Knowledge, Attitude and Perception of Human African Trypanosomiasis in Lui Hospital, Western Equatoria State of Southern Sudan

Richard Lino Loro Lako

A thesis submitted in partial fulfillment for the Degree of Master of Science in Applied Epidemiology in the Jomo Kenyatta University of Agriculture and Technology

2009

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature:……. Date……

Richard Lino Loro Lako

This Thesis has been submitted for examination with our approval as university supervisors.

Signature: …… Date: ……

Dr. Eric M. Muchiri

FELTP, Kenya

Signature: …… Date: ……..

Prof. Zipporah Ng'ang'a

JKUAT, Kenya

Signature: …… Date: ……...

Dr. Mary Mwangi

Center for Disease Control and Prevention (CDC), Kenya

DEDICATION

To my late wife Helen Poni, my Mother Angelina Poni and my beloved daughter Emmanuela Lurit for bearing with me long absence from them and the encouragement and support they accorded me through this study. .

ACKNOWLEDGEMENTS

In preparing this thesis, I acknowledge the contribution of my esteemed supervisors Prof. Zipporah Ng'ang'a, Director of Institute of Tropical Medicine and Infectious Disease Jomo Kenyatta University of Agriculture and Technology, Dr. Eric M. Muchiri Head of Division of Vector Borne and Neglected Tropical Diseases, Ministry of Public Health and Sanitation, Kenya and Dr. Mary Mwangi of Centers for Disease Control and Prevention (CDC), Kenya.

I also acknowledge the tremendous support from Ministry of Health Government of Southern Sudan, ITROMID, Field Epidemiology and Laboratory Training (FELTP) Kenya and Southern Sudan programmes. Their continuous support during dissertation, words of encouragement, and guidance from the time of the proposal development until the completion of the study.

The active role played by the field assistant researchers in making the thesis a success, both as key players for data collection and administering the questionnaire, is highly appreciated.

In-charge of Lui Hospital is thanked for his cooperation during the field work and for providing secondary data. I also extend my thanks to individual study participants whose understanding and cooperation made the accomplishment of this study possible. Thanks are also extended to Dr. Donna for her technical support in Data analysis and formatting the thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

CAR	Central African Republic
CATT	Card Agglutination Test for Trypanosomiasis
CATT-wb	Card Agglutination Test for Trypanosomiasis – Whole Blood
CI	Confidence Interval
CLN	Cervical Lymph Node
CSF	Cerebrospinal Fluid
DALYs	Disability Adjusted Life Years
DNA	Deoxyribonucleic Acid
DRC	Democratic Republic of Congo
DFMO	Difluoromethylornithin (Eflornithine)
EDTA	Ethylene Diamine Tetra acetic Acid
ELISA	Enzyme-linked immunosorbent assay
FP	Filter Paper
GalC	Galactocerebrosides
HAT	Human African Trypanosomiasis
IFA	Immunofluorescence assay
IgG	Immunoglobulin G

IgM	Immunoglobulin M
IMC	International Medical Corps
LiTat	Lyophilized bloodstream forms of *T. b. gambiense* antigen type
µl	Micro liter
MAECT	Mini Anion Exchange Centrifugation Technique
MHCT	Micro Hematocrit Centrifugation Technique
MSF-CH	Medecins Sans Frontineres Swiss
OR	Odds Ratio
PCR	Polymerase Chain Reaction
SRA	Serum Resistant associated gene (SRA)
T.b	*Trypanosoma brucei*
TgsGP	*T. b. gambiense* specific Glycoprotein gene
QBC	Quantitative Buffy Coat
USD	United States Dollar
UV	Ultra Violet light
WHO	World Health Organization

ABSTRACT

Human African Trypanosomiasis (HAT) or sleeping sickness is an endemic disease in Southern Sudan including the three States namely; Central, Eastern and Western Equatoria. The aim of this study was to determine the knowledge, attitude and perception about early detection and diagnosis of sleeping sickness in Mundri East County. A formal survey with a component of un-matched case- control study was conducted and a total of 108 participants recruited for the study. The findings reveals that majority of cases were resident in a rural area with (OR = 2.96; P value = 0.00358).

The mean ages of cases was 25.5± 5yrs and were younger than those without disease and majority were males. There was a significant difference in knowledge on signs and symptoms of HAT disease among cases than those without the disease. Similar significant differences on knowledge on disease, transmission mode, and the causes of disease diagnosis and disease management were evident between cases and controls with cases being knowledgable on the disease more than controls. Fifty two percent of cases and 75% of controls however, were not aware of mother to child transmission capacity of the disease ($\chi^2 = 5.46$; $P \leq 0.0001$). on the source of disease information, majority of both cases (66.7%) and controls (87%) got information about the disease presentation and transmission through public community meetings rather than health care provider (OR= 3.36; P value < 0.05).

Over a third of the participants (35%) had the belief that the disease is due to witchcraft

and another 56% incriminated mosquitoes in transmission of the disease. The study participants also had different perception of the disease. Over 80% of cases and controls viewed that the disease not as a health risk in the Mundri County. Analysis from several variables for identification of independent risk factors of the disease showed that fishing (OR= 2.75; P = 0.0061), hunting (OR= 2.71; P = 0.0086), herding (OR= 3.4; P = 0.0011) and fire wood collection (OR= 4.78; P < 0.0001) were significant in acquisition of the disease. Majority of the cases delayed in seeking appropriate medical attention for the condition. The mean diagnosis delay was 10.5 ± 2.5 months before contact was made with health care provider. In conclusion, there was significant difference in knowledge about disease, transmission mode and vector (tsetse flies). Younger male rural base subjects engaged in outdoor activities were more affected with HAT. Majority of cases were diagnosed at the late stage of the disease. Community health notices or campaign was the major source of information about the disease. Therefore to improve the knowledge on disease the findings of this study indicated more community involvement is needed to reduce the risk of infection.

CHAPTER ONE

1. INTRODUCTION

1.1 Background informations.

Human African Trypanosomiasis (HAT), or sleeping sickness, is a vector borne disease caused by the protozoan parasites *Trypanosoma brucei gambiense* (*T.b. gambiense*) which found in west and central Africa, extending from Angola to southern Sudan and Senegal and *Trypanosoma brucei rhodesiense* (*T.b. rhodesiense*) which occurs in eastern and south African (Figure1). Both species are transmitted solely by the tsetse fly (*Glossina* spp.); *T.b. gambiense* infections are currently responsible for over 96% of all reported HAT cases (WHO, 2006) and are characterized by a chronic progressive course which may last for months to years and may lead to death if left untreated (WHO, 2000). *T.b. rhodesiense* HAT is usually acute, and death occurs within weeks or months (Apted *et al.*, 1963).

The disease appears in two stages, the first or haemolymphatic stage, and the second or meningoencephalitic stage during which the trypanosomes invade the central nervous system (CNS). General clinical signs and symptoms are the main features of early stage HAT, and as the parasite persists and invades the CNS, the initial symptoms become more pronounced, and manifestations such as anaemia, cardiovascular and kidney disorders as well as neuropsychiatric disturbances appear. The latter are the most prominent and best-documented clinical features of the disease (Blum *et al.*, 2006). Human African trypanosomiasis is a parasitic disease unique to Sub-Saharan Africa

found between 15º degrees north and 20º degrees south latitude (Benenson, 1995). The disease is confined to sub-Saharan Africa in defined geographical foci in 36 countries. Only 20-25 000 cases are notified to WHO each year, but the true figure may now exceeded 300 000 (WHO, 1998). Given the often chronic progressive nature of infection, the fatal outcome with meningo-encephalitis and the therapeutic and diagnostic difficulties, Africa is facing a human crisis of substantial proportions to which most aid organizations and national governments are either ignorant of or unable to respond to the sleeping sickness. Resurgence and epidemics of African trypanosomiasis are associated with economic decline, civil disturbance, war, population movements and refugees. Foci of high endemicity occur in remote rural areas and are often accorded low priority by politicians and health officials. In addition, vertical disease control programmes have been progressively dismantled in preference to integrated, community-based, healthcare. While sleeping sickness has declined in a number of West African countries such as Ghana, Nigeria and The Gambia, vast areas of central Africa from southern Sudan and Uganda, through Congo-Zaire south to Angola are experiencing progressive epidemic spread. Few studies focus on the perception that people have of the screening programmes, of treatment facilities and of HAT care and its relation to health-seeking behavior (Gouteux & Malonga, 1985) describe how high cost of treatment leads to patient's refusal of care (Odiit *et al*., 2004) assessed patterns of health-seeking behavior for sleeping sickness caused by *Trypanosoma b. rhodesiense*. Other studies focus mainly on community participation in

vector control (Gouteux & Malonga, 1985; Leygues and Gouteux, 1989; Okoth *et al*., 1992). A better understanding of the factors that determine attendance in active screening is needed to identify the bottlenecks and make a HAT control programme overall more effective.

1.2 Human African Trypanosomiasis in Sudan

Sudan is the largest county in Africa (2.5 million km²) with geographical coordinate 15,000 N, 30,000 E and with considerable variation in climate, tropical in south; arid desert in north; rainy season (April to October). The country administratively has been divided into twenty six states (26) of which ten (10) are located in Southern Sudan

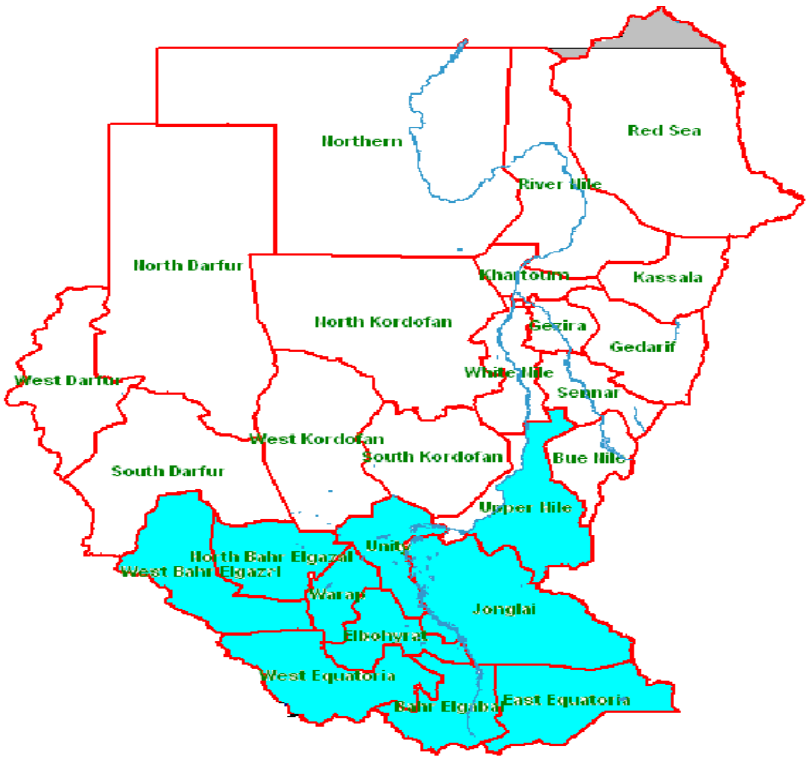

Figure 0-1 Map of Sudan showing Southern Sudan shaded in blue:

Source -Southern Sudan United Nation Joint Logistic Commission Maps. database.

Southern Sudan has been negatively affected by the First and Second Sudanese Civil Wars for all but 10 years since independence in 1956, resulting in serious neglect, lack of infrastructure development, and major destruction and displacement. More than 2 million people have died as a result and more than 4 million left internally displaced or have living as refugees in the neighboring countries (Van Mierrvenne, 1999). Human African trypanosomiasis (HAT) has been a public health problem in the country throughout most of 20th century. The epidemics have mainly been reported in the Southern and South Western parts of Sudan (confined to Equatoria and Western Bhar el Ghazal States) bordering Uganda, DRC and Central African of Republic (CAR). The foci include Raja, Yei, Kajo-Keji, Nimule, Tambura and Yambio (Duku, 1981). Outside southern Sudan, the disease historically extends to northern Uganda (Arua and Moyo districts), Democratic republic of Congo (DRC) and central Africa Republic (CAR) Figure 2.

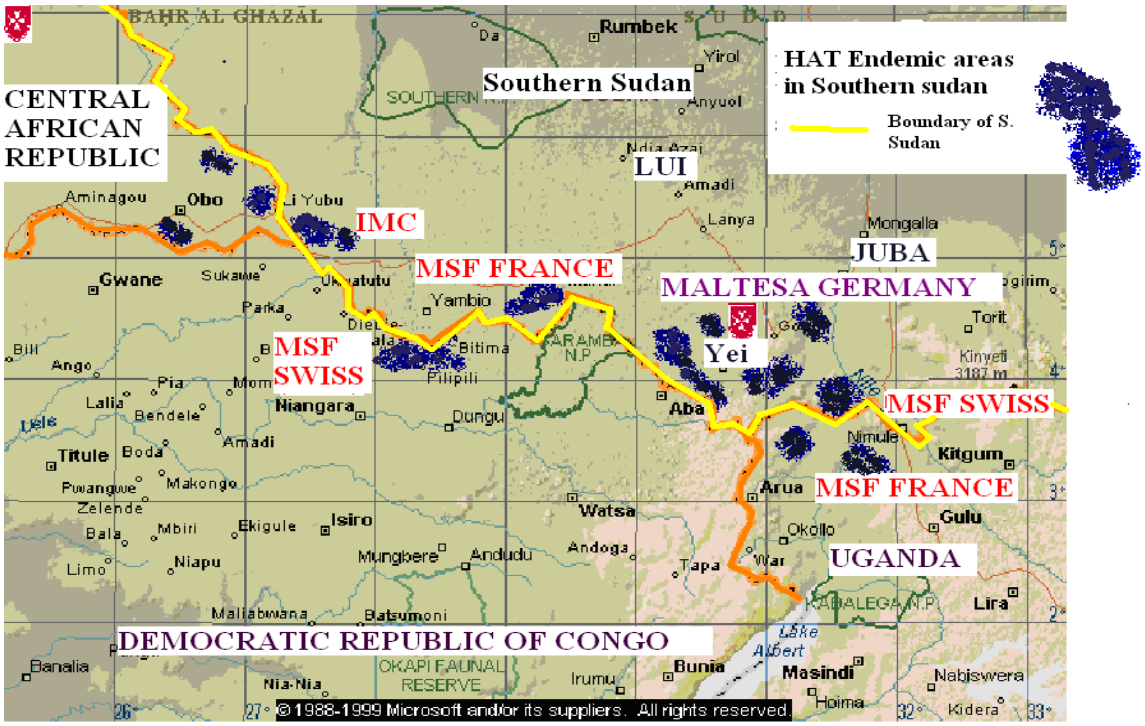

Figure 2 Sleeping sickness endemic areas in Southern Sudan.

Source: Sudan WHO - HAT Map.

In 1980 through the assistance of Belgium, Sudanese control program the incidence of the disease in Western Equatoria Province of Southern Sudan, which later collapsed when the civil war intensified in 1990. As a result the province remained without Human African trypanosomiasis control activities for more than a decade and led to resurgence of the disease with several foci reporting a prevalence exceeding 5%. During this period, control was largely depended on international organizations and non governmental organizations (NGOs). Despite un availability of accurate and empirical data on the extent of the disease but it is estimated that about 10 million people are at risk of infection and thousands of persons are infected annually (Moore *et al.*, 1999).

Several NGOs and international organizations have conducted HAT studies in Southern Sudan with support of World Health Organization but there is little information on HAT and where it exist it is restricted to a few foci and thus actual situation of human African trypanosomiasis (HAT) in Southern Sudan is therefore not clear. In Tambura County, a survey carried out between 1996 and 1997 in 16 villages showed varied prevalence ranging from 0.3% to 20.4% by village. Based on an estimated population of 25,000 in the surveyed area and with the finding of 19.4% seropositivity for the disease, it was projected that there may be 5,000 trypanosomiasis cases in the Tambura County. The high prevalence of the disease has been reported in Ezo country at (37%) and 21.5% in Yubu county, respectively (Moore *et al.*, 1999).

1.3 Study Justification

Human African Trypanosomiasis or sleeping sickness is responsible for over 300,000 new cases and 100 deaths every year in sub Saharan Africa (WHO, 1998). The disease now ranks as 7th in sub-Saharan Africa in terms of disability adjusted life years (DALYs). The DALYs lost due to sleeping sickness is 2.0 million (WHO, 2006). HAT control requires considerable resources, and budgets depend mainly on international donors (Lutumba P *et al.*, 2003) and the resource allocation by the latter is often guided by criteria such as burden of disease as expressed by DALYs (Murray, 1994; WHO, 1995) In Southern Sudan, the prevalence of the disease in reported endemic areas is not known, although a prevalence survey carried out in 1997 in 16 villages of Tambura county showed trypanosomiasis prevalence increased two orders of magnitude, and the proportion of villages affected increased from 54% to 100% in the villages affected since 1988 with high prevalence foci included Ezo (37%) in the southern part of Tambura (Moore *et al.*, 1999). A recent study has shown that Maridi County has the highest prevalence (29%) in western Equatoria state (Moore & Richer., 2001). In Mundri East County, absence of HAT educational programmes, the long distances to health care facilities and beliefs or myths surrounding HAT are the factors that hinder early detection of the disease. The county is like other parts of the country that have been badly affected by the civil war, and lies in the western Equatoria State where the disease is re-emerging. Understanding factors that influence knowledge, attitudes and perception in seeking health care is important for development of an effective, targeted

health intervention that will shorten the delay in diagnosis and treatment. Therefore this study aimed to provide valuable information for communication interventions and for policy and decision makers for development of appropriate preventive measures.

1.4 Research Questions for this study;

I. Does the Human African Trypanosomiasis continue to spread due to lack of knowledge by inhabitants about its risk factors, sign and symptoms or their health seeking behavior?

II. What influence do Socio-demographic characteristics as risk factors and clinical presentation exert on early detection and diagnosis of HAT?

1.5 Hypotheses

I. The prevalence of Human African Trypanosomiasis or sleeping sickness is not affected by lack of knowledge about its risk factors, signs and symptoms or health seeking behavior

II. Socio demographic characteristics like age, location, occupation and education have no influence on knowledge of risk factors, and symptoms for early detection of disease.

1.6 General Objective of the Study

The main objective of the study was to determine knowledge, attitude and perception for early diagnosis of human African Trypanomiasis patients and risk factors with a view to generate effective intervention measures.

1.6.1 Specific Objectives

Specifically to:

I. Determine level of knowledge on risk factors, sign and symptoms of Trypanosomiasis for early detection and diagnosis
II. Determine the association between knowledge, attitudes and perception (risk factors and symptoms) and early detection and diagnosis
III. Determine socio demographic risk factors of *trypanosomiasis*

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Historical Perspective of Trypanosomiasis

The first definitive accounts of sleeping sickness are by an English naval surgeon, John Atkins, in 1721 (Atkins, 1734) and Thomas Winterbottom, who coined the term “negro lethargy” in 1803 (Winterbottom, 1803). An appreciation of the real cause of the disease was not possible until Pasteur had established the germ theory toward the end of the 19th century. Trypanosomes had been seen in the blood of fishes, frogs, and mammals from 1843 onward, but it was not until 1881 that Griffith Evans found trypanosomes in the blood of horses and camels with a wasting disease called surra and suggested that the parasites might be the cause of this disease (Evans, 1881). These observations led to the most important discoveries about human and animal trypanosomiasis shortly afterwards.

In 1894, David Bruce, a British army surgeon investigating an outbreak of nagana, a disease similar to surra, in cattle in Zululand, was looking for a bacterial cause and found trypanosomes in the blood of diseased cattle; he demonstrated experimentally that these caused nagana in cattle and horses and also infected dogs. He also observed that infected cattle had spent some time in the fly-infested “tsetse belt” and that the disease was similar to that in humans with Negro lethargy and fly disease of hunters (Brothwell and Sandison, 1967). Trypanosomes were seen in human blood by Gustave Nepveu in 1891.

In 1902, Everett Dutton identified the trypanosome that causes Gambian or chronic sleeping sickness (*T. b. gambiense*) in humans (Dumas & Boa., 1988) and in 1910 J. W. W. Stephens and Harold Fantham described *T. b. rhodesiense*, the cause of Rhodesian or acute sleeping sickness (Kean *et al*., 1978). Trypanosoma brucei and the role of tsetse flies (Glossina) as vectors was identified in game animals in Zululand by David Bruce a century ago (Bruce, 1895). Later, morphologically identical trypanosomes were identified in the blood of a European from The Gambia, West Africa (Dutton., 1902), and transmission by riverine tsetse (Glossina palpalis) confirmed. Trypanosomes are classified under the sub-kingdom of protozoa, phylum sarcomastigophora, order kinetoplastida, family trypanosomatidae and genus *trypanosoma*. This genus has two groups, Stercoraria and Salivaria. Stercoraria contain genera in which the trypanosomes complete its development in the hindgut and transmission is by fecal contamination. The species in stercoraria includes *T. cruzi* that causes Chagas disease in South America.The salivarian group completes development in the salivary glands and transmission is by inoculation of matacyclics with the saliva. The main subgenera in this group are; *Duttonella* (species; *Trypanosoma vivax*, and *T. uniforme*; Nannomonas (species: *T. congolense* and *T. simiae*); *Pycnomonas* (species: *T. suis*) and Trypanozoom (species; *T. brucei brucei, T.b. rhodesiencse, T.b. gambiense, T. evansi* and *T. equiperdum*) (Hoare, 1970). *Duttonella, Nannomonas, pycnomonas* and some species in *Trypanozoon* (*T. brucei brucei, T. envansi* and *T. equiperdum*) causes a disease in animals. The disease is caused in man by *T. b. rhodesiense* and *T.b. gambiense*. *Trypanosomes* that

cause disease in animals are not infective to human due to their sensitivity to human serum that hinders their survival in man. However, in individuals lacking the lytic factor, *trypanosome* infection is possible. Indeed a case of human *Trypanosomasis* caused by *T. evansi* has recently been reported in India (Joshi *et al.*, 2005).

2.2 Distribution of T. b. gambiense and T. b. rhodesiense

Trypanosoma b. rhodesiense is found in East Africa while *Trypananosoma b. gambiense* is restricted to central and West Africa. The boundary between the distributions of the two parasites follows the Great Rift Valley. The separation is thought to have resulted from evolution of hominids (Welbum *et al.*, 2001). Uganda is the only country where both diseases exist and recent reports indicate that there is an overlap between *T. b. gambiense* and *T. b rhodesiense* (Enyaru *et al.*, 1999). The two parasites are morphologically indistinguishable. Currently there are two tests based on molecular markers that differentiate *T. b rhodesiense* from *T. b. gambiense,* using the Serum Resistant Associated gene (SRA) only found in *T. b. rhodesiense* and absent in *T. b. gambiense* (DeGreef *et al.*, 1989); where as for *T. b. gambiense* specific glycoprotein gene (TgsGP), is used but absent in *T. b rhodesiense* (Berberof *et al.*, 2001). Figure 3 shows the distribution of both trypanosomes in Africa and in areas suspect of overlap, the two test can be used to distinguish the infecting parasites.

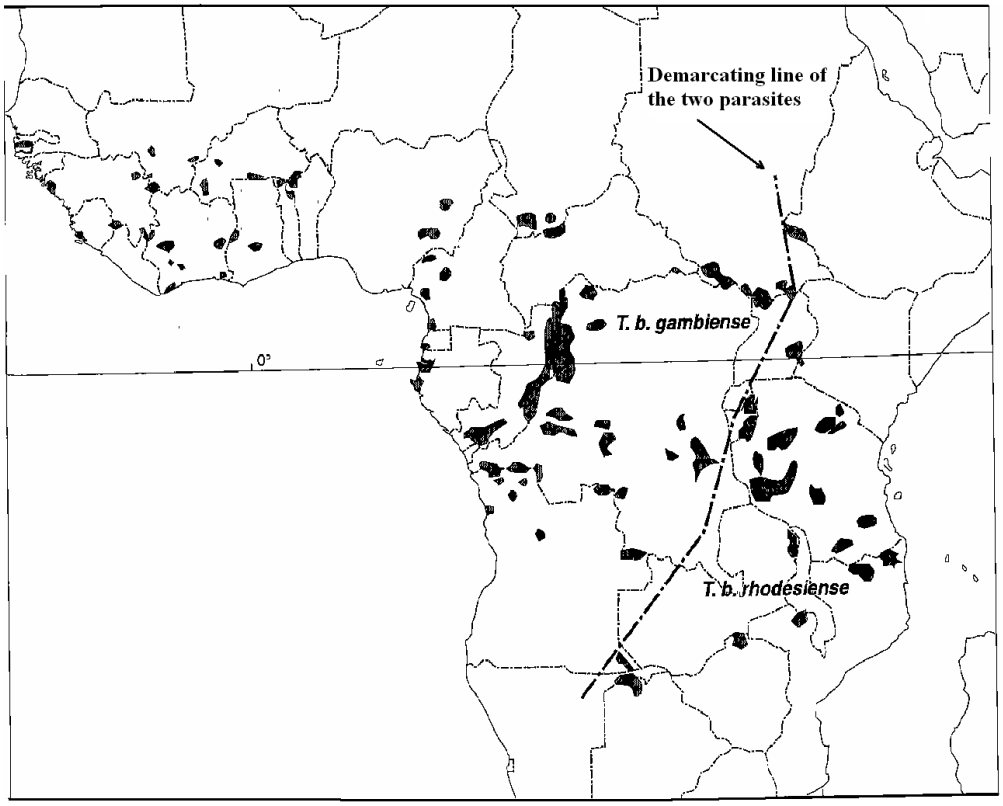

Figure 3 Geographical distributions of major endemic foci of sleeping sickness in Africa, 1995.

Source: WHO, (1998) Report.

2.3 The Vector and Life Cycle of Trypanosomiasis

Tsetse flies are the only vector of *Trypanosome brucei*. There are about 20 species of the tsetse flies but only a few transmit the disease. The flies have an average life span of between one to six months and live in warm, shady and humid areas. The parasites are ingested from an infected mammalian host by the blood- sacking vector (tsetse fly).

Once infected, the tsetse flies remain infected for life and a small number of infected tsetse flies can maintain endemic transmission cycles at relatively high levels. During feeding, the tsetse fly takes up trypanosomes from the host. In the ectoperitrophic space between midgut epithelium and the peritrophic membrane, the stumpy trypomastigotes transform to procyclics. After rapid proliferation (10-12 days) the procyclics moves to the proventriculus and subsequently migrate as epimastigotes via the hypo pharynx to the salivary glands. The attached epimastigotes further differentiate and emerge as mammal- infective metacyclic trypanosomes sfter 13- 15 days. The developmental life cycle is shown in figure 4 below.

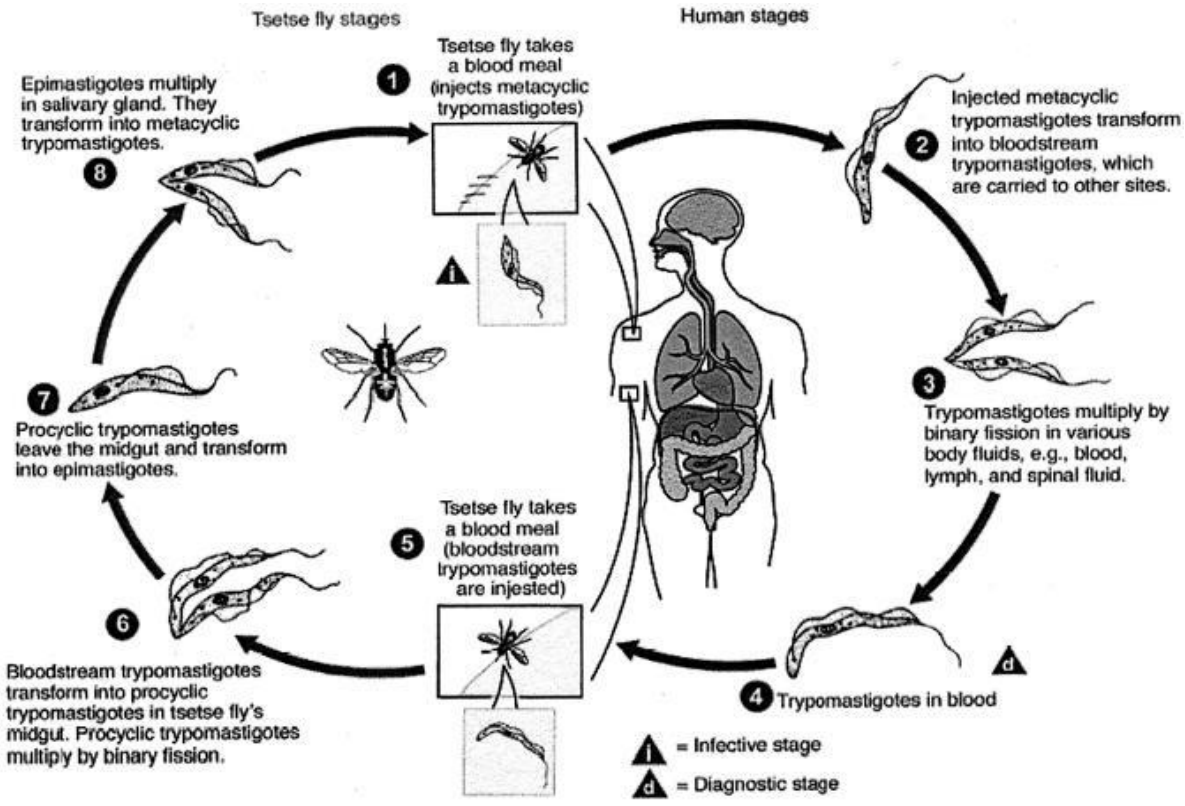

Figure 4 Life cycle of *T. b. gambiense* and *T. b. rhodesiense*

Source: Adapted from (Vickerman, 1985)

The infected fly will infect a mammalian host during feeding. Briefly, the biting tsetse fly deposits metacyclic trypanosomes in the dermal tissues of the host. The metacyclic rapidly transform into long slender bloodstream forms, which then multiply by binary fission and subsequently invade the lymphatic, blood system and later the central nervous system. Besides these long slender forms, morphologically different, non proliferating stumpy forms are observed at peak and declining parasitaemia in the blood of the host. It is these stumpy forms that are able to continue the life cycle in the insect vector. Only a small proportion of tsetse flies which have taken up trypanosomes

develop a mature infection and this takes about 3-4 weeks. It has been found that only teneral flies can develop a mature infection. The reason are not very clear but the current suggestions is that the lack of lectin and poorly developed peritroohic membrane make the teneral flies susceptible to trypanosome infection (Lehane, 1991)

2.4 Trypanosomiasis

At the site of the infective bite, parasites proliferate and, after 5 to 15 days, occasionally lead to a nodule or ulcer called a chancre (or trypanome) that spontaneously resolves within a few weeks. In African patients, chancres are generally absent at the time of diagnosis in both forms of HAT (Boa *et al.,* 1988 and Buyst, 1975). This contrasts with the high prevalence of chancres observed in European patients; in particular those infected by *T. b. rhodesiense* (Duggan and Hutchinson, 1966 & Jelinek *et al.,* 2002). In the largest published review of Europeans diagnosed with HAT, chancres were more frequently observed in patients returning from Eastern Africa (46%) than in patients returning from Western or Central Africa (23%) (Duggan and Hutchinson, 1966). At this stage, a satellite lymphadenopathy and fever with generalized malaise my be the only signs noted. As the trypanosomes invade the lymph, blood, bone morrow and tissues fluids, the patient experiences transient local oedema, sensational hyperesthesia (Kerandels sign), intermittent fever accompanied by headaches, pains in the joints, splenomegaly and lymphadenopathy (Apted, 1970). The disease progresses to the meningo-encephalitic stage (second stage) and brain tissues. This progressively leads to a variety of clinical manifestations, including headache, irritability, tremors, ataxia,

convulsions, personality changes, daytime somnolence, pronounced wasting and coma (Dumas & Boa, 1988). Both infections by *T. b. rhodesiense* and *T. b. gambiense* follow this sequence of infection stage. Generally, however, the rhodesiense disease is viewed as a compressed form because most symptoms as well as the neurological changes are the same in both forms (Kristensson *et al.*, 2002)

Human African Trypanosomasis due to *T. b. rhodesiense* infection presents as an acute (sometimes fulminant) febrile illness starting 1 to 3 weeks after the infective bite; it cannot be distinguished clinically from other tropical fevers such as malaria, enteric fever, and bacterial meningitis. Compared to *T. b. gambiense* illness, febrile episodes are more pronounced and frequent and lymphadenopathy is usually generalized. Keratitis and conjunctivitis have been observed. There is less demarcation between first- and second-stage illness, and central nervous system (CNS) involvement can be clinically limited to drowsiness and tremor. Pancarditis with congestive heart failure, arrhythmia, and pericardial effusion can kill the patient before pronounced CNS involvement becomes apparent (Stich *et al*., 2002). Most deaths (>80%) occur within 6 months of onset of illness (Odiit *et al*., 1997)

HAT due to *T. b. gambiense*, can present with intermittent nonspecific symptoms such as fever, fatigue, headaches, arthralgia, and pruritus. Transient limbs or face edema can occur. Enlarged, painless, rubbery cervical lymph nodes in the posterior cervical triangle were recognized as an alert sign for HAT long ago by Sir Thomas Masterman Winterbottom, who noted that slave traders in the late 18th century used neck swelling

as an indicator of the sleepiness or abnormal behavior that made particular slaves undesirable (Barrett *et al.,* 2003). However, enlarged cervical lymph nodes may be atypical, absent (in up to 50% of patients), or due to other causes (Pepin and Milord, 1994). Splenomegaly is another nonspecific sign and is more commonly found than hepatomegaly (Boa *et al*., 1988). This highly variable clinical picture grossly corresponds to the first, or hemolymphatic, stage of the disease. Later in the disease, neuropsychiatric symptoms and signs, due to the CNS invasion by trypanosomes and the resulting immune response (Greenwood and Whittle, 1980), gradually become more prominent. The interval between the start of the infection and the second, or neurological, stage is in the order of months or years (Burri and Brun, 2003). lassitude, headache, personality change, and overt psychiatric presentations. The neuropathogenesis of second stage HAT has been recently reviewed (Kennedy, 2004). The clinical features can be grouped into categories such as psychiatric, motor, sensory, and sleep abnormalities. The mental disturbance may include irritability, psychosis. Pyramidal (focal paralysis), extrapyramidal (rigidity and tremor), and cerebellar (dysarthria and ataxia) disorders are common. Delayed and increased sensation to pain (Kerandel's sign) can also be noted. Reversal of the normal sleep-wake cycle, with daytime somnolence alternating with nocturnal insomnia, is typical. Weight loss and endocrine abnormalities such as amenorrhea and impotence are also frequent complaints. If left untreated, patients ultimately die from the consequences of severe wasting, dysfunction of the immune system, deep coma, and seizures, often due to

bacterial infections such as pneumonia or meningitis.

2.5 Knowledge, Attitudes and perception of trypanosiamiasis

A better understanding of knowledge, attitudes and perception is needed to identify the bottlenecks and make a HAT control programme overall more effective. A cross-sectional study was conducted in Dembia district, northwest Ethiopia, in May 2001 by (Alene and Edris, 2002) to assess the knowledge, attitude and practice of the population of Dembia District towards traditional harmful health practices. Its findings reveled that educational status and religion were found to be significantly associated with the attitude of respondents towards practicing the prevailing traditional malpractices of the study area. The author concluded that an integrated health activity which includes the issue of traditional harmful health practice and its associated risks should be given due attention at grass roots level. Before 1970, great emphasis was assigned to HAT control such that concerted efforts dramatically reduced the prevalence of disease from a peak of over 120,000 cases detected in 1935 to less than 10,000 by the 1960s (WHO, 2000). At that time, diagnostic capabilities were much less sensitive and therapies less effective than they are today but the case-finding and treatment strategy are thought to have been successful because of the large-scale and frequent testing of at-risk populations achieved using coercive measures considered unacceptable today (Ekwanzala *et al.*, 1996). Few studies focus on the perception that people have of the screening programmes, of treatment facilities and of HAT care and its relation to health-seeking behaviour. Gouteux & Malonga (1985) describe how high cost of treatment leads to patient's

refusal of care. Odiit et al. (2004) assessed patterns of health-seeking behaviour for sleeping sickness caused by Trypanosoma b. rhodesiense. Other studies focus mainly on community participation in vector control (Gouteux & Malonga 1985; Leygues & Gouteux 1989; Okoth et al. 1992). A community perception study of HAT and the acceptability of interventions amongst beneficiaries of the HAT control programme in DRC to identify barriers amenable to intervention and to propose possible improvements by (Robyas et al, 2007) also showed that the disease is well known in the population and seen as a major problem. Most of the time people use a literary translation of the term sleeping sickness. Sleeping sickness is considered a 'disease of God' (maladi ya nzambi) implying that it is treatable by western medicine as opposed to 'diseases caused by people'– as a result of witchcraft (in Kikongo: ndoki). In these communities, psychiatric problems are usually associated with sorcery 'if somebody defecates in his own clothes he becomes just like somebody who is possessed'. Patients or family members ponder who may be the cause of the problem, and nearly always identify a member of the extended family. So, when somebody in the family develops sleeping sickness, first 'we think often that witchcraft (ndoki) is involved'. If a child falls sick people start saying: 'Oh, the uncle put a demon into the child'. Families may consult a traditional healer, usually somebody with visionary powers (nganga) to determine who may have caused the disease. A formal diagnosis of sleeping sickness by the health system changes this aetiologic attribution. 'If the nurses find the disease, the uncle is found innocent'. This pattern came back in nearly all focus groups. However, if

death occurs during or after HAT treatment, the issue gets more complex. In some cases, death is then seen as post-hoc evidence that the disease was in reality caused by witchcraft, in other cases only the death will be attributed to witchcraft while the disease itself is still considered as sleeping sickness. Participants know that the mobile teams come to screen for trypanosomiasis and correctly describe the sequence of technical procedures, from neck palpation and puncture, taking of blood samples for testing, to the use of the microscope. They see participation in screening as a way of protecting themselves from the disease. However, some group members confused the HAT screening with the activities of mobile vaccination teams. The fact that one must pay for the screening card is badly accepted, and people often mention that it used to be free in the past

2. 6 Laboratory Diagnosis of *T.b gambiense* infection

The diagnosis of *T. b. gambiense* HAT follows a three-step pathway: screening, diagnostic confirmation, and staging. The majority of control programs rely on active case detection through mass population screening. Screening tools therefore need to be sensitive, practical, quick, and cheap. For that purpose, the Card Agglutination Test for Trypanosomiasis (CATT/*T. b. gambiense*), currently used in most areas of endemic infection, is a more efficient screening method than the cervical lymph node (CLN) palpation and puncture. Diagnostic confirmation then relies on the finding of trypanosomes in the blood, lymph nodes, or cerebrospinal fluid (CSF). Unfortunately, it is estimated that 20 to 30% of patients are missed by the standard parasitological

techniques. Staging of the disease is a key step that allows classification of the patient into the first (hemolymphatic) or second (meningoencephalitic) stage of the disease. In the absence of reliable blood tests able to detect CNS invasion by the parasite, HAT staging relies on the cerebrospinal fluids examination for presence of parasite, level of proteins in CSF and number of while blood cells. It must be stated that the efficiency associated with implementing accurate diagnostic tools in HAT control programs based on active case finding can be offset by other crucial determinants such as a low attendance rate of the population or an insufficient proportion of patients completing treatment with subsequent cure (Robays *et al*., 2004).

2.6.1 Biological Parameters for HAT diagnosis

Biological blood parameters such as increased sedimentation rate and low hematocrit reflect the systemic chronic inflammation present in HAT patients and are therefore nonspecific. Thrombocytopenia is generally mild or absent, and features of disseminated intravascular coagulopathy are not found (Greenwood and Whittle, 1976). Liver transaminase levels, bilirubin, and renal function tests are usually within normal limits or slightly elevated (Magnus et *al.*, 1978). Protein measurements usually show decreased albumin and increased immunoglobulin concentrations, especially of IgM (Bisser *et al.*, 1997). Low serum C3 levels and split C3 products can be found, reflecting complement activation (Greenwood and Whittle., 1976). These findings are of little use in most field settings, where only the erythrocyte sedimentation rate and the hematocrit can be measured.

2.6.2 Antibody Detection

Indirect evidence for trypanosome infection can be obtained by demonstrating specific antibodies in the blood, plasma, or serum of infected hosts. Trypanosomes have a complex antigenic structure and elicit the production of a large spectrum of antibodies. *T. b. gambiense* specific IgG and IgM antibodies are present in high concentrations and are directed mainly against the immunodominant surface glycoprotein antigens of the parasite. The type of antigen(s) used greatly determines the sensitivity and specificity of the test. Current serological tests detect antibodies after 3 to 4 weeks of infection (Vanhamme *et al.*, 2001). Seropositivity must be interpreted with caution in previously treated patients since antibodies can persist for up to 3 years after cure (Paquet *et al.*, 1992). CATT/*T. b. gambiense* although not registered by any regulatory agency, the introduction of the CATT/*T. b. gambiense* (CATT) for mass population screening has been a major breakthrough in the diagnosis of *T. b. gambiense* HAT, limiting the number of parasitological examination to patients found with a positive serology. Developed in the late 1970s, the CATT is a fast and simple agglutination assay for detection of *T. b. gambiense*-specific antibodies in the blood, plasma, or serum of HAT patients (Magnus *et al.*, 2002**.**). The antigen consists of lyophilized bloodstream forms of *T. b. gambiense* variable antigen type LiTat 1.3. Antigen production is a fastidious process based on the extraction of trypanosomes from infected rat blood.

The trypanosomes are then fixed, stained with Coomassie blue, and freeze-dried. The reagent, which is produced under full quality control, is currently made only at the

Institute of Tropical Medicine in Antwerp, Belgium, and field kits containing the reagent, control sera, and a 12/220-V card rotator are available. One drop of reagent is mixed with one drop of blood and shaken for 5 min on the rotator, and the result is visible to the naked eye. Up to 10 patients can be tested at the same time, and hundreds of individuals can be screened daily. The reported sensitivity of the CATT on undiluted whole blood (CATT-wb) varies from 87 to 98%, and the negative predictive value is excellent during mass population screening (Penchenier *et al*., 2003; Robays *et al.*, 2004; Truc *et al.*, 2002; and WHO, 1998). Nevertheless, false-negative CATT results can occur, as suspected in patients infected with strains of trypanosomes that lack or do not express the LiTat 1.3 gene (Dukes *et al.*, 1992; & Enyaru *et al*., 1998). Because the CATT is not sensitive enough, because their parasites lack the LiTat 1.3 gene, or because their parasites have this gene but do not express it. This could explain the lower sensitivity of the CATT in some areas of endemic infection such as the Ethiope focus Nigeria, where an alternative serological test should be used (Edeghere *et al.*, 1989). Furthermore, when the CATT is performed on undiluted blood or serum with low dilution (<1:4), the agglutination can be inhibited, a phenomenon called prozone. To overcome this problem, which is caused by complement factors and affects the sensitivity of the test, addition of EDTA to the dilution buffer has been proposed (Pansaerts *et al.*, 1998), substantially increasing the sensitivity with only a minor loss in specificity (Simarro *et al.*, 1999). The CATT buffer supplemented with EDTA can remain stable for at least 2 years at 45°C. Despite a reported specificity of around 95%,

the positive predictive value of the CATT-wb remains limited because the test is used for mass screening in populations where the prevalence of HAT is usually below 5% (Penchenier *et al.*, 2003; Robays *et al.*, 2004; Truc *et al.*, 2002; Van Meirvenne, 1992; and Zillmann and Albiez**,** 1986). False-positive results can occur in patients with malaria and other parasitic diseases such as transient infection by nonhuman trypanosomes. The specificity of the CATT is further improved when performed on serum diluted to 1:4 (Magnus et *al.*, 1978; & Vanhamme *et al.*, 2001). This remains insufficient for diagnostic confirmation but allows a significant gain of time and financial resources by decreasing the number of parasitological investigations.

The validity of the CATT, when performed with higher serum dilutions, is discussed below. The CATT can be performed with blood-impregnated filter paper (FP). This method is particularly useful for screening individuals who cannot be reached by full mobile teams during active case finding. The micro-CATT, a protocol using small quantities of both antigen (one-fifth of the standard amount) and FP eluate (sample), showed promising results in Côte d'Ivoire (Noireau *et al*., 1991). The major constraint for widespread use of the micro-CATT is the rapid decrease in sensitivity when FP are stored for more than 1 day at ambient temperature (Truc *et al.*, 2002). Moreover, due to the minute volumes of antigen and test sample used, reading and interpretation of the agglutination patterns can be difficult with the micro-CATT. A recently modified method, the macro-CATT, was developed for testing blood-impregnated FP by using a standard amount of antigen and a higher volume of FP eluate. The macro-CATT was

evaluated in southern Sudan and showed a sensitivity of 91% and excellent stability when FP were stored for up to 2 weeks at ambient temperature (25 to 34°C) (Chappuis *et al*., 2002).

2.6.3 Other serological tests

The LATEX/*T. b. gambiense* has been developed as a field alternative to the CATT (Buscher *et al.*, 1999). The test is based on the combination of three purified variable surface antigens, LiTat 1.3, 1.5, and 1.6, coupled with suspended latex particles. The test procedure is similar to the CATT, including the use of a similar rotator. Compared to the CATT, the LATEX/*T. b. gambiense* showed a higher specificity (96 to 99%) but a lower or similar sensitivity (71 to 100%) in recent field studies conducted in several Western and Central African countries (Jamonneau *et al.*, 2000; Penchenier *et al.*, 2003; and Truc *et al.,* 2002). Further evaluations are needed before it can be recommended for routine field use. Immunofluorescence assays have been used with success for HAT control in Equatorial Guinea, Gabon, and the Republic of Congo, where they were shown to be highly sensitive and specific (Noireau *et al.*, 1988).

The availability of standardized antigen at low cost has greatly improved the reliability of the test (Magnus *et al.*, 1978). It can be used with serum or FP eluates, but the test sensitivity has been reported to be as low as 75% when used with impregnated FP (Simarro *et al.*, 1999). Enzyme-linked immunosorbent assay (ELISA) methods can be performed with serum, FP eluates, and CSF with strict standardization and quantification

(Lejon *et al.*, 1998). Interestingly, ELISA could also detect specific antibodies in the saliva from a group of 23 patients with confirmed HAT(Lejon,(A). 2003). Antibody levels were about 250-fold lower than in the serum and could not be detected by the CATT or the LATEX/*T.b.gambiense* in the vast majority of these patients. The sophisticated equipment required for IFA and ELISA methods limits their use to reference laboratories for remote testing of samples collected in the field during surveys.

2.6.4 Trypanosome Detection

Parasitological diagnosis is made by microscopic examination of lymph node aspirate, blood, or CSF. It provides direct evidence for trypanosome infection and thus allows a definite diagnosis. Unfortunately, parasite numbers in *T. b. gambiense* infection can vary between more than 10,000 trypanosomes/ml, being easily detectable, and less than 100 trypanosomes/ml, being below the detection limit of the most sensitive methods in use. Moreover, parasite detection can be rather labor-intensive. Failure to demonstrate parasites therefore does not necessarily exclude infection. Serial examination of blood on consecutive days can increase the test sensitivity but is rarely performed in practice. When possible, it is recommended to use methods that test a larger quantity of blood and/or that facilitate trypanosome visualization to improve the sensitivity of parasite detection. It is also essential to keep the time between sampling and examination as short as possible to avoid immobilization and subsequent lysis of trypanosomes in the sample. Trypanosomes are rapidly killed by direct sunlight but can survive for a few hours when

the sample is kept in a cool and dark place.

2.6.5 Chancre aspirate

Trypanosomes can be detected in the chancre a few days earlier than in the blood. The chancre is punctured, and the fluid obtained is microscopically examined as a fresh or fixed and Giemsa-stained preparation. This method is very seldom applied in the field because most infections are detected much later, when the chancre has already disappeared.

2.5.6 Lymph node aspirate

Cervical lymph node palpation should be done systematically in all patients with a positive CATT result. When enlarged CLN are present, they are punctured, the fresh aspirate is expelled onto a slide, and a coverslip is applied to spread the sample and facilitate the reading. The wet preparation is then quickly examined by microscope (magnification, x400) for the presence of motile trypanosomes. The technique is simple and cheap. The sensitivity varies between 40 and 80% depending on the parasite strain, the stage of the disease (sensitivity is higher during the first stage), and the prevalence of other diseases causing lymphadenopathy (Van Meirvenne, 1999). The yield of CLN palpation and puncture in patients with a negative CATT is very low; between 1999 and 2001, trypanosomes were observed in only 316 (0.18%) of 174,295 lymph node aspirates from CATT-negative individuals in Democratic Republic of Congo. The authors calculated that a mean of 138 h of work per new case diagnosed would be

necessary. Furthermore, all positive lymph node aspirates found in 1,000 individuals from two endemic foci located in Angola and Central African Republic were associated with positive CATT. Systematic CLN palpation and puncture is therefore not cost-effective for use with CATT-negative individuals (Lutumba, in press) unless indicative clinical signs are present.

2.6.7 Wet and thick blood films

In wet blood films, 5 to 10 µl of finger prick blood is placed on a slide and examined microscopically (magnification, x400) under a coverslip. Trypanosomes can be seen moving between the erythrocytes (the movement of the surrounding erythrocytes often attracts attention). Despite its very low sensitivity, with a detection limit as high as 10,000 trypanosomes/ml, corresponding to 1 parasite/200 microscope fields, this method is still used in some centers because of its low cost and simplicity. Giemsa- or Field's-stained thin blood films have a similarly low sensitivity. Examination of 20 µl of stained blood in a thick blood film slightly improves sensitivity, with a detection threshold of around 5,000 trypanosomes/ml. It is the technique of choice for blood examination only when no centrifuge is available, although it is quite time-consuming (10 to 20 min per slide) and requires expertise to recognize the parasite, which is frequently deformed in this preparation. Apart from trypanosomes, other parasites such as microfilaria and *Plasmodium* can be detected.

2.6.8 Microhematocrit centrifugation technique

The blood concentration technique of microhematocrit centrifugation (mHCT), sometimes referred to as the capillary tube centrifugation technique or as the Woo test, was developed more than 30 years ago and is still in use in many HAT control programs (Woo, 1970). In brief, capillary tubes containing anticoagulant are filled three-quarters full with finger prick blood. The dry end is sealed with plasticine. By high-speed centrifugation in a hematocrit centrifuge for 6 to 8 min, trypanosomes are concentrated at the level of the white blood cells, between the plasma and the erythrocytes. The capillary tubes, mounted in a special holder, can be directly examined at low magnification (x100 or x200) for mobile parasites. The sensitivity of mHCT increases with the number of tubes examined, with an estimated detection threshold of 500 trypanosomes /ml. The optimal number of tubes has not been determined with certainty, but in most programs, six to eight tubes are prepared. This technique is moderately time-consuming, and the concomitant presence of microfilaria in the blood can render the visualization of the much smaller trypanosomes very difficult. Nevertheless, this relatively simple technique can be applied during mass screening by mobile teams

2. 6.9 Quantitative buffy coat

The quantitative buffy coat (QBC; Beckton-Dickinson), initially developed for the rapid assessment of differential cell counts, has been extended to the diagnosis of hemoparasites including trypanosomes (Bailey and Smith**,** 1992; Levine *et al.*,

1989). It has the advantages of concentrating the parasites by centrifugation and, by staining the nucleus and kinetoplast of trypanosomes with acridine orange, allowing a better discrimination from white blood cells. After high-speed centrifugation of the blood in special capillary tubes containing EDTA, acridine orange, and a small floating cylinder, motile trypanosomes can be identified by their fluorescent kinetoplasts and nuclei in the expanded buffy coat. UV light is generated by a cold light source connected by a glass fiber to a special objective containing the appropriate filter. This objective can be mounted on almost every microscope. A darkroom is needed for the procedure. The relative sophistication and fragility of the material prevents its daily transport during active screening sessions. The QBC is a very sensitive technique that is very appreciated by most field laboratory workers. It also allows the diagnosis of concomitant malaria, which is very useful for patient care. With a 95% sensitivity for trypanosome concentrations of 450/ml, the QBC can detect more patients with low parasitemia than the mHCT when fewer than eight capillary tubes are used. It is as sensitive as the mini-anion-exchange centrifugation technique (mAECT) described below (Truc *et al.*, 1998). Production of the QBC kit has been abandoned, but the manufacturing of capillaries was recently resumed.

2. 6.10 Mini Anion Exchange centrifugation technique

The mAECT was introduced by (Lumsden *et al*., 1979), based on a technique developed by Lanham and Godfrey (Lanham and Godfrey**,** 1970). An initial evaluation showed that

the mAECT was more sensitive than the thick blood film and the mHCT (Lumsden *et al*., 1981). An updated version has been described by (Zillmann *et al*., 1996). The technique consists of separating the trypanosomes, which are less negatively charged than blood cells, from venous blood by anion-exchange chromatography and concentrating them at the bottom of a sealed glass tube by low-speed centrifugation. The tip of the glass tube is then examined in a special holder under the microscope for the presence of trypanosomes. The large blood volume (300 µl) enables the detection of less than 100 trypanosomes per ml, resulting in high sensitivity, but the manipulations are quite tedious and time-consuming. mAECT columns are now produced with the assistance of the Institute of Tropical Medicine—Antwerp at Kinshasa, DRC. Studies validating this newly produced version of mAECT are under way.

2. 7 Stage Determination: Cerebrospinal Fluid Examination

In the absence of sufficiently specific clinical signs and blood tests indicating the evolution from first- to second-stage HAT, staging of patients still relies on examination of CSF obtained by lumbar puncture. It is a vital step in the diagnosis process. Patients with first-stage disease receive daily intramuscular pentamidine for 7 to 10 days, a treatment associated with less than 1% mortality, whereas patients with second-stage disease are still treated in most centers with melarsoprol, an arsenical derivative associated with a 2 to 10% fatality rate (Pepin and Milord, 1994). The majority of deaths are due to treatment-related acute encephalopathies (Pepin *et al.*, 1994; and WHO**.** 1998). Eflornithine (DFMO) is a safer treatment than melarsoprol, but its

complicated schedule (four intravenous infusions per day for 14 days) and cost remain an obstacle to a wide field application. According to WHO recommendations, second-stage HAT is defined by the presence in the CSF of one or more of the following (WHO**.** 1998), raised white blood cell count (>5 cells/µl), trypanosomes, and increased protein content (>370 mg/liter, as measured by the dye-binding protein assay). As reviewed below, these criteria are not entirely satisfying and might soon be modified by recent studies' findings.

2. 7 .1 White blood cell count

The CSF white blood cell count is the most widely used technique for stage determination. After collecting the CSF sample, the cell count should be carried out as soon as possible to prevent cell lysis. Due to the small number of cells in normal CSF, cell-counting chambers should have a volume of at least 1 µl, such as the Fuchs-Rosenthal and the Neubauer devices. It is not recommended to dilute the CSF with Türck solution since this solution can lyse trypanosomes. If fewer than 20 cells /µl are counted, it is recommended to repeat the counting procedure and to calculate mean count values. The CSF pleocytosis is of lymphocytic origin, consisting mainly of B cells (Greenwood *et al.*, 1976B). The 5-cells/µl threshold for treatment decision is controversial (Lejon *et al.*, 2003B; and Miezan *et a.l*, 1998). Some countries use a threshold of 10 cells/µl (Equatorial Guinea) or even 20 cells/µl (Angola and Côte d'Ivoire) in their national protocol (Doua *et al.*, 1996; Lejon *et al.*, 2003B; & Stanghellini and Josenando, 2001). Patients with 6 to 20 cells/µl in the CSF

are sometimes referred to as being in the "early second stage" or "intermediate stage" of the illness. This group is in fact composed of individuals with or without signs of neuroinflammation, as recently demonstrated (Lejon *et al.*, 2003B). This is further illustrated by the effectiveness of pentamidine in HAT patients with 6 to 20 cells/µl of CSF in Côte d'Ivoire (Doua *et al.*, 1996), while in a study in Uganda, patients with 11 to 20 cells/µl or with evidence of intrathecal IgM synthesis (a reliable marker of neuroinflammation) had a lower cure rate, suggesting that these patients should be treated with DFMO or melarsoprol (Lejon *et al.*, 2003C). In a smaller study in Angola, the relapse rate after pentamidine treatment was similar in patients with 0 to 5 or 6 to 10 cells/µl (Ruiz *et al.*, 2002). These data support the increase of the CSF cell threshold from 5 to 10 cells /µl. Furthermore, one should take into account the higher normal cell counts in neonates (Sarff *et al.*, 1976). There is a general agreement that patients with proven HAT (trypanosomes seen in the lymph node or blood) and with >20 cells/µl in CSF should be treated as having second-stage HAT. In Médecins Sans Frontières and Malteser programs in Sudan and Uganda, serologically suspected individuals (positive CATT of 1:4) with negative parasitological examination and >20 cells/µl in the CSF are treated as second-stage HAT patients. This approach aims at partially compensating the insufficient sensitivity of trypanosome detection but exposes some non-HAT patients to unnecessary treatment for second-stage illness. It can be justified in areas with high HAT prevalence, especially where DFMO, a safer drug than melarsoprol, is used as the first-line treatment. Here again, the availability of more sensitive parasite detection

methods and more precise staging tools would solve the controversy. The morular cells of Mott, which are plasma cells with large vacuoles containing IgM, are reported to be highly indicative of HAT when found in the CSF (Greenwood and Whittle, (1973); Greenwood and Whittle, 1980; & Pepin and Donelson, 1999). Mott cells are rarely observed in the field and can also be found in other neuroinfectious diseases such as neurosyphilis (Kristensson and Bentivoglio, 1999).

2. 8 Trypanosome detection

The finding of trypanosomes in CSF allows immediate classification of a patient as being in the second stage of illness. It is important to examine the CSF immediately after lumbar puncture, because trypanosomes in CSF start to lyse within 10 min. Direct detection of trypanosomes (e.g., during cell counting) is a simple and cheap technique but suffers from insufficient sensitivity. Increased sensitivity of trypanosome detection is obtained by centrifugation of the CSF sample, especially when a double centrifugation method is used (Cattand *et al.*, 1988). The latter method is relatively time-consuming and requires two different types of centrifuges; therefore, it is not applicable in every field setting. A modified and simplified single centrifugation of CSF using a sealed Pasteur pipette has been proposed as an alternative to double centrifugation (Miezan *et al.*, 2000). Some authors challenge the value of finding CSF trypanosomes in patients with no sign of CSF inflammation (absence of elevated protein and cell count of ≤20/µl in the CSF) who were shown to respond to pentamidine treatment (Doua *et al.*, 1996;

Lejon *et al.*, 2003C; & Miezan *et al.*, 1998).

2. 9 Protein concentration

In normal healthy individuals, proteins in the cerebrospinal fluids consist mainly of albumin (70%) and IgG (30%), both originating from the serum. Protein concentrations in the cerebrospinal fluids are elevated in HAT patients and range from 100 to 2,000 mg/liter (Bisser *et al.*, 2002; & Lejon *et al.*, 2003C). Protein concentrations can also be raised in first-stage illness due to the diffusion in the cerebrospinal fluids of IgG, which can be present in very high concentrations in the serum. Recent evidence suggests that the protein concentration threshold set by WHO (370 mg/liter) is too low and should be raised to 750 mg/liter to reflect blood-brain barrier impairment, astrocyte activation, and neurodegeneration (Lejon *et al.*, 2001; Lejon *et al.*, 2003B). Despite its apparent simplicity, accurate determination of the total protein concentration in Cerebrospinal fluids is rather difficult. Cerebrospinal fluid protein concentrations obtained by different methods and different standards are not comparable. As a consequence of the sophistication of the methods, the absence of standardization, the instability of reagents, and the limited (if any) added value compared to CSF cell count (Miezan *et al.*, 1998), total protein measurement for staging HAT is no longer recommended and has been virtually abandoned in field laboratories.

2.10 Antibody detection

It has been well known for several decades that the CSF of second-stage HAT patients contains high levels of immunoglobulins, especially IgM (Greenwood *et al.*, 1973; Lambert *et al.*, 1981; & Whittle *et al.*, 1977). An increased CSF IgM concentration has thus been considered by some as a strong potential marker of second-stage HAT (Greenwood and Whittle, 1980). The demonstration of intrathecal synthesis of immunoglobulins strongly supports the diagnosis of neuroinflammatory diseases. Immunoglobulins synthesized in the CNS need to be discriminated from blood-derived immunoglobulins by calculation of the intrathecal fraction and antibody index (quantitative approach) or by detection of oligoclonal antibodies (qualitative approach) (Reiber and Peter, 2001). The origin and composition of the CSF immunoglobulins have been recently studied in experiments with large patients groups. As previously suspected (Greenwood and Whittle, 1973), these studies confirmed that the elevated immunoglobulin concentration in the CSF is due to intrathecal synthesis and that the dominant IgM presence was an early marker of CNS invasion whereas blood-CSF barrier dysfunction was found late in the course of CNS involvement (Bisser *et al.*, 2002; Lejon *et al.*, 2003D). these results were further confirmed by studies of 272 HAT patients from different areas of endemic infection, where intrathecal synthesis of IgM was found in 95% of patients with second-stage illness (Lejon *et al.*, 2003B). *Trypanosma bruci gambiense* HAT can thus be classified among neuroinflammatory diseases with a dominant IgM immune response pattern in the CNS, like Lyme

neuroborreliosis and, occasionally, neuro-syphilis (Reiber and Peter. 2001). Despite its relevance to stage determination, IgM detection in CSF has not been carried out in the field, owing to the lack of simple and robust tests. A latex agglutination test for IgM in CSF (LATEX/IgM) has recently been developed. It is designed for field use and remains stable at 45°C for more than 2 years. Following initial promising results (Lejon *et al.*, 1998B), the LATEX/IgM was evaluated with CSF samples from patients from several countries where infection is endemic (Lejon *et al.*, 2002). Cerebrospinal fluids(CSF) end titers obtained by the LATEX/IgM paralleled the IgM concentrations determined by nephelometry and ELISA. At a cutoff value of ≥1:8, the sensitivity and specificity of LATEX/IgM for intrathecal IgM synthesis were 89 and 93%, respectively.

Future prospective studies with large numbers of patients are needed for LATEX/IgM validation. Only a small proportion of the very large amount of IgM produced during HAT is specific anti-trypanosome antibody (Greenwood and Whittle, 1980). Trypanosome-specific antibodies detected in the CSF by indirect immunofluorescence are specific for second-stage illness, whereas a comparison with serum values by calculation of the antibody index is necessary when measurements are performed by ELISA (Lambert *et al.*, 1981; Lejon *et al.*, 1998; Lucasse, 1964). However, these methods are too sophisticated to be used in remote treatment centers. Unfortunately, the field-designed CATT/*T. b. gambiense* and LATEX/*T. b. gambiense* lack sensitivity when used with CSF for detection of anti-trypanosome immunoglobulins (Buscher *et al.*, 1999; Lemesre *et al.*, 1988; & Lucasse, 1964). The CSF of HAT patients

also contains antibodies with other affinities. Antibodies against brain-specific components such as neurofilaments and galactocerebrosides (GalC) have been detected and may be promising markers of second-stage illness (Ayed *et al.*, 1997; Lejon *et al.*, 2001; & Lejon *et al.*, 1999). These autoantibodies, which might result from the CNS damage and immune activation triggered by trypanosome invasion, are associated with markers of neuroinflammation such as the CSF cell count and protein and immunoglobulin concentrations (Bisser *et al.*, 2000; Lejon *et al.*, 2001). Unfortunately, anti-GalC antibodies detectable in the serum are not correlated with neuroinflammation (Bisser *et al.*, 2000).

2. 11 Management of Serologically Suspect Individuals

As previously stated, the parasitological methods have a limited sensitivity and therefore do not allow all HAT patients with an initial positive screening (usually a positive CATT on whole blood and on a 1:4 serum dilution) to be confirmed to have the disease and receive treatment (WHO, 1998). These undiagnosed patients will return home and will either be diagnosed at a later stage of the disease or simply die. Moreover, they contribute to the reservoir of parasites and thus to transmission of the disease. One option is to examine these serologically suspect individuals at regular intervals (every 3 months) for 1 to 2 years (WHO, 1998). However, compliance with follow-up visits is usually low, and the efficiency of this strategy is poor (Chappuis *et al.*, 2004). A much more promising option is to determine a subgroup of serologically suspected individuals at high risk of being infected with *T. b. gambiense* and to treat them. Of 86

serologically suspect individuals (CATT serum, ≥1:4) being monitored in Angola, 52% of individuals with a CATT plasma end titer of ≥1:16 were diagnosed with HAT during follow-up but none of the individuals with lower CATT end titers were diagnosed with disease (Simarro and Josenando, 1999). Similar results were obtained for a group of 749 serologically suspect individuals (CATT, ≥1:4) who were examined at least once during a 12-month follow-up period in Kajo-Keji county, southern Sudan (Chappuis *et al.*, 2004). Individuals with a CATT plasma end-dilution titer of ≥1:16 had a 50% person-year risk of being confirmed with HAT during follow-up, compared with a 10 and 14% person-year risk for 1:4 and 1:8 end-dilution titers, respectively. The authors of these studies recommend treatment for all serologically suspect individuals with a CATT end titer of ≥1:16, when the prevalence of HAT in the investigated population is sufficiently high. Which prevalence threshold to choose remains an open question, but it should probably be no less than 1%. In a study conducted in a low-prevalence area in Côte d'Ivoire, only one patient was diagnosed with HAT during a 2-year longitudinal follow-up of 77 serologically suspect patients (Garcia *et al.*, 2000). However, the CATT end-dilution titer was limited to 1:4 in this study. Other factors, such as poor access to care and absence or limited availability of parasitological diagnosis, should positively influence the decision to perform titration of serum and treat individuals with a CATT end titer of ≥1:16.

There is no "one-size-fits-all" algorithm for the diagnosis of HAT. The positive predictive value of any diagnostic test varies with the prevalence of HAT among the

tested population. Therefore, the diagnostic tree applied in a given area should be adapted to decreasing disease prevalence. Nevertheless, there is a need to decrease interprogram variations in the diagnostic approach. A more standard diagnostic algorithm would improve the external validity of the few studies conducted on the diagnosis and treatment of this neglected disease. An example of a diagnostic algorithm, adapted from a recent publication by WHO experts. (Simarro *et al.*, 2003). This algorithm is particularly well suited for populations with a rather high disease prevalence (above 2%), such as individuals coming on their own to the treatment center, who are more likely to have symptoms and thus the disease, or individuals from a highly active focus of infection. If the prevalence of the disease is lower (below 1%), it will be more appropriate to set a higher CATT dilution threshold before implementing the time-consuming search for parasites in the blood (1:8 instead of 1:4). As stated above, the treatment of serological suspect patients with a high CATT end-dilution titer (≥1:16) would not be justified for populations with low disease prevalence. In this case, these individuals should return home and be told to come for control visits if symptoms appear or worsen. The choice of the method for detection of bloodstream parasites will depend on the method's performance (detection threshold), the workload, the number and level of training of laboratory workers, and the financial resources. Blood concentration methods such as the mHCT or the QBC should be preferred to a wet or thick blood smear. The mAECT can be performed sequentially after a negative mHCT, but the efficiency of this controversial strategy is still being studied. As discussed above, the

CSF cell count threshold defining the stage of the disease is still subject to debate. Considering the growing evidence showing a good efficacy of pentamidine in HAT patients with ≤10 cells/µl (Doua *et al.*, 1996; & Ruiz *et al.*, 2002), a threshold of 10 cells is proposed in this algorithm. A 5-cells/µl threshold remains justified though, particularly in programs using the less toxic eflornithine as first-line therapy for second-stage illness. Current diagnostic algorithms for HAT tend to neglect investigations for other diseases such as malaria, typhoid fever, tuberculosis, syphilis, and HIV-associated diseases. These conditions can mimic or complicate *T. b. gambiense* HAT. The influence of the varying prevalence of these diseases on the validity of some key steps of the HAT diagnostic algorithm, such as the thresholds for CATT end-dilution titer and the CSF cell count, deserves further investigations.

2. 12 Other Diagnostic techniques

2.12.1 Antigen Detection Tests.

Antigen detection is an attractive concept that would allow, unlike methods detecting antibodies, a distinction between active and cured HAT. By detecting antigens released by non circulating trypanosomes sequestered in the liver, spleen, lymph nodes, or CNS, antigen detection has the potential to improve the sensitivity of current parasitological methods. Following promising results of specific antigen detection by ELISA (Nantulya *et al.*, 1992), the card indirect agglutination test for trypanosomiasis (TrypTect CIATT; Brentec Diagnostics, Nairobi, Kenya) was developed for field use. A preliminary evaluation of the TrypTectCIATT showed a high sensitivity compared to other

parasitological techniques (Nantulya, 1997), but the results of subsequent studies raised strong doubts about its specificity (Asonganyi *et al*., 1998).

2.12.2 Polymerase Chain Reaction (PCR)

Different assays now exist; however, none of them have been validated for diagnostic purposes. Polymerase Chain Reaction targeting repetitive sequences are in theory more sensitive than those targeting low-copy or single-copy sequences like the recently developed tests for distinguishing *T. b. gambiense* and *T. b. rhodesiense* (Kabiri *et al.*, 1999; Radwanska *et al.*, 2002A; Radwanska *et al.*, 2002B); Schares and Mehlitz, 1996; & Welburn *et al.*,. 2001). In principle, PCR can be applied to any patient sample that may contain trypanosome DNA, such as whole blood or buffy coat, lymph node fluid, or CSF. Samples should be stabilized in special buffers or on FP. The FTA FP produced by Whatman is particularly convenient since it is easy to handle and it protects the DNA from degradation, unlike common FP. However, the amount of sample that can be applied on filter paper is small, thus limiting the chance to contain enough DNA for detection. Samples should be protected from sunlight to avoid DNA degradation. Polymerase Chain Reaction results are not always unequivocal. Unexplained false-negative and false-positive results were observed in CATT-seropositive but parasitologically none confirmed persons and in CATT-negative controls (Garcia *et al.*, 2000; & Solano *et al.*, 2002). Also, the significance of a positive PCR on a CSF sample is unclear. (True *et al.*,1999) report that PCR is 100% sensitive compared to double centrifugation of CSF, but (Jamonneau *et al.*, 2003) clearly demonstrate that a

number of patients with positive PCR results with CSF were successfully treated with pentamidine, thus showing them to be in the first stage of the disease (Jamonneau *et al.*, 2003; & Truc *et al.*, 1999). Efforts to simplify the PCR amplification method itself, such as using an isothermal amplification reaction and visualization of the PCR product by precipitation (Kuboki *et al.*, 2003) or by oligochromatography (Mertens et al., 2004), may facilitate PCR application in African countries, but PCR is definitely not an option for field diagnosis and for the time being is restricted to research purposes.

2.12.3 Proteomic signature analysis.

Proteomic signature analysis is a promising technology that has been recently used with sera from patients with HAT and other diseases (Papadopoulos *et al.*, 2004). The accuracy of this experimental method was high (100% sensitivity and 98.6% specificity) but needs to be confirmed in prospective studies. As the authors stated, this method is impracticable in the field but could identify discriminating biomarkers that could lead to the development of more conventional and simpler tests.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study Site

Lui hospital is found in Mundri East County, one of ten counties of Western Equatoria state, which is one of 10 states of Southern Sudan. The hospital receives patients from Mundri east surrounding counties and it is a referral hospital. The County bordered by Mvolo county to the North, Terekeka county in North east, Juba county to the east, Laniya to the south and Mundri West county to the west, The county is divided into five administrative payams namely; Kediba, Witto, Lozoh, Lakamadi and Minga. The Headquarter and the seat of the highest political appointment in the county are in Lui.

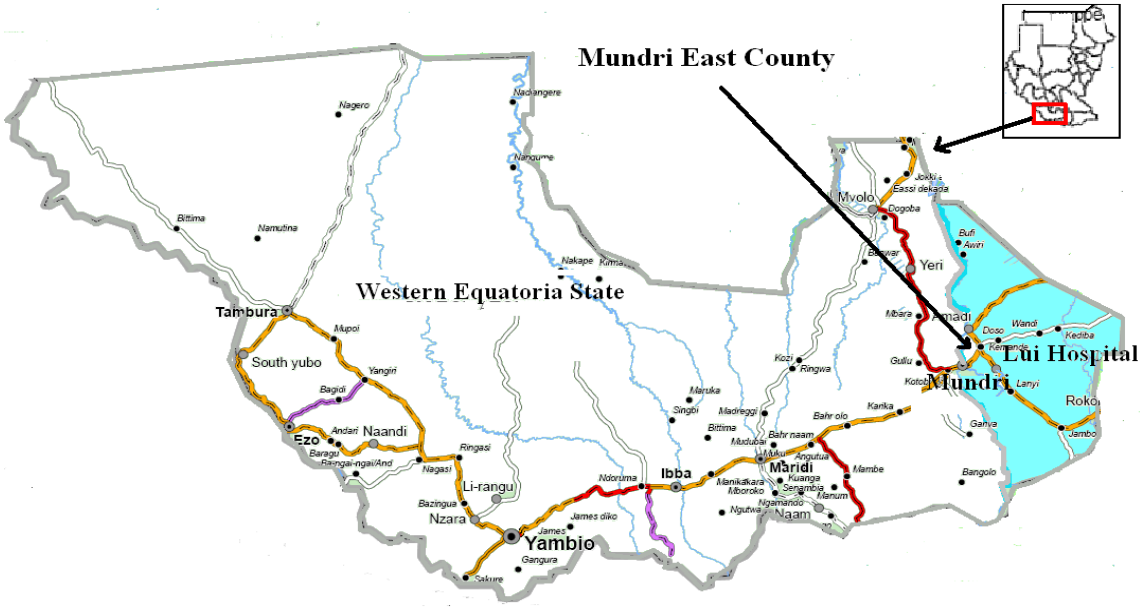

Figure 5 Map of Western Equatoria State Showing Mundri and Lui Hospital

Source: Adapted from UNJLC – Southern Sudan Office.

Like other parts of Southern Sudan; Mundri east county has been badly affected by the civil war that started since 1956, resulting in serious break down of infrastructure development, and major destruction and displacement During all this period there has been no research conducted in this war devastated areas and little information is found on the prevalence of the disease in the area. With the support of world Health Organization (WHO) and non governmental organizations (MSF), Samaritan purse and diocese of Lui management of sleeping sickness in area is taking place. MSF- F in 2004 had a treatment center in Mundri west county (Kotobi) which is now closed. According to World Health Organization Southern Sudan sub-office data of 2006 and 2007; total of 2292 persons were passively screened at Lui hospital for sleeping sickness and 81 persons were confirmed cases with incidence of 3.67%. Further, another total of 1534 persons were screened and 61 persons found positive with incidence of 3.98% in 2006 and October, 2007 respectively (WHO-Southern Sudan- HAT 2006/2007 Data Base).

The geographical distribution of these confirmed cases were from different payams of both Mundri East and Mundri West Counties although Lozoh payam of Mundri East County had high number of cases in 2007.

3.2 Study Design

This was a formal survey with a component of un-matched case-control designed. Information on the knowledge, attitudes and perception as well as risk factors was obtained through pre-tested questionnaire among subjects with disease and those without disease.

The association between risk factors was evaluated for the disease and not disease for their influence on early disease detection and diagnosis.trained research assistants and the investigator administered the questionnaire to the study subjects in local language or Arabic.

3.3 Study Population

The study population comprised of subjects who tested positive for *trypanosoma* by microscopy and CATT (Card Agglutination Test for trypanosomiasis) titration method for Human African trypanosomiasis. The controls were individuals who tested negative for sleeping sickness by CATT and were admitted in surgical ward of Lui hospital, Mundri East county. Mundri East County has estimated population of over 60,000 persons, according to WHO- Southern Sudan Data base2006-2007 for National Immunization Days(NIDs). The Muro tribe is the main ethnic group and constitutes about 90% of the population with 10% Dinkas and a mixture of other equatorial tribes. The county has a predominately agriculture-based economy and livestock keeping as the main source of income for the local population.

3.3.1 Cases

These were Human African trypanosomiasis patients aged above 2 years and confirmed for sleeping sickness by microscopy and CATT titration method. Cases were recruited from HAT clinic in Lui Hospital of Mundri East County from the month of September, 2008 to January, 2009.

3.3.2 Controls

These were subjects found negative for HAT by microscopy and CATT and were hospitalized in surgical wards for other ailments in the same hospital at the same time as cases. Controls were systematically selected from patients clinics and those admitted to surgical ward. Every fifth patient in the list for surgical ward and negative for Trypanosomiasis considered was enrolled as control.

3.4 Inclusion Criteria

Any patient diagnosed with human African trypanosomiasis and presented at Lui hospital during the study period (September, 2008 – January, 2009) was recruited for the study. Controls considered were subjects negative for sleeping sickness and in surgical ward in Lui hospital at the same study period and above 2 years of age.

3.5 Exclusion Criteria.

The study excluded all subjects who were below 2yeards and those whose results for HAT by microscopy or CATT test could not be determined. .

3.6 Sample Size

A total of 108 (54 cases and 54 controls i.e. ratio of 1:1) were recruited into the study. The Formula (Hennekens, Bury 1st addition, 1987) below was used to determine the sample size with HAT prevalence of 50%, 84% power and 95% confidence level.

$$\text{Sample size} = \frac{((P1\ q1 + P0\ q0)\ (Z\alpha + Z\beta))^2}{(P\ 1 - P0)^2}$$

Where P1 is the proportion of case among exposed (50%)

P0 is the proportion of control among unexposed (37%)

q1= (is equal to 1-P1) and

q0 = (is equal to 1-P0).

Z α is 95% confident interval (1.96)

Zβ is the power of study (84%) = 0.84

Therefore, sample size = $\frac{[(0.5 \times 0.5 + 0.37 \times 0.63)\ (\ 1.96 \times 0.84)]^2}{(0.5 - 0.37)^2} = 108$

3.7 Administeration of Questionnaire

The questionnaire was first pre-tested in Lui Hospital after translation from English to Arabic language in first week of September. All comments and observations were included and three field researchers were trained on how to administer and fill the questionnaires. Standardized, semi-structured interviewer-administered questionnaires were used to collect data from the study subjects from the time of recruitment prior to

disclosure of the results to participants. Data collected were; Socio-demographic information, knowledge on signs and symptoms, laboratory information including HAT screening and confirmation, epidemiological information (Place, Person and Time) and knowledge, attitudes and perceptions about HAT. Neither cases nor controls were made aware of the research hypothesis upfront.

3.8 Diagnosis of Trypanosomiasis

The diagnosis of *T. b. gambiense HAT* follows a three-step pathway: screening, diagnostic confirmation, and staging. Primary diagnosis is by use of card agglutination test for trypanosomiasis (CATT) (Magnus *et al.*, 1978), and confirmation is by parasitological test. This is followed by determination of the stage of the disease. In this study people who are tested positive with CATT were subjected to confirmation of the *Trypanomiasis* parasite either in their blood, cervical lymph nodes aspirates or cerebrospinal fluids by microscopic examination. The controls were only subjected to CATT test and those with negative test and admitted to surgical ward was considered for the study.

3.8.1 Diagnosis and treatment protocol for new cases of HAT in Lui Hospital

In Lui Hospital the bloods of suspected persons and controls are screened using Card Agglutination Test for Trypanosomiasis (CATT), negative persons are categorized as controls and are examined for other medical conditions. Those with positive test and with palpable cervical lymph node will be examined for the parasite in their lymph note

aspirates microscopically, if positive then is considered a case of Trypanomiasis. If the suspect has CATT positive without palpable cervical lymph node, his blood will be examined (woo test) for presence of the parasite microscopically. Positive ones are considered confirmed case, if negative the CATT dilution methods will be applied and those with positive dilution at 1/16; 1/32 are considered confirmed case while those with positive at 1/2; ¼ or 1/8 are only subject for follow up and will not be considered as confirmed cases until proved otherwise(diagram below). Confirmed cases (persons) are subjected to lumber puncture for the staging of the disease and treatment. Cerebrospinal fluids of this patient will be examined for the presence of the Trypanomiasis parasites. Patients have trypanosomes in their cerebrospinal fluids are considered at stage two and will be treated using Eflornithine while patient with negative trypanosomes in their cerebrospinal fluids are in stage one and are treated using Pentamidine.

3.9 Data Management

3.9.1 Data Storage

Data was transferred from questionnaires to the computer by a data assistant using Epi info version 3.4.3. The data was coded, stored, pass-word protected and backed-up on alternate secure storage media. Filled questionnaires were safely stored for at least 3 years.

3.9.2 Data analysis

Data was validated, cleaned and analyzed by the principal research officer using the Epi info 3.4.3 computer program for Windows.

3.9.2.1 Bivariate analysis

This was done to compare two variables to each other in contingency tables to show odds ratio (OR) as a measure of association and confidence intervals.

3.9.2.2 Measures of statistical significance

This was done using T-test for continuous variables and Chi-square for categorical variables to determine the corresponding P Values.

3.10 Ethical considerations

Permission for protocol approval was granted by both Ministry of Health Government of Southern Sudan and Jomo Kenyatta University of Agriculture and Technology. Permission for ethical clearance to Lui Hospital was also obtained to carry out the study in the Hospital. A written informed consent was sought from the study participants before enrolling them into the study. The participants were informed of the purpose of study, anticipated risk factors and benefits, confidentiality and anonymity before being asked if they wished to join the study.

CHAPTER FOUR

4. RESULTS

4.1 Description of study population

A total 54 sleeping sickness patients and 54 non-sleeping sickness patients were enrolled to the study from September 2008 to January 2009. Of the 54 cases twenty six were females (48.2%) and 28 males (51.9%). In the control group 42.6% were females and reminder 57.4% were males. The distribution by gender for both cases and controls was not significantly different Table 1. Majority of cases (38.9%) were in age group 20 – 29 years, followed by those in age group 30 – 39 years (24.4%) Figure 7. The mean age of cases was 25.5± 5 years while for the controls were 32.8± 7 years. The difference in age was significant by disease status at $\chi^2 = 8.73$; P = 0.0031. More than 50% of cases had primary education compared to 38.8% in the control group respectively. The difference between various education levels for cases and controls did not reach statistical significance ($\chi^2 = 5.28$; P = 0.071). Thirty five (68.5 %) cases were single compared to (63%) of the controls married. The difference by marital status was statistically significant further $\chi^2 = 9.51$; P = 0.0021) Table 1. Fifty one (94.4%) cases and 41 (75.9%) controls were Moru tribe and only 2 (3.7%) cases and 6 (7.4%) controls were different.

Of the 25 suspected HAT patients who had palpable cervical lymph node and underwent gland puncture one patient was found microscopically positive for trypanosomes Figure 6. The remaining 24 were negative for the trypanosomes and were subjected to CATT

whole blood test together with eighty three (83) Subjects/ Individual who had no palpable cervical lymph nodes. Fifty three (49.5%) were found positive serologically and fifty four (100%) of the controls were negative for trypanosomes by CATT method. Of fifty three serologically positive for trypanosomes by CATT nine (16.7%) of them were found positive for the parasite by Woo test while forty four (81.5%) turned negative. The 44 negative by Woo test underwent testing by CATT titration method and all gave positive results under dilution 1/16 or 1/32 and are considered confirmed cases. The confirmed fifty four cases (forty five serologically and nine parasitologically) also underwent lumber puncture to determine the stage of the disease. Cerebrospinal fluids of these patients were examined for the presence of the Trypanosmes parasites and white blood cell count for stage determination. Two cases among the 54 parasitologically confirmed cases had white blood count of less than 5-cells/µl diagnosed as stage one (the parasite is only found in blood and lymph node aspirate and known as haemolymphatic stage and was treated using Pentamidine. The remaining fifty two HAT cases were diagnosed as stage two (due to presence of one or two of the following,

Increased lymphocyte counts (≥20cells/µl), or increased protein levels (>35 mg/dl), or the presence of trypanosomes in cerebrospinal fluid) also known encephalitic stage.

Figure 6 Algorithm For cases Screening and Diagnosis Results

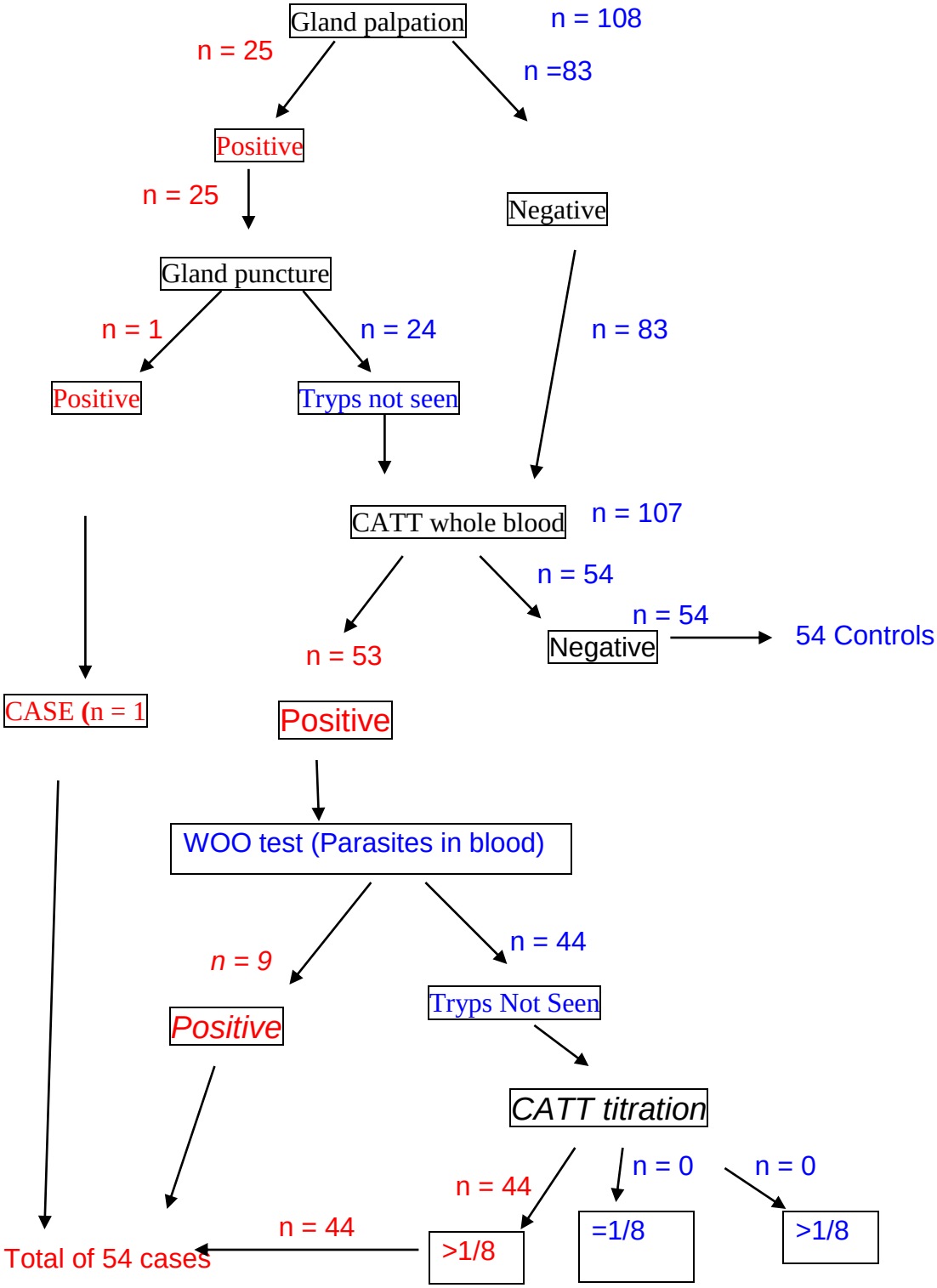

Table 1 Description of selected socio-demographic characteristics of respondents (N= 108)

Sex	No. of Cases (%)	No. of Controls (%)	Chi- square Value (χ^2)	P Value
Female	26(48.1%)	23(42.6%)	0.149	0.05
Male	28(51.9%)	31(57.4%)		
Mean Age (yrs)	25.5 ± 5	32.8± 7	8.73	< 0.0001
Occupation				
Formal employment	7(13.0%)	11(20.4%)	20.52	< 0.0001
Student	37 (68.5%)	14 (26%)		
No employment	10 (18.5%)	29 (53.7%)		
Education level				
Not educated	11 (20.4%)	22 (40.7%)	5.28	0.0712
Primary	28 (51.8%)	21 (38.9%)		
Secondary	15 (27.8%)	11 (20.4%)		
Marital status				
Married	17(31.5%)	34 (63%)	9.51	0.0020
Single	37 (68.5%)	20 (37%)		

..

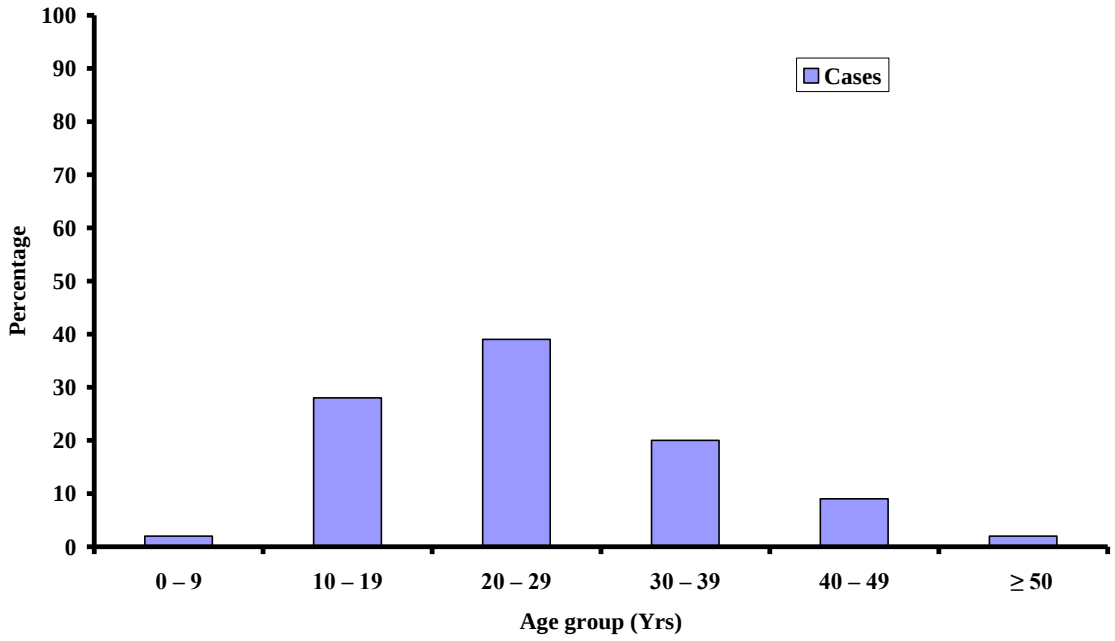

Figure 7 Proportion of cases by age in the study (N=54)

Thirty eight (70.4%) cases were resident in a rural area while 29.6% were in Urban Majority of participants lived in Mundri East (55.6%) and Mundri West 31.5%, with 59.3 % of cases and 51.9% of control coming from Mundri East and 35.2 % of cases and 27.8 % of controls from Mundri West. The remaining cases and control resided in Juba, Laniya, Nyral, Terekeka, Warrap, Wau and Yei Districts.

4.3 Knowledge on symptoms and signs of respondants

The signs and symptoms of sleeping sickness reported by participants included mood or sleep uncontrolled disturbance 16 (29.6%) by cases and 8 (14.8%) by controls (χ^2 = 2.62; P = 0.035) which is not statistically significant. Fever was reported by 51 (94.4%) cases and 19 (35.2%) controls and the distribution was significantly different (χ^2 = 39.01; P = 0.0001). Body malaise was reported more frequent among cases with 33 (61.1%) cases reporting symptom and 9 (16.7%) controls with χ^2 = 20.61, P ≤ 0.0001), Compared to 52 (96.3%) cases reported extreme headache while only 16 (29.6%) controls. Twice as many cases Twenty four (44.4%) cases had lymph node enlargement compared to 12 (22.2%) controls (χ^2 = 5.04; P = 0.008). Thirty seven (68.5%) cases and 6 (11.1%) controls mentioned pruritis with χ^2 = 34.77; P value < 0.0001) see table 2 below.

Table 2 Knowledge on Signs and Symptoms of respondents.

Signs / Symptoms	No. of Cases (%)	No. of Controls (%)	Chi- square Value (χ^2)	P Value
Fever	51 (94.4%)	19 (35.2%)	39.01	≤ 0.0001
Headache	52 (96.3%)	16 (29.6%)	48.63	≤ 0.0001
Lymphadenopathy	24 (44.4%)	12 (22.2%)	5.04	≤ 0.0001
Malaise	33 (61.1%)	9 (16.7%)	20.61	≤ 0.0001
Uncontrolled sleep disturbance	16 (29.6%)	8 (14.8%)	2.62	0.0349
Myalagia/ althralgia	46 (85.2%)	15 (27.8%)	33.90	≤ 0.0001
Edema	33 (61.1%)	3 (5.6%)	35.04	≤ 0.0001
Pruritis	37 (68.5%)	6 (11.1%)	34.77	≤ 0.0001
Sweating	30 (55.6%)	11 (20.4%)	12.73	≤ 0.0001

The HAT patients had a mean time-lag or diagnosis delay of 10.5 month (range 2 – 36 months) from the time the HAT patients developed symptoms to the time of diagnosis. Twenty nine (53.7%) cases gave no reason for delay to seek medical attention. Fifteen (27.8%) lack knowledge about need for care and 10 (18.5%) cases said poor access to health facility was the reason for the delay. Regarding attitudes of health seeking behaviours of both group, 22 (40.7%) cases and 4(7.4%) controls first visited a local pharmacy for self medication whereas 24 (44.4%) cases and 45 (79.6%) control first

sought medical attention from a facility. This means that more than 53% of cases compared to only 18% of controls do not go to health facility for health care but either go to local pharmacy or sought treatment for the ailment from a traditional healer χ^2 = 17.92; P = < 0.0001 Table: 3. There was also significant difference in attitudes and believes on avoiding going to river and forest (χ^2 = 23.9 and 16.4 respectively and P <0.0001)

Table 3 Selected Attitudes and beliefs for cases and controls on HAT

Attitudes of seeking medical attention	**No. of Cases (%)**	**No. of Controls (%)**	**Chi- square Value (χ^2)**	**P Value**
Health facility	25 (46.3%)	44 (81.5%)	17.92	≤ 0.0001
Local pharmacy or Private clinic	22 (40.7%)	4 (7.4%)		
Traditional Healer	7 (13.0%)	6 (11.1%)		
Avoid going to river				
Yes	45 (83, 3%)	19 (35.2%)	23.97	< 0.0001
No	6 (16.7%)	35 (64.8%)		
Avoid going to forest				
Yes	18 9 33.3%)	1(1.9%)	16.35	< 0.0001
No	36(66.7%)	53(98.1%)		

44.4% of cases and 74.6% of controls believe that mosquito can transmit the disease. 25% (2 cases and 25 controls) of the study population has perception that the disease is case Tsetse fly; al though 48.1 of them (49 cases and 4 controls) knew the cause is tryponsoma. 38% of study population believes that the disease can be cause by witchcraft (19 case and 19 controls). Perception of the respondents on the risk of the disease in Mundri east county was expressed as moderate 23.1%, small 41.7% and no risk at all 35.2% table 4. More than 50% of cases and 31.7% of control perceived that having family member with a disease can helps in spread of HAT disease in family.

Table 4 Perception of cases and controls on risk of staying and History of HAT in families in Mundri East county

Risk of disease in Mundri county	**No. of Cases (%)**	**No. of Controls (%)**	**Chi- square Value (χ^2)**	**P Value**
Moderate	18 (33.3%)	7 (13%)	10.26	*< 0.0059*
Small	21 (38.9%)	17 (31.5%)		
No risk at all	15 (27.8%)	30(55.5%)		
Family member with disease can be source to HAT				
Yes	28(51.9%)	17(31.5%)	35.7	*< 0.0001*
No	26(48.1%)	37(68.5%)		

4.4 Knowledge and Attitudes for HAT

Fifty three (98.1%) cases knew sleeping sickness as a disease and had its local name compared to 70.4% of the controls (χ^2 = 13.68, P value, 0.0001) table 6. Majority of cases (90.7%) knew the cause of HAT as *Trypanosoma* parasite transmitted through bites by tsetse flies compared to 7.4% of controls (χ^2 = 76.75, P value, 0.0001).

The level of knowledge on transmission between cases and controls was significantly different (χ^2 = 26.79; P value, 0.0001).Twenty six (48.1%) cases approved that sleeping sickness can be transmitted from mother to child whereas 40 (74.1%) controls disapproved the transmitted from mother to child through placenta (χ^2 = 5.46; P ≤ 0.01).

Thirty six (66.7%) cases had heard and knew about screening for sleeping sickness while 26(49.1%) controls had not or knew nothing about the screening for sleeping sickness. The difference was not significant between cases and controls; (χ^2 = 5.46; P ≤ 0.01). Twenty two (40.7%) cases and 43 (79.6%) controls had no knowledge on the types of drugs used for the treatment χ^2 = 18.85; P value < 0.0001). Table: 4

Table 5 Knowledge of HAT disease among Cases and Controls

Knowledge of HAT		No. of Cases (%)	No. of Controls (%)	Chi- square Value (χ^2)	P Value
	Yes	53 (98.1%)	38 (70.4%)	13.68	≤ 0.0001
	No	1 (1.9%)	16 (29.6%)		
Knowledge of HAT transmission					
	Yes	49(90.7%)	4 (7.4%)	76.75	≤ 0.0001
	No	5 (9.3%)	50 (92.6%)		
Bite by Tsetse flies					
	Yes	53 (98.1%)	29 (53.7%)	26.79	≤ 0.0001
	No	1 (1.9%)	25 (46.3%)		
Mother to child transmission of HAT					
	Yes	26(48.1%)	13 (25.9%)	5.46	< 0.01
	No	28(51.9%)	41 (74.1%)		
Knowledge of drugs used for HAT treatment					
	Yes	32(59.3%)	11 (20.4%)	18.85	≤ 0.0001
	No	22(40.7%)	43 (79.6%)		

4.5 Beliefs and attitudes about HAT by study participants

Forty five (83.3%) cases reported that avoiding staying or regular visit to river side reduced the risk of tsetse fly bite. On the other hand, 35 (64.8%) controls did not attribute staying or regular visit to river side with the disease with (odd ratio of 9.21; P value < 0.0001).

Eighteen (33.3%) cases had the belief that avoiding visiting the thick forest would reduce the risk of having the sleeping sickness while 53 (98.1%) of controls believed there was no association between the forest and sleeping sickness with OR = 26.5 and (95% CI = 3.38 – 207.44; P value = 0.0001).

Thirty nine (72.2%) cases had the belief that the risk of contracting sleeping sickness was greater in Mundri counties than in any other place compared to and 30 (55.6%) controls.

Nineteen (35.2%) of both cases and controls believed that sleeping sickness is caused by witchcraft. Thirty (55.6%) cases believed that sleeping sickness can not be transmitted through bite of mosquito. However, majority of the controls (79.6%) had perception that mosquito can transmit sleeping sickness. The difference in belief between the two groups was significant with OR= 4.5; P value < 0.0001 Table 5.

Table 6 Attitudes and beliefs for sleeping sickness disease and transmission in Mundri Counties between Cases and Controls

Avoid going to River	No. of Cases (%)	No. of Controls (%)	OR	95%CI	P Value
Yes	45 (83.3%)	19 (35.2%)	9.21	3.7 – 22.8	< 0.0001
No	9 (16.6%)	35 (64.8%)			
Avoid going to Forest					
Yes	18(33.3%)	1 (1.9%)	26.5	3.4 – 207.	< 0.0001
No	36(66.7%)	53(98.1%)			
Risk in staying in Mundri counties					
Yes	39(72.2%)	24(44.4%)	3. 25	1.36 – 7.9	< 0.0001
No	15(27.8%)	30(55.6%)			
Mosquito can transmit HAT					
Yes	30(55.6%)	11(20.4%)	4.5	1.6 – 12.8	<0.0001
No	24(44.4%)	43(79.6%)			

4.6 Activities that expose people to tse-tse flies bites

Thirty nine (72.2%) cases reported collecting firewood from the forest as an extra activity compared to 34 (63%) controls who did not collect firewood as an extra activity (OR = 4.42; P value < 0.0001). Twenty four (44.4%) cases and 13 (24.1%) controls reported hunting as an extra activity with higher OR = 2.5 and (95% CI = 1.10 – 5.74; P value = 0.01407). This was suggestive of association between hunting and HAT disease. Thirty 33 (61.1%) cases compared to 18 (33.3%) controls reported caring for animals as an activity with higher odds ratio = 3.14 and (95% CI = 1.43 – 6.90; P value < 0.0001). This also shows an association between animal grazing and HAT disease.

Twenty nine (53.7%) cases and 16 (29.6%) controls reported fishing as an extra activity with high OR = 2.75 and 95% CI = 1.24 – 6.08; P value = 0.0061. The frequency of fishing was significantly associated with cases than control in this study. Over 60% of the participants in the study were involved in farming activities as an extra activity. This activity was negatively associated with acquisition of disease (OR= 0.07; CI= 0.02-0.2; P < 0.0001) Faming as an activity contribute to reduction of vector breeding site and reducing the risk of the disease, and secondly the HAT patients are usually incapacitated by the disease and thus they can not perform strong physical activities such as farming. Therefore farming was associated with the disease Table: 6

Table 7 Association of selected risk factors with HAT between Cases and Controls

Extra Activity	No. of Cases (%)	No. of Controls (%)	OR	95%CI	P Value
Fishing	29 (53.7%)	16 (29.6%)	2.75	1.24-6.08	0.0061
Firewood Collection	39 (72.2%)	19 (35.2%)	4.78	2.11-1083	< 0.0001
Hunting	25 (46.3%)	13 (24.1%)	2.71	1.19- 6.18	0.0086
Herding	34 (63%)	18 (33.3%)	3.40	1.54-7.49	0.0011
Farming	21(38.9%)	48(88.9 %)	0.07	0.02 – 0.2	< 0.0001
History of HAT in the family					
Yes	48 (88.9%)	21 (38.9%)	12.5	4.6 – 34.5	<0.0001
No	6 (11.1%)	33 (61.1%)			
Access To health education					
Community notice	36 (66.7%)	47 (87.0%)	3.36	1.2- 10.02	<0.0001
Health care provider	18 (33.3%)	7 (13.0%)			

Of 108 people interviewed, forty eight (68.6%) cases and 21(38.9%) controls had had a relative with HAT disease before(OR= 12.5; P < 0.0001). This means that having history of HAT case in the family gave 12 times risk than in families without previous case of HAT.

In terms of where information was obtained about HAT disease 67% cases obtained information about the disease through community notices/ campaigns whereas seven (13%) controls had heard about HAT disease through community health education OR = 3.36; 95% CI = 1.16 – 10.02; P value < 0.0001 Table 7

CHAPTER FIVE

5. DISCUSIONS AND CONCLUSIONS

5.1 Discussion

5.1.1 Socio-demographic and Risk factors

In this study, it was found that participants were aware of the activities that exposed individuals to tsetse bites and therefore the risk of contracting HAT. There was a significant different in the level of awareness between cases and controls that could be attributed to health education from health care personnel (Kinung'hi *et al*., 2006). This is also in agreement with another risk factors assessment conducted in northern Uganda by Organization of African unity/ scientific, technical and research commission , that Cases spent more time outside their village of residence than controls, and more cases than controls collected firewood in the forest. Water collecting and bathing points, farms and gardens and firewood collecting points cases had more cases of sleeping sickness in the family.

Findings of this study showed that majority of participant recruited were from rural setting and they had three times the risk of acquiring the infection than those in urban area 70.4% vs. 29.6% respectively. Majority of cases were in age group 20 – 40 years, although the distribution of cases was across all the age groups. This is contrast to the study conducted among urban residence in Kinshasa, Congo by (Robyas *et al.,* 2004) which reported an increasing number of human African trypanosomiasis (HAT) cases in urban residence of Kinshasa, Democratic Republic Congo. A few risk

factors were independently associated with HAT. These included fishing, hunting, firewood collection, and herding in this study. Involvement in farming turned to be protective in this study and was in contrast to field cultivation activity as reported in Kinshasa by (Robyas et al., 2004). Previous studies have associated gender with HAT cases about infection and transmission. In this study 52 percent of the cases were males and the finding is in agreement with what was reported by (Kinung'hi et al., 2006) that adult males were likely to suffer from HAT disease and formed larger proportion of HAT patients in Tanzania. Other studies have associated the risk of acquiring HAT to some activities such as honey gathering, timbering, fishing, hunting and herding which exposing an individual to tsetse bite. The findings of this study concur with these outdoor activities which suggest that involvement in activity increased risk of acquiring infection.

Collecting firewood, an activity which was reported by majority of the cases had the highest association with acquiring of infection (OR= 78; $P < 0.0001$). Other factors which turned to be independent risk factors included hunting (OR= 2.71, $P< 0.05$), herding (OR= 3.40, $P < 0.05$) and history of HAT cases in the family (OR = 12.5, $P < 0.0001$). The mean age of HAT patient was 25.5 ± 5 years and had a mean time lag for seeking diagnosis or diagnosis delay was 10.5 ± 2.5 months. More than 96% of the confirmed HAT cases were diagnosed at stage two compared to only two cases diagnosed at stage one. This predominance of HAT second-stage has previously been reported elsewhere (Triolo *et al*., 1985). Studies by (Cramet, 1982; Debroise *et al*.,

1968; Ngandu-Kabeya, 1976; and Triolo *et al*., 1985) have reported that majority of HAT patients usually seek diagnosis late and findings of this study concur. Late diagnosis has also been reported by other authors including (Kazumba *et al.*, 1993; Triolo et al., 1985), as the clinical presentation in *T. Gambiense* can be difficult to differentiate from other febrile illnesses. Odiit *et al.*, 2004, in a study in Eastern Uganda reported similar results that the median total diagnosis delay, from onset of the illness to presentation of cases to health facility for diagnosis, was about 60 days (2 month) and this delay was associated with late stage sleeping sickness. Several factors have been attributed to diagnosis delay for HAT patients and these included failures of health service provider to diagnose sleeping sickness accurately, lack of equipments such as microscopy especially in rural health centers, and not easy mechanism of collecting appropriate specimens for use in diagnosis.

More than half of cases (53.7%) in this study gave no good reason for delay to seek medical service though a few attributed delays to seek medical attention to poor access to health facility, or had visited a traditional healer for possible remedy. These results are in agreement with Sudan Household survey, 2006 which reported several persons in southern Sudan had attitudes that inadequate maintenance of health facilities, denied them from accessing quick medical attention. Secondly, lack of modern health facilities pushed the inhabitants to seek consultation from traditional healer for possible herbal treatment or use of local pharmacy for self medication.

This study also revealed significant association between history of HAT case in a family and presence of case, that having history of HAT case in family increased the risk of infection by 13 folds compared to case which did not a have a previous case of HAT in the family. This too also in agreement with (Kinung'hi *et al.*, 2006) who reported that 1.1% and 2.1% reported at least one family member suffered from the disease in 2005 and 2006 respectively.

One third (33.3%) of the cases reported to have received the information about HAT disease from Health workers compared to only (13%) of control who got the same information from Health care providers. The difference between the two groups was significant and the finding is in agreement with sources of information amongst the respondents in Usinga (close to Ugala Game Reserve) in Tanzania, 2006 that HAT patients are the major source and followed by health care provider.

5.1.2 Knowledge, Attitudes and Believes

Findings of this study showed that participants in Lui hospital had some level of knowledge of human African trypanosomiasis because they had a local name for the disease as “Adravo Uduro”. Its local name they reported, was derived from sleep “disease that makes one sleep during day time” This is in agreement with a study conducted in Congo by (Robays *et al.*, 2004) who reported that HAT disease is well known among the population and was seen as a major health problem. In this study, a few respondents literary translated the term sleeping sickness: ‘maladi ya mpongi’ or ‘

maladi ya ketol' as the local name of the disease. However, despite some knowledge of HAT among respondents, there was no significant difference in the level of education of respondents between cases and controls groups. The main source of HAT information to the participants was reported to be community notices using mega phones and health education to patients at health facilities. The kind of information frequently received was the symptoms and signs. This indicates that, there is a gap of knowledge delivery from reliable sources such as health care professionals to enable the community understanding the appropriate prevention techniques.

The findings of this study further indicated that the attitude of avoiding going to tsetse infested forests and river bank was significantly associated with the risk of disease.

Over a third of the participants (35%) had the belief that the disease is due to witchcraft and another 56% incriminated mosquitoes in transmission of the disease. The study participants had different perception of the disease with over 80% of cases and controls reporting that the disease was not a health risk in the Mundri County. This demonstrated that there is lack of knowledge about vector, and transmission like shown in other studies (Kinung'hi *et al.*, 2006). Several Activities were frequently mentioned by respondents and turned to be independent risk factors significantly associated with the disease in this study. These included fishing, farming, hunting, bearing for animals (herding) and fire wood gathering. Farming appear a protective factor of HAT disease this was attributed to incapacitation of sleeping sickness patients by infection and probably explain farming activities were less frequently reported by cases as

compared to controls. The rest of the activities were positively associated with HAT disease. This was also in agreement with the study done in Urambo District, Tanzania (Kinung'hi *et al.*, 2006). The study also found significant association between the family history and the HAT disease.

This study also found that there was majority of the controls had not heard of HAT screening campaigns before compared to the response gotten from the cases group. This finding is also in line with what was reported by (Robyas *et al*., 2007) in Congo that majority of Participants knew of mobile teams which screened for trypanosomiasis and correctly described the sequence of technical procedures, from neck palpation and puncture, taking of blood samples for testing, to the use of the microscope (Robyas *et al*., 2007) . They reported that participation in screening was perceived a way of protecting themselves from the disease. However, a few of respondents did not distinguish HAT screening from the activities of mobile vaccination campaigns. Thirty five percent of both cases and control attributed the cause of sleeping sickness to witchcraft. Although our results turned not to be significant, a different between cases and controls in a study in Congo (Robyas, *et al*, 2004) reported that Sleeping sickness was considered a 'disease of God' (maladi ya nzambi) in Congo implying that it is treatable by western medicine as opposed to 'diseases caused by people' or as a result of witchcraft (in Kikongo: ndoki). 'If they inject you and you get better, you know it is not witchcraft but it is a disease of God'.

Overall, there was some level of knowledge about HAT among the study population

though, it may not have been sufficient to act as deterrent to acquiring of the HAT infection. The relationship of witchcraft and the cause of disease indicated, participants attitudes were shaped to some extent by their local beliefs. The signs and symptoms of the disease were much more known by the study participants than the cause of the disease. A few participants attributed the cause of disease to mosquito bites and also to activities viewed irrelevant by the investigators such soaking of cassava in the river.

5.2 Conclusions

Based on the findings of this study I accept the Hypotheses of this study and concluded that,

I. There is some knowledge about tsetse flies and HAT disease. However, this was a gap in knowledge about the mode of transmission, causes, screening and diagnosis, treatment and risk factors for HAT by the respondents
II. Majority of HAT patient diagnosed were at the second stage of the disease and the reasons were attributed to either poor access to health facility or poor seeking health behavior.
III. .Majority of HAT patients are resided in rural areas and were younger males in active age group (20 – 39)
IV. Most of the respondents received information about HAT through community notice.

5.3 Recommendations

Before communities are engaged in active programmes for controlling tsetse and HAT, it is important that their knowledge, attitudes and practices are understood. Even in situations where the communities are sufficiently aware of trypanosomiasis and its consequences, and/or where successful tsetse and HAT control operations have been carried out, there may still be a need to increase community's awareness about known control techniques to facilitate their participation in future tsetse and HAT control activities. It is recommended that Ministry of Health Government of southern Sudan to

I. To Formulate policy to guide health education and active early screening
II. To provide further education to the community on the disease and control measures in Mundri East and surrounding counties. Through other means including media, schools, churches to disseminate information's on HAT
III. Scaling up of the Trypanosomiasis control program with active community involvement.
IV. Further research on mapping new foci areas for HAT including health seeking behaviors to provide more information.
V. Involvement of various public sector including health, livestock, agriculture professionals and allied the experts is important in order to improve community health seeking behaviors and participation in appropriate HAT and tsetse fly control interventions

REFERENCES

Apted, F. I. C., Ormerod, W. E., Smyly, D. P., and Stronach, B. W., (1963). A comparative study of the epidemiology of endemic Rhodesian sleeping sickness in different parts of Africa. *J. Trop. Med. Hyg*. 66:1-16.

Apted, F.I., (1970). Clinical manifestations of sleeping sickness in: The African trypanosomiasis. Ed.Mulligan HW (ed). George Allen and Unwin, *London*, pp.661-683

Asonganyi, T., Doua. F., Kibona S. N., Nyasulu, Y. M., Masake, R., and Kuzoe, F., (1998). A multi-centre evaluation of the card indirect agglutination test for trypanosomiasis (TrypTect CIATT). *Ann. Trop. Med. Parasitol*. **92:**837-844.

Atkins., (1734). The navy surgeon or a practical system of surgery. Caesar Ward and Richard Chandler, London, United Kingdom

Ayed, Z., Brindel, I., Bouteille, B., Van Meirvenne, N., Doua, F., Houinato, D., Dumas, M., and Jauberteau, M. O., (1997). Detection and characterization of autoantibodies directed against neurofilament proteins in human African trypanosomiasis. *Am. J. Trop. Med. Hyg.* **57:**1-6.

Bailey, J. W., and Smith, D. H., (1992). The use of the acridine orange QBC technique in the diagnosis of African trypanosomiasis. Trans. R. Soc. *Trop. Med. Hyg*. 86:630

Barrett, M. P., Burchmore, R. J., Stich, A., Lazzari, J. O., Frasch, A. C., Cazzulo, J. J., and Krishna, S., (2003). The trypanosomiases. *Lancet* 362:1469-1480

Benenson, A., (1995) Control of communicable disease Manual 16th edn. American *Public health association*, Washington DC.

Berberof, M., Perez-Morga, D., & Pays, E., (2001). A receptor-like flagellar Pocket glycoprotein specific to Trypanosoma brucei gambiense.*Med.Biochem.Parasitol*. 113, 127-138.

Bisser, S., Bouteille, B., Sarda, J., Stanghellini, A., Ricard, D., Jauberteau, M. O., Marchan F., Dumas, M., and Breton, J. C., (1997). Contribution of biochemical tests in the diagnosis of the nervous phase of human African trypanosomiasis. *Bull. Soc. Pathol. Exot*. 90:321-326.

Bisser, S., Ayed, Z., Bouteille, B., Stanghellini, A., Breton, J. C., Dumas, M., and Jauberteau, M. O., (2000). Central nervous system involvement in African trypanosomiasis: presence of anti-galactocerebroside antibodies in patients' cerebrospinal fluid. Trans. R. Soc. *Trop. Med. Hyg*. **94:**225-226.

Bisser, S., Lejon, V., Preux, P. M., Bouteille, B., Stanghellini, A., Jauberteau, M. O., Büscher, P., and Dumas, M., (2002). Blood-cerebrospinal fluid barrier and intrathecal immunoglobulins compared to field diagnosis of central nervous system involvement in sleeping sickness. *J. Neurol. Sci*. **193:**127-135.

Blum, J., Schmid, C., Burri, C., (2006). Clinical aspects of 2541 patients with second stage Human African Trypanosomiasis. *Acta Trop*. 97, 55–6.

Boa, Y. F., Traore, M. A., Doua, F., Kouassi-Traore, M. T., Kouassi, B. E., and Giordano, C., (1988). The different present-day clinical picture of human African trypanosomiasis caused by T. b. gambiense. Analysis of 300 cases from a focus in Daloa, Ivory Coast. *Bull. Soc. Pathol*. Exot. Fil. 81:427-444.

Brothwell, D., and Sandison, A. T., (ed.). (1967). Diseases in antiquity. Charles C Thomas, *Springfield*, Ill.

Bruce, D., (1895). Preliminary Report on the Tsetse fly Disease or Nagana in Zululand. Durban.

Burri, C., and Brun, R., (2003). Human African trypanosomiasis, p. 1303-1323. In G. C. Cook and Z. Alimuddin (ed.), Manson's tropical diseases, 21st ed. Elsevier Science, Edinburgh, United Kingdom

Buscher, P., Lejon, V., Magnus, E., and Van meirvenne, N., (1999), improved latex agglutination test for detection of antibodies in serum and cerebrospinal fluid of *trypanosoma brucei gambiense* infected patients. *Acta Trop*. 73:11-20.

Buyst, H., (1975). The diagnosis of sleeping sickness in a district hospital in Zambia. Ann. Soc. Belg. *Med. Trop*. 55:551-557.

Cattand, P., Miezan, B. T., and de Raadt, P., (**1988).** Human African trypanosomiasis: use of double centrifugation of cerebrospinal fluid to detect trypanosomes. *Bull. W. H. O*. **66:**83-86.

Chappuis, F., Pittet, A., Bovier, P. A., Adams, K., Godineau, V., Hwang, S. Y., Magnus, E., and Büscher, P., (2002). Field evaluation of the CATT/*Trypanosoma brucei gambiense* on blood-impregnated filter papers for diagnosis of human African trypanosomiasis in southern Sudan. *Trop.* Med. *Int. Health* **7:**942-948.

Chappuis, F., Stivanello, E., Adams, K., Kidane, S., Pittet, A., and Bovier, P. A., (**2004).** Card agglutination test for trypanosomiasis (CATT) end-dilution titer and cerebrospinal fluid cell count as predictors of human African trypanosomiasis (*Trypanosoma brucei gambiense*) among serologically suspected individuals in southern Sudan. *Am. J. Trop. Med*. Hyg. **71:**313-317.

Cramet, R., (1982). Sleeping sickness in children and its long-term aftereffects. Apropos 110 personal observations at Fontem Hospital (Cameroon). *Med. Trop*. 42, 27–31

Debroise, A., Debroise-Ballereau, C., Satge, P., Rey, M., (1968). African trypanosomiasis in young children. *Arch. Franc¸aise Ped*. 25, 703–720**.**

DeGreef, C., Imberechts, H., Matthyssens, G., Van, M. N., & Hamers, R., (1989). A gene expressed only in serum- resistant variants of trypanosome bruceirhodesiense *Mol.Biochem.Parasitol*, 169-176

Doua, F., Miezan, T. W., Sanon Singaro, J. R., Boa Yapo, F., and Baltz, T., (**1996).** The efficacy of pentamidine in the treatment of early-late stage *Trypanosoma brucei gambiense* trypanosomiasis. *Am. J. Trop. Med*. Hyg. **55:**586-588

Duku, O. M., (1981). Human African trypanosomiasis in the Southern Sudan, Present Situation control Measures. Proceedings of the 16th Congress of international scientific council of *Trypanosomiasis* research and control (ISCTRC) in Yaoundé, Cameroon. 139-145p.

Dukes, P., Gibson, W. C., Gashumba, J. K., Hudson, K. M., Bromidge, T. J., Kaukus, A., Asonganyi, T., and Magnus, E., (1992). Absence of the LiTat 1.3 (CATT antigen) gene in Trypanosoma brucei gambiense stocks from Cameroon. *Acta T*rop. 51:123-134

Duggan, A. J., and Hutchinson, M. P., (1966). Sleeping sickness in Europeans: a review of 109 cases. *J. Trop. Med.* Hyg. 69:124-131

Dumas, M., & Boa, F., (1988). Human African trypanosomiasis. In: Microbial Disease: Handbook of Clinical Neurology pp, 339-343

Dutton, J. E., (1902). Preliminary note upon a trypanosome occurring in the blood of man. Thompson Yates Lab. Rep. 4:455-468.

Edeghere, H., Olise, P. O., and Olatunde, D. S., (1989). Human African trypanosomiasis (sleeping sickness): new endemic foci in Bendel State, Nigeria. *Trop. Med. Parasitol*. 40:16-20

Enyaru, J. C., Matovu, E., Akol, M., Sebikali, C., Kyambadde, J., Schmidt, C., Brun, R., Kaminsky, R., Ogwal, L. M., and Kansiime, F., (1998). Parasitological detection of Trypanosoma brucei gambiense in serologically negative

sleeping-sickness suspects from north-western Uganda. *Ann. Trop. Med. Parasitol*. 92:845-850

Enyaru, J. C., Odiit, M., Winyi-Kaboyo, R., Sebikali, C. G., Matovu, E., Okitoi, D., & Olaho-Mukani, W., (1999). Evidence for the occurance of trypanosome brucei rhodesiense sleeping sickness outside the traditional focus in south- east Uganda. *Ann.Trop.Med.Parasitol*. 93, 817-822

Evans, G., (1881). On a horse disease in India known as 'Surra' probably due to a haematozoon. *Vet. J. Ann. Comp. Pathol*.> 13:1-10, 82,-88, 180,-200, 326.-333

Ekwanzala, M., (1996) In the heart of darkness: sleeping sickness in Zaire. *Lancet*, 348(9039): p. 1427-30.

Georgina pagey, MBBS. DA. (UK). DipObs RANZCOG, FRACGP. (2003). Resurgence of sleeping Sickness in Southern Sudan, J *Ru. Rem. Env.H.* 2(2); 60-65

Garcia, A., Jamonneau, V., Magnus, E., Laveissiere, C., Lejon, V., N'Guessan, P., N'Dri, L., Van Meirvenne, N., and Büscher, P., (2000). Follow-up of Card Agglutination Trypanosomiasis Test (CATT) positive but apparently aparasitaemic individuals in Cote d'Ivoire: evidence for a complex and heterogeneous population. *Trop. Med. Int. Health* **5:**786-793.[Medline]

Gouteux, J. P., & Malonga, J. R., (1985). Socio-entomologic survey in human trypanosomiasis focus of Yamba (Peoples Republic of Congo). *Medecine Tropicale* 45,

259–263.

Greenwood, B. M., and Whittle, H. C., (1973). Cerebrospinal-fluid IgM in patients with sleeping-sickness. *Lance*t **ii:**525-527

Greenwood, B. M., and Whittle, H. C., (A). (1976). Coagulation studies in Gambian trypanosomiasis. *Am. J. Trop. Med. Hyg*. 25:390-394

Greenwood, B. M., and Whittle, H. C., (B). (1976). Complement activation in patients with Gambian sleeping sickness. *Clin. Exp. Immunol*. 24:133-138

Greenwood, B. M., Whittle, H. C., Oduloju, K. O., and Dourmashkin, R. R., (1976). Lymphocytic infiltration of the brain in sleeping sickness. Br. *Med. J*. **2:**1291-1292

Greenwood, B. M., and Whittle, H. C., (1980). The pathogenesis of sleeping sickness. *Trans. R. Soc. Trop. Med. Hyg*. 74:716-725

Hoare, C., (1970). systematic description of mammalian trypanosomiasis of Africa. In: The African Trypanosomaisis ed. Mulligan HW, *George Allen and Unwin London* ltd., London, pp.222-259.

Jamonneau, V., Truc, P., Garcia, A., Magnus, E., and Büscher, P., (2000). Preliminary evaluation of LATEX/*T. b. gambiense* and alternative versions of CATT/*T. b. gambiense* for the serodiagnosis of human African trypanosomiasis of a population at risk in Cote d'Ivoire: considerations for mass-screening. *Acta Trop.* 76:175-183.

Jamonneau, V., Solano, P., Garcia, A., Lejon, V., Dje, N., Miezan, T. W., N'Guessan, P., Cuny, G., and Büscher, P., (2003). Stage determination and therapeutic decision in human African trypanosomiasis: value of polymerase chain reaction and immunoglobulin M quantification on the cerebrospinal fluid of sleeping sickness patients in Cote d'Ivoire. *Trop. Med. Int. Health* **8:**589-594

Jelinek, T., Bisoffi, Z., Bonazzi, L., van Thiel, P., Bronner, U., de Frey, A., Gundersen, S. G., McWhinney, P., and Ripamonti, D., (2002). Cluster of African trypanosomiasis in travelers to Tanzanian national parks. Emerg. *Infect. Dis*. 8:634-635

Joshi, P. P., Shegokar V. R., Power, R. M., Herder, S, Katti, R., Salkar, H.R., Dani V.S., Bhargava, A., Jannin, J., & Truc, P., (2005). Human trypanosomiasis caused bt trypanosome evansi in India: the first case report. *Am.J.Trop.Med.Hyg.*73, 491-495

Kabiri, M., Franco, J. R., Simarro, P. P., Ruiz, J. A., Sarsa, M., and Steverding, D., (1999). Detection of *Trypanosoma brucei gambiense* in sleeping sickness suspects by PCR amplification of expression-site-associated genes 6 and 7. *Trop. Med. Int. Health* **4:**658-661.

Kazumba, M., Kazadi, K., Mulumba, M. P., (1993). Characteristics of Trypanosomiasis in children. Apropos of 19 case reports at the CNPP (Neuro-Psycho-Pathology Center), University Hospitals of Kinshasa, Zaire. *Ann. Soc. Belge Med. Trop*. 73, 253–259.

Kean, B. H., Mott, K. E., and Russell, A. J., (ed.). (1978). Tropical medicine and parasitology: classic investigations. *Cornell University Press, Ithaca*, N.Y.

Kennedy, P. G., (2004). Human African trypanosomiasis of the CNS: current issues and challenges. *J. Clin. Investig*. 113:496-504.

Kinung'hi, S. M., Malele, II., Kibona, S. N., Matemba, L. E., Sahani, J. K., (2006) Knowledge, attitudes and practices on tsetse and sleeping sickness among communities living in and around Serengeti National Park, Tanzania. *Tanzan Health Res Bull* 8: 168–172.

Kristensson, K., and Bentivoglio, M., (1999). Biological diagnosis of human African trypanosomiasis, p. 157-181. *In* M. Dumas, B. Bouteille, and A. Buguet (ed.), Progress in human African trypanosomiasis, sleeping sickness. *Springer-Verlag, Paris*, France.

Kristensson, K. Mhanga, J. D., & Bentivoglio, M., (2002). Parasites and the brain: neuro-invasion, immunopathogenesis and neuronal dysfunctions *Curr.Top.Microbiol.Immunol*. 265, 227-257

Kuboki, N., Inoue, N., Sakurai, T., Di Cello, F., Grab, D. J., Suzuki, H., Sugimoto, C., and Igarashi, I., (**2003).** Loop-mediated isothermal amplification for detection of African trypanosomes. *J. Clin. Microbiol*. **41:**5517-5524

Lambert, P. H., Berney, M., and Kazyumba, G., (1981). Immune complexes in serum and in cerebrospinal fluid in African trypanosomiasis. Correlation with

polyclonal B cell activation and with intracerebral immunoglobulin synthesis. *J. Clin. Investig*. **67:**77-85.

Lanham, S. M., and. Godfrey, D. G., (1970)., Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. *Exp. Parasitol*. **28:**521-534.

Lehane, M. J., & Msanfi, A. R., (1991). lectin and peritrophic membrane development in the gut of Glossina m. morsitans and a discussion of their role in protecting the fly against trypanosome infection. *Med.Vet.Entomol*. 5, 495-501

Lejon, V., Büscher, P., Magnus, E., Moons, A., Wouters, I., and Van Meirvenne, N. A (1998). A semi-quantitative ELISA for detection of *Trypanosoma brucei gambiense* specific antibodies in serum and cerebrospinal fluid of sleeping sickness patients. *Acta Trop*. **69:**151-164.

Lejon, V., Büscher, P., Sema, N. H., Magnus, E., and Van Meirvenne, N. B (1998). Human African trypanosomiasis: a latex agglutination field test for quantifying IgM in cerebrospinal fluid. *Bull. W. H. O*. **76:**553-558.

Lejon, V., Rosengren, L. E., Büscher, P., Karlsson, J. E., and Sema, H. N., (1999). Detection of light subunit neurofilament and glial fibrillary acidic protein in cerebrospinal fluid of *Trypanosoma brucei gambiense*-infected patients. *Am. J. Trop. Med. Hyg*. **60:**94-98.

Lejon, V., Legros, D., Rosengren, L., Gastellu Etchegorry, M., and Büscher,

P., (2001). Biological data and clinical symptoms as predictors of astrogliosis and neurodegeneration in patients with second-stage *Trypanosoma brucei gambiense* sleeping sickness. Am. *J. Trop. Med. Hyg*. **65:**931-935.

Lejon, V., Legros, D., Richer, M., Ruiz, J. A., Jamonneau, V., Truc, P., Doua, F., Dje, N., N'Siesi, F. X., Bisser, S., Magnus, E., Wouters, I., Konings, J., Vervoort, T., Sultan, F., and Büscher, P., (2002). IgM quantification in the cerebrospinal fluid of sleeping sickness patients by a latex card agglutination test. *Trop. Med. Int*. Health **7:**685-692.

Lejon, V., Kwete, J., and Büscher, P., A (2003). Towards saliva-based screening for sleeping sickness? *Trop. Med. Int. Health* **8:**585-588.

Lejon, V., Reiber, H., Legros, D., Dje, N., Magnus, E., Wouters, I., Sindic, C. J., and Büscher, P., B(2003).. Intrathecal immune response pattern for improved diagnosis of central nervous system involvement in trypanosomiasis. *J. Infect. Dis*. **187:**1475-1483.

Lejon, V., Legros, D., Savignoni, A., Etchegorry, M. G., Mbulamberi, D., and Büscher, P., C (**2003).** Neuro-inflammatory risk factors for treatment failure in "early second stage" sleeping sickness patients treated with pentamidine. *J. Neuroimmunol*. **144:**132-138.

Lejon, V., Sindic, C. J., Van Antwerpen, M. P., Doua, F., Dje, N., Solano, P., Jamonneau, V., Wouters, I., and Büscher, P., D (2003).. Human African

trypanosomiasis: quantitative and qualitative assessment of intrathecal immune response. *Eur. J. Neurol*. **10:**711-719.

Lemesre, J. L., Noireau, F., Makoundou, M. L., Louembet, M. T., and Frezil, J. L., (1988). Contribution of serologic technics to the analysis of the cerebrospinal fluid in Congolese patients with sleeping sickness. *Bull. Soc. Pathol. Exot*. Fil. **81:**506-510.

Levine, R. A., Wardlaw, S. C., and Patton, C. L., (1989). Detection of haematoparasites using quantitative buffy coat analysis tubes. *Parasitol. Today* 5:132-134.

Leygues, M., & Gouteux, J. P., (1989). A community battle against a tropical endemic disease: supernatural beliefs and tsetse fly traps in the Congo. *Social Science & Medicine* 28, 1255–1267.

Lucasse, C., (1964). Fluorescent antibody test as applied to cerebrospinal fluid in human sleeping sickness. *Bull. Soc. Pathol. Exot. Fil*. **57:**283-292

Lumsden, W. H., Kimber, C. D., Dukes, P., Haller, L., Stanghellini, A., and Duvallet, G., (1981). Field diagnosis of sleeping sickness in the Ivory Coast. I. Comparison of the miniature anion-exchange/centrifugation technique with other protozoological methods. *Trans. R. Soc. Trop. Med*. Hyg. 75:242-250.

Lumsden, W. H., Kimber C. D., Evans D. A., and Doig, S. J., (1979). *Trypanosoma brucei*: miniature anion-exchange centrifugation technique for detection of low

parasitaemias: adaptation for field use. *Trans. R. Soc. Trop. Med*. Hyg. **73:**312-317.

Lutumba, P., Robays, J., Miaka, C., Kande, V., Molisho, D., Declercq, J., etnAl.,(1993–2003). Trypanosomiasis control, Democratic Republic of Congo**,** *Emerg Infect Dis*. 2005; 11:1382–8

Magnus, E., Vervoort, T., and Van Meirvenne, N., (1978). A card-agglutination test with stained trypanosomes (C.A.T.T.) for the serological diagnosis of *T. b. gambiense* trypanosomiasis. Ann. *Soc. Belg. Med*. Trop. 58:169-176.

Magnus, E., Lejon, V., Bayon, D., Buyse, D., Simarro, P., Verloo, D., Vervoort, T., Pansaerts, R., Büscher, P., and Van Meirvenne, N., (2002).. Evaluation of an EDTA version of CATT/Trypanosoma brucei gambiense for serological screening of human blood samples. *Acta Trop*. 81:7-12

Miezan, T. W., Meda, H. A., Doua, F., Yapo, F. B., and Baltz T., (1998). Assessment of central nervous system involvement in gambiense trypanosomiasis: value of the cerebro-spinal white cell count. *Trop. Med. Int*. Health **3:**571-575.

Miezan, T. W., Meda, H. A., Doua, F., Dje N. N., Lejon V., and Büscher, P., (2000). Single centrifugation of cerebrospinal fluid in a sealed Pasteur pipette for simple, rapid and sensitive detection of trypanosomes. *Trans. R. Soc. Trop. Med. Hyg*. **94:**293.

Murray, CJL., (1994). Quantifying the burden of disease: the technical basis for

disability-adjusted life years. *Bull World Health Organ*.; 72: 429– 445.

Moore, A., & Richer M., (2001). Re-emergence of epidemic sleeping sickness in southern Sudan. *Trop.Med. Int.health* 6, 342-347

Moore, A., Richer, M., Enrile, M., Losio, E., Roberts, J., & Levy, D., (1999). Resurgence of sleeping Sickness in Tambura county, Sudan. *Am J Trop.med.hyg*.61,315-318

Nantulya, V. M., Lindqvist, K. J., Stevenson, P., & Mwangi, E. K., (1992). application of a Monoclonal antibody-based antigen detection enzyme-linked immunosorbent assay (antigen ELISA) for field diagnosis of bovine trypanosomiasis at Nyguruman, Kenya. *Ann.Trop.med.parasitol*.225-230

Nantulya, V. M., Doua, F., and Molisho, S., (1992). Diagnosis of *Trypanosoma brucei gambiense* sleeping sickness using an antigen detection enzyme-linked immunosorbent assay. *Trans. R. Soc. Trop. Med. Hyg*. **86:**42-45.

Nantulya, V. M., (1997). TrypTect CIATT—a card indirect agglutination trypanosomiasis test for diagnosis of *Trypanosoma brucei gambiense* and *T. b. rhodesiense* infections. *Trans. R. Soc. Trop. Med. Hyg.* **91:**551-553.

Nantulya, V. M., (1998). immunodiagnostics of rhodesiense sleeping sickness: detection of circulating trypanosomal antigens in sera and cerebrospinal fluid by enzyme immunoassays using a monoclonal antibody. *Bull. Soc. Pathol.exot.Filiales*.81, 511-512.

Ngandu-Kabeya, G., (1976). Study of the symptomatology of African Trypanosomiasis in children (apropos of 24 cases). *Ann. Soc. Belge Med. Trop*. 56, 85–93.

Noireau, F., Lemesre, J. L., Nzoukoudi, M. Y., Louembet, M. T., Gouteux, J. P., and Frezil, J. L., (1988). Serodiagnosis of sleeping sickness in the Republic of the Congo: comparison of indirect immunofluorescent antibody test and card agglutination test. *Trans. R. Soc. Trop. Med. Hyg*. **82:**237-240.

Noireau, F., Force-Barge, P., and Cattand, P., (1991). Evaluation of Testryp CATT applied to samples of dried blood for the diagnosis of sleeping sickness. *Bull. W. H. Or*. **69:**603-608

Odiit, M., Kansiime, F., and Enyaru, J. C., (1997) Duration of symptoms and case fatality of sleeping sickness caused by Trypanosoma brucei rhodesiense in Tororo, Uganda. *East Afr. Med. J*. 74:792-795

Odiit, M., Shaw, A., Welburn, S.C., Fe`vre, E. M., Coleman, P. G., & McDermott, J. J., (2004) Assessing the patterns of health-seeking behaviour and awareness among sleeping sickness patients in eastern Uganda. Annals of *Trop Med Parasitol* 98, 339–348.

Okoth , J. O., Kirumira, E. K., & Kapaata, R., (1992). A new approach to community participation in tsetse control in the Bugosa sleeping sickness focus, Uganda. A preliminary report. *Annals* of *Trop Med Parasitol* 85(3), 315–322.

Organisation of African Unity/Scientific, Technical and Research Commission

(OAU/STRC) Risk factors assessment for sleeping sickness acquisition in S.E. Uganda: a case-control study

Pansaerts, R., Van Meirvenne, N., Magnus, E., and Verhelst, L., (1998). Increased sensitivity of the card agglutination test CATT/Trypanosoma brucei gambiense by inhibition of complement. *Acta Trop*. 70:349-354

Papadopoulos, M. C., Abel, P. M., Agranoff, D., Stich, A., Tarelli, E., Bell, B. A., Planche, T., Loosemore, A., Saadoun, S., Wilkins, P., and Krishna, S., (**2004**). A novel and accurate diagnostic test for human African trypanosomiasis. *Lancet* **363:**1358-1363.

Paquet, C., Ancelle. T., Gastellu-Etchegorry, M., Castilla, J., and Harndt, I., (1992). Persistence of antibodies to Trypanosoma brucei gambiense after treatment of human trypanosomiasis in Uganda. *Lancet* 340:250

Penchenier, L., Grebaut, P., Njokou, F., Eboo Eyenga, V., and Büscher, P., (2003). Evaluation of LATEX/T.b. gambiense for mass screening of Trypanosoma brucei gambiense sleeping sickness in Central Africa. *Acta Trop*. 85:31-37

Pepin, J., and Milord, F., (1994). The treatment of human African trypanosomiasis. Adv. Parasitol. 33:1-47

Pepin, J., Milord, F., Khonde, A., Niyonsenga, T., Loko, L., and Mpia, B., (1994) Gambiense trypanosomiasis: frequency of, and risk factors for, failure of melarsoprol therapy. Trans. *R. Soc. Trop. Med. Hyg.* **88:**447-452.

Pepin, J., and Donelson, J. E., (1999). African trypanosomiasis (sleeping sickness), p. 774-796. *In* R. L. Guerrant, D. H. Walker, and P. F. Weller (ed.), *Tropical infectious diseases, vol. I. Churchill Livingstone, Philadelphia, Pa.*

Radwanska, M., Chamekh, M., Vanhamme, L., Claes, F., Magez, S., Magnus, E., de Baetselier, P., Büscher, P., and Pays, E., A (2002). The serum resistance-associated gene as a diagnostic tool for the detection of *Trypanosoma brucei rhodesiense*. *Am. J. Trop. Med. Hyg*. **67:**684-690.

Radwanska, M., Claes, F., Magez, S., Magnus, E., Perez-Morga, D., Pays, E., and Büscher, P., B (2002). Novel primer sequences for polymerase chain reaction-based detection of *Trypanosoma brucei gambiense*. *Am. J. Trop. Med. Hyg*. **67:**289-295.

Reiber, H., and Peter, J. B., (2001). Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. *J. Neurol. Sci*. **184:**101-122.

Robays, J., Bilengue, M. M., Van der stuyft, P., and Boelaert, M., (2004). The effectiveness of active population screening and treatment for sleeping sickness control in Democratic republic of Congo. *Trop.Med. int. health* 9:542-550

Robays, J., Kadima, A. E., Lutumba, P., Bilenge, C. M. M., Mesu, V. K. B. K., De Deken, R., Makabuza, J., Deguerry, M., Van der Stuyft, P., & Boelaert, M., (2004). Human African trypanosomiasis amongst urban residents in Kinshasa: a case-control study**.** *Tropical Medicine and International Health***,** 9 (8): 869-875

Robays, J., Lefe`vre, P., Lutumba, P., Lubanza, S., Kande Betu Ku Mesu, V.,

Van der Stuyft, P., and Boelaert, M., (2007) Drug toxicity and cost as barriers to community participation in HAT control in the Democratic Republic of Congo, *Trop. Med. Int. Health*. *12: 290–298.*

Ruiz, J. A., Simarro, P. P., and Josenando, T., (2002). Control of human African trypanosomiasis in the Quicama focus, Angola. *Bull. W. H. Or*. 80:738-745.

Sarff, L. D., Platt, L. H., and McCracken, G. H. Jr., (1976). Cerebrospinal fluid evaluation in neonates: comparison of high-risk infants with and without meningitis. *J. Pediatr*. **88:**473-477

Schares, G., and Mehlitz, D., (**1996).** Sleeping sickness in Zaire: a nested polymerase chain reaction improves the identification of *Trypanosoma (Trypanozoon) brucei gambiense* by specific kinetoplast DNA probes. *Trop. Med. Int. Health* **1:**59-70.

Simarro, P., Franco, J. R., and Ndongo, P., (1999). Field evaluation of several serological screening tests for sleeping sickness (T. b. gambiense). *Bull. Liais. Doc*. OCEAC 32:28-33

Simarro, P. P., Ruiz, J. A., Franco, J. R., and Josenando, T.,. (1999). Attitude towards CATT-positive individuals without parasitological confirmation in the African trypanosomiasis (*T.b. gambiense*) focus of Quicama (Angola). *Trop. Med. Int. Health* **4:**858-861

Simarro, P. P., Louis, F. J., and Jannin, J., (**2003**). Sleeping sickness, forgotten illness: what are the consequences in the field? *Med. Trop*. **63:**231-235.

Solano, P., Jamonneau, V., N'Guessan, P., N'Dri, L., Dje N. N., Miezan,T. W., Lejon, V., Büscher, P., and Garcia, A., (2002). Comparison of different DNA preparation protocols for PCR diagnosis of human African trypanosomosis in Cote d'Ivoire. *Acta Trop*. **82:**349-356.

Stanghellini, A., and Josenando, T., (2001). The situation of sleeping sickness in Angola: a calamity. *Trop. Med. Int. Health* **6:**330-334.

Stich, A., Abel, P. M., and Krishna, S., (2002). Human African trypanosomiasis. *Br. Med. J.* 325:203-206

Triolo, N., Trova, P., Fusco, C., Le Bras, J., 1985. Report on l7 years of studies of human African trypanosomiasis caused by T. gambiense in children 0–6 years of age. *Med. Trop*. 45, 251–257**.**

Truc, P., Jamonneau, V., N'Guessan, P., Diallo, P., B. and Garcia, A., (1998). Parasitological diagnosis of human African trypanosomiasis: a comparison of the OBC and miniature anion-exchange centrifugation techniques. *Trans. R. Soc. Trop. Med. Hyg*. **92:**288-289.

Truc, P.,. Jamonneau, V., Cuny, G., and Frezil, J. L., (1999). Use of polymerase chain reaction in human African trypanosomiasis stage determination and follow-up.

Bull. W. H. Or. **77:**745-748.

Truc, P., Lejon, V., Magnus, E. Jamonneau, V., Nangouma, A., Verloo, D., Penchenier, L., and. Büscher, P., (2002). Evaluation of the micro-CATT, CATT/*Trypanosoma brucei gambiense*, and LATEX/*T. b. gambiense* methods for serodiagnosis and surveillance of human African trypanosomiasis in West and Central Africa. *Bull. W. H. O.* **80:**882-886.

Van Meirvenne, N., (1992). Diagnosis of human African trypanosomiasis. *Ann. Soc. Belg. Med. Trop*. 72(Suppl. 1):53-56.

Van Miervenne, N., (1999). Biological diagnosis of human African trypanosomiasis, p. 235-252.

Vanhamme, L., Pays, E., McCulloch, R., and Barry, J. D., (2001). An update on antigenic variation in African trypanosomes. *Trends Parasitol*. 17:338-343

Vickerman, K., (1985) developmental cycles and biology of pathogenic trypanosomes *Br.Med.Bull*. 41, 105-114

Welbum, S.C., Fevre, E. M., Coleman, P. G., Odiit, M., & Maudlin, I., (2001). sleeping sikness: a tale of two diseases. *Trends parasitol*. 17, 19-24

Welburn, S. C., Picozzi, K., Fevre, E. M., Coleman, P. G., Odiit, M., Carrington, M., and Maudlin, I., (**2001).** Identification of human-infective trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum-resistance-

associated (SRA) gene. *Lancet* **358:**2017-2019.

Whittle, H. C., Greenwood, B. M., Bidwell, D. E., Bartlett, A., and Voller, A., (1977). IgM and antibody measurement in the diagnosis and management of Gambian trypanosomiasis. *Am. J. Trop. Med.* Hyg. **26:**1129-1134.

Winterbottom, T. M., (1803). An account of the native Africans in the neighbourhood of Sierra Leone to which is added an account of the present state of medicine among them, vol. 2:, p. 29-31. C. Whittingham, London, United Kingdom

Woo, P. T., (1970). The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Trop*. **27:**384-386.

World Health Organization,(1995). Expert Committee on the Control and Surveillance of African Trypanosomiasis WHO Expert Report.

World Health Organization, (1998). Control and surveillance of African Trypanosomiasis. WHO. WHO Technical Report.

World Health Organization. Report, (2000) Health systems improving performance Geneva.

World Health Organization, (2006). Human African Trypanosomiasis (sleeping sickness): epidemiological update. WHO *Weekly Epidemiological Record*, 8, 71–80. .

World Health Organization, (**2006**)/ southern Sudan polio National Immunization Days, estimated population data 2006.

World Health Organization / Southern Sudan. (2006 & 2007). Human African Trypanosomiasis (sleeping sickness) data base...

Zillmann, U. and Albiez, E. J. (1986). The Testryp CATT (Card Agglutination Test for Trypanosomiasis): a field study on gambiense sleeping sickness in Liberia. *Trop. Med. Parasitol*. **37:**390-392.

APPENDICES

Appendices: 1. Informed consent

Dear Sir (Madam) – Richard Lako from Ministry of health Government of Southern Sudan and Resident trainee of Applied Epidemiology Training Program/ Kenya program, is conducting a survey to assess knowledge, attitude, beliefs and early detection practices among human African Trypanomiasis patients and controls to examine factors influencing responses in Lui hospital in your county. OF which the participation is voluntarily and he therefore asks for your help on data collection in order to development recommendation for both ministry of health Government of southern Sudan and State Ministry of health to better improve on treatment and prevention.

Do you agree to participate in this study? _____ _____

Signature of participant

If No, _____

Thank you very much for participation.

Researcher's name _____ Translator's Name _____

Date _____ 2008.

Appendices: 2. Questionnaire

Questionnaire No

Status: 1. Case ☐ 2. Control A ☐ B ☐

DEMOGRAPHIC INFORMATION

1. Name

_____

2. Present Address:County _____Village _____

   Boma _____ Gold Man _____

3. Previous Address: County _____ Village

   _____

   Boma _____ Gold Man _____

4. Gender M ☐ F ☐ 4. Tribe _____

5. Birth date ___ _____ ____ (day, month, year of birth)

6. Age _____

7. Education 1 – Primary school 2 – Intermediate

3 – Secondary School 4 – High

5 – Currently at day care 6 – No education

8. Marital status: 1- Single 2- married 3- Separated/ Divorced 4- Cohabiting

9. Occupation: **1** – Currently employed **2** – Student and employed

3 – Student **4** – Unemployed and not a student

10. Place of employment _____

11. Place of study _____

CLINICAL AND LABORATORY INFORMATION

12. Symptoms and signs:

Malaise---------- ☐ Yes ☐ No

Headache---------- ☐ Yes ☐ No

Fever ---------- ☐ Yes ☐ No

Myalgia/Arthralgia---------- -☐ Yes ☐ No

Lymphadenopathy---------- ☐ Yes ☐ No

Sweating ---------- ☐ Yes ☐ No

Purities ---------- ☐ Yes ☐ No

Mood/sleeping disturbance ---------- ☐ Yes ☐ No

Edema ---------- ☐ Yes ☐ No

Other (specify) _____

13. Date of onset of first symptom DD_____MM _____ 2008

14. Date of hospitalization DD_____MM _____ 2008

15. Date of specimen collected DD_____MM _____ 2008

16. Date of screening DD_____MM_____/2008

17. lymph node aspirate CATT ☐ Negative ☐ Positive ☐ not done

18. Screening result. ☐ Negative ☐ positive

19. CATT whole blood test ☐ Negative ☐ positive

20. CATT titration ☐ > 1/8 ☐ = 1/8 ☐ < 1/8

21. Microscopic examination LN/Blood ☐ positive ☐ Negative ☐ not done

22. Microscopic examination CSF ☐ positive ☐ Negative ☐ not done

23. Double centrifugation of CSF ☐ WCC < 5 cells/µl ☐ WCC > 5 cells/µl

Facility name _____

EPIDEMIOLOGICAL INFORMATION

13. How long have you stayed in Mundri?

1. 1 - 6 months ☐

2. 7 – 12 months ☐

3. 1 year and more ☐

14. Have you ever leave out side your Home village? Yes ☐ No ☐

if no skip to Q 29

15. If **yes in which parts of the country?**

Within Western Equatoria State ☐

Within Equatoria Region ☐

Outside Equatoria Region ☐

16. Or which Neighboring Countries? Uganda ☐ Congo ☐ Central Africa Republic ☐ Others _____

17. Source of water for domestic use?

River ☐ Bore Hole ☐ Un protected well ☐ Swamp ☐

Others specify _____

18. Do you Have any animal in your household or near by crawl? Yes ☐ No ☐

If **yes**, specify animal type:

Cattle	☐ Yes	☐ No	Number _____
Sheep	☐ Yes	☐ No	Number _____
Goat	☐ Yes	☐ No	Number _____
Dog	☐ Yes	☐ No	Number

_____

Pig ☐ Yes ☐ No Number _____

Other (specify) _____

19. What other extra activity do you perform ?

Collecting water ---------- ☐ Yes ☐ No

Farming---------- - ☐ Yes ☐ No

Fishing----------☐ Yes ☐ No

Collecting firewood---------☐ Yes ☐ No

Soaking cassava----------☐ Yes ☐ No

Hunting----------☐ Yes ☐ No

Going to market---------- ☐ Yes ☐ No

Caring for livestock ------- ☐ Yes ☐ No

Others ----------☐ Yes ☐ No

20. Do you know what sleeping Sickness is? Yes ☐ No ☐

a) If **yes**, how do you called it in your Language? _____

b) How do you get the sleeping sickness? _____

21. How Do you protect your self against the sleeping sickness?_____

22. Where Do you go for treatment and why?

_____

_____

_____

23. Any of your family members get sick with sleeping Sickness? Yes ☐ No ☐

a) If **Yes**, When and How old is he? _____

b) How Close To You? Mention _____

c) Where did he go for treatment? _____

d) What happen to him? _____

24. Do you use Insecticide treated mosquito net (ITN) at your home?

Yes ☐ No ☐

25. What do you think could be the cause of the sleeping Sickness?

_____
_____

26. First contact with health provider _____Weeks/Month

27. First contact_____ Traditional Healer

Drug shop ☐

Health Unit/center/Hospital ☐

Private clinic ☐

28. Reasons for delay in consulting a health provider

Underestimated their symptoms ☐

Too busy ☐

Poor access to health care ☐

No reason given ☐

Not delayed ☐

Others mention _____

Knowledge, Attitudes and belief

29. Can People reduce chance of getting by sleeping sickness by not going to River Bank?

Yes ☐

No ☐

Don't Know ☐

30. Can people get sleeping sickness parasite through bite of mosquito?

Yes ☐

No ☐

Don't know ☐

31. Can sleeping sickness cause by Witchcraft?

Yes ☐

No ☐

Don't know ☐

32. If one of family member has sleeping sickness does the rest will also have a disease?

Yes. ☐

No ☐

Don't know ☐

33. Is it possible to healthy individual to have a sleeping Sickness?

Yes ☐

No ☐

Don't know ☐

34. Do you thing the chance of you getting sleeping sickness are small , moderate or no risk at all?

Small ☐

Moderate ☐

No risk at all ☐

35. What are the main communications from which you receive the sleeping sickness information?

1. Radio ☐ 2. T.V ☐ 3. Film ☐ 4. Drama ☐

5. Community notice ☐ 6. Health Education by Health workers ☐

7. Friends ☐ 8.Family ☐ 9. Political leaders ☐

10. Religious leaders ☐ 11. Traditional leaders ☐

36. Can sleeping sickness be transmitted from mother to Child?

Yes ☐

No ☐

Don't know ☐

37. What drugs do you know for treatment of sleeping sickness?

Pentamidine ☐

DFMO ☐

Marlosoporal ☐

None ☐

38. For how long does the sleeping sickness treatment Last?

Less than one month ☐

1- 6 month ☐

7- 12 month ☐

More than one years ☐

Rest of life ☐

39. Have heard about screening for sleeping sickness?

Yes ☐

No ☐

Don't Know ☐