups (P<.05) (Table 18).

(Fig. 11) showed that the Hb level of group (1) decreased gradually after the 2nd week post infection during the course of experiment to reach the minimum values at week 14. The control group showed no significant changed during the course of the experiment.

Table 18: Hb concentration of infected and non infected calves.

<table>
<thead>
<tr>
<th>Animals group</th>
<th>Mean Hb concentration</th>
<th>Std. error</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
</tr>
<tr>
<td>Infected</td>
<td>5.775</td>
<td>.220</td>
<td>5.237</td>
</tr>
<tr>
<td>control</td>
<td>8.023</td>
<td>.220</td>
<td>7.486</td>
</tr>
</tbody>
</table>

(P<.05)
Figure 10

Mean PCV of *T. VIVAX* infected and control calves

Mean PCV %

Infected  Control

Time

PCV %
Figure 11

**Mean HB concentration of *T. VIVAX* of Infected and non Infected Calves Groups**

Bar chart showing the mean HB concentration over weeks for infected and control groups.

**Mean HB concentration of *T. VIVAX* of Infected and non Infected Calves Groups**

Line graph showing the mean HB concentration over weeks for infected and control groups.
**White Blood Cells (WBCs):**

The mean values of the white Blood Cells was 5001.696 ± 162.725 for the infected group 1 and 7543.571 ± 162.725 for the control in (group 2). The statistical analysis showed significant difference between the two groups P< .05 (Table 19).

Fig. (12) showed the white blood cells counts of the infected and non infected calf group, the infected group (1) showed rapid decrease in WBCs values at the first 4 weeks post infection then it slightly increased and again it decreased to reach the minimum values by week 14 or before death.

**Table 19: WBCs counts of infected and non infected groups**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Mean</th>
<th>Std. error</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
</tr>
<tr>
<td>Infected control</td>
<td>5001.696</td>
<td>7543.571</td>
<td>4603.523 7145.398 5399.870 7941.745</td>
</tr>
</tbody>
</table>

**Red Blood Cell (RBCs):**

The mean values of the Red Blood cells were 3554018 ± 172889.0 in group 1 and 7500377.5 ± 172889.0 in group 2 the non infected group statistically higher RBCs counts than the infected group P<.05. (Table 20).

Fig. (13) showed the rapid decline in RBCs values of the infected which dropped immediately post infection until week 4 then it increased slightly and decreased again to reach the minimum level at week 14.
Table 20: RBCs count of infected non infected calf groups

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Mean</th>
<th>Std. error</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower bound</td>
<td>Upper bound</td>
<td></td>
</tr>
<tr>
<td>Infected control</td>
<td>3554018</td>
<td>172889.0</td>
<td>3130973.673 3977062.041</td>
</tr>
<tr>
<td></td>
<td>7500375</td>
<td>172889.0</td>
<td>7077330.816 7923419.184</td>
</tr>
</tbody>
</table>
Figure 12

Mean WBCs of T VIVAX of infected and non-infected calves groups

Mean WBCs of T VIVAX of infected and non-infected calves groups

- Infected
- Control
Figure 13

Mean RBCs of T VIVAX of infected and non Infected Calves Groups

Mean RBCs of T VIVAX of infected and non Infected Calves Groups
Clinical parameters:

Temperature:
All the infected groups of animals became febrile (40.5 –41.5)°C during peaks of parasitaemia. At low parasitaemia animals showed normal temperature and there were no significant difference between the control groups.

Heart Rate:
There was an increase in the numbers of heart beats (79-80) beat/minutes during the peak of parastaemia when animals were also febrile. At low level of parastaemia animals showed slight increase in heart rates. There were no significant difference between the control and the infected groups.

Respiratory Rate:
The infected animal showed increase in the respiratory rates to reach (26-28) inspiration per minute when animals were febrile and the parastaemia at high level. At low level parastaemia the respiratory rates were normal (18-24) inspiration per minute. In chronic course of infection animals showed mild increase in respiratory rate.

Histopathology

Macroscopic findings:
At necropsy animals showed emaciation anaemia, enlargement of lymph nodes spleen, liver and heart, congestion petechial and ecchymotic haemorrhages in the mucosal and serosal surfaces, in
heart, spleen, kidney brain and testicles, Serous fluid in the lung and heart.

**Microscopic findings:**

**Brain:**

Generally the predominant cellular reaction in the brain were lymphoid, plasma and histocytes. Histopathologically, there were congestion of most blood vessels, vasculitis with prevascular cuffing of mononuclear cells (Fig. 14) malacies lesion were decrivable subendymal tissue where ependymal layer was desquamated, these areas were infiltrated with mononuclear cells interning led with proliferated glial cells and large cell which contain large amount of pink cytoplasm and eccentric nuclear morula cells (Fig. 15). Adjacent to these necrotic foci there were rarefaction of encephalon stroma and increase in cellularity by proliferated glial cells. Occasionally, the superficial layers of cerebral hemisphere below the sulci showed focal areas of coagulative necrosis. At the vicinity this necrotic foci many neuron, were collapsed, distorted and revealed chromatolysis and some were disintegrated.

**Heart:**

Endocarditis, pericarditis and myocarditis were the predominant cardiac lesions, these focal to diffuse areas of interstitial oedema and infiltration of lymphoid plasma and histocytes were regularly encountered (Fig. 16). Most of lymphatic vessels in vicinity of lesions were filled with pink protienous material.
Fig 14: Section of Brain, perivasicular cuffing of mononuclear cells, proliferation of glial cells rarefaction. H& E

Fig 15: Section of Brain, detachment of ependymal layer, malacic lesions, infiltration of mononuclear morula cells and rarefaction. H& E
Fig 14: Section of Heart, diffuse infiltration of mononuclear cells, oedema and lymphatic vessels filled with pink proteicous material. H&E

Fig 15: Section of Lung, oedema and infiltration of mononuclear cells. 10×H&E
Fig 16: Section of Kidney, intense effusion of mononuclear cells in interstitial tissue. H&E ×10

Fig 16: Section of Kidney, pink proteicous material Bowman’s space. Necroed nephrons with dissociated lining epithium H&E
Lung:
This organ presented (Fig. 17) oedema and effusion of cellular exudate which consisted of lymphoid cells, plasma and histocytes.

Liver:
No evidence of microscopical lesions apart of mild lymphocytic reaction and fibroplasia in portal area.

Kidney:
There were interstitial glomerulonephritis, which evidenced by intense infiltration of cellular exudate in the interstitial tissue (Fig. 18) and the glomerular tuft. The predominant cellular exudate were lymphoid plasma and histocytes. Some of the glomeruli showed dilate Bowman’s capsule which in many contained pink proteinous material (Fig. 18). In many nephron the lining epithelium were detached from basement membrane and some cells were necrosed and disintegrated.

Lymphnodes:
The microscopically changes presented by this organ included atrophic lymphoid follicles, oedema and accumulation of lymphoid plasma and histocytes in the medullary sinuses (Fig. 19) many of lymphatic vessels were distended with pink proteinous material (Fig. 19).

Testis:
The most prominent testicular lesions were orchitis and epididymitis. The semineferous tubules were atrophic to varying degree with severity and stage of degenerative changes. Most of
these tubules were detached from the basement membrane which was thin and discontinued (Fig. 20) though the basal layer of germinal cell of many seminiferous tubules consisted of varying number of germinal cells, the majority of them were lined entirely by sertoli cells (Fig. 21). The spermatogenesis was suppressed partially or completely according to the severity of degenerative changes and dissolution of germinal cells in the seminiferous tubules. There were complete spermatogenesis where the seminiferous tubules were atrophic and distorted (Fig. 21) and in advance changes where the atrophic obliterate tubules were collapsed with obstructed Lumina (Fig. 22). These changes were accompanied with diffuse infiltration of lymphoid plasma and histocytes in the interstitial connective tissues, between the degenerated and dissociated tubular cells (Fig. 20 and in the rete testis tubules and connective tissue where there was fibroplacia (Fig. 23) focal areas when the normal rete testis tubules tissue was replaced by the accumulation of cellular exudate (Fig. 24). The epididymitis was evidenced with necrosis and detachment of tubular lining epithelium, fibrotic reaction and effusion of mononuclear cells (Fig. 25). The ductus efferent showed vacuolation and erosions of lining epithelium and intense infiltration of mononuclear cells in preiductal area (Fig. 26).
Fig 19: Section of Lymphnodes, oedema effusion of mononuclear cells in medulary sinuses and lymphatic containing pink proteous materials. H&E ×10
Fig 20: Section of Testis, interstitial infiltration of mononuclear cells, degenerated detached semineferous tubules. This discontinued basal membrane. H& E ×10

Fig 21: Section of Testis, distorted detached semineferous tubules, some lined by sertolic cells and spermatogenous. H& E ×10
Fig 22: Section of Testis, distorted, collapsed seminiferous tubules. H&E ×10

Fig 23: Section of Testis, infiltration of cellular exudate in rete testis tubules and in the interstitial tissue, fibroplasias in connective tissue. H&E ×10
Fig 24: Section of Testis, replacement of rete testis tissue with accumulation of cellular exudate. H&E ×10

Fig 25: Section of Testis, epididymous, necrosis and desquamation of lining epithelium, fibroplasia and mononuclear cells in connective tissue. H&E ×10
Fig 26: Section of Testis, ductus efferentes, necrosis and erosion of lining epithelium, accumulation of cellular exudate in subductal area. 
H& E ×10
Discussion

This study started by a general trypanosomosis survey conducted in a known trypanosomosis endemic area (A/Rahman, 2002 and Abdelsalam, 1996) far away from the known tsetse belts of the Sudan. The survey was conducted twice in the same localities both in the dry and the rainy season. The ecology of this area is described by Rahman (2002) who also conducted intensive entomological surveys in this area and showed that although the area is tsetse free, yet biting flies are very numerous and may play an important role in the mechanical transmission of the disease. The only trypanosomosis species found infecting cattle during this survey was *T. vivax*. The wide distribution of *T. vivax* in tsetse free areas of Sudan was reported by Ulienburg (1998) who diagnosed *T. vivax* in sedentary cattle herds al along the white Nile from Malakal in southern Sudan up into semi-desert of Khartoum Province, thousands of kilometers from any known tsetse belt in the Sudan. in the recent years and following the development that occurred in the animal production sector specially in dairy farming the problem of bovine trypanosomosis due to *T. vivax* started to show up.

The results of this survey indicated that the infection rate of *T. vivax* varied between 6.5 to 2.2% with a mean infection rate of 1.2% during dry season (March-June). This result is similar to that obtained by Abdel Salam (1996) from Singa in the same area.
Such figures indicated that bovine trypaosomosis is under enzootic stable condition.

The survey conducted during the rainy season at the same localities of the dry season survey showed infection rates that varied between 1.43% and 6.35 with a total infection rate of 1.7% which is twice the infection rate at the dry season. The same findings were reported by Kalu and Lawani (1996) from Kano State, Nigeria, who showed that the infection rates of ruminant trypanosomosis was twice during the rainy season in comparison with the average during the dry season. The increase of infection rate during the rainy season might be due to the increase in biting flies densities during the rainy season of heavy rains and flood. Outbreaks of trypanosomosis were reported to cause heavy losses among cattle population in the Sudan. Karib (1961) reported that heavy rains and flooding of the year 1946 resulted in 50% deaths among Shilluk cattle resident along the White Nile far from tsetse infestation. Also an outbreak of bovine trypanosomosis occurred in Khartoium after high rainfall and floods of the year 1988 (Musa et al., 1990). Abdel Rahman (2002) reported high prevalence of the disease in Singa area following heavy rainfall of the year 1993.

During this study the trypanosomes were diagnosed by the conventional parasitological methods which are not sensitive enough to detect low parasitaemic animals. As most of the infected animals showed low parasitaemias in the field, this might
be the reason for the low trypanosomes infection rates reported during this survey. Amna Al-Nur (1997) in Khartoum State and Babkier (2000) in Gazeira State found similar results when they examined cattle parasitologically, but they obtained high \textit{T. vivax} infection rates that exceeded 17% in serological examinations. Entomological surveys conducted during this study showed that Tabanid flies were found in high densities in the study area. Suliman (1992), Abdel Salam (1996) and A/Rahman (2002) found similar results in their previous work in this area. During late rainy season the nomads move from tsetse areas of Khor Yabus avoiding the biting flies of the South, usually reaching the study area (Singa, Sinnar) by September (A/Rahman, 2002). They get mixed with resident cattle in the area till they leave towards the south in November, when it starts to get dry and some of them remain till December to graze the post harvest products of the agricultural projects (Abdel Salam, 1996). Kheir et al. (1995) found those migratory herds cattle harbouring trypanosomes in their blood. A/Rahman (2002) stated that the presence of tabanids and stomoxys flies at their peak of abundance, together with the nomadic cattle among which some are harbouring trypanosomes provide optimum conditions for mechanical transmission to take place between migratory and resident cattle. A/Rahman (2005) conducted that it is now an established fact that all cattle rearing areas of the Sudan are endemic for bovine trypanosomosis and
biting flies are playing a major role in the mechanical transmission of the disease in those areas.

Ethidium bromide has been used as a curative drug of cattle trypanosomosis in the Sudan since 1953 (Karib, 1961). It was primarily a curative compound and its protection was observed by (Deswitz, 1957; Leach et al., 1955, White side, 1960), Ethidium appears to be particularly effective against *T. vivax* infections in both East and West Africa (Mwambu, 1971; Njogu et al., 1985 and Stevenson et al., 1995). In the past it was reported to give protection to cattle for a period up to 10 month (Mwambu and Mayonde, 1971).

Chemotherapy is the only available trypanosomosis control method used for the control of mechanically transmitted trypanosomosis. In this study the sensitivity of *T. vivax* stocks circulating outside the tsetse areas to ethidium bromide was tested in goats using the recommended treatment dose and double that dose. The results obtained showed that, the *T. vivax* stocks circulating outside the tsetse areas (mechanically transmitted) are resistant to both doses. These results are in agreement with that obtained by Abel Gadir et al. (1981) who showed that the major trypanosome species in tsetse belts of the Sudan were resistant to 0.5mg/kg and 1ml/kg Homiium bromide. The same findings were also reported by Mohamed Ahmed et al., (1992) who studied trypanosomes stocks collected from the tsetse belts of Bahr Al Arab. However, this study is the first to investigate the resistance
of the mechanically transmitted *Trypanosoma vivax* to Ethidium bromide which is commonly used in these areas.

Abdel Razig *et al*. (1968) stated that Berenil is a “sanative” drug in the Sudan, and advised the use of Berenil to destroy Ethidium resistant stain. Unfortunately Mohamed Ahmed *et al*. (1992) reported drug resistance against Diminazene aceturate among *T. vivax*, *T. congolense* and *T. brucei* stocks isolated from Bahr Al Arab tsetse belt. In this study *T. vivax* experimentally infected goats, treated with the recommended (3.5mg/kg) and twice the recommended dose of diminazine aceturate, relapsed after treatment. This indicates the presence of drug resistance among the mechanically transmitted *T. vivax* stocks circulating outside the tsetse belts. The results obtained in this study are in agreement with the studies of Whitelaw *et al*. (1988) in west African goats infected with *T. vivax* which showed relapse 6 weeks after diminazine aceturate treatment. Similar findings were also reported by Logan *et al*. (1984) who got relapses 10 to 25 days after diminazine aceturate treatment to *T. vivax* or mixed infection of *T. vivax* and *T. congolense* under fly free conditions.

In the contrary (Leach, 1961) noted that a dose of 7 mg/kg Berenil was sufficient to cure infections with *T. vivax* and *T. congolense*. Also Njogiu and Heath (1986) successfully treated the Isometamedium resistant haemorrhagic infection of *T. vivax* with 7 mg/kg diminazine aceturate. Joshua *et al*., (1995) noted that the
Diminazine aceturate which is commonly used is gradually losing its efficiency because of the emergence of drug resistance. In North Ivory Coast *T. vivax* resistance to 0.5 and 1mg/kg Isometamedium and novidium chloride were effectively treated with 6mg/kg diminazine aceturate (Kupper and Walter, 1983). Other authors confirmed the diminazine resistance to *T. vivax* in west Nigeria Jones-Davis (1967; 1968; Mclenan and Na’Isa, 1970) and in Uganda (Mwamba and Mayenda, 1971) and in Kenya (Schillinger and Röttcher, 1984).

The long period of extensive use to these drug among various populations of the trypanosomes in the Sudan, besides the problems of sub-dosing by the owners might be the main reason for the development of drug resistance. Generally, drug resistance has been closely associated with easy availability and improper use of trypanocides which are losing their potency (Williamson, 1979; Braide, 1987; Joshua, 1988) and no new trypanocide has been developed in the past three decades (Connor, 1992). Therefore it is essential to control the field application of drugs to ensure that, if resistance to one drug appears, an effective alternative drug is available, such a combination called “sanative pair” (Losos, 1986).

Relapses or reappearance of trypanosomes in the blood circulation might be due to emergence of trypanosomes from sites within the host to which the drug has no access, Jennings *et al.* (1979). The same observations were reported by Whitelaw *et al.* (1988) who
experimentally demonstrated the presence of *T. vivax* in aqueous humor and cerebrospinal fluid of goats treated with 10mg/kg of diminazine aceturate ad relapsed 6 days later. It was suggested that these might be sites which are inaccessible to trypanocides and from which the trypanosomes could invade the blood circulation.

On the other hands, in *T. vivax* experimentally infected calves may be able to control parasitaemia, it would appear that selfcure of infection was related to the ability of the calves to control parasitaemia as well as severity of anaemia (Uzoigwe, 1986). The fluctuation of trypanosomes observed in the blood are characteristic of most forms of trypanosomosis, and the periodic peak of parasitaemia suggested rapid changes in the circulating populations. Obvious cyclic waves of parasitaemia were observed about every 6 days in *T. vivax* (Maxie *et al.*, 1979). In this study, the experimentally infected goats showed fluctuating parasitaemia with peaks coinciding with periods of fever and death., these observations were the same as those noted by Losos (1986) who observed the high peaks of parasitaemia to be associated with elevation of temperature and death or development of chronic disease. In this study significant and rapid fall in PCV values occurred in all infected groups in calves and goats. Similar results were obtained by Van den Ingh *et al.*, 1976a; Sarror, 1980; Ugochutkwu, 1986; Losos, 1986; Muray *et al.*, 1988; Sekoni,
1990; Gossens et al., 1998; Silva et al., 1998) in *T. vivax* and *T. congolescense* infections in goats and cattle.

West Africa dwarf and red sokoto goats infected with *T. congolescensis*, showed sever drop in PCV 9-11% within 11 to 13 days post infection, followed by leukocytosis and death Adah et al. (1993). Masiga et al. (2002) noted that trypanosomosis infected sheep and goats, whose PCV fall below 15% rarely recovered. In both calves and goats in this study there is significant drop in Hb concentration observed as that reported by Van den Ingh et al. (1976) and Sarror (1980) in *T. vivax* and *T. congolescense* infection in goats and cattle. Opasina (1985) observed a significant correlation between PCV and Hb values in *T. vivax* infected sheep.

This study, also showed significant reduction in the RBCs values in all of infected calves and goats, the same observation were reported by Ugochukwu (1986) who found *T. vivax* and *T. congolescense* infection in cattle to cause significant fall in Hb concentrations and erythrocytes counts. These findings were also described by Gardiner et al. (1989) in Ayrshire cattle infected with *T. vivax* in Kenya. Others reported the destruction of RBCs in *T. vivax* infection (Mamo and Holmes, 1975; Jennings, 1976; Kobayashi et al., 1976; Valli et al., 1978; Dragie, 1979; Table et al., 1979; Facer et al., 1982). Nadia (2005), reported significant reduction in RBCs counts in *T. vivax* infected black Nubian goats of Sudan. Previous studies showed that the presence of anaemia,
and the haematological values of infected animals will not change to normal values after treatment with trypanocidal drugs. Murray et al. (1988) stated that the anaemia persists in the absence of detectable parasitic in the blood, while the response to trypanocidal drugs treatment is slow or not at all. The same observation were reported by Masiga et al., (2002) who studied anaemia caused by trypanosomosis in both sheep and goats.

In this study a significant reduction in the WBCs counts was found in all goats and calves studies. These findings are in agreement with (Naylor, 1971, Losos et al., 1973; Maxie et al., 1979; Valli and Mills 1980; Sarror et al., 1981; Ellis et al., 1987; Katunguka et al., 1992; Bengally, 1993) who noted leukopaenia in goats accompanying the first waves of parastaemia, then pancytopaenia developed. In the contrary Nadia (2005) noted, no significant difference in WBCs in T. vivx experimentally infected goats.

In this study most of the animals died due to infection. The principal sign of trypanosomosis in livestock is anaemia. Persistent anaemia and congestive heart failure due to myocardial damage are the major causes of mortality of trypanosomes infected animals (Sanousi, 1979, Murray et al., 1988; Silva et al., 1999).

Undulating fever is a well recognized feature of bovine African trypanosomosis which is usually associated with a trypanolytic crises (Fiennes, 1954). In this study infected animals showed
increased in temperature, heart rate and respiratory rate which is positively correlated with high parasitaemia. These findings are in agreement with Van den Ingh *et al.* (1976) who also observed elevation in temperature heart and respiratory rates during period of high parasitaemia in *T. vivax* infected animals. Maxie *et al.* (1979) note that infected cattle with *T. vivax* showed fever, increased heart and respiratory rates, anorexia, emaciation. Parasitaemia and body temperature were positively correlated and associated with anaemia, leucopenia and thrombocytopenia. Losos (1986) observed the high peaks of parasitaemia to be associated with elevation of temperature and death often occurs during these periods. Ismail (1988) found the persistence of elevated temperature, respiration and heart rates to correlate with parasitaemias. He related that to reduction in RBCs which caused a decreased in oxygen carrying capacity and thus a probable stimulation of respiration and heart rate.

On the contrary Venenal *et al.* (1976) found no correlation between the changes in the heart rate and the temperature rise during fever produced by *T. vivax* infection in goats. Van dam *et al.*, (1996) noted that the body temperature was higher in goats infected with *T. vivax*. Most of the clinical signs observed in this study were also described in other studies by Losos and Ikede (1972); Gardiner (1989) and Ulinberg (1998) in *T. vivax* infected cattle and goats. In west Africa the severity of the disease and time of death varied between individual animals, Maxie *et al.*
The clinical signs of bovine trypanosomes observed in Bolivia, were fever, anaemia, abortion, lethargy and progressive emaciation (Silver et al., 1999).

In this study, the calves infected with *T. vivax* showed characteristic and significant clinical and histological changes. There was obvious emaciation, anaemia, enlargement of lymph nodes, spleen liver ad heart. Petechial and echymotic haemorrhages were observed in mucosal and serosal surfaces of visceral organs and congestion in most of the vital organs was observed. Oedema and serous fluids in the lungs were also found at necropsy. Isoun (1975) found the basic histological lesions to be the generalized fibrin thrombus formation in blood vessels of the heart lungs spleen and brain.

In this study, the histological findings confirmed the gross findings where congestion, haemorrhages and degenerative changes were observed in most tissues and organs. The brain showed lymphoid cells, histocytes and mononuclear cells infiltration, proliferative glial cells and necrotic foci. These findings were realized by Gardiner et al. (1989) who reported the presence of mononuclear cells infiltration and gliosis. Nadia (2005) in her studies in *T. vivax* infected goats also noted the presence of glial cells in the brain of infected goats besides changes in the heart showing endocarditis, pericarditis, myocarditis, in the lungs interstitial oedema, filtration of lymphoid plasma and histocytes were observed. The same
findings were reported by Van den Ingh (1976) De Neija and Baker, (1979) who noted pericarditis and myocarditis associated with the invasion of trypanosomes to solid tissues, this is particularly seen in *T. brucei* infection. Lesions were also observed in the heart with myocardial fibrous degeneration and necrosis, mononuclear cell infiltration during *T. vivax* infection in goats (Masake, 1980). The changes in the lungs were edema, pulmonary exudate, and lymphoid cell infiltration. The liver showed mild reaction. The changes observed in the liver and lungs and the pulmonary oedema were described by Van den Ingh *et al.* (1976a) in *T. vivax* infection in goats and Gardiner *et al.* (1989) in cattle. The kidneys showed interstitial glomerulonephritis, infiltration of lymphatic cells. This finding was noted by Gardiner, (1989) an Van den Ingh (1976) in goats and cattle infected with *vivax* and by Nadia (2005) in *T. vivax* infected goats. The atrophic lymphoid folicles, oedema the infiltration of lymphatic cells with pink proteinous material were observed in this study in the lymph nodes. This was also described by Masake (1980) who noted marked generalized lymph nodes enlargement and splenomegaly, which was attribute to marked lymphoid proliferative response. In parallel studies van den Ingh ad others (1976b) reported proliferative lymphoid changes in *T. vivax* infected goats.

In this study, the tissue damage was wide spread, it appears that the cardiac damage and the changes in the brain and the haematic
changes were of particular significance and evidence of congestive heart failure. However, Losos and Ikede (1972) did not find heart lesions or extravascular trypanosomes in *T. vivax* infected cattle.

In this study, the testis showed marked significant change prominent orchitis, epididimitis, atrophic somniferous tubules, degenerative changes and intense infiltration of lymphatic cells. These findings were reported previously by Isoun and Anosa (1974) and Masake (1980). Who realize orchitis and abnormal sperm formation in *T. vivax* infection in goats.

Testicular degeneration and cellular infiltration, in *T. vivax* infection of sheep and goats were noted by Anosa and Isoun, 1980).
Conclusion:
This study confirmed the wide spread of *Trypanosoma vivax* outside the tsetse area, and indicated the importance of mechanically transmitted animal trypanosomosis.

The mechanically transmitted *T. vivax* stock were found resistant to both Homidium bromide and diminazine aceturate, the most commonly used trypanocides in Sudan. Therefore, trypanosomosis is becoming a serious animal health problem, in animals outside the tsetse areas specially in dairy farms and in animals kept in the irrigated agricultural project.

The pathological studies conducted in this work showed that the mechanical transmitted *T. vivax* is very invasive and virulent in animals affecting all the tissues with the subsequent effect on their productivity.

Therefore the control of this disease is very necessary and the following parameters for its control are recommended:

1- Use of effective trypanocidal drugs.
2- Treatment of animals coming from tsetse area with effective trypanocidal drug before leaving the tsetse area to non-tsetse area specially trade cattle.
3- Perfect and quick diagnosed methods are required in different veterinary centres.
4- Training of veterinarians in the field for microscopic diagnosis of *T. vivax*. 
5- Extension is very important in the role of mechanical transmission in trypanosomosis epidemiology, both among animal owner, para vets and veterinarians.

6- Control of biting flies in dairy farm is recommended specially within the integrated best manage approach.

7- Ethidium bromide, which extensively used in this country, is 100% resistant to *T. vivax* in both therapeutic and twice recommend dose and should be replaced for sometime with other effective drugs.
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haemorrhage-causing *T. vivax* in N’Dawa and Boran cattle.


