Prevalence of Trypanosomosis in Cattle in Juba Area, Central Equatoria State, Sudan.

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Dedication

To my husband Alier Deng, my daughter and my sisters
With love and gratitude
Acknowledgments
Firstly my praise to Almighty God for giving me the blessing and strength to accomplished this work.

I would like to express my deepest thanks and gratitude to my supervisor Dr. Khitma Hassan Elmalik for her keen guidance and continuous encouragement to me throughout this study.

I am particularly grateful to Dr. Ahmed Hussain, Dr. Raffaele C. Mattioli and Ahmed Eltaib for helping in collecting the data used in this study.

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My debt to all my friends and colleagues, who participate by any way to help finishing this work as they gave hope and moral support throughout my ill health episode.
Abstract

A survey was conducted in selected localities in Central Equatoria State, South Sudan to avail the information about the prevalence of African animal trypanosomisos.

220 blood films were prepared during January 2009 from Rajaf West (Khor Romla), Rajaf East (Gumba), and Kamaron, of Juba county of Central Equatoria State.

The results from the parasitic survey showed, T. vivax in most of the positive smears and two T. brucei mixed with T. vivax and only one T. congolense The prevalence rate in Rajaf west (Khor Romla) was the highest (5.19%) followed by Rajaf East (Gumba), (4.17%), then Kamaron with the prevalence rate of (2.28%). Old animals tend to have a higher infection rate than the younger, but for other age groups there was no marked difference, Also there was no difference between the sexes in acquiring the disease, where (4.69%), and (4.08%), were the infection rates for males and females respectively.

The packed cell volume was significantly different between infected and non infected animals. From the results of this survey this area need more research to be done and more sensitive diagnostic techniques to be used, to detect the actual prevalence of trypanosomes in different seasons

The result from interviewing the owners about the Knowledge of trypanosomosis revealed that, they have no Knowledge about the treatment and the control of (lobit) the local name for trypanosomosis, but they used some remedies to treat some other diseases.
As this is one of the few surveys conducted, it is also needed that other animal species be included to have a full view of infection in livestock in the area.

ملخص الأطروحه

تم أجراء مسح ميداني في مواقع مختارة لولاية الاستوائية الوسطى بجنوب السودان بغرض تحديد وجود نسب الأصابة بأنواع طفيليات المنتقبيات (Trypansoma) في الإبل والتمكن من إنشاره.

5
تم تحضير 220 شريحة دم خلال يناير 2009م من كل من الرجاف غرب (خور رملا) ورجاف شرق (قومبا) ومنطقة كامرون محافظة جوبا بولاية الاستوائية الوسطى. وقد أوضحت نتائج المسح المجهرى أن أغلب الشرايين المرتبطة كانت من نوع T. vivax بينما كانت في حالة تبين T.vavix نوع المختلط T.brucei T.congolence كانت معدل الإصابة في غرب الرجاف (خور رملا) تمثل النسبة الأعلى (9.19%) وتليها منطقة شرق الرجاف نسبة الإصابة (4.17%) ثم منطقة الكامرون بنسبة إصابة (2.28%).

وجد أن الحيوانات الكبير افتراضها من الحيوانات الصغير. ولكن في بعض الاحصاء الأخرى لا توجد أختلافات ملموسة بين الجنسين في إكتساب المرض. في الذكور كانت نسبة الإصابة بالمرض (6.9%) والإناث (4.08%).

كان حجم كبد كريات الدم الحمراء (PCV) في الحيوانات المصابأ مختلف عنها في الحيوانات غير المصابة.

أوضحت النتائج أن مصدريات الحيوانات أنهم يفتقرن أو غير ملمومين بطرق العلاج والتحكم في المرض (لوبيد) ولكنهم يستخدمون بعض مستخلصات النبات المحلة لعلاج بعض الأمراض الأخرى.

من نتائج هذا المسح الميداني أجريت أن هذه المنطقة تحتاج لمزيد من البحوث واستعمال وسائل تشخيصية تقنية ذات سهولة عالية للكشف عن النسبة الحقيقي لداء المثلوبدي في المواسم مختلفة. ويعتبر هذا البحث القليل الذي أجريت في المنطقة. لذا يجب إجراء بحوث أخرى في الانواع المختلفة من الحيوانات لحصول على نتائج شاملة لكل فصائل الحيوانات في المنطقة.
TABLE OF CONTENTS

DEDICATION ... i
Acknowledgements ... ii
Abstract ... iii
Arabic Abstract ... iv
Table of contents ... v
List of tables ... ix
List of plates ... x
Introduction ... 1

CHAPTER ONE: LITERATURE REVIEW
1.1 Trypanosoma ... 4
1.2 Classification ... 4
1.3 Host range ... 5
1.4 Transmission of trypanosomes ... 6
  1.4.1 Cyclical transmission ... 6
  1.4.2 Mechanical transmission ... 7
    1.4.2.1 By biting insects ... 7
    1.4.2.2 By iatrogenic means ... 8
  1.4.3 Transmission by other means ... 8
1.5 Tsetse fly Distribution ... 10
1.6 African animal trypanosomosis ... 10
1.7 Life cycle of trypanosomes ... 11
1.8 Pathogenesis ... 12
1.9 Clinical signs ... 12
1.10 Economic implication of African animal trypanosomosis ... 15
1.11 Trypanosomes outside of tsetse belt in Sudan ... 17
1.12 Diagnosis of trypanosomes ... 17
  1.12.1 Clinical diagnosis ... 18
  1.12.2 Laboratory methods ... 18
1.12.2.1 Parasitological diagnosis
1.12.2.2 Haematocrit centrifugation
1.12.2.3 Animal sub-inoculation
1.12.2.4 Xenodiagnosis
1.12.2.5 In vitro culture methods

1.12.3 Serological diagnosis
1.12.3.1 Complement Fixation Test
1.12.3.2 Indirect haemagglutinations (IHA)
1.12.3.3 Indirect fluorescent antibody (IFA) test
1.12.3.4 Enzyme linked immunosorbent assay (ELISA)
1.12.3.5 Antigen-detection ELISA (Ag.ELISAs)

1.12.4 Monoclonal antibodies (MoAbs)

1.12.5 Molecular methods for Detecting Livestock- infective Trypanosomes

1.12.6 The card agglutination test (CATT)

1.13 Control of trypanosomiasis
1.13.1 Chemotherapy
1.13.2 Trypanotolerance
1.13.3 Vector control
1.13.3.1 Land clearing
1.13.3.2 Slaughter of animals
1.13.3.3 Pesticides
1.13.3.4 Trapping
1.13.3.5 Sterile insect technique (SIT)
1.13.3.6 Community base tsetse control

1.13.4 Vaccination

CHAPTER TWO: Materials and methods
2.1 Bloods samples collection
2.1.1 Study area location
2.1.2 Vegetation
2.1.2.1 Open Savannah Woodland
2.1.2.2 Derived Savannah woodland
2.1.3 Temperature
2.1.4 Rainfall
2.1.5 Soil
2.1.6 Animal species and breeds
2.1.7 Husbandry practices
2.1.8 Availability of veterinary services
2.2 Blood collection and examination
2.2.1 General parasitological methods used in this study
2.2.2 Wet mount
2.2.3 Thin and thick blood films
2.2.4 Haematocrit Concentration Technique (HCT, Woo, 1970)
2.3 Traditional diseases diagnosis
2.4 Traditional treatment of the diseases
CHAPTER THREE: Results
3.1 Prevalence of trypanosomosis in cattle in cattle
   3.1.1 The infection rate in cattle in different sites
      3.1.1.1 Infection rate in Rajaf West
      3.1.1.2 Infection rate in Rajaf East
      3.1.1.3 Infection rate in Kamaron
3.2 Trypanosome species identification
3.3 PCV values
3.4 Diseases diagnosis and ranking
3.5 Traditional treatment
3.6 The veterinary services
CHAPTER FOUR: Discussion
REFERENCES

LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The infection rate by age and sex Juba county</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>The infection rate in cattle in different sites</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>Infection rate in Rajaf West</td>
<td>47</td>
</tr>
<tr>
<td>4</td>
<td>Infection rate in Rajaf East, Gumba</td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>Trypanosoma Species</td>
<td>49</td>
</tr>
<tr>
<td>6</td>
<td>Average and range of PCV</td>
<td>50</td>
</tr>
<tr>
<td>Page</td>
<td>Topic</td>
<td>Page</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>7</td>
<td>Infection rate in Kamaron</td>
<td>52</td>
</tr>
<tr>
<td>8</td>
<td>Common diseases in Juba County and their local names</td>
<td>54</td>
</tr>
</tbody>
</table>
**LIST OF PLATES**

<table>
<thead>
<tr>
<th>photo</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nilotic cattle Breeds</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>Case of bovine trypanosomosis</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>Trypanosoma vivax</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td>Trypanosoma brucei</td>
<td>51</td>
</tr>
</tbody>
</table>
INTRODUCTION

Animal trypanosomosis is one of the major constrains to socio-economic development in Africa. The disease is spreading over an area of approximately 10 million km² across the continent between 29°S and 14°N. This area is infested by tsetse flies the vector of nagana. Tsetse infestations affect 38 countries and the 147 million cattle living within these countries. The tsetse-infested area in Sudan is estimated to be about 300,000 km² (Lewis 1949a.; Ford, 1976). It is estimated that approximately 30% of the 147 million cattle in countries, affected by tsetse flies are exposed to trypanosomes infection (FAO/WHO/OIE 1982; Murray and Gray 1984). The trypanosome that causes nagana in livestock mainly in cattle, sheep, and goat include trypanosome vivax, T. congolense and T. brucei. In camels, the main pathogenic species is T. b. evensi, which causes a disease generally referred as “Surra”. In horses, T. equiperdum causes dourine while in pigs T. simiae and T. suis are the main pathogenic species. The disease in man (human African trypanosomes) is commonly known as sleeping sickness, which can exist in an acute or chronic form and caused by T. b rhodesiense and T. b gambiense.

The parasites concerned are protozoa belonging to the trypanosoma, Genus. They are transmitted to by tsetse fly (Glossina Genus) bites, which have acquired their infection from human beings or animals harboring the pathogenic parasites. Tsetse flies are found in sub-Saharan Africa, only certain species transmit the disease and different species
have different habitats. They are mainly found in vegetation by the rivers, lakes, gallery-forests and wooded savannah.

Sleeping sickness occurs only in sub-Sahara Africa in the regions where there are tsetse flies that can transmit the disease for reason that are so far unexplained. There are many regions where tsetse flies are found, but sleeping sickness is not found.

The rural population living in regions where transmission occurs and which depend on agriculture, fishing, Animal husbandry or hunting are the most exposed to the bite of the tsetse fly and therefore to the disease. The sleeping sickness generally occurs in remote rural areas where health systems are weak or non-existent. The disease develops in the area whose size can range from village to an entire region within a given area; the intensity of the disease can vary from village to the next.

Pathogenic trypanosomes cause disease in all species of domesticated livestock throughout many of the tropical and subtropical regions of the world. In Africa *Trypanosoma brucei*, *T. vivax* and *T. congolense*, occur wherever the tsetse fly vector is found. *T. evensi*, which is transmitted mechanically by several different species of haematophagous biting flies, is found in North Africa, the Near East, the Far East and Central and South America where it, is transmitted mechanically by biting flies.

The livestock sector is an important contributor to the overall economy of Sudan; it provides over 20% of the country’s foreign exchange and 100% of meat and 80% of milk supplies, (FAO, 2006).
Systemic surveys for animal trypanosomosis in the equatorial region of Sudan had been carried out for long period. After the comprehensive peace Agreement, communities of returnees are establishing with their livestock in the area. To predict health hazards, this study is planned to survey one of the diseases that might threaten livestock keeping.

The objectives of this study are:

1- To carry out a cross–sectional study to estimate trypanosomosis prevalence rate in Central Equatoria State.

2- To identify morphologically the *Trypanosoma* species in the study area.

3- To identify areas where Trypanosomosis is most likely a threat to animals.
CHAPTER ONE
LITERITRE REVIEW

1.1. Trypanosoma

Trypanosomosis is one of the most important problem facing many areas in the world especially Africa.

Trypanosomes are of the class Kinetoplastida, a monophyletic group of unicellular parasitic protozoa. They are obligate parasites; that multiply in the body fluids especially blood stream and tissue fluid of the vertebrate host; and live in the digestive tract of the invertebrate host which is generally a biting fly (Itard, 1981).

Trypanosomosis is a disease caused by the hemoflagellate protozoa classified as trypanosoma spp. It is a disease in man causing sleeping sickness, as well a disease of animal causing animal trypanosomes. They are frequently found in the blood of various animals’ species in many regions of the world; the majorities are not pathogenic, but some species are of considerable economic important causing disease in man and animals.

The disease generally is a chronic condition, which is usually fatal if not appropriately treated. It leads to a considerable loss of weight and anemia; various symptoms are exhibited including fever; edema; dermatitis and nervous disorders (Finelle, 1983).

1.2. Classification of trypanosome

Hoare (1957, 1964) proposed a classification of the mammalian trypanosomes into two sections, the 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.

The salivarian trypanosomes are those transmitted by insect vectors through saliva either cyclically by tsetse flies (Glossina species) or mechanically by other biting flies. The subgenus Nannomonas contains group of small trypanosomes with medium size marginal kinetoplast no free flagella and poorly developed undulating membrane, e.g. T.congolense. Members of subgenus Duttonella are group of trypanosomes with large terminal kinetoplast, distinct free flagella and inconspicuous undulating membrane e.g. T.vivax. The subgenus trypanozoon includes the trypanosome species that cause sleeping sickness to human beings and surra to camels and equines, species of this group are polymorphic, which occur in three forms, the long slender, the short stumpy and the intermediate forms, T. brucei group and T. evensi are examples.

The stercorarian trypanosomes are mostly non-pathogenic parasites, which undergo cyclic development, but the infective forms are deposited in the feces of the vector (Kettle, 2000). The example of these trypanosomes is the T. theileri, which occur in cattle all over the world and is transmitted by tabanids flies and probably by ticks (Uilenberg, 1998).

1.3. Host range

Cattle, sheep, goats’ horses, camels, dogs’ pigs and monkeys are susceptible to African animal trypanosomosis and may suffer syndromes
ranging from sub clinical, mild or chronic infection to acute fatal disease.

Rats, mice, guinea pigs, and rabbits are useful laboratory species. More than 30 species of wild animals can be infected with pathogenic trypanosomes, and many of these remain carrier of the organisms.

Ruminants are widely known to be active reservoirs of trypanosomes; wild Equidae, Lions, Leopards, and wild pigs are all susceptible and can be as carriers of trypanosomes.

1.4. Transmission of trypanosomes

There are three major type of transmission of trypanosomes.

1.4.1 Cyclical transmission
Cyclical transmission is restricted to Glossina species (tsetse fly) only. When a tsetse fly hatches from its pupal case it is free from trypanosomes, until takes its first blood meal it is called a teneral fly, it acquire trypanosomes infection when feeding on a parasitaemic mammalian host. The trypanosomes undergo a cycle of development and multiply in the digestive tract of the fly until the infective metatrypanosomes stages develop in different regions of the digestive tract of the fly (Mulligan, 1970, Hajduk, 1992).

The metacyclic trypanosomes occur either in the biting mouthpart or in the salivary glands. The period from ingesting infected blood to the appearance of these infective forms varies from one to three weeks, (Uilenberg, 1998); once the infective metatrypanosomes are present, the fly remains infective for the remainder of its life. During the act of
feeding, the fly penetrates the skin with its proboscis. By the rupture of small blood vessels a pool of blood is formed in the tissues and the fly inject saliva to prevent coagulation.

Infection of the host takes place at this stage with infective metacyclic trypanosomes in the saliva. The infective metatrypanosomes undergo development and multiplication at the site of infection where a swelling, or chancre may be detected and the mature blood trypanosomes (trypomastigotes) are released via lymph vessels and lymph nodes into the blood circulation.

Reproduction in the mammalian host occurs through a process of binary division. Animal trypanosomosis in the Northern Sudan is believed to be transmitted mechanically by biting flies other than Glossina e.g. Tabanidae, Stomoxy (El Karib 1961, Yagi and Abdel Razing 1972).

1.4.2. Mechanical transmission

1.4.2.1. By biting insects.

The process is purely mechanical. A biting insect passes the blood from an infected animal to another in the course of interrupted feeding. The time between the two feeds is crucial for effective transmission because the trypanosomes die when the blood dries. The importance of this mode of transmission is variable from place to place, depending on the numbers of hosts and biting insects present, and on the species of trypanosome.
Large biting insects such as *tabanids* carry more blood and are more likely to act as mechanical vectors than for example mosquitoes. Tsetse flies themselves can of course also act as mechanical vectors. This mode of transmission has proved to be sufficiently effective to maintain *Trypanosome vivax* and *trypanosome evensi* in South and Central America, and the latter species in North Africa and Asia as well. No tsetse flies occur outside tropical Africa, apart from small tsetse pockets which had occurred in the Southwest of the Arabian Peninsula (Ouma *et al.*, 2006) Many insects are involved in this type of transmissions, *Tabanids, stomoxys*, and tsetse flies themselves. Jordan (1974) stated that within areas infected with *Glossina* spp., it is difficult to distinguish between the mechanical and cyclical transmission.

**1.4.2.2. By iatrogenic means.**

This can occur when using the same needle or surgical instruments on more than one animal, at sufficiently short intervals that the blood on the needle of instruments does not dry. It is not uncommon occurrence when animals are vaccinated or treated by injection, or when blood is collected from several animals in a row, without changing, or disinfecting needles or pins. It may also occur when several animals are subjected at short intervals to a surgical intervention (dehorning, castration, etc) without properly disinfecting the instruments. (Uilenburg 1998).

**1.4.3. Transmission by other means**

It is well known that carnivores may be infected with *T. evansi* and *T. brucei* by ingesting meat or organs from infected animals, as long as
these are still sufficiently fresh to contain live trypanosomes (Uilenburg, 1998). Infection occurs through the mucosa of the mouth. Transmission of *T. evansi* in Latin America by bites of vampire bats is common. These bats become infected by ingesting blood from infected horses or cattle. The *trypanosomes* multiply in the bats and these are thereafter able to transmit the disease to healthy animals. The trypanosomes apparently pass readily through the oral mucosa of the bat in both directions (Uilenburg, 1998).

All trypanosome species are occasionally transmitted congenitally, from the mother to offspring, through the placenta either while the fetus is still in the uterus, or when bleeding occurs during birth. Congenital transmission of *T. vivax*, for example, has been observed in Latin America as well as Africa (Uilenberg, 1998).


Venereal transmission is the normal means by which dourine of equines, caused by *Trypanosoma equiperdum* is propagated. Because of its presence in the mucous exudates of penis and sheath of the stallion and the vaginal mucous of the mare, copulation from an infected to healthy animal and its geographical distribution is not restricted to specific climatic condition. This species is essentially a tissue parasite and causes at most very low parasitaemias in the circulating blood of equines (Uilenberg, 1998; Abdel Rahman, 2002).
1.5. Tsetse fly Distribution

The tsetse fly is found exclusively in Africa. The northern limit of its range lies approximately along the 10°N latitude, the southern limit responds roughly to latitude 20°S, but curves down to eastern coast of Africa to 30°S, the ecoclimate generally corresponds to more than 1000 mm of rainfall (Itard, 1981).

However, trypanosomes occur outside the tsetse belt in Africa; wells (1972), stressed that the occurrence of *T. vivax* in areas where tsetse flies are absent is widely attributed to mechanical transmission. Ford (1964) found that in Rhodesia *T. congolense* culminated in cattle adjacent to the tsetse area, whereas *T. vivax* was found in areas further away. This support the finding of Ziemann (1905), who showed that in Cameroon tsetse were not the only vectors of *T. vivax*, some result was found in Uganda (Ford, 1986 and in Nigeria, Folkers and Jones – Davis, 1966, and in Sudan (Al Rahman, 2002).

1.6. African animal trypansomosis

Trypanosomes are usually chronic disorder, the duration and symptom of which vary with animal host species and the pathogen. Three groups can be distinguished. Disease due to typically African trypanosomes (*T. vivax, T. congolense, T. brucei, T.suis, T. uniform*), are all cyclically transmitted by *Tsetse* fly, this groups of diseases are sometimes generally termed (Nagana), which was originally used exclusively for trypanosomes due to *T. brucei*. Biting insects other than tsetse flies mainly transmit trypanosomes of *Camelidae* and *Equidae* due
to *T. evensi* is known as surra. In addition, Dourine is contagious trypanosomes of equines due to *T. equiperdum*. (Hirumi, 1977).

1.7. **Life cycle of trypanosomes**

Transmission from one vertebrate host to another, is carried out by blood-sucking invertebrates usually an insect, but with amphibian trypanosomes (e.g. *T. inopinatum*), the vector is leech. Shortly after infected blood is drawn into the insect gut, the trypomastigote forms become transformed into procyclic epimastigote forms, the later give rise (directly or by fission) to further trypanosomal forms, usually referred to as trypomastigote metacyclic trypanosomes. The mode of transmission, just outlined, is known as cyclical to distinguish it from mechanical transmission. In this process, trypanosomes survive, for a short time, probably only minutes, on and about the mouthparts before an insect bites again, without undergoing any developmental cycle (Zeledón Rodrigo. 1999).

It is important to distinguish between these two types of transmission. If trypanosomes are transmitted by bites that rapidly, succeed one another, this is mechanical transmission. Those transmitted by the cyclic method cannot be transmitted until sufficient time has elapsed to enable them to reach an infective stage by a particular developmental sequence in the insect vector, a cycle usually requiring 15 – 35 days. Cyclical development may culminate in two sites within the insect, the hindgut (technically referred to the posterior station’), or the foregut (the ‘anterior station’), and this environmental discrimination has
resulted in the division of the Family Trypanosomatidae into two sections as follow:

(a) Stercoraria, which contains genera, which complete their development in the ‘posterior station’, that is to say that infective form appear in the faeces and hosts are infected by the contaminative route.

(b) Salivaria, which contains genera that complete their development in the ‘anterior station’ (i.e. in the salivary system) and transmission takes place by inoculation of the metacylic stage

1.8. Pathogenesis

Initial replication of trypanosomes is at the site of inoculation in the skin, this cause a swelling and a sore (chancre), trypanosomes then spread to the lymph nodes and continue to replicate. *T. congolese* localized in the endothelial cells of small blood vessels and capillaries. *T.b. brucei* and *T. vivax* localized in tissue. Antibody developed to glycoprotein coat of the trypanosome kills the trypanosome and result in the development of immune complexes. Antibody however does not clear the infection because the trypanosome has genes that code for many different surface- coats glycoprotein and change its surface glycoprotein to evade the antibody (Masake and Morrison, 1981).

1.9. Clinical signs

The cardinal clinical sign observed in AAT is anemia, within a week of infection with hematic trypanosomes (*T. congolense* and *T. vivax*, there is usually pronounced decrease in packed cell volume, hemoglobin, red blood cell, and white blood cell level, intermittent fever, edema and
loss of condition. Abortion may be seen and infertility of males and females may be sequel.

The severity of the clinical response is dependent on the species and breed of affected animal and the dose and virulence of the infecting trypanosome (Taylor, 1998).

*T. congolense* is hematic trypanosome found only in blood vessels of the animals it infects. It does not localize and multiply outside blood vessels.

*T. vivax* has variable incubation period and it consider less virulent.

In infection with *T. congolense* mortality of over 50% can occur. *T. brucie* brucie has relatively short incubation period and causes sever to fatal infection in horses, dogs and cats. It usually causes mild, chronic, or sub-clinical disease in cattle, sheep and goats, chronically infected animals show immune suppression associated with depletion of lymphoid cell. In acute stage of the disease, the lymph nodes and spleen are packed with proliferating germinal centers, necrotic foci are found in the brain, heart and skeletal muscles (Losos and Ikede, 1972).

The heart is often affected by myocarditis and heart failure is often the direct cause of the death. Dargie *et al.* (1979) stated that the infection might be acute causing rapid death due to congestive heart failure or chronic, causing remittent fever or emaciation.

The reproductive system is frequently affected and abortion and fertility are common in areas of high trypanosomes challenge (Yagil, 1982), oedemas are also often presents in trypanosomes particularly in horses and dogs. There is evidence of increased permeability of blood
capillaries, and therefore leakage of blood plasma leading to the swelling (Uilenberg, 1989). Wasting and loses of condition are particularly common in chronic trypanosomes.

Surra in camels (local name given to trypanosomes in some area) and equines is manifested by an elevation of body temperature which is directly associated with parasitaemia and progressive development of anemia, loss of condition and weakness. Recurrence of fever occurs during the course of the disease, oedema particularly in the dependent part of the body, urticarial plaques and petechial hemorrhages in serous membrane may be observed. The disease may be fatal within months or may last for few years. Spontaneous recovery is rare (Hornby, 1952, Stephen, 1970).

The disease is often rapidly fatal for camels, dogs and horses, but it can be mild in bovines, donkeys, goats and sheep. Molyneux et al.,( 1984) indicated that in the sleeping sickness, (HAT) the disease is characterized by three stages the:

(1) Chancre:
Is primary lesion at the site of the infection (in T.b. gambiense disease), which characterized by localized erythematic, swelling tenderness localized sensation of heat, desquamation and hyperpigmentation.

(2) Haematolymphatic:
Periodic fever, headache, joint pain and muscles ache, lymphadenopathy, loss of weight, pruritus tympani and anemia, edema,
peripheral or pericardial and pleural effusion, cardiac disorder, specific ECG changes congestive heart failure (e.g. pulmonary edema).

(3) Meningoencephalitic:
Nervous system disorders, brisk reflexes deep hyperesthesia, paraesthesia convulsions, mental disorder, insomnia, somnolence, ataxia, slurred speech paresis paralysis, archaic reflexes in *T. gambiense* (Molyneux *et al.*, 1984).

The success of the trypanosomes as pathogenesis is due to their ability to undergo antigenic variation this enabling them to establish persistent infection by evading host immune response (Gray, 1965; Veckreman, 1978).

1.10. Economic implication of African animal trypanosomosis
Trypanosomosis has been a serious constraint to agriculture development and human settlement in Africa since the turn of the 20th century (FAO, 1992).

Trypanosomosis currently causes annual losses of some USD 1.5 billion and, over the long run, has had the effect of limiting Africa’s agricultural income to some USD 4.5 billion a year below its potential level, (PAAT, 1997-2007, FAO, IAEA, AU, WHO, 1999). The program against African Trypanosomosis (PAAT, 1998-1999) estimated the number of cattle population at risk of AAT in Sudan by 3,208,907 of cattle out the total estimated number of cattle population in Sudan (22,500,000 of cattle), the program also estimates the number of cattle not kept because of the AAT by 5,440,724 of cattle.
These infections threaten an estimate of 60 million people and about 50 million head of cattle. Currently about 50,000 cattle and 70,000 people are estimated to be infected. Every year, animal trypanosome causes about three million deaths in cattle and approximately 35 million doses of trypanocidal drugs are administered. Nagana has a severe impact in agriculture in Sub-Saharan Africa. In susceptible cattle breeds, it has been reported that trypanosomosis reduce the calving rate by up to 20%, and causes the death of another 20% of calves that are born even in the trypanotolerant animals FAO (2005). Data collected from Ethiopia Ghibe Valley (an area where tsetse control has been successful) revealed that drought oxen in high-risk area with tsetse infestation were 33% less efficient than those in low-risk ones. In Gambia studies indicated, that trypanosomosis reduces milk production by 26%, reducing lambing, and kidding rates by as much as 37% (FAO 2005). Thus, trypanosomosis has limited the use of animal traction in much of Sub-Saharan Africa. Farm sizes are often smaller in tsetse-infested area and transporting crops to the market is difficult. The lack of livestock also reduces the amount of manure available to fertilize fields. In this way, trypanosomosis continues to have a major impact on agricultural productivity and overall rural development.

Trypanosomosis is a serious problem, as one of Africa’s greatest constrain to socio-economic development, severely affecting human and livestock health limiting, land use, causing poverty.
1.11. Trypanosomes outside the tsetse belt in Sudan

Ismail (1975) reported the presence of *T. vivax* in Damazin (Blue Nile). In Kosti district Ismail and Tag Eldin (1976) reported *T. congolense* and *T. vivax* on the western side of the White Nile River, mixed infection was also found. Homedia (1993) recorded *T. vivax* in Sennar. Surveys conducted in South Darfur area along Bhar El Arab site at the tsetse fringe and South Kordofan revealed that the species of trypanosomes prevalent to be *T. vivax*, *T. brucei* and *T. congolense*, (Hall *et al.*, 1983; Rahman *et al.*, 1990). The surveys which conducted by Rahman confirm that *T. vivax* is enzootic in the wide area of Sudan more than 2,000 km away from the known tsetse belt of the country found *T. vivax* infected cattle along the Nile.

Out breaks of bovine trypanosomosis were reported due to *T. congolense* Buxton (1950), reported *T. congolense* as far north as Kosti area along the white Nile more than 1,000 km² north of the tsetse belts. He related the transmission of the disease to enormous population of *Tabanidae* and biting flies.

1.12. Diagnosis of trypanosomes

The type of diagnostic test used in the detection of infections caused by the animal trypanosomes vary according to the epidemiological characteristics of the disease and the strategy for control. Where tsetse transmitted trypanosomes occurs and where the disease prevalence is high, even test of low diagnostic sensitivity will suffice if chemotherapy or chemoprophylaxis is administered on the herd basis. Many parasitological and serological techniques have been developed, these
techniques have been reviewed extensively (Molyneux, 1975; Nantulya, (1990).

1.12.1. Clinical diagnosis

The clinical signs of acute bovine trypanosomosis include anemia, weight loss, roughness of the hair coat, enlargement of peripheral lymph nodes, pyrexia, and abortion, reduce milk yield, in the absence of treatment, disease, cases progressing to a more chronic disease state may be characterized by anemia, cachexia, poor productivity and infertility. The clinical picture depends on the species of infecting trypanosomes and the geographical location. Hyper acute disease associated with trypanosoma vivax may resemble an acute septicemia or resolution a hemorrhagic syndrome, cases of which may often be found dead. Classically in West Africa, acute T. vivax has been considered more important than Trypsamosa congolense has, whereas in central of east Africa T. congolense was considered the more important parasite. T. congolense infections tend to be less acute and less dramatic, and in that sense are less pathogenic than T. vivax infections, although the result is almost as lethal (Stephen, 1986). Trypanosoma brucei infection are generally regarded as being of low pathogenicity for African cattle which may be infected without showing overt clinical signs, but exotic breads of cattle and their crosses may be more susceptible (Jordan, 1986).

1.12.2. Laboratory methods

1.12.2.1. Parasitological diagnosis

Achieved by the examination of blood films and Giemsa- stained, thick and thin fixed blood films with aid of the light-microscope.
With the wet blood film, a drop of blood can be examined next to the animal. Thin and thick blood smears fixed in methanol or acetone and stained with Giemsa may be used in the laboratory to detect blood parasites to determine the trypanosome species. However, these techniques are not sensitive enough to detect the low parasite levels, characteristic of the disease in large animals. Goel Singh (1971) reported that thick smear method was more sensitive and effective than the thin smear method in case of low parasitemia in *T. evansi* infection.

The best chance to detect the parasite is better to collect the blood sample early in the morning and from the peripheral capillaries, e.g. from the ear or the underside of the tail.

Although glandular fluid collected from the prescapuler lymph node and fluid expressed from the chance may also contain trypanosomes (Robson and Ashkar, 1972), it is often more practical to collect blood in sufficient quantity to allow the use of a concentration method. In addition, the cerebrospinal fluid (CSF) examination is widely used in the diagnosis of human trypanosomes, (WHO, 1986).

In addition, wet preparation of lymph node aspirate is examined on a glass with a cover slip at a magnification of 10x46.

### 1.12.2.2. Haematocrit centrifugation

It is used in mild clinical, or sub clinical cases (carrier) with low parasitimia in which it is difficult to demonstrate the parasites, concentration methods become necessary. Blood is collected into heparinised capillary tubes (75x1.5mm), which are sealed at one end and centrifuged, sealed end down, then the capillary tube is placed under the
microscope and the buffy coat/plasma junction is checked for trypanosome as described by (Woo1970). The Buffy coat examination technique (BCT) is by cutting the capillary tube, expressing the Buffy coat/plasma interface on a microscope slide and using dark-ground or phase-contrast illumination (Murray et al., 1977, Wernery et al., 2001). The advantage of these two methods is that diagnostic sensitivity increase, due to a concentration of parasites following centrifugation at the same time the packed red cell volume (PCV) can be determined as a measure of anemia (Paris et al., 1982).

The (BCT) method has an additional advantage in that the three most important trypanosomes species can be often identified, due to their characteristic movement patterns, and an estimation of the parasitaemia can be made using scoring system (Paris et al., 1982).

A method using miniature anion-exchange columns for the separation of trypanosomes from erythrocytes prior to concentration by centrifugation (Lumsden et al., 1979), was also tried.

**1.12.2.3. Animal sub-inoculation**

Sub-inoculation of blood from suspected cases or subclinical cases into another species (especially laboratory rodents) has been widely used, though not all trypanosomes are infectious for these species. Immunosuppression of laboratory rodents either by irradiation or by using chemical immuno-suppressants such as cyclophosphamid may increase the proportion of trypanosomes, resulting in infection. Inoculation of susceptible rodents may be more effective for some
trypanosomes species, particularly Trypanzoon species than others, Robson and Ashker (1972) found more cases of T. brucei infection in Kenya mouse sub inoculation using blood examination but for T. congolense infections mouse sub-inoculation revealed only half as many positive animals and failed to pick up any T. vivax infection. For this, species-sub- inoculation of domestic ruminants (sheep or goats), is recommended T. vivax may vary in its ability to give rise to parasitaemia in goats depending on the geographical origins of the parasites (Peregrine et al., 1991)

1.12.2.4. Xenodiagnosis

Xenodiagnosis is the feeding of a clean susceptible vector species on a suspected case of trypanosomosis, after which it is either dissected and examined for the presence of infection, or allowed to feed on a clean animal, which is examined for the development of infection. It is an extremely sensitive method Eisler et al. (1998).

1.12.2.5. In vitro culture methods

Zweygarth and Kaminsky (1990) described a method for isolating T. brucei brucei and T.evansi directly in culture from host animals with low trypanosomosis which were in some cases not detectable on wet blood film or HCT. A kit for the in Vitro isolation of trypanosoma gambiense from humans has been shown to be useful in diagnosis as it may demonstrate the presence of this organisms at very low levels in patients’ circulation. The method has also been assessed for use in domestic animals and shown to detect more T. brucei infection then conventional parasitological techniques (McNamara et al., 1995a). However, this
method is relatively expensive and not practical for routine diagnosis of bovine trypanosomoses.

1.12.3. Serological diagnoses
The aim of serological tests is to detect specific anti-bodies developed by the host against the infection. The detection of anti-bodies indicates that there has been infection, but as antibodies persist for some time, on the other hand circulatory trypanosomal antigens are eliminated quickly after the disappearance of the trypanosomes and their presence therefore may show usually that, live trypanosomes are present in the animals. Serological tests are mainly used as tools for research for monitoring trypanosomosis control programmes and for surveys.

1.12.3.1. Complement Fixation Test
The complement fixation (CF) test has been used for the diagnosis of *T. equiperdum* the cause of dourine in horses. Serology has played a major role in diagnosis of this disease. Since trypanosomes are rarely found in blood or other fluids. The complement fixation test was used successfully in the control and eradication of dourine in North America, (Watson, 1920), and was used in the diagnosis of surra in buffalo in the Philippines (Randall and Schwartz, 1936).

1.12.3.2. Indirect haemagglutination (IHA) test
It was used in the diagnosis of *T.evansi* in camels (Jatkar and Sinhg, 1974), and in control programmed for buffalo and cattle (Shen, 1974). In the test with *T. vivax* it was consider unreliable (Clarkson *et al.*, 1971).
1.12.3.3. The Indirect fluorescent antibody (IFA) test

This test is used for detection of trypanosomal antibodies. The (IFAT) has been shown to be both sensitive and specific in the detection of bovine anti-trypanosomal antibodies (Luckins and Mehlitz, 1978).

Although a degree of cross-reactivity between *T. brucei*, *T. congolesi* and *T. vivax* indicated that IFAT is not very reliable, species-specific cross-reactivity is not complete. All three antigens must be used for maximum efficiency (Lukins, 1993).

1.12.3.4. Enzyme linked immunosorbent assay (ELISA)

An immunodiagnostic method based on a direct sandwich enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies. It has been used in the field or under resourced laboratories (Lukins 1992), and it has been used for monitoring tsetse control and eradication programmes and were used for diagnosis of both human and animal trypanosomiasis (Lukins *et al.*, 1978; Molyneux, 1975; Mahmoud and Elmalik, 1978).

The indirect ELISA was adapted for a number of protozoan diseases, including trypanosomosis (voller *et al.*, 1976) and it was shown to be capable of detecting specific antibodies in trypanosome-infected cattle (Luckins and Mehlitz, 1978). It was also shown to detect more serological positive cattle than the IFAT, cross-reactivity between the three major tsetse-transmitted trypanosome species occur in the indirect ELISA using crude antigen preparation. Sera must be screened against all three antigens for optimum sensitivity (Lukins and Mehlitz, 1978).
In the last decade (1990-2000) interest increased in the Ab-ELISA for bovine trypanosomosis prevalence and risk. The technique has recently found use in many regions of sub-Saharan Africa, for example, under the food and Agriculture organization/ international Atomic Energy Agency (FAO/IAEA) co-coordinated Research Programme (D winger and Hall, 2000).

1.12.3.5. Antigène- détection ELISA (Ag. ELISA)
The aim of the development of these tests was to increase the sensitivity of diagnosis in both the analytical sense and in the epidemiological sense. In addition, it was hoped to maximize the specificity of the test. In practice, this was equated to lack of cross-reactivity of each species-specific test trypanosomes (Nantulya and Lindquist, 1989).

1.12.4. Monoclonal antibodies (MoAbs)
It has been recently developed to distinguish T. brucei, T.congolense and T. vivax (Nantulya et al., 1987). The monoclonal antibodies (MoAbs) opens a new avenue for diagnosis of African trypanosomosis, when these MoAbs are used in an antigen-trapping ELISA, diagnosis is sensitive and specific enabling many latent infection to be detected.

1.12.5. Molecular methods for Detecting Livestock- infective Trypanosomes
The molecular methods, based on the detection and amplification of nucleic acids (DNA and RNA). The detection and identification trypanosomes by molecular means should be based upon stable, parasite-
specific genetic characteristic specific to that parasite that can withstand environmental influences exerted by either the host or the vector. (Masake *et al*., 2002) indicated that when use PCR was used to identify multiple copy segments of DNA, the presence of parasite DNA equivalent to one trypanosome in 10ml of host blood could be detected. In experimental studies, the PCR can detect trypanosomes in cattle as early as 5 days after an infective tsetse bite (Masak *et al*., 2002).

1.12.6. The card agglutination test (CATT)
Card agglutination test for trypanosomosis adapted to assist diagnosis of West African human sleeping sickness, it relies on the presence of antitrypanosomal antibody to agglutinate intact, stained preserved trypanosomes. Whilst with some modifications, this system has been found useful in the diagnosis of *T. evansi* infection (Magnus *et al*., 1978b).

1.13. Control of trypanosomosis

1.13.1. Chemotherapy
Chemotherapy is the treatment of the disease by the use of chemical drugs. Such drugs are curative; they block one or more vital processes, which are essential to the invading microorganism. Certain compounds have specific effects on some enzyme system. Trypanosomes are immunosuppressive, but by using chemotherapy which stop the multiplication of the trypanosomes and help the immune system to overcome the infection. Chemotherapy is being
Widely used based on usage of various types of trypanocidal drugs. Most of these trypanocidal drugs have been in use for many years. Their effectiveness has been widely reduced and trypanosomes developed what is known as drug resistance (Lukins, 1999; El Rayah, 1992).

Trypanocidal drugs commonly used include Diminazene aceturate (Berenil), Homodium bromide (Ethidium), Homodium chloride (Novidium), Isometamidium (Samorin), Quinapyramine sulphate (trypanocide), and Quinapyramine prosalt.

Diminazene, homidium and Isometamidium are used mainly in cattle goats and sheep. The Quinapyramine was withdrawn from the market in 1977 because of the emergence of widespread resistance among trypanosomes in cattle; it was reintroduced in 1985 mainly to treat *T.evansi* infection in camels and horses. Although suramin is the oldest trypanocide it is still used to treat early cases of human sleeping sickness as well as *T. evansi* infection in camels.

Diminazene is active against *T.congolense* and *T. vivax* at the dosage rate of 3.5mg/kg. The phenanthridium related compounds homodium and isometamidium are active against *T.congolense* and *T. vivax* but less against the *T.brucel* group. Mustafa (2004) treated experimentally infected sheep with quinapyramine sulphate (antrycide) at the dose of 3mg/kg body weight with complete disappearance of trypanosomes from the peripheral blood. Rahman (2002) indicated that samorin at 1mg/kg body weight had given significant long prophylactic period in cattle at challenge, index of 450-1750 than a dose of 0.5mg/kg.
Homidium is mainly used for its therapeutic affects but does have prophylactic activity for several weeks (Dolan et al., 1990). It is administered by deep intramuscular injection. Quinapyramne sulphat is soluble in water and administered by subcutaneous injection to treat *T. evensi* and *T. brucei* infection, the use of quinapyramine was the suggested cause of the multiple drug-resistance problems in the Ghibe valley of Ethiopia. Intravenous administration of isometamidium as 1% w/v solution has been successfully to treat trypanosomiasis in cattle (Dowler et al., 1989). Isomitamidium, which is widely consider being the drug of choice in the prevention of bovine trypanosomiasis is administered by deep intramuscular injection, but the disadvantage of isometamidium is that an encapsulated lesion forms within the muscle from which the drug is slowly released to give prolonged protection from infection by trypanosomes. Peregrine et al. (1977) indicated that the duration of protection appears to be largely dosed related, the higher the dosage rate, the longer is the period of protection. Effectiveness of Ascofuranone (an antibiotic isolated from Ascochyta visiae) as a tool for chemotherapy against African trypanosomiasis in animals was studied by Yabo et al. (2006).

Carloin et al., (2004) found that megazol could cure sheep infected with *T. brucei*, but oral administration was found not to be effective route.

The use of a sanative drug to eliminate trypanosomal infections is of great importance. Cross-resistance between diminazene and isometamidium has been reported. Consequently, the alternate use of
these two compounds, as sanative pair is widely practiced. Caution is
needed when using diminazene and isometamidium to avoid cumulative
toxic effects. Isometamidium should not be given less than one week
after diminazene, and at least one month should lapse after treatment
with isometamidium before is injected.

Further, more frequently repeated trypanocidal treatments have been
associated with toxicity problems (Dolan, 1993; Eisler et al., 1997). In
addition, it has been shown in other areas that there is a strong correlation
between the treatment frequency and the rate of development of resistant.
The widespread, unsupervised and under dosed users, the few
compounds developed resistance on the part of the parasite (Afewerk et al., 2000), which retains its resistance after cyclic transmission by tsetse
(Gray and Roberts, 1971)

Abdel Gadir et al. (1981) showed that Sudanese isolates of three
major trypanosomes species *T. vivax*, *T. congolense* and *T. brucei*
expressed resistance to 1.0 mg/kg homodium bromide, the relapse
infections were all susceptible to 1.0 mg/kg body weight diminazine
aceturat.

So far, resistance to one or more of the three-trypanocidal drugs
used in cattle has been reported in at least 11 countries in sub-Saharan
Africa including Burkina Faso, Chad, Cote di Voire, Ethiopia, Kenya,
Nigeria, Somalia, The Sudan, the United Republic of Tanzania, Uganda,
Zimbabwe reported by Peregrine (1994). The Central African Republic
(Finelle and Yvore, 1962), were also reported.
Therefore, it has been recommended that in high tsetse challenge areas control of trypanosomosis should not rely solely on drugs but that an integrated approach should be adopted using vector control to reduce the tsetse challenge along with reduced frequency of drug dosing. Where such measures have been adopted the result have been impressive (fox et al., 1993; Peregrine et al., 1994). In West, and Central Africa the prefer use of trypanotolerant livestock to drugs may be appropriate in areas of high tsetse challenge. In order to delay the development of resistance the following measures are recommended:

- Reduce the application of trypanocides by integrating their use with other control methods. This lessens selection pressure by the drug. There is definitely a strong correlation between the scale and frequency of treatment and the development of resistance.
- Avoid the use of quinapyramine in cattle. It was widely used in cattle in Africa during the period 1950 to 1970. In 1976, it was withdrawn from sale, for cattle use because of problems with toxicity and resistance development, it is still available for use in camels.
- Consider the use of sanative pair of drugs isometamidium, or -ethidium and diminazene.
- Avoidance of the exposure of trypanosomes, to sub therapeutic drug concentration ((Whiteside, 1960).
1.13.2. Trypanotolerance

Trypanotolerance is a phenomenon present in some African cattle and small ruminant breeds. It is defined as the ability to survive and to be productive in tsetse-infested areas without the aid of treatment.

Trypanotolerance is usually attributed to the African Boss Taurus Taurus breeds particularly the N’Dama and the West African short horn, present mainly in west and central Africa. When trypanotolerant cattle breeds generally show slightly lower mortalities then trypanosusceptible breeds (up to 10% for trypanotolerant breeds compares with 10% to 20% for trypanosusceptible ones, and lower reduction in calving rates (1 to 12% compared with 11 to 20%), (FAO 2000). Some cattle are more trypanotolerant than others, for example west N’Dama and muturu cattle, are more resistant than the West African zebu, when infected they do not develop anemia (Murray et al., 1992)

In Sudan (Rahman 2002) reported that western Baggara cattle survived natural tsetse challenge, and show a better ability to control parasitaemia compare to other indigenous zebu breeds existing in the Sudan.

1.13.3. Vector control

Many techniques have been used to reduce tsetse population with earlier, crude methods being replaced in more recent times by methods which are more cheaper, more directed and ecologically better considered, they includes
1.13.3.1. Land clearing
Complete removal of any bush or woody vegetation from the area, tsetse fly tends to rest on the trunks of trees, so the removal of woody vegetation made the area inhospitable to the flies, but the clearing of woody vegetation has come to be seen as an environmental problem more than a benefit.

1.13.3.2. Slaughter of animals
One of the early techniques is slaughter of all the wild animals on which tsetse fed. For example, the Island of Principe off the west coast of Africa was entirely cleared of feral pigs in 1930s, which led to the extirpation of the fly. While the fly eventually re-invaded in the 1950s, the new population of tsetse was free from disease.

1.14.3.3. Pesticides
Pesticides have been used to control tsetse, initially during the early part of the twentieth century in localized efforts using the inorganic metal-based pesticides. Expanding after the Second World War into massive aerial and ground based campaigns with organochlorine pesticides such as DDT, applied as aerosol spray at ultra-low volume rates, later, more targeted technique used pour-on formulation, in which advanced organic pesticides were applied directly to the back of cattle’s.

Insecticides (delta-metherines and alphacypermethrins), were used on host and as impregnated traps and targets in Tanzania, Kenya, Botswana, and Zanzibar (Allsopp, 1990).
The use of specific insecticide formulation applied to artificial attractive devices and cattle is an efficient and sufficiently specific method to suppress tsetse target population in most situations, Bauer *et al.*, (1995).

Success largely depends on the density, and placement of the impregnated attractive devices in the fly habitat, the availability of attractants for the target tsetse species (Hall and smith, 1995); the size of the control area; reinvasion pressure and the population dynamic of tsetse population in the adjacent areas (Bauer *et al.* 1995) and tsetse host preference (Clausen *et al.*; 1998).

In Uganda (Nyeko *et al.;* 1993), reported that trypanosomosis control is still relying mainly on insecticides application (aerial or ground spraying) against tsetse and on treatment of suspected clinical cases in livestock, as a result over 41800 square miles of land was reclaimed from tsetse infestation, and made available for human settlement.

In the Sudan control and eradication has been carried out as earlier as 1960s. Abdel Razig *et al.;* (1969) reported eradication of tsetse flies in the Jur Narrows by means of game destruction together with the use of insecticides used on bait animals. Rahman (2002), cited Yagi and Abdel Razig, 1969), have successfully eradicated tsetse flies form the isolated tsetse pocket in Koallib Hills using insecticides baited animals and selective ground spraying of resting sited and breeding sites of tsetse. Although tsetse flies are being incriminated for more than 80% of trypanosomosis infection in the Sudan (Rahman, 2002), yet no clear policies and plans have been put in place for their control.
1.13.3.4. Trapping

Tsetse population can be monitored and effectively controlled by using traps. The recent traps are simple sheets or have a bioconical form. The NG2G trap used to catch Glossina pallidipes in Kenya, F3 and Epsilon traps for the G. pallidipes in Zimbabwe. The bioconical traps for the Riverine tsetse flies, vavouatrap is used for large-scale control of riverine tsetse, the canopy is for Horse flies and dear flies. Some of these traps are impregnated with insecticides to insure the death of the fly, and use of chemicals attractants to lure tsetse to the trap or the synthetic version of these chemicals can be used to create artificial odors. Belete et al., (2004), demonstrated effectiveness of cow urine kept for several days as attractant to be used in traps, in the community based tsetse control programs in Ethiopia, cited by Springer Netherlands (2007).

1.13.3.5. Sterile insect technique (SIT)

This technique involves the rearing of large numbers of tsetse flies, separation of males, and irradiation of these males fly with large doses of gamma rays to make them sterile and then release them into the wild, at regular intervals. Since females only mate a few times in their life, generally once, any mating with sterile male will prevent that female from giving birth to any offspring.

The sterile insect technique (SIT), has recently been used on Zanzibar, an island off the coast of East Africa.

All of these control methods has been criticized either as none environmentally friendly technique (killing of wild animals, spraying of
insecticides and removal of vegetation), or very expensive such as the sterile male technique (Saini 2003).

1.13.3.6. Community base tsetse control

It is an approach for the tsetse flies control been adapted recently to include community in the control of the fly (community participation), also it called community base tsetse control (Barrett and Okali, 1998). Locals are trained to make their own flytraps and work with scientists to eradicate the fly from their areas. Community education and mobilization are the most important tools for involving a community in development activity.

Another approach for control of the flies, is a secure fly-proof housing of livestock, can provide effective relief from the effect of biting flies. Livestock owners worldwide practice movement of animals to avoid tabanid attack on either a daily or a seasonal basis.

In the Sudan, Hall et al., (1984) concluded that, the objective evidence for the implication of tabanids in mechanical transmission of bovine trypanosomosis was lacking, but that tabanids were a major factor in causing the seasonal migration of cattle.
1.13.4. Vaccination

Up to now, there is no vaccine produced for the control of trypanosomosis because their variant surface glycoprotein keeps on switching. In addition, it has been a common phenomenon that, a mixed infection occurs in most of the trypanosomes infection, hence making it difficult for vaccine development, (Uilenberg, 1998).

CHAPTER TWO
MATERIALS AND METHODS

2.1 Blood samples collection

Cattle in Rajaf (East, Khor Romla and West Rajaf, Gumba), and Kameron, Juba County of Central Equatoria State were sampled during January 2009. A conventional sample of Nilotic cattle breed were included (plate I). Animals were selected to be examined parasitologically. They were including both males and females from 4 age group as follows:

1. Younger 1≤ one year
2. Heifers and young bull's from 2-4 years
3. Adult from 5-8 years.
4. Old more than 8 years.

Animals which were in ill health and in bad condition and whose owners were willing were chosen (plate II). The sites covered all
directions, South, North (east and west of Bahr el Jabel), Juba town being the centre.

Blood samples were collected from the jugular vein in heparinated tubes for parasitological and hematology examinations. Screening of the blood for trypanosomes was carried out using the standard detection techniques (wet, thin and thick film) for quick assessment and by haematocrit centrifugation technique (HCT) for accurate diagnosis. Also, the packed cell volume (PCV) of all animals was recorded using the microhaematocrit method.

Plate I  Nilotic cattle breeds
2.1.1. Study area location

This study was carried out in Juba county, central Equatoria State, Republic of the Sudan. The county lies between Latitude 04°:52, Longitude 31°: 36 E, Alt. 460m. There is a wide range in climate. Topographic and vegetation characteristics, within the three broad ecological zones, the Ironstone plateau, the central Hill, the green belt and Mountain.

2.1.2 Vegetation

The vegetation varies considerably, the scrub savannah of the lower elevation of the Ironstone Plateau, grades into mosaic of high
Savannah, broad leaf forest area and open grassland, the green belt. They are the Agro-ecological Zones in central Equatoria. The Savannah Zone intercalated with scattered several varieties of trees and species of perennial and annual grass, this area has a soil which is acidic in nature. The most dominant feature of the area is River Bahr El Jebel which links the White Nile through Uganda with Lake Victoria. Patches of evergreen thickets and double storey gallery forests grow along the banks of the river. There are two main types of vegetation predominant.

2.1.2.1. Open Savannah Woodland

In this zone the soil is of loamy and loamy-clay types transected by a vast numbers of streams and rivers, the area is relatively dry. The vegetative cover is comprised of *Acacia nilotica*, *A. sibriana*, *A. seyal* and *A. mellifera* and the deciduous trees of *Anogeissus leio* carpus and *Combretum glutinosum*. The main grasses are of the *Imperata*, *Panicum* and *Andropogon* species. Along the water courses, there in the evergreen trees consist of *Ficus religiose*, *Tamarindus indica*, *Azanirachta indica*, *Anogeissus leiocarpus* together with *combretum* spp. and various climbers and grasses.

2.1.2.2 Derived Savannah woodland:

This is known as Roga-loka ecological zone, an ironstone zone with broad-leaf woodland forest, and many seasonal streams but no swamps. The main evergreen trees are of *Ficus religiose*, *Spina-christi*,

49
Tamarindus indica, Khaya senegalensis, Anogeissus leiocarpus and Combretum glutinosum.

2.1.3 Temperature
Maximum mean temperature is 33.9°C the minimum mean temperature is 21.1°C. The annual mean temperature is 29°C.

2.1.4. Rainfall
Rainfall varies from 900 - 1.300 mm distributed over six to eight months on most of the Ironstone Plateau. Within the greenbelt, at the extreme southern limits of the Ironstone plateau rainfall averages 1.300 - 1.600 mm over eight to nine month period. The central hills have about the same total precipitation as the Ironstone plateau, with more variable rainfall patterns, the mountains slopes and foothills have steep precipitations gradient rang from 2.000 mm at the high elevations to 800 mm in the foothills.

2.1.4. Soil
Soil types in the Equatoria zones, range from shallow, highly leached, latertic soil on the Ironstone plateau to deep medium fertility loams and sandy loams in the high plateau and mountains.

2.1.5. Animal species and breeds
Livestock rearing is one of the major human activities in addition to cultivation and fishing. The cattle breed in the area is predominantly the Nilotic breeds known with a relatively small size (250-400 kg. for adult), big horns and the coat can be in many different colours (plate, I)
The estimation of livestock, poultry, and pets population in Juba County was as shown below:

<table>
<thead>
<tr>
<th>Species</th>
<th>Number x 10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>166,007</td>
</tr>
<tr>
<td>Goat and sheep</td>
<td>170,800</td>
</tr>
<tr>
<td>Chickens</td>
<td>84,202</td>
</tr>
<tr>
<td>Ducks</td>
<td>12,574</td>
</tr>
<tr>
<td>Dogs</td>
<td>1,430</td>
</tr>
<tr>
<td>Cats</td>
<td>409</td>
</tr>
</tbody>
</table>

2.1.6. Husbandry practices

The main livestock production system practiced in the study area is sedentary system; they used to herd their animals around their villages for the whole year.

This type of grazing has resulted in low production of rangeland. Livestock owners reported that they faced various constrains in keeping livestock. This constrains are, disease, and lack of inputs and there is no vaccination.

2.1.7. Availability of veterinary services
There was a very little infrastructure in Juba County to support the large scale livestock intervention. Apart from a number of NGOs who are active in the area and network of community animal health workers, there are very few skilled personal. Farmers reported the lack of inputs and information on husbandry as constrains of livestock keeping.

2.2 Blood collection and examination

2.2.1 General parasitological methods used in this study

For parasitological examination blood samples were collected in the morning (7:30 -10) am, from the jugular vein of the selected animals. The samples were collected into heparinized tubes and directly into slide as wet mount, thick and thin blood films.

2.2.2. Wet mount

A wet mount was done from a drop of blood on the slide, covered with a cover slip, and examined microscopically for motility of the parasite at 10x40 magnifications.

2.2.3. Thin and thick blood films

The slides were air-dried, labeled, fixed in absolute methanol for two minutes and air dried. The smears were stained using 10% Giemsa stain solution for half an hour, rinsed with water for seconds and allowed
to air dry. The film was examined under oil emersion objective for
detection of trypanosome and further species differentiation.

2.2.4. Haematocrit Concentration Technique (HCT, Woo, 1970):
heparinised capillary tubes and Blood sample were drown into
centrifuged for 5 minutes at 12000 RPM using microhaematocrit
centrifuge (Howkslay and sons Ltd., England). Then the buffy coat layer
and neighboring red blood cells in each sample were extruded on a glass
slide, covered with a cover slip (22 x 22 mm), and examined under light
microscope with x40 phasecontrast objective (Olympus England) for
presence of motile trypanosomes, (Murray and Urquhart 1977).

2.3. Traditional disease diagnosis
The livestock owners whose herds were examined were
interviewed during the survey on how they diagnose the diseases
traditionally. They were asked to describe the symptom of the disease,
and if related to specific season, whether rainy or dry season. Farmers
were also interview if they linked diseases with ticks. They were
interviewed on their ranking of diseases according to importance.

2.4. Traditional treatment of the diseases:
The owners were asked about the diseases they know and their local
names. They were asked how they treat some of the diseases, about the
use of plants, how they prepare the remedies. Other traditional ways of
treatment were described to relieve certain symptoms like retained
placenta, diarrhea, arthritis, bloat and some tick remedies and swollen
lymph nodes.
Chapter three
Results

3.1 Prevalence of trypanosomosis in cattle:
A total of 220 head of cattle were examined during January 2009.
The total prevalence of *trypanosomal* infection was 4.09% in the study area where 9 heads were found infected.
Infection by age is shown in (Table I), for all groups. All age groups had similar infection, although the old group was found the highest infected group.
3.1.1. The infection rate in cattle in different sites

In this survey it appeared that the prevalence of *trypanosomosis* infection is higher in Rajaf West (5.19%) followed by *Kamaron*, (4.17%) and Rajaf East, with the prevalence of (2.8%). Results are shown in (Table II).

3.1.1.1. Infection rate in Rajaf West:

Infection was detected in 4 animals out of 77(5.19), of those 3 were females and one male. By age 2(3.7) were young, one was a heifer and none of the adults was infected and two were from the old group (3.33%) (Table III).

3.1.1.2. Infection rate in Rajaf East

Infection was detected in 2 animals out of 71(2.8%) being one female and one male. By age none of younger's, or adults were infected. One bull (14.3%) and one bull (33.3%) were infected (Table IV).
(Table I). Infection rate in different age groups.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Females</th>
<th>Males</th>
<th>Total infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>+ve (%)</td>
<td>No. examined</td>
</tr>
<tr>
<td>Younger</td>
<td>85</td>
<td>2 (2.41)</td>
<td>43</td>
</tr>
<tr>
<td>Heifer(Bull)</td>
<td>29</td>
<td>1 (3.57)</td>
<td>22</td>
</tr>
<tr>
<td>Adult</td>
<td>31</td>
<td>1 (3.33)</td>
<td>2</td>
</tr>
<tr>
<td>Old</td>
<td>8</td>
<td>2 (3.33)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>153</td>
<td>6 (4.08)</td>
<td>67</td>
</tr>
</tbody>
</table>
(Table II). Infection rate in cattle in different sites

<table>
<thead>
<tr>
<th>Sex</th>
<th>Site</th>
<th>Females</th>
<th>Males</th>
<th>Infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>+ve</td>
<td>No. examined</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(%)</td>
<td></td>
<td>(%)</td>
</tr>
<tr>
<td>Khor Romla, Rajaf west</td>
<td>53</td>
<td>3 (2.8)</td>
<td>24</td>
<td>1 (6.3)</td>
</tr>
<tr>
<td>Rajaf East, Gumba</td>
<td>48</td>
<td>1 (2.3)</td>
<td>23</td>
<td>1 (4.52)</td>
</tr>
<tr>
<td>Kamaron</td>
<td>52</td>
<td>2 (4)</td>
<td>20</td>
<td>1 (5.26)</td>
</tr>
<tr>
<td>Total</td>
<td>153</td>
<td>6 (4.08)</td>
<td>67</td>
<td>3 (4.69)</td>
</tr>
</tbody>
</table>
(Table III). Infection rate in Rajaf west

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Females</th>
<th></th>
<th>Males</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. examined</td>
<td>+ve (%)</td>
<td>No. examined</td>
<td>+ve (%)</td>
<td>No. examined</td>
<td>+ve (%)</td>
</tr>
<tr>
<td>Calves</td>
<td></td>
<td>37</td>
<td>1 (2.8)</td>
<td>17</td>
<td>1 (6.3)</td>
<td>54</td>
<td>2 (3.7)</td>
</tr>
<tr>
<td>Heifers (bulls)</td>
<td></td>
<td>8</td>
<td>1 (14.3)</td>
<td>5</td>
<td>0 (0.0)</td>
<td>13</td>
<td>1 (7.7)</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td>5</td>
<td>0 (0.0)</td>
<td>2</td>
<td>0 (0.0)</td>
<td>7</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Old</td>
<td></td>
<td>2</td>
<td>1 (50)</td>
<td>3</td>
<td>0 (0.0)</td>
<td>3</td>
<td>2 (33.3)</td>
</tr>
</tbody>
</table>
(Table IV). Infection rate in Rajaf East, Gumba:

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Females No. examined</th>
<th>+ve (%)</th>
<th>Males No. examined</th>
<th>+ve (%)</th>
<th>Total No. examined</th>
<th>+ve (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calves</td>
<td>17</td>
<td>0 (0.0)</td>
<td>15</td>
<td>0 (0.0)</td>
<td>32</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Heifers (bulls)</td>
<td>13</td>
<td>0 (0.0)</td>
<td>8</td>
<td>1 (14.3)</td>
<td>21</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>14</td>
<td>0 (0.0)</td>
<td>0</td>
<td>0 (0.0)</td>
<td>14</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>4</td>
<td>1 (33.3)</td>
<td>0</td>
<td>0 (0.0)</td>
<td>4</td>
<td>1 (25)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>48</td>
<td>1 (2.13)</td>
<td>23</td>
<td>1 (4.5)</td>
<td>71</td>
<td>2 (2.8)</td>
</tr>
</tbody>
</table>
3.1.1.3. Infection rate in Kamaron

Infection was detected in 3 animals out of 72 (5.26%) of those 2 were females and one male. By age 2 (4.8%) were young, one adult (8.33%), none of the heifers or old animals were infected (Table VII).

3.2. Trypanosoma species identification

Microscopic examination of stained blood smears showed the presence of the following species: 8 samples had *T. vivax*, (3.63%) two of those were mixed infection with *T. brucei*. (0.9) *T. congolense* (0.45) was identified in one smear. (plates III, IV) (Table V).

(Table V) TRYPANOSOMA SPECIES:

<table>
<thead>
<tr>
<th>Species</th>
<th>No infected</th>
<th>Infected rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. vivax</em></td>
<td>8</td>
<td>3.63%</td>
</tr>
<tr>
<td><em>T. brucei</em> mixed</td>
<td>2</td>
<td>0.90%</td>
</tr>
<tr>
<td><em>T. congolense</em></td>
<td>1</td>
<td>0.45%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>9</strong></td>
<td><strong>4.45%</strong></td>
</tr>
</tbody>
</table>

* *T. vivax* was in a mixed infection with *T. vivax*  
** * Infection from all examined animals (220)

3.3. PCV values:

Animals had a PCV average value for all examined animals of 27.036, ranging between 42 -18.
The infected group had a range of PCV of 21–18, with an average of 20.89, while the parasitologically negative had a range of 42 – 21, and an average of 27.29 (Table VI)

**Average and range of PCV (Table VI)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Range</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>All animals</td>
<td>42 – 18</td>
<td>27.036</td>
</tr>
<tr>
<td>Non infected</td>
<td>42 - 21</td>
<td>27.29</td>
</tr>
<tr>
<td>Infected</td>
<td>21 -18</td>
<td>20.89</td>
</tr>
</tbody>
</table>
Plate III Trypanosoma vivax

Plate IV Trypanosoma brucei
(Table VII) Infection rates in Kameron

<table>
<thead>
<tr>
<th>Sex</th>
<th>Females</th>
<th>Males</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>+ve (%)</td>
<td>No. examined</td>
</tr>
<tr>
<td>Calves</td>
<td>31</td>
<td>1 (3.3)</td>
<td>11</td>
</tr>
<tr>
<td>Heifers(bulls)</td>
<td>8</td>
<td>0 (0.0)</td>
<td>9</td>
</tr>
<tr>
<td>Adults</td>
<td>12</td>
<td>1 (9.09)</td>
<td>0</td>
</tr>
<tr>
<td>Old</td>
<td>1</td>
<td>0 (0.0)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>2 (4)</td>
<td>20</td>
</tr>
</tbody>
</table>
### 3.3. Disease diagnosis and ranking:

The diseases according to symptoms described, by owners, and tentatively given veterinary names are shown in (Table VII).

The disease ranking by owners according to their importance, was *Twanti lo juba* was ranked as the most important disease in Juba County of Central Equatoria. *Twanti lo juba* was defined as swelling of the parotid, precrural and prescapular lymph nodes, high mortality rate in calves, lachrymation, salivation, and in the terminal stages of severe cases, diarrhea was noted just prior to death. The disease was not reported to affect adult animals. Farmers reported that on post mortem, they find enlarged watery lungs. This description of *Twanti lo juba* is clinically and epidemiologically consistent with East Cost Fever (ECF).

The next important disease was *Lokido* (CBPP) and *Lokido lo ldin* (CCPP). It was defined as wasting, difficult breathing and farmers reported at on the post mortem, they fined lungs whitish in color, firm, lungs found adhered on rib cage and difficult to detect when animal is alive and it affect all ages.

Ticks (*masiret/maser*) were reported as the next most important livestock health problem. Interestingly, tick were not associated with any disease, but were only seen as damaging the skin at the site of infestation.

*Brucellosis* (*Lokusan/Loyop/yango chang*), which affect cattle and goats at any stage. Symptoms are describes as animal can abort more than once, the farmers think that it is caused by infected bulls, and the control it by castration of infected males.

( Table VIII). **Common diseases in Juba County and their local names**
<table>
<thead>
<tr>
<th>Scientific disease name</th>
<th>Local disease name</th>
<th>Tribe</th>
</tr>
</thead>
<tbody>
<tr>
<td>BQs</td>
<td>Logepera</td>
<td>Bari</td>
</tr>
<tr>
<td>CBPP</td>
<td>Lokido</td>
<td>Bari</td>
</tr>
<tr>
<td>CCPP</td>
<td>Lokido lo ldin</td>
<td>Bari</td>
</tr>
<tr>
<td>ECF</td>
<td>Twanti lo juba</td>
<td>Bari</td>
</tr>
<tr>
<td>Heart water</td>
<td>Lokumere</td>
<td>Bari</td>
</tr>
<tr>
<td>Trypanosomosis</td>
<td>Lobit</td>
<td>Bari</td>
</tr>
<tr>
<td>Haemorrhagic septicemia</td>
<td>Twanti lo lubugu</td>
<td>Bari</td>
</tr>
<tr>
<td>Coccidiosis</td>
<td>Yaka</td>
<td>Bari</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Buloto</td>
<td>Bari</td>
</tr>
<tr>
<td>FMD</td>
<td>Lukwak</td>
<td>Bari</td>
</tr>
<tr>
<td>PPR</td>
<td>Pale Buloto</td>
<td>Bari</td>
</tr>
<tr>
<td>Gumburo</td>
<td>Jabo</td>
<td>Bari</td>
</tr>
<tr>
<td>Salmonella infection</td>
<td>Buloto pela</td>
<td>Bari</td>
</tr>
<tr>
<td>Orf/Caprine pox</td>
<td>Logurum</td>
<td>Bari</td>
</tr>
</tbody>
</table>

*Trypanosomosis (Lobit)*, which is described as chronic wasting syndrome (emaciation, weight loss), tail hair falls off, affect all age groups but adults are affected more, affect both cattle and goats, mortality is not common, abortion, the post mortem finding, lungs attached to the
ribs, enlarged lungs, the cause is unknown. It seasonality mostly during the dry season there is no control.

3.4. Traditional treatment:
The owners were asked how they treats some of the disease, they reveal that they used pounding seeds of *lalok* tree, and soaking these in boil water and the resultant liquid is cooled and given as a drench to expel retained placenta.

Peeling and soaking of the fleshy back of sycamore tree (Billing) in boiled water and give as cooled drench to cure diarrhea and arthritis, crushing and soaking seeds of *Ardeb* (*Kite*) in boiled water and the resultant liquid is cooled and given as drench to cure bloat/constipation.

Also they control ticks infestation by manual removal of the ticks, piercing the ticks with thorn and burning the site to prevent pus formation.

They use hot iron to burn the swollen lymph nodes, and in addition to that they use of sap from a plant known as *Loreng* to burn the swollen lymph nodes.

3.5. The veterinary services

Although there are a lot of veterinarians, still there is no governmental veterinary settled clinic or hospitals in Juba county of Central Equatoria State.

Non Governmental Organization in the field of livestock in the area:

V.S.F. (Belgium, Swiss, Germany).

Norwegian people Aids.

Vet. Work Sudan.

A.D.R.A.

DOT, (Dioceses of Torite).
All the above mentioned organization is adopting the community based animal health system and there was no scheduled vaccination in the state. But right now are expecting to receive 20 mobile clinics for the Ministry of Animal Resources and Fisheries (MARF), Southern Sudan.
Chapter four
Discussion

The overall aim of this study was to avail base line information about the prevalence of the Animal African Trypanosomosis, and to identify morphologically the trypanosomes species in cattle in Juba County, Central Equatoria State Southern Sudan. The study revealed an overall trypanosomosis prevalence rate of (4.09%). This agrees with Kialla (2005). Who found an average infection rate of 3.4%. The rates in that study varied between 5.0%, 4.9% and 2.8%. The results of this study revealed that there is no big difference in trypanosomosis infection rates in different animals age groups. It was clear that, the rate tend to be higher in old animals (25%) than in the younger, while the prevalence of trypanosomosis in animals age from (2-4) is (3.92%), followed by adults age 5-8 years (3.03%). Although the study was a cross sectional, yet the amount of infection was alarming. Kialla (2005). detected 7.5% in cattle up to 2 year of age. She however did not include old cattle. The high infection rate may be due to the lack of veterinary drugs, which lead the livestock owners to use traditional herbs for treatment of their animals, and there is no proper monitoring and supervision. This is typically tsetse transmitted trypanosomosis. The trypanosomes species diagnosed in the three sites of the study area were T.vivax, mixed T. brucei and T. vivax and T. congolence which are either mechanically transmitted by biting flies from Tabanus and Stomoxys species and/or Tsetse flies. These results are in agreement with previous studies by Musa at al., (1990). They considered T. vivax to be the major cause of morbidity and mortality among cattle' in the Sudan. It also confirmed the establishment of T.vivax infection in different parts, of the Sudan much far from tsetse fly belt as reported by Abdoon et al., (2001),
who isolated \textit{T. vivax} from Khartoum, and Sennar States of Sudan. Uilenberg (1998), reported the detection of trypanosomosis infection along the River Nile from Malakal to Khartoum. The detection of \textit{T. brucei} in Juba County may raise alarm as it could be from the sleeping sickness group. Knowing that \textit{Glossina} species are present in the area Mohamed, (2005), it could lead to an outbreak if not properly handled. Also the detection of \textit{T. congoense} is of significances. \textit{T. congoense} is supposed to be the most pathogenic and symptoms may be misdiagnosed as being ECF. Trypanosomosis presence in Southern Sudan has been reported earlier in the 1940s of the last century (EL Karib, 1961). Since that time the trypanosomosis established itself in many areas in Southern Sudan, due to abundance of the biting flies and also due to the improper control measures which includes absence of accurate diagnosis and treatment, the disease continued to pose a threat on livestock in the area. Anemia is Known to be the major characteristic of animals trypanosomosis and the Packed Cell Volume (PCV) value are regarded as a reliable indicator of anemia (Murray and Dexter, 1988). Although there is a difference between the PCV average of infected (20.89%), and non infected (27.29%), but the productivity could be affected and consequently reduced when animals are kept under traditional management conditions and nutritional deficiency and concurrent uncontrolled diseases (Dwinge \textit{et al.}, (1994), such condition could influence PCV values as well, (Hall \textit{et al.}, (1983). But it was clear in this study that positive animals had a lower PCV than negative ones. Furthermore trypanosomosis are known to induce immunosuppression (Clarkson, 1971), which enhances the contraction of other infectious diseases. The effect of nutrition and environmental condition was typically reflected in animals examined during this survey.
Although some animals had high PCV, of over 40, but the majority were within the lower end of the normal range. The results of this study showed no significant difference between infection among males (4.69%) and females (4.08%). This could be explained by the biased sampling since fewer males were investigated due to the fact that livestock owners tend to keep females whereas males were sold with the intention to obtain living necessities in return. Even though, the infection is not expected to vary between sexes, as there is no evidence of sex linkage of infection.

Results obtained in this study confirmed the establishment of the disease in Central Equatoria State, and also proved that, the biting flies (Tabanus, Stomoxys and Glossina), are the most and the only vectors known so far, that are responsible for the transmission of the trypanosomosis in the study area. Other studies (Yasir et al., 2002) had mapped the advancement of Glossina in Central Equatoria State. During this study the trypanosomes were diagnosed by the conventional parasitological methods, which are not sensitive enough to detect low parasitaemic animals, but 9 out of 220 examined animals were parasitologically positive. This indicates that the prevalence could be higher if more sensitive methods were used. As most of the infected animals showed low parasitaemia in the field, this might be the reason for the low trypanosomes infection rate reported during this survey.

(Anma Al-Nor, 1997) in Khartoum State and Babkier (2000) in Gazeira State found similar results when they examined cattle parasitologically, but they obtain high T.vivax infection rate that exceeded 17% by serological examination. Only Stomoxys, Tabanus, were observed and described by Owners, no Tsetse flies had even been described. This does not exclude the
presence of tsetse flies which had actually been reported by other workers. The owners knowledge is not supported by awareness raising efforts by the concerned scientists.

These areas, need evaluation and introduction of modern diagnostic techniques for trypanosomosis and vector control are highly recommended, such as anti-trypanosomal agents, vector control, pour-on or impregnated traps and screens. The responsibility is mainly of veterinary services, which are to be technically supported.

More Studies need to be carried out for determination of the impact of the disease on the livestock, and also to determine the best ways to control the disease, in the study area.

The participatory epidemiological methods are very important and also involvement of the local communities in the control and eradication strategies. It is also an opportunity to introduce new concepts and technologies that can be adopted in the field. It is therefore recommended that:

1- The infection could be of a higher prevalence rate than the one recorded in this study. The incidence should be calculated at different seasons. More detailed studies including incidence studies are recommended.

2- More sensitive diagnostic method to be applied, because the disease is usually diagnosed during the chronic stage.

3- A genuine study of the ethno-veterinary practice in South Sudan is to be conducted. Some remedies could be adopted and refined to be used in modern veterinary medicine.
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