SEASONAL OCCURRENCE OF NON-TSETSE TRANSMITTED TRYPANOSOMOSIS IN MALAKAL COUNTY, UPPER NILE STATE- SUDAN

By

AWNOUR ADENG NGWOUK
B. V. MED. UNIVERSITY OF CAIRO/EGYPT 1992

SUPERVISED BY,
DR. KHITMA HASSAN ELMALIK

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE UNIVERSITY OF KHARTOUM FOR THE DEGREE OF MASTER OF VETERINARY SCIENCES

DEPARTMENT OF PREVENTIVE MEDICINE & VETERINARY PUBLIC HEALTH AND FACULTY OF VETERINARY MEDICINE SEPTEMBER 2006
Dedication

This study is dedicated to the soul of my late father, who reserved no effort to get me where I am now. It is also dedicated to my mother, my brothers and sisters, my son Adeng and to my beloved wife Nyakuna, who all were in support and assistance until this work was finalized.
TABLE OF CONTENTS

Dedication---------------------------------------------------i
Table of contents------------------------------------------ii
List of tables---------------------------------------------iv
List of figures---------------------------------------------vi
List of plates---------------------------------------------viii
Acknowledgement-------------------------------------------ix
Abstract--------------------------------------------------xi

Chapter one---------------------------------------------------1
Introduction-----------------------------------------------1
Chapter two--------------------------------------------------7

Literature review------------------------------------------7
2.1. Trypanosomes-----------------------------------------7
2.1.1. Morphology and classification----------------------7
2.1.2. Transmission and life cycle------------------------9
2.2. The vectors-----------------------------------------11
2.2.1. Biological vectors-------------------------------11
2.2.2. Mechanical vectors-------------------------------13
2.2.2.1. Tabanids-------------------------------------14
2.2.2.2. Muscids-------------------------------------15
2.3. Transmission dynamics-------------------------------16
2.3.1. Essentials for establishment of infection-------16
2.3.2. Experimental transmission of trypanosomosis----18
2.4. Distribution and prevalence of trypanosomosis-----18
2.4.1. Distribution of trypanosomosis in Africa--------18
2.4.2. Distribution of trypanosomosis in Sudan---------20
2.4.3. Distribution of trypanosomiasis outside Africa--22
2.5. Pathogenesis of trypanosomosis---------------------23
2.6. Clinical signs and post mortem----------------------25
2.7. Diagnosis of trypanosomosis------------------------28
2.7.1. Parasitological examinations---------------------28
2.7.2. Biological examinations--------------------------29
2.7.3. Biochemical examinations-------------------------30
2.7.4. Serological examinations-------------------------31
2.8. Impact of trypanosomosis---------------------------33
2.9. Control of trypanosomosis---------------------------34
2.9.1. Control of infection-----------------------------34
2.9.10. Control of the vectors---------------------------36
2.9.11. Use of the innate immunity (Trypanotolerance)--38
2.9.12. Vaccination------------------------------------40
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Contents</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Annual rainfall data in the study area as from 2003 - 2005.</td>
<td>50</td>
</tr>
<tr>
<td>4.2</td>
<td>Number and species of biting flies trapped per month in Detang area during the study period.</td>
<td>55</td>
</tr>
<tr>
<td>4.3</td>
<td>Monthly prevalence of trypanosomiasis in the two selected sites (Malakal and Detang) and the overall prevalence during study period.</td>
<td>64</td>
</tr>
<tr>
<td>4.4</td>
<td>Wet blood examinations according to age group.</td>
<td>65</td>
</tr>
<tr>
<td>4.5</td>
<td>Results of blood examinations using different parasitological methods during, January 2005, May 2005 and August 2005.</td>
<td>66</td>
</tr>
<tr>
<td>4.6</td>
<td>Diseases names as mentioned by the different ethnic groups interviewed during PDS in the study area and their corresponding veterinary terms.</td>
<td>70</td>
</tr>
</tbody>
</table>
4.7 Result of Disease ranking by different ethnic groups according to Prevalence using Pile scoring Method.

4.8 Result of Disease ranking by different ethnic groups according to Importance using Pile scoring Method.

4.9 Disease ranking according to prevalence.

4.10 Disease ranking according to prevalence.
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Content</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Average monthly temperature, Relative humidity and the rainfall data recorded during the study period (Sep. 2004- Aug. 2005).</td>
<td>49</td>
</tr>
<tr>
<td>4.2</td>
<td>Rainfall data in the study area as from 2003 to 2005.</td>
<td>51</td>
</tr>
<tr>
<td>4.3</td>
<td>Monthly abundance of biting flies according to species in the study area.</td>
<td>56</td>
</tr>
<tr>
<td>4.4</td>
<td>Abundance &amp; Species of Biting Flies trapped in Detang area during the study period.</td>
<td>57</td>
</tr>
<tr>
<td>4.5</td>
<td>Relationship between species of biting flies trapped during the study period.</td>
<td>58</td>
</tr>
<tr>
<td>4.6</td>
<td>Percentage of Tabanidae Species trapped during the study period.</td>
<td>59</td>
</tr>
<tr>
<td>4.7</td>
<td>Monthly abundance of Tabanidae flies trapped during the study period in Detang area.</td>
<td>60</td>
</tr>
</tbody>
</table>
4.8 Monthly abundance of Stomoxys flies in Detang area during the study period.

4.9 Correlation between biting flies abundance and trypanosomosis prevalence in the study area.

4.10 Correlation between rainfall, biting flies abundance and prevalence rate in the study area during the study period.

5.11 Disease ranking by different ethnic groups in the study area according to prevalence in Upper Nile-state, Malakal.

5.12 Disease ranking by different ethnic groups in the study area according to importance in Upper Nile state Malakal.


**LIST OF PLATES**

<table>
<thead>
<tr>
<th>Plate</th>
<th>Content</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Modified F3 Traps used for flies trapping in Detang area during the study period.</td>
<td>54</td>
</tr>
</tbody>
</table>

viii
Acknowledgement

Without God’s help nothing could have been achieved, may God accept our effort and bless this work (Amen).

I would like first to express and extend my deepest thanks and gratitude to my supervisor Dr. Khitma Hassan El Malik, Faculty of Veterinary Medicine, University of Khartoum, for the courage and guidance she has been giving to me throughout the days of this study. In fact without her encouragement and support this study would have not been carried out. I am also indebted to Dr. Ahmed Hussein Abdel Rahman in the Central. Vet. Res. Laboratories for his generous and unlimited support provided during this study.

My thanks would also go to Dr. Dominic Peter and Dr. Mansour Issa Bunduki from Veterinary Department and Regional Veterinary Laboratory/Malakal respectively, for the valuable assistance they have been giving to me throughout the study period.

My thanks and appreciation would also go to the chiefs, Deng Maluk and Hamadi Hanu of Dinka herders in Detang area and Messeriya community in Malakal town respectively for the help and
understanding they had towards this study.
I am also very grateful to the Food and Agriculture Organization (FAO), for all the help and understanding I have received particularly from the administration and all the colleagues.
Finally my thanks go to the CAHWs, Obaai and Kwanyireth in Detang village for the assistance they have given.
Abstract

This study was conducted in two locations in Makal County in Upper Nile state, Sudan. This is an area believed to be transitional between the tsetse zone and the fringe of the semi-arid zone. The aim of the study was to determine the incidence and species of circulating trypanosomes in the area. Total of 34,664 biting flies were caught using five modified F3 traps. Tabanidae were 99% and Stomoxys 1%. The most abundant fly caught was A. agrestis 65% followed by T. taenula 34% then Stomoxys 1%. Both A. agrestis and Stomoxys peaked in September while T. taenula peaked in November. No Glossina flies were trapped. 200 blood samples from randomly selected sedentary Nilotic cattle from different age groups were examined monthly for trypanosomes presence for twelve months, using wet mount technique. 422 out of 2,400 samples examined were found positive giving a prevalence rate of 17.6%. Of the positive animals, 65% were under 4 years old. The incidence rates ranged between 8% and 29% in March and October respectively.
The prevalence rate using Buffy coat examination was 63.5%. Morphologically, the only *Trypanosoma* species detected in the two locations was *T. vivax*.

Participatory techniques used revealed cattle owners knowledge of the disease to be extensive, ranking it second in prevalence 29% after CBPP 33% and third in importance 19% after CBPP 32% and Rinderpest 21%.

This study provided information on non-tsetse transmitted trypanosomosis in the area and the factors contributing to its occurrence. Positive correlation between rainfalls, biting flies’ abundance and the prevalence rate was evident. However it is suggested that more work has to be done to cover other areas not covered by this study, which include the socioeconomic impact of the disease in the area and a practical package for disease control to be tried in a participatory manner that would involve the livestock owning communities.
"From the beginning of Arab and European influence in the hinterland of tropical Africa, Trypanosomosis of man and animals has curbed the realization of human ambitions and the mobilization of the continent vast resources".

This is a quotation from Herbert. S. Gasser (1963), which captures the importance of African Trypanosomosis and its influence on the life and livelihood of Africans. Trypanosomosis has always been a major barrier to economic and social development of the African Continent. It is a disease complex that affects a wide range of mammals. The disease is caused by blood-borne protozoa called trypanosomes, which occur in blood of a wide range of vertebrates from fish to mammals (Kettle, 2000). Few may also invade tissue cells (Soulsby, 1968).


In Africa, the problem of animal trypanosomosis (also called nagana) was recognized by African stockman
long before the cause of the disease was known, and many pastoralists have always associated the disease with the presence of tsetse flies. *Trypanosoma* species responsible for animal trypanosomosis include the pathogenic trypanosomes, *T. congolence*, *T. vivax* and *T. brucei*. All are transmitted cyclically by several tsetse flies each of which is adapted to different climatic and ecological conditions (Ford 1971 cited by Okeleng 1996). Some *Trypanosoma* species have lost their ability for biological transmission and have completely adapted themselves for mechanical transmission. These include *T. evansi*, which is mechanically transmitted by biting flies (*Tabanus* & *Stomoxys*) and *T. equiperdum*, which is transmitted venereally. *T. vivax* is transmitted both biologically and mechanically.

Tsetse flies are only found in the African continent inhabiting a very wide area that involves more than 37 countries representing about 1/3 of the continent. In Africa tsetse flies (*Glossina* species) are considered to be the principal vectors of trypanosomosis. They are responsible for biological transmission of the disease, in which the parasites undergo cyclic transformation before they could be infective. The tsetse belt covers about 40% of tropical Africa, an area of 10 million square km, being larger than the United States of America, but
it is not in a uniform sufficient density to pose a fly problem (Kettle, 2000). Outside Africa and outside the tsetse belt, biting flies are been incriminated for being the transmitters of the disease. They transmit the disease mechanically whereby the parasite does not undergo cyclical development inside the flies. Mechanical vectors include various species of biting flies from the *Tabanus* and the *Stomoxys* genera. This usually occurs in case their feeding is interrupted. Tsetse flies are also capable of transmitting the trypanosomes mechanically.

Animals infected with one or more of the pathogenic Trypanosomes suffer from acute infection that can result in death within several days or weeks or in chronic cases, infection persists for months or even years ending by animals becoming carriers for the disease. Clinically infected cattle suffer from anemia, emaciation with good appetite, reproductive impairment and intermittent fever characterized by a wave-like pattern as a result of domination of one variant during each parasitaemia (Kettle 2000). The degree of anemia is determined by the level and duration of parasitaemia, which in turn is dependent on the species and particular serodeme of the trypanosome involved (Abdalla, 1996).
The history of trypanosomosis in Sudan goes back to the beginning of the Twentieth century. The disease was first reported by the Sudan Veterinary Department as early as 1904. It has been regarded by Anon (1904) and El Karib (1961) to be the most serious disease problem of animals in Sudan. Since early sixties up to the moment, extensive work had been done in the Sudan on the disease. These included work done in Darfur region by ElKarib (1961), Abdalla & Ismail (1972), Hall et al., (1983) and Rahman (2002), in Kurdofan by Anon (1973) & Hall et al., (1990-1991), in Kassala & Blue Nile by Abdalla (1996), Rahman (2002), in Sennar by Suleiman (1992), Homeida (1993), and Rahman (2002), in Kosti & Eldoeim by Homeida (1993) and in Khartoum by Ahmed (1997) and Rahman (2002). Results obtained from these studies were different, with prevalence rates varying from area to area. The most obvious result was the correlation between density of biting flies (flies abundance), rainy seasons and incidence of trypanosomosis (Abdalla, 1996).

Uilenberg (1998) reported that he had diagnosed T. vivax in late 1950s in sedentary cattle far away from the tsetse belt, along the White Nile from Malakal to Khartoum.

Diagnosis of trypanosomosis is exclusively dependant on the detection of the trypanosomes in the blood of
the affected animal. This is through examination of wet blood and other body fluids preparation, blood preparations stained by Geimsa and Buffy coat preparations under the microscope. Recently other screening methods are being used indirectly for the detection of the parasite through detection of either their antibodies (serology) such as ELISA and CATT or their antigens like PCR and DNA probes.

The economical impact of trypanosomosis is represented in that it has inhibited the development of livestock production industry in much of tropical Africa (Uilenberg 1998). Trypanosomosis is one of the major constraints on animal production in areas of Africa that have greatest potentiality for significant increases in livestock population and livestock productivity (Guy d’leteren & Kimani 2004), and even cattle-ranching is impractical in much of Africa Savana land. It also causes sever diseases in both human (Sleeping Sickness) and domestic animals (Nagana).

Livestock owners in Upper Nile area have always been ranking animal Trypanosomosis to be among the first five diseases prevailing in the area, (Awnour & Jacob 2003). This has resulted in a wide-spread usage of anti-trypanosomal drugs mainly Homidium salts (chloride and bromide), Diaminzene Aceturate and others. In most cases this is done without examining
the blood for the presence of the parasite, a situation that may result in drug waste as some of the assumed to be trypanosomosis may actually be other wasting diseases. In fact no specific studies were previously carried out on that particular subject in the study area, as a result of this ambiguity, this study is been planned with the objective of clarifying whether trypanosomosis is a real problem in the area as stipulated by cattle owners and to what level is it prevailing. The study would be carried out among resident Nilotic cattle and specifically is designed to look into the following objectives:

1. To determine the prevalence and incidence rate as well as the causing agent of trypanosomosis in Malakal County, Upper Nile state.

2. To study the correlation between the biting flies abundance and the incidence of trypanosomosis in the study area.

3. To assess the validity of Participatory Epidemiology as an effective method for assessing disease prevalence.
CHAPTER TWO
LITERATURE REVIEW

2.1. The Trypanosomes

Trypanosomes are blood-borne protozoa that belong to the Genus *Trypanosoma*, Family *Traypanosomatidae* and Order *Kinetoplastida*. They inhabit blood and tissue fluids of wide range of Mammals, both domesticated and Wild and including Man. Some trypanosomes may occasionally invade body tissues (Soulsby, 1968 and Uilenberg, 1998).

2.1.1. Morphology & classification

Trypanosomes are elongated unicellular organisms mostly leaf-like in shape containing a nucleus which is more or less situated centrally. They vary in size from 8 – 50 micron. They are obligatory parasites of body fluids especially blood and tissue fluids of many vertebrate hosts plus the digestive tract of invertebrate host, which is mainly a biting fly, Abdalla (1996). A Trypanosome possesses a single flagellum, which originates from a small granule (the flagellar pocket near to Kinetoplast) near the posterior end of the body. The flagellum runs anteriorly attached to a fold of pellicle called the undulating membrane. It is the motor organ of the organism by which it moves forward. When stained with
a Romanowsky stain, the cytoplasm stains purple or blue while the nucleus and the kinetoplast stain red. Mammalian Trypanosomes were classified by Hoare (1957 and 1972) into two sections depending on the site of development of the trypanosome in the vector and mode of transmission as follows:

<table>
<thead>
<tr>
<th>Section</th>
<th>Subgenus</th>
<th>Type Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Salivaria</td>
<td>i - Duttenlla</td>
<td>T. (D) vivax</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. (D) uniforme</td>
</tr>
<tr>
<td></td>
<td>ii - Nannomonas</td>
<td>T. (N) congolense</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. (N) Simiae</td>
</tr>
<tr>
<td></td>
<td>iii - Trypanozoon</td>
<td>T. brucei brucei</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. (b). rhodesiense</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. (b). gambiense</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. (b). evansi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. (b) equiperdum</td>
</tr>
<tr>
<td></td>
<td>iv - Pycnomonas</td>
<td>T. (p) suis</td>
</tr>
<tr>
<td>2. Stercoraria</td>
<td>i - Scizotrypanum</td>
<td>T. cruzi</td>
</tr>
<tr>
<td></td>
<td>Ii - Megatrypanum</td>
<td>T. theileri</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. ingens</td>
</tr>
</tbody>
</table>

The Salivarian trypanosomes are those transmitted by insect vectors through saliva either cyclically by the tsetse flies (*Glossina* species) or mechanically by other biting flies from the *Tabanidae* and *Muscidae* species. The subgenus *Nannomonas* contains group of small trypanosomes with medium - sized marginal
kinetoplast, no free flagella and poorly developed undulating membrane. While members of subgenus *Duttonella* are group of trypanosomes with large terminal kinetoplast, distinct free flagella and inconspicuous undulating membrane. Under the wet-mount blood smears members of this group are very active. The subgenus *Trypanozoon* includes the *Trypanosoma* 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. All these forms are encountered during the infection.

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). They are large parasites, which gained their importance in the fact that they can confuse parasitological diagnosis of the pathogenic trypanosomes. Example of these trypanosomes is the *T. theileri*, which occur in cattle all over the world and transmitted by tabanids flies and probably also by ticks (Uilenberg 1998).

### 2.1.2. Transmission and life cycle

In Africa, the major pathogenic *Trypanosoma* species in livestock are transmitted by tsetse fly (genus
Glossina) and include T. congoensis, T. vivax, T. brucei brucei and T. simiae. The subspecies of T. b. brucei, i.e. T. b. rhodesiense and T. b. gambiense cause sleeping sickness in man.

The life cycle of trypanosomes depend mainly on the means of transmission. In biological transmission, which is mainly associated with the tsetse flies Glossina species, the infected fly injects metacyclic trypomastigotes when it feeds on host blood, then the metacyclic trypomastigotes transform into trypomastigote and reproduce asexually in the blood stream. When another non-infected fly come and feeds on the infected blood it ingests trypomastigotes, which reproduce asexually in the fly’s gut, migrates to fly’s salivary glands, and transforms into infective metacyclic trypomastigotes. In case of mechanical transmission the trypomastigotes are transmitted from host to host via contaminated mouth parts of the vector. In this case there is no development of the parasite in the vector.

Abdel Karim (1989), observed that blood meal analysis of engorged tsetse flies showed mixed meals from wide range of hosts including horses, donkeys, bovine, camels, dogs, sheep, goats and other mammals.
2.2. The Vectors

2.2.1. Biological vectors

Tsetse belt in Africa covers an area of about 10 million km² i.e. around 1/3 of the continent between latitudes 15° N and 25° S (Hoare 1957, 1972). The area extends from Ethiopia in the east through southern Sudan, Northern Kenya, Uganda, southern Chad, Republic of central Africa and Niger, in central Africa to southern Mali, Mauritania, Senegal, and Gambia. In all these countries, tsetse flies are considered to be the major vectors for the transmission of trypanosomosis.

Both sexes of tsetse flies are haematophagous and therefore are potential vectors of trypanosomosis to man and domestic animals. They belong to the genus *Glossina*, which is virtually confined to the Afrotropical region although Elsen et al., (1990) have recently found two species in south-west Saudi Arabia. The tsetse flies when infected become infective throughout their life. The genus *Glossina* contains 23 species and 8 subspecies (Uilenberg 1998).

Host finding by tsetse flies involves visual and chemosensory stimuli. They can locate hidden hosts from distances of 50 m or even 90 m (Kettle 2000). They have a clear host preference when obtaining
their blood meals, for example the savannah species of *Glossina (moristans group)*, which feeds mainly on mammalian hosts particularly bovids and suids, are considered to be the better vectors for pathogenic trypanosomes. A blood meal analysis showed that 4 games animals (wart hog, bush buck, bush pig and kudu) are the preferred animals by tsetse flies (Nyindo 1992).

In Sudan tsetse surveys had been started early in the beginning of the twentieth century. They occupy an area of about 300,000 km² of the good fertile soil. The nomadic livestock used to be around this area in the dry season (more than five months) posing them to be vulnerable to trypanosomosis infection, which they disseminate to the sedentary cattle back home on return during the dry season, through the abundant biting flies making 90% of Sudan livestock at trypanosomosis risk (Rahman 2002). Lewis (1949) and (1950) had described tsetse belt in Sudan to be like a horse shoe around the Nile swamps with three gaps, the valley of Bahr Elghazal, the arid country east of Kapoeta and the Sobat area where the tsetse fringe passes through Ethiopian territory. He reported occurrence of seven species in the country, which include:

*G. m. submorristans, G. f. fuscipes, G. tachinoides, G. longipennis, G. fusca fusca, G. pallipedes, G.*

2.2.2. Mechanical vectors

The role of mechanical vectors in the transmission of African livestock trypanosomes has always been controversial relative to tsetse flies (their cyclical vectors), Desquesens and Dia (2003). Many flies have been incriminated for having being mechanical vectors for trypanosomes infection. These include the tabanids and the muscids. This had made El Karib (1961) to consider trypanosomosis in the Sudan to be essentially a problem of mechanical transmission, with particularly the tabanids playing a major role (Okeleng 1996). Foil (1989) revised the role of tabanids as vectors of viral, bacterial, protozoal and filarial pathogens. Rahman (2002) also reported that, high disease prevalence coincided with the periods of high abundance of Tabanids and Stomoxys flies in studies carried out in Blue Nile area between 1992 and 1994.

2.2.2.1. Tabanids

Foil (1989) reported 37 genera and 4254 species of tabanids all over the world (Rahman 2002). In the
Sudan Lewis (1953) had identified (75) species of tabanid flies in different locations of the country. More four species were added by Yagi (1968). From that time more studies were carried out by many researchers on the tabanids and their relationship to animal trypanosomosis (Yagi and A/Razig 1972b, Hall et al., 1984, Abdel Karim 1980, Hall et al., 1990-91, Suliman 1992, Abdalla 1996 and Ahmed 1997). Areas of tabanids distribution in Sudan were described by Abu Shama (1993), to be extending from El Gadarif in the east passing through Sennar up to South Kordofan in the west. Reid 1957, studied the abundance of Tabanids in southern Sudan using Harris traps (Ahmed 1997). Although ten species were trapped, only $T.\ taeniola$, $T.\ par$ and Atylotus species were caught in high numbers.

The role of tabanids as vectors of diseases was reviewed by Krinsky (1976). Although they are widely incriminated for being vectors of many viral, bacterial and protozoa diseases, yet only 5 studies were carried out on their role in disease transmission. Most females of tabanids feed on blood so they are the ones responsible for trypanosomosis disease transmission. Species of Tabanus were more efficient vectors than those of Chrysops and Haematopota, and all tabanids are better vectors than mosquitoes or biting muscids, such as Stomoxys. It is
being believed that \textit{T. evansi} and \textit{T. vivax} are transmitted mechanically, and tabanids are the most important of the mechanical vectors, but it is more difficult to evaluate the importance of mechanical transmission where there are alternative routes of infection (Kettle 2000). Mechanical transmission of \textit{T. vivax} by \textit{T. importunus} was successfully carried out experimentally in French Guiana. Zebu bull calves were inoculated with a \textit{T. vivax} isolate originally from French Guiana and they were kept together with the recipient calves in boxes, then all were subjected to \textit{T. importunus}. Ten days later, \textit{T. vivax} was found in blood of the recipient calves (Raymond 1991).

2.2.2.2. Muscids

Lewis (1954) gave a full account on the \textit{Muscidae} in the Sudan, where he mentioned thirteen species of \textit{Musca}, three species of \textit{Stomoxys} and two species of \textit{Haematobia}. The implication of these flies on disease transmission was discussed by many authors including Stealman (1976), Burg (1990) and Suliman (1992). Buxton (1955) commenting on outbreak of trypanosomosis among Shilluk cattle in Upper Nile (south Sudan) in 1949 had directed the attention to the presence of muscids other than \textit{Glossina} that suck blood, and their possible role in transmission of
trypanosomes. Ahmed (1997) cited Field (1966) referring to the effect of the non-biting haematophagus Diptera and their significance in transmitting diseases. He was able to record that as they congregate on remnant drops of blood made by other biting flies especially tabanids, when they feed on parasitaemic animals, they were able to suck and pass motile trypanosomes to non-infected animals in their saliva. Stomoxys, being persistent biters, which are often engaged in interrupted feeding together with the fact that they feed more than once per day, fits them to be mechanical vectors of blood dwelling pathogens including Trypanosomes. They contribute to the spread of T. evansi, which causes the disease surra in a wide range of hosts (Kettle 2000).

2.3. Transmission dynamics
Transmission of pathogenic trypanosomes to mammals occurs mainly through vector insects, where transmission is either cyclical or mechanical.

2.3.1. Essentials for establishment of infection
Many factors contribute to the establishment of trypanosomal infection but the presence of tsetse vectors headed all. This was proved by a study carried out in Malawi, whereby trypanosomal
infections were detected in cattle sampled adjacent to known tsetse foci (Van den Bossche et al., 2000). In a study carried out in Ghide valley, southwest Ethiopia, it was found that a relationship is present between the tsetse relative density, proportion of feeds taken by the tsetse and the seasonal changes. These factors were all related to the monthly rainfall (Leak et al 1993). Host preferences also play a great role in transmission and distribution of tsetse-born trypanosomosis. Magona et al (2000) in Uganda reported that trypanosomosis disease in T. vivax infection is associated with the age of the animal while the opposite is true for animals infected with T. congolense.

The epidemiology of non-tsetse transmitted trypanosomosis (T. evansi and T. vivax) is influenced by many factors. There may be seasonal outbreaks, where the populations of biting flies (Tabanids, stable flies, etc.) are influenced by important climatic differences. In the dry season the chronic disease become more apparent as a result of immuno-depressive factors such as the poor nutritional status of the animal, which diminish its defenses. The epidemiology is also greatly influenced by host preferences and diurnal (daily) behavior patterns of the various local species of tabanids and other biting flies (e.g. whether the hours that they are
active allow much contact with livestock or not), (Uilenberg 1998).

2.3.2. Experimental transmission of trypanosomosis
Abdoon et al., (2001) studied the possibility of propagating T. vivax in white mice. They concluded that the stocks in study were poorly transmissible between rodents. However a successful demonstration of T. vivax transmission by tabanid flies (A. agrestis) was carried out experimentally in Borkina Faso. The study proved un-equivocally the role of tabanids in mechanical transmission of T. vivax (Desquesens and Dia, 2003).

2.4. Distribution and prevalence of trypanosomosis
Distribution of African Trypanosomosis in both animals and man is more or less related to the distribution of the tsetse fly vector; however trypanosomosis in domestic animals may be extended beyond tsetse belts in the presence of large numbers of biting flies. Horse flies (Tabanus) and stable flies (Stomoxys) are particularly important as mechanical vectors (Uilenberg 1998).

2.4.1. Distribution of trypanosomosis in Africa
The impact of trypanosomosis extends in sub-Saharan Africa over (10) million km square (a one third of
the continent). Of these, some (3) million are covered by equatorial rain forest; the remaining area contains some very good grazing areas. The distribution of African Animal Trypanosomosis (Nagana) coincides largely with the distribution of its biological vectors, the tsetse flies and the disease tends to die out in their absence. However some trypanosomine species have adapted themselves for mechanical transmission resulting in extension of their infection beyond the tsetse belts. For example *T. vivax* in Ethiopia is commonly found in highland too cold for tsetse survival. Also *T. evansi* has been spread widely by biting insects outside tsetse infested regions in Africa. It is present in tropical and sub-tropical areas of Africa north of the equator. It is also relevant to mention that in the past *T. vivax* has been present on the Indian Ocean island of Mauritius, without tsetse, but has been eradicated (Uilenberg 1998).

Epidemiology of bovine trypanosomosis was investigated parasitologically and serologically, in two districts of Northern Ghana. The results revealed that the parasitological and serological prevalence of bovine trypanosomosis was significantly higher in (16%, 53% and 8%, 24% respectively) in the two districts (Mahama et al., 2004). In Zambia (Sinyangwe et al., 2004) found the overall mean prevalence of
trypanosomosis to be 14.4%, with 96% infection caused by *T. congolense*, *T. evansi* 2% and *T. brucei* 2%. Nyeko et al., (1993) carried out trypanosomiasis surveys in some parts of Uganda and found a prevalence of 8.6%. Another cross-sectional study was undertaken by Wasiwa and Katunguka-Rawkishaya (2004) in south-western Uganda in which blood samples were examined from 10 different localities by wet blood smears, thin stained blood smears, and by Buffy coat examination of centrifuged capillary blood samples. The prevalence was found to be 6.42%. Among the positive 84.5% were infected with *T. vivax*. In Chad, prevalence of *T. evansi* infection (Sura) was studied and a prevalence rate of 5.3% was reported using Buffy coat technique and 30.5% with serology. (Arnaud and Abdesalam 2004), the real prevalence was estimated at 16.9% ± 1.4 (α 5%). In a study in Ethiopia the overall prevalence of bovine trypanosomiosis was found to be 15%. Among the positive animals 71.1%, 28.4% and 0.6% were due to *T. vivax*, *T. congolense* and mixed infection (*T. vivax* and *T. congolense*), respectively (Kidanemariam et al. 2002).

2.4.2 Distribution of trypanosomosis in Sudan

Tsetse fly distribution in the Sudan was documented by many researchers (Ensor, 1908, Archibol, 1927,
Lewis 1949 and 1950, Abdel Razig et al., 1977) as cited by Abdalla (1996). Yagi and Razig ((1972) noted changes in the tsetse belt beyond the previously known limits to new areas northward. This was confirmed later by Hall et al (1984). Trypanosomosis outside known tsetse belt in Sudan was first described by Anon (1904) in Bahr Elghazal in cattle brought from Upper Nile. Anon (1947) reported diagnosis of T. congolense at Malakal Laboratory in 1943. Elkarib (1961) attributed the out break that occurred in 1946 to infection with T. congolense due to mechanical transmission. El Karib (1961) and Musa et al., (1990) considered T. vivax to be a major cause of morbidity and mortality in cattle in Sudan. This goes together with the findings of Hall et al., (1983), who found T. vivax to be the predominant infecting species after they have examined blood samples from over 4,000 migratory cattle during a survey in southern Darfur. Hall et al., (1990) found T. vivax predominating in herds in south Kordufan, which increased significantly as they move away from the known tsetse foci. Abdel Razig et al., (1977) recorded 7.1% trypanosome infection rate in Sudanese zebu cattle in Wadi Umbelasha in south darfur. Tagelsir (1992) reported that T. vivax in recent years became a real constraint to livestock production especially in dairy farming. Anoon (1993)
reported a trypanosomosis prevalence of 52.8% in Singa area in cattle infected with *T. vivax*. Uilenberg (1998) stated that he has diagnosed *T. vivax* in the late 1950s in sedentary cattle herds all along the White Nile from Malakal in southern Sudan up into the semi-desert of Khartoum province, hundreds of kilometers from any tsetse belt. He also reported the probability of presence of *T. equiperdum* in the Sudan. El Malik and Elnasri (2005) have reported 10% infection with *T. vivax* in Unity state in a study carried out in 2003-2004. Prevalence Abdoon et al., (2001) isolated 5 stocks of *T. vivax* from different areas in Sennar and Khartoum states of the Sudan. Saeed et al., (2002) showed that *T. evansi* infection is endemic among camels in Butana plain, eastern Sudan. Boxton (1955) cited by Abdalla (1996) attributed trypanosomosis outbreak in Upper Nile in 1949 to be related to *Muscidae*. This out break was reported by El karib (1961) to have had killed over 50% among Shilluk cattle in Upper Nile, south Sudan (Rahman 2002).

### 2.4.3. Distribution of trypanosomosis outside Africa

*T. evansi* and probably *T. equiperdum* which appear to have been derived from *T. brucei* have adapted to mechanical and venereal transmission respectively. *T. evansi* has been spread widely by biting insects
outside tsetse infested regions in Africa, and also outside Africa; it is present in Asia and in South and Central America from Panama to Argentina. *T. equiperdum* infection, as a venereal disease is even less restricted by climate and in the past has spread as far as Russia in the northern hemisphere, and as far to the south as Chile and South Africa. It has been eradicated from North America and most of Europe, but has made a sort of comeback or rediscovered in Europe (Italy, Russia and other countries) as it is still present in parts of Asia including Uzbekistan and China. No reliable information from South America though sometimes its presence had been reported, (Uilenberg, 1998).

2.5. Pathogenesis of trypanosomosis

When the tsetse fly injects infective metacyclic trypanosomes into the skin of the host, there is a phase of local inflammation and a swelling, which lead to a so-called chancre development. The metatrypanosomes divide and multiply in the chancre and give rise to the typical blood forms which invade the lymphatic vessels and nodes, and then the blood stream. Trypanosomosis like any other infectious diseases starts with a rise of body temperature (hyperthermia). This results from the contact between the multiplying trypanosomes in the host and the
defense system of the host (Uilenberg, 1998). The surface proteins of the trypanosomes provoke the host in making specific antibodies against these proteins, and after few days almost all of the trypanosomes in the blood are destroyed by these antibodies and the body temperature drops. However, few parasites survive as they have been able to replace their surface proteins by different ones, against which the antibodies cannot act. These trypanosomes are able to multiply and cause a new peak of parasitaemia and hyperthermia, until the body of the host makes specific antibodies against the new surface proteins. This process continues for a long time as the trypanosome is able to make an almost unlimited number of antigenic variants, and the host will continue responding to each of them, until either the antigenic repertoire of the trypanosome is finally exhausted, in which case self-cure of the host follows, or the ability of the host to react to all of the antigenic variants is overwhelmed, and the host dies (Uilenberg, 1998).

The mean PCV of animals infected with trypanosomosis is always less than that of the normal animals, (Wasiwa and Katunguka-Rwakishaya 2004). The most significant and complicating factor in the pathogenesis of trypanosomosis is the profound immunosuppression. This marked immunosuppression
lowers the host’s resistance to other infections and thus leaving them vulnerable to secondary infection, which greatly interferes with vaccination against other diseases (www.vet.edu/gray_book), Verloo et al., (2000) and Onah et al., (1993). Infection of animals with chronic T. brucei, with T. congolense leads to increased parasitaemia of T. bucei (Van den Bossche 2004). Concurrent infection of Trypanosomosis and Sarcocystosis was reported to have resulted in further pathologic effects on experimental calves than if by trypanosomosis alone (Rahman et al., 2001). Okeleng (1996) studied effect of concurrent infection with T. evansi and Haemoncus contortus on Sudanese indigenous goat. The infected goats with Haemonchus contortus showed sever decline in PCV, Hb concentration and MCHC. The goats died earlier than those with single T. evansi infection. Changes in serum protein (particularly Igm) of trypanosome experimentally infected Sudanese camels were reported. It was found that they reached twice their pre-infection levels (Boid et al., 1981)

2.6. Clinical signs and Post Mortum
Clinical signs vary mainly depending on the degree of challenge, host susceptibility and the virulence of the infecting trypanosome strain. Van den Bossche et
al., (2006) isolated 31 strains of *T. congoense* of different strains in cattle in Zambia. The highly virulent strains had median survival time of infected animals, ranging from 5 to 9 days, moderate between 10 to 30 days and low virulent strains of more than 30 days median survival time.

After inoculation, the pathogen goes through an incubation period that ranges from few days to few weeks, depending on the infecting trypanosome species, then fever develops.

Typically trypanosomosis is a wasting disease in which there is slow progressive loss of condition accompanied by increasing anaemia and weakness (although the appetite is good), to the point of extreme emaciation, collapse and death). However there is variation from acute disease in pigs caused by *T. simiae* to the usually mild condition caused by *T. brucei* and *T. evansi* in cattle (Uilenberg 1998). Subcutaneous oedema as well as symptoms of CNS infection is oftenly seen in horses infected with trypanosomes of the subgenus *Trypanozoon* (*T. brucei, T. evansi and T. equiperdum*) (Losos and Ikede (1972). In Asia *T. evansi* was reported to had caused abortion in Buffaloes (OIE 2000). Some *T. vivax* strains were reported from east Africa causing acute disease in cattle, with what is known as haemorrhagic syndrome,
which is characterized by fever, anaemia, generalized haemorrhage of the viscera and mucosal surface and even blood stained diarrhea and death 2-4 days after infection (Rahman 2002) and Abdalla (1996). *T. evansi* is reported to produce mild disease in sheep, goats, cattle and donkeys (Mahmoud and El Malik 1978 and Mahmoud and Gray 1980). El Qarawi et al., (2004) studied the effect of trypanosomosis infection on reproduction. They demonstrated deterioration in semen characteristics in naturally infected dromedary (*camelus dromedaries*) bulls and a significant decline in testosterone plasma level.

No pathogonomic lesion is seen in trypanosomosis infections, however pale mucus membranes (due to anaemia), Oedema and serous fat atrophy are commonly seen. Ascites, hydropericardium and hydrothorax are also noted beside spleenomegally, hepatomegally and enlargement of the Lymph Nodes in the acute disease. Blood microscopic examination show regenerative changes as it tries to compensate the lost blood due to infection (anisocytosis, normoblasts and basophilic punctuations). In chronic trypanosomosis, the carcass is emaciated and often dehydrated. The skin may show pressure sores and ulcers and closely adherent to the underlying muscles and bones. Other
organs tend to be normal or smaller in size (Uilenberg 1998).

2.7. Diagnosis of trypanosomosis
Diagnostic procedures for trypanosomosis vary not only according to the tools available but often even more to what one wishes to know (Uilenberg, 1998). For instance if the purpose for diagnosis is for treatment, then its not necessary to know the species causing the disease since the control measures for all trypanosomes species are generally similar.

2.7.1. Parasitological examinations
The aim of these examinations is to demonstrate presence of the parasite in the blood and/or other body fluids of the host directly by using different microscopic examinations, which include; wet blood examination in which a drop from fresh blood preferably from the ear vein (Greig et al., 1979), is put between a glass slide and a cover slip then examined using medium magnification. Trypanosomes are seen either directly wriggling between the blood cells or indirectly as they actively agitate the blood cells. Thick and thin blood and lymph smears stained with Geimsa are also used in addition to the concentration method (Woo 1970). However wet blood methods alone were proved not enough to be used for
the diagnosis of trypanosomosis (Robson and Ashkar, 1972). The Buffy coat examination method (haematocrit method) is considered to be more sensitive and reliable than the other direct microscopic examinations even if parasitaemia is low as 5 trypanosomes/ml blood (Woo 1970, Kalu et al 1986). However its efficiency in low parasitaemia is in question. Mustafa (2004) considered parasitological tests insufficient to detect trypanosomes recommending more sensitive tests to be used. (Woo (1970) and Singh (1971) found that thick smear examination is more sensitive than thin smear examination when parasitaemia is low in T. evansi infection (Abdalla 1996). Bruce 1910 indicated that T. vivax can be diagnosed based on its distinctive motility (cited by Rahman 2002).

2.7.2. Biological examinations

Laboratory Animals inoculation was used for diagnosis of trypanosomosis and it proved some value (Godfrey Killick-Kendrick 1962. Baker (1970) indicated that best animals to be used for different trypanosomes isolation include goats for T. vivax and rodents (mice and rodents) for T. brucei, T. conglenese and T. evansi. In Uganda 45 trypanosome isolates, were sub-inoculated into laboratory mice and 21 isolates produced patent parasitaemia (Nyeko et al., 1993).
However, due to infrequent availability of these animals in the field they have not been used regularly in the field. Some researchers concluded that use of laboratory animals for diagnosis of trypanosomosis is of no value due to difficulty of handling them under field conditions and presence of some refractory species or strains of trypanosomes, in addition to the long time required for getting results (El Malik, 1976). Some species like *T. vivax* are difficult to be dealt with in the laboratory due to their inability to adapt to laboratory animals. However Leeflang (1976) isolated 3 strains of *T. vivax* naturally infective to mice and are now in world-wide use for experimentation in rodents.

2. 7. 3. Biochemical examination

Abdalla (1996) and Mustafa (2004) cited a good number of chemical tests that had been used for detection of *T. evansi* infection, which depended on the increase in serum globulin content. They include the Mercuric Chloride Test MCT (Bennett and Keeny 1929), the Formol Gel test FGT (Plantureux 1923 and Knowles 1927) and the Thymol Turbidity Test TTT (Abdel Gaffar 1960. All these tests/methods were not specific for trypanosomosis hence were not reliable for accurate diagnosis (Pegram and Scott 1976, El Malik 1976, Hakimdar 1987 and Oshake 1988).
2. 7. 4. Serological examination

Many indirect or serological methods were used for the diagnosis of trypanosomosis. They assess the presence and level of circulating antigens and/or antibodies in the blood of the host. They include Complement Fixation Test (CFT) Schoening (1924), Sabanshiev (1973), Indirect Haemagglutination Test (IHATT) (Jatkr and Singh, 1971), Precipitation Test (PT) (Bansal and Pathak, 1971), Latex Fixation Test (LFT) (Mohammed and Kreier, 1972, ISCTRC 1995).

Immunofluorecent Antibody Test (FAT) and Enzyme Linked Immuno-Sorbent Assay (ELISA) were used for diagnosis of both human and animal trypanosomosis (Mahmmoud and El Malik 1978, Molyneux 1975, Luckins et al., 1978). Gibson (1994) considered Polymerase Chain reaction test (PCR) to be a highly sensitive test for detection of trypanosomes in very small numbers in the host or fly mouth parts. Majiwa PA., et al., (1994), has used non-radioactive DNA probes in combination with polymerase chain reaction (PCR) to detect African trypanosomes in the blood of mammalian hosts and the saliva of live tsetse flies. Salano and Amester-Delafosse (1995) used PCR for detection of *T. congolense* in *T. taeniola* and *A. agrestis* in Burkina Faso (Mustafa 2004).
In Egypt, while parasitological examinations failed to detect *T. evansi* infection in 200 blood samples from water buffaloes, 24% were found positive when Card Agglutination test (CATT.*T. evansi*) was used (Hilali et al., 2004). Reid and Copeman (2003) compared ELISA using AS 40-50% fraction with two commercial tests: the Card Agglutination Test for Trypanosomosis (CATT/*.evansi*) and Suratex. They found that CATT/*.evansi* at ¼ serum dilution had higher sensitivity and the ELISA had higher specificity. They recommended that there is no likely benefit in combining antibody detection tests to improve the accuracy of diagnosis. Mustafa (2004) detected 44% *T. evansi* infection in camels and 20% in sheep using CATT/*.evansi* while only 11% were detected in camels using microhaematocrit centrifugation technique (MHCT) and it failed to detect any in the sheep.

2.8. Impact of trypanosomosis

Trypanosomosis has been a serious constraint to agriculture development and human settlement in Africa since the turn of the century (FAO 1992). Being associated with tsetse flies, it prevent utilization of potential resource areas in the African continent. A considerable concern was that presence of tsetse flies in Okavango District, Botswana would result in return of human sleeping sickness which could affect the tourist industry in the area (over $10 million per year), (PAAT 2000). In susceptible cattle breeds, it has been reported that trypanosomosis reduces the calving rate by up to 20%, and causes the deaths of another 20% of calves that are born even in the trypanotolerant animals FAO (2005). In Gambia studies indicated that trypanosomosis reduces milk production by 26%, and reducing lambing and kidding rates by as much as 37% (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 that those in low risk ones. The negative impact of trypanosomosis on livestock production and livelihood of vast majority of livestock keeping populations in Sudan as well as elsewhere in Africa can not be escaped.
2.8. Control of trypanosomosis

Control of trypanosomosis in animals is done either directly through controlling the infection (causative agent) or indirectly through control of the fly vectors (Glossina and other biting flies). Also rearing of trypanotolerant livestock breeds is now gaining ground.

2.8.1. Control of the infection (causative agent)

Chemotherapy is being widely used based on usage of various types of trypanocidal drugs. Most of these trypanocidal drugs have been in used for many years their effectiveness has widely reduced and trypanosomes developed what is known as drug resistance (Luckins 1999, El Rayah 1992). Trypanoocidal drugs commonly used include, Homidium compounds (Ethidium and Novidium), Diaminizene aceturate (Berenil), Quinapyramine sulphate (Antrycide), Isometamidium chloride (Samorin) and arsenical compound (cymelarsan or mel cy). Currently Diaminazene and isometamidium are most widely used in cattle because they have no cross resistance, while equines and camels are treated with quinapyramine (Kettle; 2000). Mustafa (2004) treated experimentally infected sheep with quinapyramine sulphate (antrycide) at a dose of 3 mg/kg body weight with complete disappearance of trypanosomes from the
peripheral blood. Rahman (2002) indicated that Samorin at 1 mg/kg body weight had given significant long prophylactic period in cattle at challenge index of 450-1750 than at a dose of 0.5 mg/kg. Leeflang et al., (1976) reported existence of T. vivax resistant stocks to recommended doses of Berenil and Homidium chloride in Nigeria. However Abdel Gadir et al., (1981) showed that, while Sudanese isolates of the three major trypanosomes species; T. vivax, T. congolense and T. brucei expressed resistance to 1.0 mg/kg homidium bromide, the relapse infections were all susceptible to 1.0 mg/kg bw diminazine aceturate. However a similar study was carried out in Uganda by (Nyeko et al., 1993), in which infected cattle were treated with Berenil at 7mg/kg bw and later examined for relapse infections. Four out of 89 had positive infections within 30 days of treatment. Treatment with Samorin at 2mg/kg bw effected complete cure. There were no drug-resistant trypanosomes detected in the isolates examined. Effectiveness of Ascofuranone (an antibiotic isolated from Ascochyta visiae) as a tool for chemotherapy against African trypanosomosis in animals was studied by Yabu et al., (2006). Carloine et al., (2004) found that Megazol could cure sheep infected with T. b. brucei but oral administration was found not to be an effective route. In a study carried out in eastern province of
Zambia, it was concluded that isometamidium chloride (Samorin) and diaminizene aceturate (Berenil) are still expected to be effective as a sanative pair in that area since not more than 1 stabilate of 71 investigated showed evidence of resistance to both drugs (Sinyangwe et al., 2004). Mel cy (cymearsan) was found to be effective in curing T. brucei group infections in laboratory animals (Raynaud et al., 1989 cited by Rahman 2002).

2.8.2. Control of the vectors

Many methods are being used for the control of tsetse flies and the biting flies. These include game destruction and eviction, bush clearing, insecticides application (ground and aerial) and sterile insect technique (SIT), (Ford 1970, Burret 1970). Insecticides (delta-metherines and alpha-cypermethrins) were used on host and as impregnated traps and targets in Tanzania, Kenya, Butswana and Zanzibar (Allsopp, 1997). 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 the 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, commercial ranching and large scale sugar
cane plantation by 1970. Other measures were indeed used including bush clearing and game hunting, because measures for controlling trypanosomiasis should however be aimed at reducing the level of host/parasite/vector interaction not just the vector population. Lummamba et al., (2004), evaluated the efficacy of a 1% pour–on formulation of cyfluthrin (Cyclence, Bayer) in controlling bovine trypanosomiasis in eastern Zambia, and found that treatment of adult cattle at 7 weeks interval at a dosage of 15 ml/100 kg has resulted in an increase in the average PCV though the decrease in incidence of trypanosomal infections was a bit prolonged. Sterile insect technique has been used for eradication of tsetse (Glossina austini) in Zanzibar after initial suppression of flies’ population by persistent application of insecticide impregnated screens (Salih et al., (1997). After release of the sterilized male flies by aircraft, wild flies disappeared completely in the area after 35 days.

Cherenet et al., (2006) in a study carried out in a tsetse-infested and tsetse-free zone in Ethiopia suggested that trypanosomosis control can not be achieved by tsetse control alone. They recommended supplemental measures that should include drug therapy and biting fly control. Belete et al., (2004) demonstrated effectiveness of cow urine kept for
several days as an attractant to be used in traps in community based tsetse control programs in Ethiopia. In Sudan control of trypanosomosis has been going on since the twentieth century. Tsetse flies control and eradication in Sudan has been carried out as earlier as 1960s. Abdel Razig et al., (1968) reported eradication of tsetse flies in the Jur Narrows by means of Game destruction together with use of insecticides used on bait animals. Yagi and Abdel Razig (1969) have successfully eradicated tsetse flies from the isolated tsetse pocket in the Koallib Hills using insecticides baited animals and selective ground spraying of resting sites and breeding sites of tsetse flies, cited by Rahman (2002).

Control of other biting flies (particularly Tabanidae and Muscidae) in Sudan as well as in other parts of the world had and still been controversial. Although they are being incriminated for more than 80% of trypanosomosis infection in Sudan (Rahman 2002) yet no clear policies and plans have been put in place for their control.

2.8.3. Use of the innate immunity (Trypanotolerance)

Some cattle are more trypanotolerant than others, for example West African N'dama and Muturu cattle are more resistant than the West African Zebu. When infected they don’t develop anemia (Murray et al.,
Murray, M. and Trail, J., C. (1987) reported that N’Dama and West African shorthorn were much more productive than originally believed despite their small stature. In a study by Guy & Kimani (2004), it was found that the natural innate resistant possessed by breeds of cattle such as the N’Dama and West African shorthorn to trypanosomosis and to several other important infectious diseases should be an increasingly important component of national and regional disease control programs, because they had been demonstrated to be economically viable at both public and private levels. In Sudan (Rahman 2002) reported that western Baggara cattle survived natural tsetse challenge, and showed a better ability to control parasitaemia compared to other indigenous zebu breeds existing in the Sudan. He indicated that these animals can still produce well (pcv 30% with 210 gm daily bw gain) even when they are at risk in Bahr El Arab area throughout the rainy season, despite that they are kept under strategic chemotherapy using suitable trypanocidal drugs (samorin) and anthelmintic (Ivermectine). Katusuka – Rawakishaya et al., (1999) considered the level of nutrition to be among the factors that can make infected animals withstand adverse effects of trypanosomes infection (T. congoense).
2.8.4. Vaccination

Up to the moment there is no vaccine produced for the control of trypanosomosis due to the fact that, their variant surface glycoprotein keeps on switching, as a result they can not be used as vaccine targets. Also it has been a common phenomenon that, mixed infection occurs in most of the trypanosomes infections, hence making it difficult for vaccine development. (Uilenberg 1998).
Chapter THREE
MATERIALS AND METHODS

3.1. Study Site description;
This study was carried out in Malakal county, Upper Nile state, Republic of the Sudan. The state lays in the Savannah region between longitude 30°.7" and 34°.2" E and Latitude 7°.9" and 12°.3" N. The soil is of black cotton type, which is covered by Savannah trees mostly Acacia species (A. nubica (El laot), A. Senegal (El Hashab), A. mellifera (elkitir), A. seyal (El Taleh), Balanties aegyptica (Elhiglig), and Tamarindus indica (El Aradaeb), with most of the land covered by grasses and small vegetation. The study was specifically carried out in two sites in the area in the vicinity of Malakal town, the capital of the state, which lies along the River White Nile just some 10 Kms north of the Juncture of the Sobat and Bahr Eljabel Rivers.
The first site was the Detang village on the western bank of the Nile, about 3 Kms northwest of Malakal town. Detang village lies in a plain land covered with green vegetation with many palm trees around.
The second site was Bam residential area inside Malakal town in the southern zone.
The two sites are about Eight Kilometers apart. The selection of the two sites was based on the results
of a Participatory Disease Search (PDS) exercise carried out in the area in 2003. The exercise revealed Trypanosomosis disease to be the third prevailing disease in the area.

3.2. The meteorological data:
Meteorological data collected included the following:
1. Minimum, Maximum and Average Monthly Temperature, and Minimum, Maximum and Average Monthly Relative Humidity. (Department of Meteorology, Malakal)
2. Monthly Rainfall Data for the period of the study, which extended from September 2004 up to August 2005 and Monthly Rainfall Data for the last (3) years as from 2003 to 2005, (State Ministry of Agriculture, Malakal).

3.3. The study design and schedule:
This study was carried out from September 2004 and continued for twelve months up to August 2005. 2,400 blood samples, were randomly collected and examined from cattle from the Nilotic Breed from different age groups (1,200 samples from animals under 4 Years old and 1,200 samples from animals above 4 Years old). Biting Flies were also trapped, sorted according to species and then counted biweekly. The monthly abundance was summed and recorded.
Meteorological data (Temperature, Relative Humidity and the Rainfall) were also collected on monthly basis throughout the study period, from Department of Meteorology and State Ministry of Agriculture in Malakal.

3.4. Ethnic groups in the area of study:
The study area is being inhabited by the Nilotic tribes mainly the Shilluk (the original inhabitants) plus Dinka, Nuer and other tribes from all over the Sudan. There is also a well established community of Messeyria inside Malakal town who keep a big number of livestock, cattle in particular..

3.5. Husbandry practices and animal movements:
The animals in the two sites are of the Nilotic Sanga breed. They are almost sedentary, as they are kept during the rainy season within the homesteads and only released for grazing in the mornings and brought back in the evenings, while in the dry season they are moved to the Kaals (dry season grazing areas) along river sides and in the islands near the villages. They don’t travel long distances away from their original villages. However around November/December every year nomads from Southern Kurdofan, Northern Kurdofan, White Nile and Blue Nile
states arrive to the area and stay there up to May/June the next year, when they go back after the start of the rains. Although they are kept separately still they exhibit a sort of coming together during watering and when grazing.

3.6. Parasitological examinations:
Blood samples from 200 randomly selected Nilotic cattle at 2 different age groups (over and under 4 years old) were collected and examined repeatedly on monthly basis for the presence of trypanosomes in the two study sites, 100 per each, to form, a total of 2,400 samples in the 12 months of study. The following techniques were used:

3.6.1. Wet blood examination technique:
This was performed by collecting a drop of fresh blood on a blood slide from the ear vein and covered by a cover-slip, then examined under light binocular microscope using 10x40 magnification power for detection of trypanosomes.

3.6.2 Micro-haematocrit centrifugation technique:
Blood samples were collected in heparinized haematocrit capillary tubes (HCT, sealed at one end with clay, spun at 1500 rpm for 5 minutes in an electric micro-haematocrit centrifuge, then the
supernatant was discarded and the Buffy layer with some adjacent red blood cells were examined for the presence of motile trypanosomes. Only 600 samples were examined using this technique in the two study sites, 100 samples per site in January 2005, May 2005 and August 2005. This was applied as a comparative method to wet blood examination. Power failure limited the use of this technique throughout the study.

3.6.3. Geimsa stained blood samples examinations:
Using thin and thick blood smears prepared from a drop of blood, spread over a glass slide using either another glass slide in case of thin blood smears, or spread over the glass slide using an edge of another slide in case of thick blood smears. Both blood smears were left to dry and the thick blood smears were de-haemoglobinized by immersion in distilled water. Both smears were then fixed in absolute methanol for 2-3 minutes before staining by immersion in a solution of Geimsa stain diluted at 1:10 with buffer water for 45 minutes then the stained films were rinsed in buffer water and left to dry in air before examination under oil immersion using 10x100 magnification. Trypanosoma species was determined morphologically in the stained smears.
3.7. Flies trapping
5 modified F3 traps made of blue Polyester Cotton cloth with 4 Iron poles, modified by Central Veterinary Research Laboratories were used for fly’s collection. They were erected 24 hours in Detang cattle camp 500 meters apart and flies were collected every 3 days at 04:00 PM. Then the trapped flies were identified and counted. The monthly abundance is then calculated.

3.8. Participatory disease searching:
Participatory Disease Searching was carried out using Participatory Techniques, which include:

3.8.1. Semi structured interviews
Which is a guided kind of interview using check lists instead of direct questions and the informants are given chance to elaborate on the topic of animal diseases as relevant as they are. Four informant groups were interviewed in the two study sites. Group size was of at least 5 individuals.

3.8.2. Disease ranking
Informants were asked to rank the 5 diseases they have mentioned during the semi structured interviews as the most common diseases in their respective sites according to both their prevalence and importance.
3.8.3. Proportional piling

The informants were asked to rank the common diseases in their sites according to their prevalence and importance using scores piling method. They were given a pile of 20 stones for each individual and asked to divide them against different diseases once according to prevalence and again according to importance.

3.9. Data analysis

Data collected were analyzed directly using the computer (Microsoft Office EXEL Version 2003). Statistical significance was also calculated using t-test method.
CHAPTER FOUR
RESULTS

4.1. Climatic Factors (Meteorological Data)

4.1.1. Temperature and humidity
Data collected included the maximum and minimum daily temperatures and the average monthly temperatures. The daily temperature ranges from 26 °C and 34 °C. The maximum daily temperature recorded was 34 °C, which was recorded in April, while a minimum daily temperature of 26 °C was recorded in September. The relative humidity was between 13% in January and March, and 80% in September. The monthly Mean Values of the temperature and the relative humidity during the study period are illustrated in Figure (4.1).

4.1.2. The rainfall
The Rainfall started in April during the period of study and ended in October. Maximum and minimum rains were recorded in August 255.5 mm and April 10 mm respectively as shown in Table (4.1) together with the records of rainfalls for the last 3 years starting from 2003 up to 2005 and also illustrated in Figure (4.1).
Fig. (4.1) Average monthly temperature, relative humidity and the rainfall data recorded during the study period.
Table (4.1)

Annual rainfall data in the study area as from 2003 up to 2005*:

<table>
<thead>
<tr>
<th>Year</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>102.5</td>
<td>230</td>
<td>155</td>
<td>114.8</td>
<td>59.0</td>
<td>54</td>
<td>55</td>
<td>0</td>
<td>803.3</td>
</tr>
<tr>
<td>2004</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>40.4</td>
<td>102</td>
<td>102.7</td>
<td>216.3</td>
<td>51.5</td>
<td>94</td>
<td>0</td>
<td>0</td>
<td>639.9</td>
</tr>
<tr>
<td>2005</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>98.8</td>
<td>111</td>
<td>149.1</td>
<td>255.5</td>
<td>238.5</td>
<td>64.8</td>
<td>0</td>
<td>0</td>
<td>927.7</td>
</tr>
</tbody>
</table>

* Source, State Ministry of Agriculture (SMOA), Upper Nile State/Malakal
Fig. (4.2) The Rainfall data in the Study area, as from 2003 to 2005
4.2. Flies Trapping and Identification

Total number of biting flies trapped using 5 modified F3 traps, Plate (4-1), in the selected trapping site (Detang area) was (34,446) flies. Trapped flies were identified to be *Tabanus taeniola*, *Atylotus agrestis*, *Musca domestica* and *Stomoxys* species. The numbers of *Musca* flies trapped were not included, because the subject of the study was the biting flies.

4.2.1. Tabanidae

The total number of Tabanidae flies trapped was 34,216 making 99% of the total biting flies trapped. The species identified were *T. taeniola* and *A. agrestis*, making 34% and 66% of the total *Tabanus* flies trapped respectively. These results are summarized in table (4.3) and illustrated in Figures (4.3), (4.4), (4.5) and (4.6). The monthly abundance of Tabanids is illustrated in Figure (4.7).

4.2.2. Stomoxys

Few *Stomoxys* flies were trapped; constituting 1% of the total biting flies trapped. Their abundance ranged from (43) in September to (1) in March as shown in table (4.3) and illustrated in figures (4.3), (4.4), (4.5) and (4.8).
4.2.3. *Musca Domestica*

Quite big numbers from this species were trapped but were not considered since the biting flies are the subject of the study.
Plate (4.1) Modified F3 Traps used for flies trapping in Detang area during the study period.
Table (4.2)

Number and species of biting flies trapped per month in Detang area during the study period:

<table>
<thead>
<tr>
<th>Month</th>
<th>T. taeniola °</th>
<th>A. agrestis</th>
<th>Stomoxys</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>September</td>
<td>2421</td>
<td>4866</td>
<td>43</td>
<td>7330</td>
</tr>
<tr>
<td>October</td>
<td>2249</td>
<td>4505</td>
<td>31</td>
<td>6785</td>
</tr>
<tr>
<td>November</td>
<td>2485</td>
<td>3797</td>
<td>22</td>
<td>6304</td>
</tr>
<tr>
<td>December</td>
<td>1034</td>
<td>2453</td>
<td>17</td>
<td>3504</td>
</tr>
<tr>
<td>January</td>
<td>339</td>
<td>788</td>
<td>20</td>
<td>1147</td>
</tr>
<tr>
<td>February</td>
<td>164</td>
<td>452</td>
<td>10</td>
<td>626</td>
</tr>
<tr>
<td>March</td>
<td>43</td>
<td>136</td>
<td>1</td>
<td>180</td>
</tr>
<tr>
<td>April</td>
<td>95</td>
<td>229</td>
<td>18</td>
<td>342</td>
</tr>
<tr>
<td>May</td>
<td>235</td>
<td>845</td>
<td>17</td>
<td>1097</td>
</tr>
<tr>
<td>June</td>
<td>195</td>
<td>986</td>
<td>8</td>
<td>1189</td>
</tr>
<tr>
<td>July</td>
<td>333</td>
<td>987</td>
<td>19</td>
<td>1339</td>
</tr>
<tr>
<td>August</td>
<td>2037</td>
<td>2542</td>
<td>24</td>
<td>4606</td>
</tr>
<tr>
<td>Total</td>
<td><strong>11630</strong></td>
<td><strong>22586</strong></td>
<td><strong>230</strong></td>
<td><strong>34446</strong></td>
</tr>
</tbody>
</table>
Fig. (4.3) Monthly abundance of biting flies according to species in the study area.
Fig. (4.4) Abundance & Species of Biting Flies trapped in Detang area during the study period.
Fig. (4.5) Relationship between species of biting flies trapped during the study period.
Fig. (4.6) Percentage of Tabanidae species trapped during the study period.
Fig. (4.7) Monthly abundance of Tabanidae flies trapped during the study period in Detang area.
Fig. (4.8) Monthly abundance of Stomoxys flies in Detang area during the study period.
4.3. Parasitological examination of blood samples

4.3.1. Wet Blood samples results
Out of 2,400 animals randomly examined for presence of trypanosomes during the study period, 422 were found positive giving a total prevalence rate of 17.6%. The monthly incidence rate, ranged from 8% in March to 29% in October. The prevalence rate in Malakal town was 13.7% while it was 21.4% in Detang area. The prevalence rate in animals less than 4 year’s old was 22%, while it was 12% in animals over 4 year’s old (P >0.05). Among the positives animals, the under 4 years old, constituted 65% while the over 4 year’s animals constituted 35%. Strong correlation between rainfalls, biting flies abundance and prevalence rate was obvious. Results are shown in Tables (4.3), (4.4) and (4.5), and illustrated in Figures (4.9), (4.10).

4.3.2. Geimsa stained blood samples results
This method was used for confirmation and identification of the trypanosomes detected with Wet Mount Technique based on their morphology. Only *T. vivax* was identified during the study period.

4.3.3. MHC technique results
Out of 600 blood samples examined using the microhematocrit Centrifugation Technique, 381
samples were found positive giving prevalence rate of 63.5%, Table (4.5). The Results were statistically significant when compared to results obtained using wet mount technique (P >0.005).

4.3.4. Identification of trypanosomes

The tentative identification of the trypanosomes was based on observing the activity and style of movement of the trypanosomes during the wet blood examination and also on the morphology of the trypanosomes in a Geimsa stained samples. T. vivax was identified.
Table (4.3)
Monthly Prevalence of Trypanosomosis* in the two selected sites (Detang and Malakal town) and over all prevalence during the study period:

<table>
<thead>
<tr>
<th>Month</th>
<th>Detang area</th>
<th>Malakal town</th>
<th>Total animals examined</th>
<th>Total positive (Prevalence %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># of animals examined</td>
<td># of positive animals (%)</td>
<td># of animals examined</td>
<td># of positive animals (%)</td>
</tr>
<tr>
<td>Sep. 04</td>
<td>100</td>
<td>34 (34%)</td>
<td>100</td>
<td>22 (22%)</td>
</tr>
<tr>
<td>Oct. 04</td>
<td>100</td>
<td>34 (34%)</td>
<td>100</td>
<td>24 (24%)</td>
</tr>
<tr>
<td>Nov. 04</td>
<td>100</td>
<td>28 (28%)</td>
<td>100</td>
<td>20 (20%)</td>
</tr>
<tr>
<td>Dec. 04</td>
<td>100</td>
<td>21 (21%)</td>
<td>100</td>
<td>17 (17%)</td>
</tr>
<tr>
<td>Jan. 05</td>
<td>100</td>
<td>18 (18%)</td>
<td>100</td>
<td>12 (12%)</td>
</tr>
<tr>
<td>Feb. 05</td>
<td>100</td>
<td>14 (14%)</td>
<td>100</td>
<td>12 (12%)</td>
</tr>
<tr>
<td>Mar. 05</td>
<td>100</td>
<td>11 (11%)</td>
<td>100</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>Apr. 05</td>
<td>100</td>
<td>12 (12%)</td>
<td>100</td>
<td>8 (8%)</td>
</tr>
<tr>
<td>May 05</td>
<td>100</td>
<td>14 (14%)</td>
<td>100</td>
<td>12 (12%)</td>
</tr>
<tr>
<td>June 05</td>
<td>100</td>
<td>21 (21%)</td>
<td>100</td>
<td>11 (11%)</td>
</tr>
<tr>
<td>July 05</td>
<td>100</td>
<td>24 (24%)</td>
<td>100</td>
<td>8 (8%)</td>
</tr>
<tr>
<td>Aug. 05</td>
<td>100</td>
<td>26 (26%)</td>
<td>100</td>
<td>14 (14%)</td>
</tr>
<tr>
<td>Total</td>
<td>1200</td>
<td>257 (21.4%)</td>
<td>1200</td>
<td>165 (13.7%)</td>
</tr>
</tbody>
</table>

*Only T. vivax was identified.

** Overall prevalence during the study period.
Table (4.4)

Wet blood examinations results according to animal age group:

<table>
<thead>
<tr>
<th>Animal Age Group</th>
<th>Number animal examined</th>
<th>Positive animals</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 4 Years</td>
<td>1,200 (50%)</td>
<td>273 (50%)</td>
<td>22.7%</td>
</tr>
<tr>
<td>Over 4 Years</td>
<td>1200</td>
<td>149</td>
<td>12.4%</td>
</tr>
<tr>
<td>Total</td>
<td>2400</td>
<td>422</td>
<td>17.6%</td>
</tr>
</tbody>
</table>

* Statistical significance, P <0.05 (t. value 1.85).
Table (4.5)

Results of blood examinations using different parasitological methods during, January 2005, May 2005 and August 2005:

<table>
<thead>
<tr>
<th>Month</th>
<th>Number of animals examined</th>
<th>Prevalence of trypanosome using different methods</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wet Mount Technique (P* %)</td>
<td>Micro-haematocrit Technique (P %)</td>
</tr>
<tr>
<td>January</td>
<td>200</td>
<td>30 (15%)</td>
<td>124 (62%)</td>
</tr>
<tr>
<td>May</td>
<td>200</td>
<td>26 (13%)</td>
<td>112 (56%)</td>
</tr>
<tr>
<td>August</td>
<td>200</td>
<td>40 (20%)</td>
<td>145 (72.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>600</td>
<td>99 (16.5%)</td>
<td>381 (63.5%)</td>
</tr>
</tbody>
</table>

*Prevalence%

** Statistical significance, P>0.005 (t. value 9.045).
Fig. (4.9) Correlation between biting flies abundance and Trypanosomosis prevalence rate in the study area.
Fig. (3.10) Correlation between rainfall, biting flies abundance and prevalence rate in the study area during the study period.
4.4. Participatory Disease Searching Results

Trypanosomosis was mentioned by the cattle owners among the prevailing and important diseases in the area, Table (4.6). The disease was ranked by the informants to be among the first prevailing five diseases in the area. It was graded differently by the different ethnic groups, however was graded second by the all informants collectively, according to importance and third according to prevalence as is shown in Tables (4.7), (4.8), (4.9) and (4.10), illustrated by Figures (4.11) and (4.12).
Table (4.6)

Diseases names as mentioned by the different ethnic groups interviewed during PDS in the study area and their corresponding veterinary terms.

<table>
<thead>
<tr>
<th>Dinka</th>
<th>Shilluk</th>
<th>Nuer</th>
<th>Mesyreia</th>
<th>English</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nyatek</td>
<td>Keno</td>
<td>Nyapec</td>
<td>Jedery/Thaoon</td>
<td>Rinderpest (RP)</td>
</tr>
<tr>
<td>About pio</td>
<td>Jwok Obou</td>
<td>Jok pout</td>
<td>Abuganait</td>
<td>Contagious Bovine PleuroPneumonia (CBPP)</td>
</tr>
<tr>
<td>Jok Rem</td>
<td>Jwok Remo</td>
<td>Yeith</td>
<td>Eltasamum</td>
<td>Haemorrhagic Septicaemia (HS)</td>
</tr>
<tr>
<td>Lwac</td>
<td>Waat</td>
<td>Noi</td>
<td>Eldhoban</td>
<td>Trypanosomosis</td>
</tr>
<tr>
<td>Dat</td>
<td>Dat/Ahwok</td>
<td>Dat/Jol</td>
<td>Abulisan</td>
<td>Food &amp; Mouth Disease (FMD)</td>
</tr>
<tr>
<td>Jong acom</td>
<td>Twongo</td>
<td>Potpot</td>
<td>Didan/Hulla</td>
<td>Liver Fluke/Worms</td>
</tr>
</tbody>
</table>
Table (4.7)

Result of Disease ranking by different ethnic groups according to Prevalence using Pile scoring Method:

<table>
<thead>
<tr>
<th>Name of Disease</th>
<th>Dinka (1 Gx5 Px20S)</th>
<th>Shilluk (1Gx5Px20S)</th>
<th>Nuer (1 Gx5Px20S)</th>
<th>Mesyreia (1Gx5Px20S)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scores</td>
<td>Rank</td>
<td>Scores</td>
<td>Rank</td>
</tr>
<tr>
<td>Rinderpest</td>
<td>03</td>
<td>5</td>
<td>04</td>
<td>5</td>
</tr>
<tr>
<td>CBPP</td>
<td>31</td>
<td>1</td>
<td>37</td>
<td>1</td>
</tr>
<tr>
<td>HS</td>
<td>19</td>
<td>4</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>Trypanosomosis</td>
<td>26</td>
<td>2</td>
<td>37</td>
<td>2</td>
</tr>
<tr>
<td>FMD</td>
<td>21</td>
<td>3</td>
<td>06</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total iles used</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

NB. G= Group
     P= Persons
     S= Stones
     (N)= Rank
Table (4.8)

Result of Disease ranking by different ethnic groups according to Importance using Pile scoring Method:

<table>
<thead>
<tr>
<th>Name of Disease</th>
<th>Dinka (1 Gx5 Px20S)</th>
<th>Shilluk (1Gx5Px20S)</th>
<th>Nuer (1 Gx5Px20S)</th>
<th>Mesyreia (1Gx5Px20S)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scores</td>
<td>Rank</td>
<td>Scores</td>
<td>Rank</td>
</tr>
<tr>
<td>Rinderpest</td>
<td>23</td>
<td>3</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>CBPP</td>
<td>26</td>
<td>1</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>HS</td>
<td>13</td>
<td>4</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>Trypanosomosis</td>
<td>25</td>
<td>2</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>FMD</td>
<td>13</td>
<td>5</td>
<td>06</td>
<td>5</td>
</tr>
<tr>
<td>Total iles used</td>
<td>100</td>
<td></td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

NB. G= Group  
P= Persons  
S= Stones  
(N)= Rank
Table (4.9)

Disease ranking according to prevalence:

<table>
<thead>
<tr>
<th>Rank</th>
<th>Type of Disease</th>
<th>Number of piles scored</th>
<th>Percentage of piles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CBPP</td>
<td>132</td>
<td>33%</td>
</tr>
<tr>
<td>2</td>
<td>Trypanosomosis</td>
<td>116</td>
<td>29%</td>
</tr>
<tr>
<td>3</td>
<td>FMD</td>
<td>84</td>
<td>21%</td>
</tr>
<tr>
<td>4</td>
<td>HS</td>
<td>60</td>
<td>15%</td>
</tr>
<tr>
<td>5</td>
<td>Rinderpest</td>
<td>8</td>
<td>2%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>400</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table (4.10)

Disease ranking according to Importance:

<table>
<thead>
<tr>
<th>Rank</th>
<th>Type of Disease</th>
<th>Number of piles scored</th>
<th>Percentage of piles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CBPP</td>
<td>128</td>
<td>32%</td>
</tr>
<tr>
<td>2</td>
<td>Rinderpest</td>
<td>84</td>
<td>21%</td>
</tr>
<tr>
<td>3</td>
<td>Trypanosomosis</td>
<td>76</td>
<td>19%</td>
</tr>
<tr>
<td>4</td>
<td>HS</td>
<td>64</td>
<td>16%</td>
</tr>
<tr>
<td>5</td>
<td>FMD</td>
<td>48</td>
<td>12%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>400</td>
<td>100%</td>
</tr>
</tbody>
</table>
Fig. (4.11) Disease ranking by different ethnic groups in the study area according to prevalence in Upper Nile/Malakal
Fig. (4.12) Disease ranking by different ethnic groups according to importance in the study area during the study period.
CHAPTER FIVE
DISCUSSION AND CONCLUSION

The total flies trapped during the study period in Detang area was 34,446. The highest number of flies trapped was in September 21%, and the least was in March (0.5%). The Tabanids constituted the majority of the trapped flies with few numbers of Stomoxys flies. Species of Tabanidae trapped were A. agrestis and T. taeniola. Other species of Tabanus plus other flies were not trapped because they were not attracted to the traps, although seen frequently in the area. These included T. biguttatus, T. gratus and Hippobosca species. These are out of the Seventy species reported by Lewis (1953). Identification was done in the study site and the confirmation was done in the Central Veterinary Research Laboratories, Soba, Khartoum/Sudan. Large numbers of Musca flies were also trapped. These species of tabanids plus others (T. Sufis and T. gratus) have been reported earlier in many locations in the Sudan, as in Khartoum by Lewis (1953) and Veterinary Research Department (1991-1992). The results are also in agreement with the results of studies carried out by Yagi & Abdel Razig (1972), Abdel Karim (1980), Hall et al., (1984), Suliman (1992) and Ahmed (1997). They all found that the numbers of Tabanus species
particularly *T. sufis* and *A. agrestis* peaked in the rainy season, mostly around October. *A. agrestis* was the most abundant fly trapped in the study area making 65% of the total flies, and 66% of the total Tabanids trapped. *T. taeniola* made up to 34% of the total flies trapped. *A. agrestis* peaked in September while *T. taeniola* peaked in November, and both least in March. *Stomoxysis* flies were also trapped but in few numbers and their number increased in September, and decreased in March. These results are not different from what was reported earlier by Abdel Razig and Yagi (1975) in Rahad Turda, Abdel Karim (1980) in Kordufan and Suliman (1992) in Sinnar. The abundance of *Tabanidae* in the study area confirmed the reports and conclusions reached upon by Uilenburg (1998) and Yagi (1968), which incriminated the Tabanids for being the principal vector of trypanosomosis in areas outside the Tsetse belt. However the large numbers of *T. taeniola* that has been trapped during this study showed that this species is being attracted to F3 traps unlike what was reported by Ahmed (1997), who only observed *T. taeniola* on animals but were not trapped using Canopy traps and F3 traps in Khartoum state. Nevertheless this study had confirmed the findings of Ahmed (1997) with
regards to the fact that A. agrestis is well attracted to F3 traps. Muscids, although said to have been responsible for the trypanosomosis outbreak that occurred in Upper Nile in 1949 (Buxton 1955), yet their presence in the study area during the study period was very poor as per the trapping results. They only made (1%) of the total biting flies trapped, which might have no big significance on the transmission and spread of the disease in the study area. However some bias in the trapping results is not excluded because of the different methods and materials used, so this might have been improved by addition of attractants, which might have had impact on the trapping exercise in terms of numbers and/or species of biting flies trapped.

During this study, no Glossina flies were trapped, which suggested their absence in the study area. This agreed with the fact that the study area is far away from the tsetse belt and its ecology particularly the vegetation and soil texture does not suit tsetse flies establishment. There were no previous reports or evidence of Tsetse presence in the study area. However tsetse presence is being reported from the eastern borders of Upper Nile along the Sudanese - Ethiopian borders up to the
Upper-Blue Nile states borders at Yabus (Rahman 2002). This result also confirmed that biting flies were responsible for transmission and spread of the disease in the study area. It goes with the reviews of Mahmoud and Gray (1980) who reported that seasonal outbreaks of *T. evansi* were in correlation with increase in Tabanids population during the rainy seasons in Tropical Africa, a fact that was later pinpointed by Rahman (2002) by saying that, it is an epidemiological fact in Sudan, when there are floods the biting fly's populations increase and there are more chances for mechanical transmission of trypanosomosis to occur.

This study revealed an overall trypanosomosis prevalence of 17.6% in tested animals in the study area over a period of twelve months using wet blood examination. The incidence rate ranged from 8% in March and 29% in October. Fluctuation in incidence rates in correlation with abundance of biting flies was evident. These results proved the establishment of the disease in the study area as reported earlier by Buxton (1955), ElKarib 1961 and Uilenberg (1998). A prevalence of 63.5% was found using Micro-Haematocrit Centrifugation Technique (P >0.005). These are in line with the findings of Abdalla (1996) and Homeida (1993), who demonstrated
sensitively of Micro-haematocrit Centrifugation Technique over the Wet Blood Technique. They also proved the perception of cattle owners regarding presence of trypanosomosis disease in the study area. This was clear from the Participatory Disease Searching Exercise carried out during this study, which revealed extensive usage of anti-trypanosomal drugs by the cattle owners in response to what they assume to be trypanosomosis. However their assumption might have not been necessarily true in all cases but nevertheless proved the endemicity of the disease in the study area.

The results of this study revealed great difference in Trypanosomosis infection rates in different animal’s age groups. It was clear that the prevalence rate tend to be higher in younger animals than in older ones. In this study 65% of the positive animals were from the age group less than 4 Years old, while it was 35% in those over 4 Years old (P >0.05). This is because of the old animals becoming more or less resistant to the disease with the age and repeated treatment with anti-tryposomal drugs (Ethidium, Novidium and Berenil), hence not acquiring the disease on exposure, or the disease in many cases turned to be chronic hence making animals rarely becoming parasitaemic, and as such making
chances of detecting trypanosomes very narrow if not difficult unless more delicate techniques are applied, like the serology. These findings are similar to what was found by Abdalla (1996), who confirmed that infection is more prevalent in calves (70.5%) than in adult cattle (29.4%).

The only trypanosome species diagnosed in the two sites of the study area was *T. vivax*, which is mechanically transmitted by biting flies from *Tabanus* and *Stomoxys* species. These results are in agreement with previous studies by Rahman (2002) and Musa et al., (1990). They all considered *T. vivax* to be the major cause of morbidity and mortality among the cattle in the Sudan. It also confirmed the establishment of *T. vivax* infection in various parts of the Sudan much far from the tsetse fly belt as reported by Abdoon et al., (2001, who isolated 5 stocks of *T. vivax* from different areas in Sennar and Khartoum states of the Sudan, and Uilenberg (1998) who reported detection of Trypanosomosis infection along the white Nile from Malakal to Khartoum. However other species of Trypanosomes reported by Homeida (1993) and Hall et al., (1990-1991) were not detected, which proved that the trypanosomes infection in the study area is a purely mechanically borne.
Trypanosomosis presence in south Sudan has been reported earlier in the 1940s of the last century (El Karib, 1961), when an outbreak of the disease almost killed 50% of the Shilluk cattle along the White Nile in 1946. Since that time the disease established itself in many areas in south Sudan, due to the abundance of the biting flies and also due to lack of proper control measures, which includes accurate diagnosis and treatment. In most of the cases this has been done by the cattle owners themselves leading in many occasions to development of drug resistance status. No evident of tsetse flies presence was reported in the area of this study, however movement of livestock (Cattle, Sheep and Goats) have been continuing since quite long time for trade (From Eastern borders of Upper Nile) and as a result of nomadism (Arab and Felata Nomads from Southern Kurdofan and White Nile states). This might have resulted in introduction of the parasite into the area and as a result of other biting flies’ abundance (Tabanids and Muscids) the disease got established in the area and subsequently have spread all over.

The overall prevalence rate in the study area was 17.6%. The prevalence in Detang area, where flies
trapping was carried out, was 21.4% and the incidence rate ranges from 11% in March and 34% in September and October. The prevalence rate in Malakal town was 13.7%, with incidence rate ranging between 5% in March and 24% in October. These results showed some variation in trypanosomosis prevalence in the two sites, in which it is less in Malakal town than in Detang area. This may be attributed to the fact that animals in Malakal town are being kept within a close area in houses in the residential areas, and as such no suitable breeding sites would be available for the biting flies, leading to existence of small populations of biting flies, while the cattle in Detang area, are kept in an open grassy land surrounded with water sources which gives good conditions for flies breeding and multiplication. Also the animals in Malakal town are under direct care and supervision of their owners who tend to be more concerned about drug administration to their stock than the herders in the cattle camps and villages, hence tend to look for veterinary services from the Veterinary Department as soon as they suspect disease. Also the availability of veterinary services in town is handier than in the village. The correlation between rainfalls, biting flies’ abundance and the infection rate was obvious. The
climate (Temperature and relative Humidity) was observed to have had more impact on the flies’ abundance as well as on the prevalence rate of trypanosomosis. The prevalence rate increases with the increase in the abundance of the flies, which in turn increases with the increase in the Rainfalls. This relationship was reported by Abdalla (1996) and Ahmed (1997) in Sennar and Khartoum states respectively and also by Mahmmoud and Gray (1980).

The Participatory Disease Search (PDS) results obtained in this study are in agreement with the results of the studies carried out in other parts of the Sudan in general and South Sudan in Particular (El Malik and Elnasry (2005), Catley et al.,(2002) and Awnour and Jacob (2003)). They all revealed cattle owners knowledge about the disease and also showed how they prioritize the disease among other diseases prevailing in the Sudan. In fact some tribesmen interviewed during this study were found to be more knowledgeable of livestock diseases (Nuer and Mysseria), than others (Dinka and Shilluk). The informants interviewed prioritized trypanosomosis to be the Second in prevalence and the Third in importance among the prevailing diseases in the area. These results were in agreement with the other results obtained during this study using the
conventional methods (Blood examinations). FAO (1992) reported the same statement saying that Tsetse-transmitted trypanosomosis figures among the first three priority veterinary diseases in the 37 affected sub-Saharan countries in Africa. The results also confirmed that, cattle owners concern about the disease has been real; as a result they widely use anti-trypanosomal drugs in an attempt to control the disease, which may be unjustifiable in some cases. This has had bad consequences on the disease control programs in terms of drug resistance and also might have lead to drug toxicity. However the results obtained in this study proved that Participatory Disease Search (PDS) is an important exercise for disease searching within animal herds by involving the communities in a participatory manner. It does not ignore the conventional methods for intelligence gathering but however consolidates them, (Catley et al., (2002).

Results obtained in this study confirmed the establishment of the disease in Upper Nile state and they also proved that, the biting flies (Tabanus and Stomoxys) are the most and the only vectors known so far that are responsible for spread and transmission of the disease in the study area. However more studies are to be carried out for determining the exact impact of the disease on the
livestock in the area, health wise as well as socio-
economically. Also more studies are required to
determine how best the disease can be controlled,
given the fact that, to have a successful and
sustainable control of the disease, we should
effectively in the control
programs. It is worth mentioning here that, cattle
owners in the study area have been widely and
traditionally using dried-dung smokes for chasing
away the biting flies, a practice that seems to have
been going on for quite long time but with an un-
proved impact. These areas need in-depth evaluation
and introduction of modern agents for trypanosomosis
and vector control are highly encouraged and
recommended such as anti-trypanosomal agents, vector
control pour-On or impregnated traps and screens.

In conclusion of this study and if we need this job
to be well done (the effective control of
trypanosomosis), we should take the advise given by
Okoth (1999) that community participation using
appropriate technologies such as low-cost
traps/targets and integrating farming activities
with tsetse control activities seem to be the most
appropriate approach in south Uganda. So we should
give the livestock owning communities the required
tools in form of awareness raising and proper as
well as appropriate control packages and involve them in the control programmes such that they finish the job as Churchill once said, "Give us the tools and we will finish the job", FAO (1992).

It is recommended that:

1. Extension services packages, that target the rural communities should be devised, to raise their awareness and actively involve them in control efforts, particularly in vector control and proper drug use.

2. Haematocrit Centrifugation Technique (HCT) should be adopted, as a routine diagnostic method for trypanosomosis confirmation given that equipment necessary for its usage in the field are availed.
References


Anon (1973) Veterinary Report Kordofan.


Carloine, Bernard Bouteille et al., 2004 (Plasma kinetics and efficacy of oral megazol treatment in *Trypanosoma brucei brucei* – infected sheep. Veterinary Parasitology, Volume 121 Issues 3-4 May 2004).


Guy d’leteren & Kamau Kimani 2004/ Indigenous genetic resources: A sustainable and environmentally friendly option for livestock production in areas at
risk from trypanosomes, Science in Africa, Issue 1, June 2004).


Luckins, A. G., Gray, A. R. and Rae, P. c. (1978). Comparison of the diagnostic value of serum immunoglobulin levels, an enzyme linked immunosorbent assay and fluorescent antibody test in


Mustafa, I. O. (2004). The role of sheep in the epidemiology of camel trypanosomiasis in Kordufan state. M. V. Sc., Thesis submitted to University of Khartoum, Faculty of Veterinary Sciences


خلاصة

بغرض دراسة نسبة الإصابة بمرض الذبابة (التربانسوما) و معرفة أنواع التربانسوما السببية له، أجريت هذه الدراسة بمقاطعة ملكال بولاية أعلي النيل بالسودان، و هي منطقة تعتبتر انتقالية بين حزام النسيم وسي الحزام الغربي الجاف. تم القبض على 4.644 ذبابة للدغة باستخدام خمسة إشراك معدل من نوع (F3). كانت 90% من نسبة الجمع من عائلة التبانيدى (ذبابة السرية) و 1% من الاستمؤكس. أكثر نسبة قبض كانت من نوع ذبابة الاكتيلوس افريستيس 65 %، التباناس تنيولا 34% و الاستمؤكس 1%.

كانت قمة تواجد الاكتيلوس افريستيس والاستمؤكس في شهر سبتمبر و التباناس تنيولا في شهر نوفمبر.

لم يتم القبض على ذبابة النسيم تسي في منطقة الدراسة. تم فحص 200 عينة دم من الأبقار الفيلة المقيمة من فئات عمرية مختلفة شهرياً و لمدة 12 شهر بواسطة الاختبار المجهرى المباشر (WMT) 122 من جملة 2400 عينة تم فحصها و جدت موجبة بنسبة إصابة 17.6%.

65% من العينات الموجبة كانت من الفئة العمرية تحت 8 سنوات. تراوحت نسبة الإصابة بين 0% و 29% في شهري مارس و أكتوبر على التوالي. نسبة الإصابة باستخدام الاختبار الترسيبي باستعمال الأنابيب العشرية كانت 63.5%.

ظاهرياً تم الكشف فقط على نوع الترانسوما فايافاكس بالمنطقة. طرق البحث بالمشاركة التي أجريت بالمناطقين أظهرت معرفة واسعة لأصحاب المواشي بالمرض حيث قاموا بترطيب ثانيا (29% من حيث الإصابة بعد الالتهاب الرئيسي البري الساري (33%))، و ثالثا من حيث الأهمية بعد الالتهاب الرئيسي البري الساري (32%) و الطاعون البقرى (21%).

الدراسة قدمت معلومات عن مرض الذبابة الغير المنقولة بواسطة ذبابة النسيم تسي و العوامل المؤثرة في حدوثه بالمنطقة، حيث وجدت بان هناك تواجد ايجابي بين كمية الأمطار، كثافة الذباب اللادغ و نسبة الإصابة بالمرض.

عليه فإن الدراسة اقترحت عمل دراسات إضافية لتغطية الجوانب التي لم تشمل في هذه الدراسة كالألغاز الاجتماعية و الاقتصادية للمرض، و إيجاد حزمة عملية يمكن تطبيقها لمكافحة المرض بالمنطقة و ذلك بمشاركة ملاك المواشي.