SERODIAGNOSIS OF HUMAN AFRICAN TRYPANOSOMOSIS AMONG HUMAN IMMUNODEFICIENCY VIRUS PATIENTS IN ANKPA GENERAL HOSPITAL, KOGI STATE

BY

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DECLARATION

I declare that the work in the thesis entitled, **'Serodiagnosis of Human African Trypanosomosis among Human Immunodeficiency Virus Patients in Ankpa General Hospital, Kogi State'** has been carried out by me in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously been presented for another degree or diploma at this or any other institution.

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Yusuf WADA Signature Date

CERTIFICATION

This Thesis titled "**SERODIAGNOSIS OF HUMAN AFRICAN TRYPANOSOMOSIS AMONG HUMAN IMMUNODEFICIENCY VIRUS PATIENTS IN ANKPA GENERAL HOSPITAL, KOGI STATE**" by **Yusuf WADA** meets the regulations governing the award of **Master of Science Degree** in Veterinary Public Health and Preventive Medicine of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my beloved mother Rabi Abu, no doubt, I am indeed, what I am today because you have always been there for me and also for your undying love.

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ABSTRACT

Human African Trypanosomosis is a serious disease of man and animal. The present study was aimed at investigating the occurrence of Human African Trypanosomosis among Human Immunodeficiency Virus patients in Ankpa General Hospital, Kogi State. *Trypanosoma brucei gambiense* infection decreases the specificities of antibody detection test for HIV diagnosis. Human African Trypanosomosis symptoms are nonspecific, variable and inconsistent, and alone are insufficient for diagnosis. Moreover, HAT symptoms can be confused with those of malaria, enteric fever, tubercular meningitis and HIV. Ethical clearance was obtained from the State Ministry of Health. Blood samples were collected from HIV patients at the Ankpa General Hospital after their consent was sought. Subjects to be sampled were selected using convenience sampling with the sex, age and occupation of the HIV patients recorded. Four hundred and sixty five blood samples were collected from HIV patients. They were screened serologically using Card agglutination test for *T.b gambiense* (CATT) and parasitologically using the wet mount and haematocrit centrifugation technique (HCT). The overall sero-prevalence of HAT in Ankpa General Hospital among HIV patients was 3.01% (14/465). Sero-prevalence among the females and males was 3.60% (12/333) and 1.52% (2/132) respectively. There was no significant association ($p>0.05$) between the infection and sex. There was a sero-prevalence of 3.63% (12/330) and 2.04% (2/98) among the age groups of 18-45 years and above 45 years respectively which were not statistically significant ($p>0.05$). There was no significant association ($p>0.05$) between the infection and occupation, farmers had the highest sero-prevalence of 5.00% (10/200). No parasites were however detected in the blood samples. The questionnaire survey showed that 79.35% (369/465) of the respondents were aware of HAT and 20.64% (96/465) were not. There was a significant association ($p<0.05$) between education, age, occupation and awareness to HAT. There was no significant association between sex and awareness to HAT

($p>0.05$). This study has established a serological evidence of HAT among HIV patients in Ankpa General Hospital, Kogi State. It is recommended that tsetse fly control measure should be put in place. It is also recommended that specific validation on three HIV test should be carried out to increase specificity. It is also recommended that public campaign on HAT should be carried out to increase both the awareness and knowledge of the populace to HAT in order to alert the people on the impact of the disease and how best to avoid it.

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ABBREVIATIONS

aaMφ	Alternatively activated macrophages
AIDS	Acquired Immunodeficiency Deficiency Syndrome
ART	Anti Retroviral
AU	African Union
B cells	Bone marrow cells
C	Complex
caMφ	Classically activated macrophages
CATT	Card agglutination Test for *Trypanosoma brucei gambiense*
CDC	Center for Disease Control and Prevention
CD 1...8	Cluster of differenciation 1…8
cm	Centimeter
cNOS	Calcium-dependent Nitric oxide synthase
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
Con A	Concanavalin A
DC	Double Centrifugation
DNA	Deoxyribonucleic acid
DRC	Democratic Republic of the Congo
ECG	Electrocardiogram
EDTA	Ethylene Diamine Tetra-acetic Acid
EGF	Epidermal Growth Factor
ELISA	Enzyme Linked Immunosorbent Assay
et al.	Et alia (and others)

FIND	Foundation for Innovative New Diagnostics
FMoH	Federal Ministry of Health
G.	*Glossina*
HAT	Human African Trypanosomosis
HCT	Haematocrit Centrifugation Technique
HDA	Helicase Dependent Amplification
HIV	Human Immunodeficiency Virus
IFN	Interferon
Ig	Immunoglobulin
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
iNOS	Calcium independent Nitric Oxide Synthase
KM	Kilometer
LAMP	Loop-mediated isothermal Amplification
LED	Light-emitting diode
LGA	Local Government Area
m-AECT	Miniature anion exchange centrifugation technique
MHC	Major Histocompatibility Complex
ml	Millilitre
mRNA	Messenger Ribonucleic acid
MSC	Modified Single Centrifugation
NACA	National Agency for the Control of AIDS
NASBA	Nucleic Acid Sequence Based Amplification
NASBA-OC	Nucleic Acid Sequence Based Amplification Oligochromatographic

NEACA	National Expert Advisory Committee on AIDS
NEAR	Nicking Enzyme Amplification technology
NK	Natural Killer
NO	Nitric oxide
NOS	Nitric oxide synthase
NPC	National Population Commission
PATTEC	Pan-African Tsetse and Trypanosomiasis Eradication Campaign
PCR	Polymerase Chain Reaction
PFR	Paraflagellar Rod
PG	Prostaglandins
RIME	Ribosomal Mobile Element
RNI	Reactive Nitrogen Intermediates
ROI	Reactive Oxygen Intermediates
RPA	Recombinase Polymerase Amplification
scFv	Single-chain variable fragment
SIT	Sterile Insect Technique
s.l	sensu lato
SRA	Steroid Receptor RNA Acceptor
T.	*Trypanosoma*
T.b	*Trypanosoma brucei*
TGF	Transforming Growth Factor
TgsGP	*T. b. gambiense* uses primers amplifying the single copy glycoprotein gene
Th	T helper
TLR	Toll like receptors
TLTF	Trypanosome- Released Triggering Factor

TMA	Transcription Mediated Amplification
TNF	Tumor Necrosis Factor
UNAIDS	United Nation Programme on HIV/AIDS
UNGASS	United Nations General Assembly
UNDP	United Nations Developmental Project
USD	United State Dollar
VAT	Variable antigen type
VSG	Variant Surface Glycoprotein
W.H.O	World Health Organization
^{0}C	Degree Centigrade
β	Beta
%	Percentage
α	Alpha
γ	Gamma
<	Less than
>	More than

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND INFORMATION

Human African trypanosomosis (HAT), also known as sleeping sickness, is a vector-borne parasitic disease. The parasites are transmitted to humans by the tsetse fly (Diptera: Glossinidae), which is found in Sub-Saharan Africa. Sleeping sickness is considered as a re-emerging and neglected disease (Cattand *et al*., 2001). At the end of the 1990s, and up to 2001, 60 million people were estimated to be living in high risk infection areas and that between 300,000 to 500,000 people were infected (WHO, 2001). However, the epidemiological situation has improved notably over the last four years due to the increase of surveillance activities. Therefore, it is currently estimated that the number of new cases per year has fallen to 17,500 and that the accumulative rate stands between 50,000 and 70,000 cases worldwide (WHO, 2006). In the past few years the reported number of cases of sleeping sickness has again reduced due to increased control measures, although the exact number of cases is uncertain because of poor health services in most of the affected areas (Brun, 2010).

Two subspecies of *Trypanosoma brucei* can infect humans. *T. b.gambiense* causes a generally chronic form of sleeping sickness in West and Central Africa. *T. b. rhodesiense*, found in Eastern and Southern Africa, generally causes a more acute form of the disease. In both forms the disease is characterized by two clinical stages related to the propagation of the parasite in the infected host. In the first stage, when trypanosomes multiply in the haemolymphatic system, infected individuals experience intermittent episodes of fever and develop lymphadenopathy, and other non-specific signs such as hepatomegaly, spleenomegaly and skin rashes (Stich, 2002). In the

second stage of the disease, trypanosomes reach the Central Nervous System resulting in a chronic meningoencephalitis with headaches and extensive neurological changes, which result in severe sleep disturbances resembling narcolepsy, convulsions, semi-coma, and death (Stich, 2002).

Among available serological tests, the most commonly used in the field is the Card agglutination Test for Trypanosomiasis or CATT: *T. b. gambiense* (CATT) (Magnus *et al*., 1978) developed for detection of trypanosome specific antibodies. Bloodstream form trypanosomes of *T. brucei* spp. contain both somatic (common) and variable antigens (Seed, 1974; Le Ray, 1975). Studies on variable antigen type (VAT) repertoires (Van Meirvenne *et al*., 1975, 1977) revealed that some predominant VATs such as LiTat 1.3 are particularly widespread in *T. b. gambiense*.

Concentration techniques such as the mini-haematocrit centrifugation technique (Woo, 1970) originally developed for avian trypanosomes (Bennett, 1962) or the miniature anion exchange centrifugation (m-AECT; Lumsden *et al.,* 1979), improve detection levels to 500 and 100 parasites per mL by microscopy, respectively (WHO, 1998).

The first two cases of HIV and AIDS in Nigeria were identified in 1985 and were reported at an international AIDS conference in 1986 (UNAIDS, 2004). In 1987 the Nigerian health sector established the National AIDS Advisory Committee, which was shortly followed by the establishment of the National Expert Advisory Committee on AIDS (NEACA).

In 2010, National Agency for the Control of AIDS (NACA) launched its comprehensive National Strategic Framework to cover 2010 to 2015, which requires an estimated N756 billion (around USD 5 billion) to implement (All Africa, 2010). Some of the main aims included in the framework are to reach 80 percent of sexually active adults and 80 percent of most at-risk populations with HIV counseling and testing by 2015; ensure 80 percent of eligible adults and

100 percent of eligible children are receiving Anti Retroviral (ART) drugs by 2015; and to improve access to quality care and support services to at least 50 percent of people living with HIV by 2015 (NACA, 2009).

Matete and Kajejo, (2005) reported an interaction between infections of *T.b rhodesiense* and HIV/AIDS in Western Kenya. Meda *et al*., (1995) also reported a prevalence of 4.3% of HIV infection in HAT patients in Cote d'ivoire.

1.2 STATEMENT OF RESEARCH PROBLEM

HAT caused by *T. b. gambiense* is debilitating and complex parasitic zoonoses transmitted by tsetse flies. The disease has re-emerged in the 1990s as a serious public health problem in sub-saharan Africa including Nigeria (Stitch *et al*., 2003; Waiswa *et al*., 2003), and poses a major health risk to tourists visiting Tropical Africa (Conway-Klaassen *et al*., 2002; Jelinek *et al*., 2002).

In Nigeria, human infection with *T. b.gambiense* in Gboko endemic area of Benue State was first reported in 1974, and since then, no successful attempt has been made to control the tsetse vector in the division (Aiyedun and Amodu, 1974). Osue *et al*. (2008) and Karshima, (2010) reported an active transmission of the Gambian type sleeping sickness in Abraka, Delta State and Taraba State respectively, with a prevalence of 9.8% in 491 samples and 1.8% in 400 samples respectively using the Card Agglutination Test for Trypanosomosis (CATT).

Early diagnosis of HAT is important in interrupting the transmission cycle of the parasite and progress of the disease to the chronic stage. Treatment of patients with late-stage disease, when parasites have invaded the Central Nervous System (CNS), is difficult due to the cost and long treatment schedules, which normally require hospital admission. Melarsoprol, the only drug that is effective for the late-stage *T. b. rhodesiense* form of disease, causes a post-treatment reactive

encephalopathy in an estimated 10% of patients, half of which are fatal (Pe´pin and Milord, 1994; Kennedy, 2004) Treatment of early stage HAT is much easier and safer, although some side effects have been reported when pentamidine or suramin are used (Lejon *et al.,* 2003).

Studies on wild animals revealed the involvement of numerous animals such as non-human primates, reptiles, antelops and wild bovids (Herder *et al*., 2002; Njiokou *et al*., 2006; Simo *et al*., 2006), as well as domesticated animals such as pigs, goats, sheep and cattle (Nkinin *et al*., 2002; Simo *et al*., 2006) as suitable reservoirs for *T.b. gambiense* and consequently, their role in the transmission of the disease.

In Nigeria, an estimated 3.6 percent of the population is living with HIV and AIDS (UNGASS, 2010). Although HIV prevalence is much lower in Nigeria than in other African countries such as South Africa and Zambia, the size of Nigeria's population (around 162.5 million) means that by the end of 2009, there were an estimated 3.3 million people living with HIV.(UNAIDS, 2010; UNDP, 2011).

Approximately 220,000 people died from AIDS in Nigeria in 2009 (UNAIDS, 2010). An overall HIV prevalence of 5.80% was reported for Kogi State during the 2003 national sentinel survey (FMoH, 2004).

Lejon *et al*., (2010), in their study showed that *T. b gambiense* infection decreases the specificities of antibody detection test for HIV diagnosis. Unless tests have been validated for interference with HAT, HIV diagnosis using classical algorithms in untreated HAT patients should be avoided (Lejon *et al*., 2010). Specific validation on three HIV test can increase specificity (Lejon *et al*., 2010).

Harms and Feldmeier (2005) showed that HIV and tropical disease including HAT affect each other mutually. They further stated that HIV infection may alter the natural history of tropical

infectious disease, impede rapid diagnosis or reduce the efficacy of anti parasitic treatment. Also, tropical disease may facilitate the transmission of HIV and accelerate progression from asymptomatic HIV infection to AIDS (Harms and Feldmeier, 2005).

Kagira *et al*., (2011) in their study showed that HAT co- infections with other disease including HIV exist and that multiple co infections may influence the disease pathogenesis and complicate management of HAT.

HAT symptoms are nonspecific, variable and inconsistent, and alone are insufficient for diagnosis. Moreover, HAT symptoms can be confused with those of malaria, enteric fever, tubercular meningitis and HIV (Chappuis *et al.,* 2005).

Compared with immunocompetent people with HAT, HIV-infected people appear to have higher risk for treatment failure and worse outcome of both HAT and HIV infection (Blum *et al*., 2001). No such investigation has been carried out in Ankpa Local Government and the State as a whole.

1.3 JUSTIFICATION OF RESEARCH

There is no information on HAT in Ankpa Local Government Area of Kogi State, despite the presence of factors that can facilitate the occurrence of the disease such as the presence of forested vegetation which is a good breeding ground for the tsetse flies, the Fulani herdsmen and wildlife species that can serve as reservoirs (Herder *et al*., 2002; Njiokou *et al*., 2002; Simo *et al*., 2006). There is no information on occurrence of HAT among HIV patients in Ankpa Local Government and the State as a whole.

In Nigeria, HIV is known to coexist with malaria and tuberculosis, however, information on the co-infection of HAT and HIV has not been documented.

Traditionally, the field of tropical medicine focused on diseases caused by protozoa, helminths, and arboviruses. The triumvirate of pathogens currently devastating the tropics—*Plasmodium falciparum*, HIV, and *Mycobacterium tuberculosis* includes only one of such pathogen.

No HIV-related alterations in epidemiology, natural history, or therapeutic response have been identified in the case of many tropical pathogens, including *T.b. gambiense*. This should not be construed as strong evidence for the absence of such effects. In regions where coinfection with HIV and non-endemic tropical diseases is marked by low prevalence, subtlety of interaction, diagnostic difficulty, or low research priority, the interactions are likely to be overlooked. It took 15 years for the first significant interaction between infections with the high profile pathogen *P. falciparum* and infection with HIV to be demonstrated, a relative lack of benefit of increasing parity among pregnant women in the control of malaria. (Steketee *et al*., 1996).

Ankpa Local Government also happens to share boundaries with Benue State which has reported endemic HAT areas (Aiyedun and Amodu, 1974).

1.4 AIM AND OBJECTIVES OF THE STUDY

1.4.1 Aim:

This study seeks to investigate the occurrence of HAT among HIV patients in Ankpa General Hospital, Kogi State.

1.4.2 Objectives:

The objectives of this study were to;

- Detect HAT among HIV patients using serological and parasitological technique in Ankpa General Hospital

- Determine the age, occupation and sex-specific prevalence rates of HAT among HIV patients in Ankpa General Hospital
- Assess the level of knowledge and awareness to HAT among the HIV patients in Ankpa General Hospital

1.5 RESEARCH QUESTION

- Does HAT occcur among HIV patients in Ankpa Local Government?
- What is the difference in disease occurrence among age groups, sex and occupation?
- What is the level of knowledge and awareness to HAT among HIV patients in Ankpa Local Government?

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Human African trypanosomiasis, which is fatal if left untreated, affects rural populations in sub-Saharan Africa. Its prevalence has changed during the past 100 years largely because of control and intervention programmes. After major outbreaks at the beginning of the 19th century, the disease was almost eliminated in the mid-1960s, followed by resurgence in the late 1990s and a fall in the number of cases in recent years. Although the present number of cases (50000–70000) seems negligible on a worldwide scale, the characteristics and focal distribution of the disease can have a great socio economic effect on affected villages. Diagnosis and treatment is unsatisfactory and needs more research and development. As one of the most neglected tropical diseases, African trypanosomiasis could catch the attention of initiatives and public–private partnerships. With new methods to diagnose and treat patients and to control transmission by the tsetse fly, elimination of the disease might be possible (Brun *et al*., 2010).

2.2 EPIDEMIOLOGY

The geographical range of human African trypanosomiasis (sleeping sickness) is restricted to sub-Saharan Africa, where there are suitable habitats for its vector, the tsetse fly. Together with the animal form of African trypanosomiasis, known as *nagana*, human disease is a major cause of rural underdevelopment in sub-Saharan Africa. Although the disease has also been reported in Urban and peri urban areas, (Robays *et al*., 2004) it mainly affects poor and remote rural regions. Disease transmission occurs in children and adults during activities such as farming, hunting, fishing, or washing clothes along the rivers.

The African trypanosomes pathogenic for man belong to the species *Trypanosoma brucei,* which has three subspecies: *T. b. gambiense*, which causes endemic disease in central and West Africa; *T. b. rhodesiense*, which causes more acute disease in east and southern Africa and *T. b. brucei*, which usually infects domestic and wild animals but not man. The strict geographical separation between *T.b. gambiense* and *T.b. rhodesiense* could soon change; however, because the continued spread of *T. b. rhodesiense* in Uganda towards the northwest might cause an overlap of the distributions of the two forms of disease (Picozzi *et al*., 2005). Sporadic reports have appeared of disease in man caused by non human-pathogenic trypanosome species. These species are *T. b. brucei* (Deborggraeve *et al*., 2008) *T. congolense* (Truc *et al*., 1998) and *T. evansi* (Joshi *et al*., 2005). Three major epidemics have ravaged the continent in the past century (Steverding, 2008). The first, which largely affected Tropical Africa (region of sub Saharan Africa) took place between 1896 and 1906, and killed an estimated 800000 people (Louis and Simarro, 2005). A second major epidemic between 1920 and the late 1940s prompted the colonial powers to invest in vector control and mobile teams to undertake active surveillance of the population—two strategies that are still the pillars of control. These control mechanisms were initially effective, and the disease was almost eradicated in the early 1960s. However, after the advent of independence there was a collapse of surveillance and control activities in most endemic countries, often exacerbated by civil conflicts. This collapse led to a progressive re-emergence of the disease, which reached a peak in the late 1990s in the Democratic Republic of the Congo (DRC), Angola, Central African Republic, southern Sudan, and Uganda (Smith *et al*., 1998; Barret, 1999; Pasquet *et al*., 1995; Moore and Richer, 2001; Stanghellini and Josenando, 2001; Ekwanzala *et al*., 1996; Van Niuewenhove, 2001).

Since this peak of infection in the 1990s, increased control activities have succeeded in rolling back disease related to *T. b. gambiense* in several countries (Barret, 2006; Abel *et al*., 2004; Lutumba, 2003). In the 24 countries regarded as endemic for such disease, there was a 69% reduction in the number of reported cases between 1997 and 2006 (from 36585 to 11382). However, *T. b. rhodesiense* disease, which contributed only 4% (n=486) of all reported cases in 2006, showed no similar decrease because only a few endemic countries implemented control programmes (Simarro *et al*., 2008). However, these figures should be regarded with caution since under-reporting is known to mask the true burden of *T. b. rhodesiense* disease (Odiit *et al*., 2005; Fevre *et al*., 2008). The decrease in reported *T. b. gambiense* infections is encouraging, but because African trypanosomiasis mainly affects remote rural communities in regions with poor health infrastructures, many cases certainly remain undiagnosed or unreported, and the true burden of disease in Africa remains unknown. WHO used a ratio of 1:3–4 to calculate the figure of 50000 –70000 new cases in 2004 (WHO, 2006). Hidden pockets of highly endemic

T. b. gambiense disease remain, as shown by the high number of patients (n=1800) diagnosed and treated by Medecins Sans Frontieres between July, 2007, and June, 2008, in two remote areas of Central African Republic (Batangafo) and DRC (Doruma and Banda) (CF, unpublished data). Similarly, the local burden of *T. b. rhodesiense* illness on individuals and health services remains high in some affected areas (Fevre *et al*., 2008). Human African trypanosomiasis due to *T. b. gambiense* is very rare in short-term tourists, but has been reported in immigrants, refugees, and expatriates resident for long periods in rural areas (Bisoffi *et al*., 2008; Iborra *et al*., 1999; Ezzedine *et al*., 2007; Lejon *et al*., 2003). Because of its long incubation time and chronicity, the disease should be considered even if a patient's last stay in an endemic region occurred many years ago. The number of tourists infected in countries that report the most local patients is low,

probably because these countries are rarely visited by travelers. By contrast, disease due to *T. b. rhodesiense* has been reported in short-term tourists travelling to East African game reserves, mainly in Tanzania (Jelinek *et al*., 2002; Mendonca *et al*., 2002; Moore *et al*., 2002; Moore *et al*., 2002) but also in Botswana, Rwanda, Kenya, and Malawi (Spencer *et al*., 1975)

2.3 CLINICAL FEATURES

The disease appears in two stages, the first haemolymphatic stage and the second meningoencephalitic stage, which is characterised by invasion of the CNS. The penetration of trypanosomes through the blood-brain barrier is an active process (Masocha *et al*., 2007) and occurs at or near intracellular junctions. Disease caused by either of the two parasites leads to coma and death if left untreated. *T. b. gambiense* infection is characterised by a chronic progressive course. According to models based on survival analysis, the estimated average duration of such infection is around 3 years, which is evenly divided between the first and second stages (Checchi *et al*., 2008). *Trypanosoma brucei rhodesiense* disease is usually acute, and death occurs within weeks or months (Odiit *et al*., 1997). A trypanosomal chancre (a reaction at the location of the tsetse fly bite) is rarely seen with *T. b. gambiense*, but occurs in 19% of patients infected with *T. b. rhodesiense*.

The leading signs and symptoms of the first stage are chronic and intermittent fever, headache, pruritus, lymphadenopathy, and, to a lesser extent, hepatosplenomegaly. In the second stage, sleep disturbances and neuropsychiatric disorders dominate the clinical presentation. Fever is intermittent, with attacks lasting from a day to a week, separated by intervals of a few days to a month or longer (Duggan and Hutchinson, 1966) and is rarely seen in the second stage (Blum *et al*., 2006). The febrile episodes correspond to a type 1 inflammatory reaction associated with activation of macrophage-1 cells and high concentrations of interferon γ, tumor necrosis

factor, reactive oxygen intermediates or metabolites, and nitric oxide. This reaction controls parasite invasion and proliferation, but the exacerbated immune response can induce collateral tissue damage (Stijlemans *et al*., 2007). To alleviate parasite elicited pathological changes the host can mount type 2 immune responses consisting of sequential production of interleukin 10 and interleukin 4 or interleukin 1 that can induce macrophage-2 cells with anti-inflammatory properties (Stijlemans *et al*., 2007). The mechanism of antigenic variation on the surface of the parasite allows it to persist and to elicit new parasitic waves. Sleep disorder is a leading symptom of the second stage and is the one that gave the disease its name. Somnographic studies have shown that the disease causes dysregulation of the circadian rhythm of the sleep/wake cycle and a fragmentation of the sleeping pattern rather than the frequently reported inversion of sleep (Buguet *et al*., 2001). In severe cases the circadian rhythm of prolactin, renin, growth hormone, and cortisol secretion disappears (Buguet *et al*., 2001; Lundkvist *et al*., 2006). The neurological symptoms include tremor, fasciculation's, and general motor weakness, paralysis of a limb, hemi paresis, akinesia, and abnormal movements such as dyskinesia or chorea-athetosis. There might be Parkinson-like movements due to muscular hypertension, non-specific movement disorders, and speech disorders. Abnormal archaic reflexes can also arise. These disorders are rarely seen during the first stage and increase in frequency with the duration of the disease (Blum *et al*., 2006; Kennedy, 2006). Psychiatric symptoms such as irritability, psychotic reactions, aggressive behavior, or inactivity with apathy can dominate the clinical picture (Kennedy, 2006).

In Europe, infected immigrants have sometimes been wrongly admitted to psychiatric clinics (Bedat-Millet *et al*., 2000). Cardiac involvement documented by electrocardiogram (ECG) changes is frequently seen in *T. b. gambiense* disease, but is rarely of clinical relevance. The most frequent ECG changes are QTc prolongation, repolarisation changes, and low voltage. In

T. b. rhodesiense infection, myopericarditis can be more severe (De Raadt and Koten, 1968; Koten and De Raadt, 1969). By contrast with heart problems in Chagas disease, which is caused by another trypanosome, *T. cruzi,* arrhythmias, conduction problems and blocks, and congestive heart failure are rare in African trypanosomiasis (Blum *et al*., 2007; Blum *et al*., 2008). Endocrine disorders of the thyroid and adrenocortical function can take the form of hypofunction or hyperfunction, but rarely demand specific treatment (Blum *et al*., 2007). These symptoms are more pronounced in *T. b. rhodesiense* disease (Reincke *et al*., 1998).

The symptomatology of human African trypanosomiasis in travelers is strikingly different from the usual textbook descriptions of African patients, and is similar for both *T. b. gambiense* and *T. b. rhodesiense* infections (Duggan and Hutchinson, 1966) presenting as an acute febrile disease with temperatures up to 40–41°C (WHO, 2006; Fevre *et al*., 2008). In travelers, a chancre at the inoculation site (Iborra *et al*., 1999) and a trypanosomal rash (Fevre *et al*., 2008) are more frequently seen, and results of laboratory tests show greater abnormalities than in patients from endemic countries. Severe haematological disorders, impaired kidney function, electrolyte disturbances, high concentrations of C-reactive protein and liver enzymes have been described (Lejon *et al*., 2003; Jelinek *et al*., 2002; Oscherwitz, 2003; Ripamonti *et al*., 2002).

A great diversity of clinical pictures can be seen in patients infected by *T. b. gambiense*, ranging from acute disease to chronic forms. Asymptomatic carriers are also reported (Jamonneau *et al*., 2002; Jamonneau *et al*., 2000). Whereas an initial genetic characterization of the infecting *T. b. gambiense* in patients in Cote d'Ivoire showed little genetic polymorphism (Jamonneau *et al*., 2002) a PCR analysis done some years later did detect DNA of *T. brucei.* sensu lato in

healthy carriers. These parasites might represent a distinct and previously unrecognized genetic group of trypanosomes, other than *T. b. gambiense* or *T. b. rhodesiense*. An infection or coinfection with these *T. brucei.* sensu lato could cause a slow and gradual development of morbidity (Jamonneau *et al*., 2004). Additionally, infections of people with trypanosomes thought to be non-pathogenic for man have been described in case reports (Blum *et al*., 2005). Typically, *T. b. rhodesiense* disease progresses very rapidly; however, in the southern countries of east Africa, in particular Malawi, a more chronic form has been reported. These two distinct clinical forms have been associated with two genotypes of the Steroid Receptor RNA Acceptor (SRA) gene. Additionally, tumor necrosis factor α concentration was high in early-stage patients in Uganda, whereas in Malawi high concentrations of transforming growth factor β concentrations were seen (MacLean *et al*., 2004). However, the apparently much slower progression into the second disease stage in the chronic form hampers direct comparison of the populations (Brun *et al*., 2010).

2.4 LIFE CYCLE

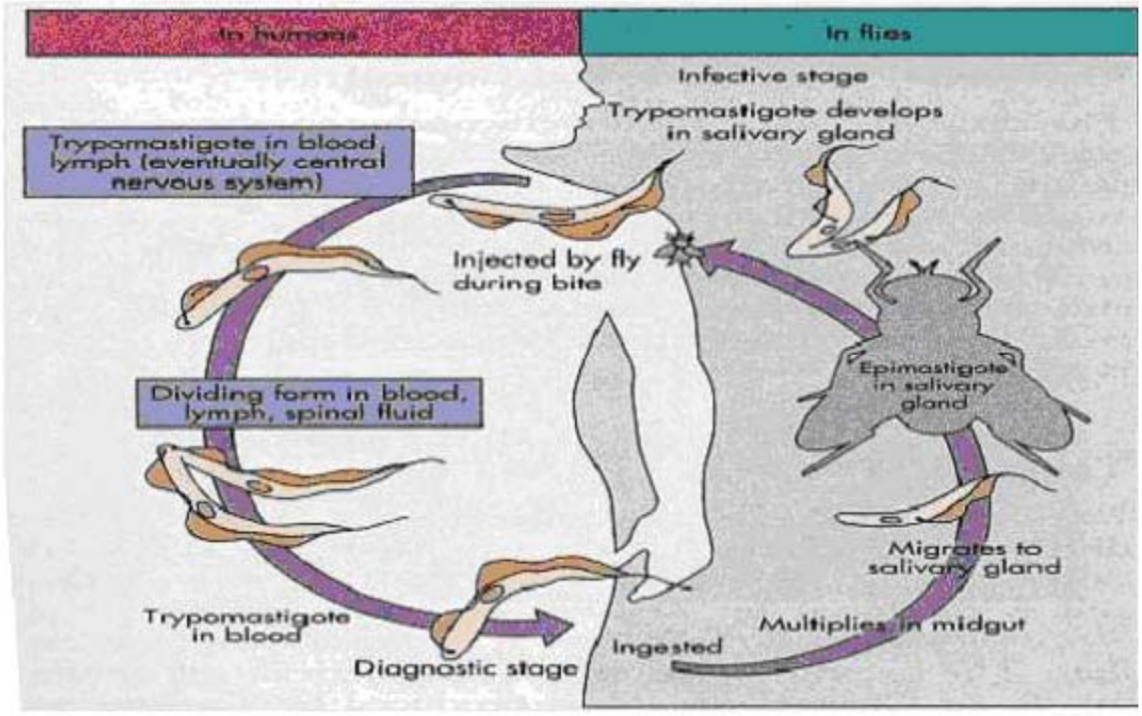

Fig 1: Life cycle of the *Trypanosoma brucei* parasites(Assafa *et al*., 2004)

The tsetse fly (genus *Glossina*) is a large, brown biting fly that serves as both a host and vector for the trypanosome parasites. While taking blood from a mammalian host, an infected tsetse fly injects metacyclic trypomastigotes into skin tissue. From the bite, parasites first enter the lymphatic system and then pass into the bloodstream. Inside the mammalian host, they transform into bloodstream trypomastigotes, and are carried to other sites throughout the body, reach other blood fluids (e.g., lymph, spinal fluid), and continue to replicate by binary fission.

The entire life cycle of African trypanosomes is represented by extracellular stages. A tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected

mammalian host. In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission.

The entire life cycle of the fly takes approximately 3 weeks.

In addition to the bite of the tsetse fly, the disease can be transmitted in the following ways: Mother to child infection, the trypanosome can sometimes cross the placenta and infect the fetus (Olowe, 1975). Laboratories: accidental infections, for example, through the handling of blood of an infected person and organ transplantation, although this is uncommon, Blood transfusion and Sexual contact (Rocha *et al.*, 2004)

2.5 IMMUNOLOGY AND IMMUNOPATHOGENESIS

The inoculation of trypanosomes into the mammalian hosts triggers a series of events involving, at first, innate immunity and, secondarily, specific immunity (Philippe and Bernard, 2006). The latter requires an efficient presentation of parasitic antigens, activation of T and B cells implying specific antigen receptor recognition, and the development of effectors cells and molecules. These mechanisms are highly regulated by multiple signals delivered through a large number of receptors transduced across the plasma membrane and processed. During co-evolution with their hosts, trypanosomes have learnt to cope with host immune systems, by penetrating, diverting, and altering the numerous steps leading to the generation of an effective immune response. Major modifications of immune systems have been observed in trypanosomiasis: lymphadenopathy, spleenomegaly (up to thirty times the normal size) with destruction of lymphatic tissue architecture and hypergammaglobulinemia. However, their effectiveness is

limited as, most of the time, parasites cannot be eliminated and immunopathological phenomena, which induce tissue alterations, appear (Philippe and Bernard, 2006).

One of the major characteristics of trypanosomes is the presence of the Variant Surface Glycoprotein (VSG) which covers nearly all the membrane of trypanosomes in mammals and is the predominant surface antigen of African trypanosomes. VSG constitutes an important molecular interface between trypanosomes and the host immune system. VSG prevents trypanosome lyses by complement alternative pathway, and above all, enables them to avoid the specific immune response via the phenomenon of antigenic variation (trypanosomes sequentially express antigenically distinct VSG). VSG also has several effects on immune elements such as induction of auto antibodies and cytokines, in particular tumor necrosis factor (TNF) - α (Tachado and Schofield, 1994; Okomo-Assoumou *et al*., 1995a; Magez *et al*., 2002). Other trypanosome components and soluble factors, such as a trypanosome- released triggering factor (TLTF) which triggers interferon (IFN)-γ production by T cells, are also involved in modulation of the immune system by acting on the synthesis of immune elements (Olsson *et al*., 1991; Vaidya *et al*., 1997). Furthermore, increased levels of circulating endotoxins are a feature of human and experimental trypanosomiasis. These endotoxins, potent immunomodulatory molecules, participate to the immune disorders observed in trypanosomiasis (Nyakundi *et al*., 2002). Elaboration of escape mechanisms to host immune defenses and induction of parasite growth factor production are well developed by trypanosomes.

In a recently discovered escape mechanism, host arginase induction, trypanosomes decrease immune response efficiency and increase the production of L-ornithine, an essential growth factor (Gobert *et al.* 2000, Vincendeau *et al.* 2003).

Understanding of the immune response was recently advanced by the discovery of the T and B subpopulations and, especially, of the T helper (Th) subsets, as well as the cytokines synthesized by each Th1 and Th2 subset. These factors control different aspects of the immune response, in peculiar the synthesis of nitric oxide, which is probably involved in several steps in the immune mechanisms. The role of γ δ T cells should also be taken into account as they have been implicated in other parasitic diseases such as malaria and leishmaniasis. Most of the data concerning African trypanosomiasis have been obtained in animal diseases or experimental animal models. Few studies have concentrated only on the immunology of HAT. The recent knowledge of the entire genome of *T. brucei* is an essential breakthrough to investigate immunology and immunopathology of HAT (Berriman *et al*., 2005).

2.6 IMMUNE RESPONSES DURING INFECTION WITH AFRICAN TRYPANOSOMES

The immune response of vertebrates consists of two arms: the innate immune response which has a low specificity and the adaptive immune response which is antigen specific. The immunology of infections by the African trypanosomes is a complex process and has been recently reviewed (Philippe and Bernard, 2006; Stijlemans *et al*., 2007). Being an extracellular parasite, the African trypanosome encounters both the innate as well as the adaptive immune response from the host.

2.6.1. Innate Immune Response against African Trypanosomes.

Once in the bloodstream of the mammalian host, the trypanosomes encounter the innate host immune system as the first barrier. As already mentioned, human and some other primates have trypanolytic factors in their serum that aid the primary defense mechanism. In a cellular innate immune response, different host cells are activated by different trypanosomal factors, initiating

an acute inflammatory response (Janeway and Medzhtov, 2002; Takeda *et al*., 2003). Among many molecules, the trypanosomal DNA that might be released from the dead trypanosomes has been shown to activate macrophages in a process called classical activation, to secrete pro inflammatory molecules like TNF, IL-12 and NO (Nitric Oxide) (Shoda *et al.,* 2001; Harris *et al.,* 2006). In this regard, the involvements of toll like receptors (TLR) and in particular the TLR9, in parasitemia control (Drennan *et al*., 2005), would suggest that the DNA from trypanosomes plays a role in disease progression. The GPI anchor of the VSG also interacts with the macrophages (via a putative receptor which is still elusive) and induces secretion of pro-inflammatory cytokines (Almeida *et al*., 2000; Coller *et al.,* 2003; Tachado and Schofield, 1994). So, the first response of the host immune system consists of classically activated macrophages (caMφ) secreting pro-inflammatory molecules such as TNF, IL-1, IL-6, NO (Duxbury *et al*., 1972; Mosser and Roberts, 1982; Pan *et al*., 2006). The caMφs can phagocytose antibody-opsonised parasites (Shi *et al*., 2004) as well as secrete trypanotoxic molecules such as TNF and NO (Mabbot *et al*., 1994; Sternberg and Mabbot, 1996; Kaushik *et al*., 2000; Magez *et al*., 1997) that are involved in the control of the first peak of parasitemia.

2.6.2. Adaptive Immune Response.

The initial inflammatory response is beneficial to the host at the early stage of the infection, but a sustained inflammation can cause pathology. Hence, it is essential for the host to reduce the inflammation which is obtained by down regulating the caMφ and their pro- inflammatory cytokines. Production of type II cytokines such as IL-4, IL-10 and IL-13 which can modulate the macrophages to become more anti-inflammatory type alternatively activated macrophages (aaMφ) are involved in a longer survival of the host. So a type I inflammatory response at the

beginning of the infection and a shift to the type II immune response in the late stage of the infection are correlated with the capacity of the host to control the parasite and the pathology respectively. In a murine model, it has been shown that the VSG specific cytokine responses associated with the resistance to the murine African trypanosomosis are infection-stage dependent, with the type-I cytokine responses being critical during the early stage of infection while the type-II cytokine responses to be more important during the late and chronic phases of the disease (Namangala *et al*., 2009). Several studies suggest that the cytokine responses influence the outcome of African trypanosomiasis (Hertz *et al*., 1998; Uzonna *et al*., 1999; Inoue *et al*., 1999; Mertens *et al*., 1999).

However, the precise role of the individual cytokines is still equivocal and may be dependent on the parasite strain, the mouse model or both. In this context the role of IFN-γ (Hertz *et al*., 1998) for resistance against *T. b. rhodesiense* and the role of IFN-γ and NO together with the antibody response have been shown to be crucial in the control of *T. congolense* infection (Magez *et al*., 2006). However, in the *T. evansi* model even though TNF, IFN and NO levels are elevated in the early stage of infection, none of these molecules seem to be important for the parasitemia control as well as the survival of the host (Baral *et al*., 2007). In another trypanosome model, *T. borelli*, a blood parasite of carp, NO hinders antibody clearance from the surface of the parasite and increases susceptibility to the complement lysis (Forlenza *et al*., 2009). Moreover, Magez *et al*. (1993; 1999) demonstrated that TNF plays a key role in both parasitemia controls as well in the development of pathology in *T. brucei* infections. Concerning the role of type II cytokines, some have shown that CD4+ T cell regulated IL-4 production was crucial for controlling *T. b. gambiense* infections in mice (Inoue *et al*., 1999) and a role for IL-4 in resistance to bovine

trypanosomiasis was also proposed (Mertens *et al*., 1999) while others (Hertz *et al*., 1998) reported that IL-4 knockout mice do not show any alteration in the parasite control. Namangala *et al*. (2000) showed that during the chronic stage of infection a Th2 cytokines production as well as an IgG1 antibody response to the trypanosome antigens is linked to the longer survival of the host in *T. brucei* infection model. Moreover, the levels of IL-10 and IL-6 in the brain have been shown to be associated with the protection from neuroinflammatory pathology of HAT (Sternberg *et al.,* 2005; Kennedy, 2009)

2.6.3. Humoral Responses

During the trypanosome infection a dominant humoral response of the host is expected, since the location of the parasite is extra-cellular. Both the murine and bovine trypanosomiasis is characterized by a polyclonal B cell activation as evidenced by an increased number of B cells and a significant elevation in plasma Igs (Hudson *et al*., 1976; Luckins and Mehlitz, 1978; Luckins and Mehlitz, 1976). Because of the polyclonal B cell activation, a significant component of the resultant antibody is either polyspecific or auto reactive (Buza *et al*., 1997; Williams *et al*., 1996; Kobayakawa *et al*., 1979). Although the VSG molecules are highly immunogenic for all mouse strains upon immunization, dramatic differences in the ability of animals to mount the VSG-specific B cell response occur after infection (Schleifer *et al*., 1993). It is shown in different independent studies that specific antibodies directed against the trypanosome VSG mediate the destruction and clearance of parasites in successive parasitemic waves and hence contribute to antibody-mediated trypano tolerance (Uzonna *et al*., 1999; Williams *et al*., 1996; Morrison and Murray, 1985; Taylor *et al*., 1996). Animals immunized with the irradiated trypanosomes or the VSG antibodies directed against the specific surface exposed epitopes of the

VSG coat opsonize the parasites and the immune complexes are efficiently phagocytosed and destroyed, mainly in the liver, by the macrophages (Kupffer cells) (Shi *et al*., 2004; Mosser and Roberts, 1982; Pan *et al*., 2006). During African trypanosomiasis, the VSG-specific B cell responses can occur in a T-cell independent manner (Reinitz and Mansfield, 1990). However, the T-cells improve the B-cell responses, mainly by secreting cytokines mediating antibody class switching. In this context an increased IL-4 mRNA level and a concomitant increase in the IgG1 antibodies against the VSG was observed in the trypanotolerant N'dama cattle infected with *T. congolense* but not in the trypano susceptible Boran cattle (Mertens *et al*., 1999).

In animal trypanosomiasis, trypano tolerance is a combination of the humoral response needed for parasite control as well as the ability to control the immunopathology which is the cause for loss of productivity. Schofield *et al*. (1999) described a rapid major histocompatibility complex (MHC)-unrestricted antibody response to the diverse pathogens including the trypanosomes. These authors demonstrated the CD1-restricted IgG formation in response to *Plasmodium* and *Trypanosoma* GPI anchored antigens mediated by IL-4 producing CD4+, NK1.1+ helper T-cells (NKT cells) and proposed that this may represent a general mechanism for a rapid response to the GPI-anchored surface antigens and the parasite control. Although the trypanosome-specific antibodies are produced in the early stage of infection and may be protective as they mediate parasite clearance (Macaskill *et al*., 1980; Dempsey and Mansfield, 1983; Levine and Mansfield, 1984; Black *et al*., 1986; Mahan *et al*., 1986), remove immune complexes (Lambert *et al*., 1981), and possibly neutralize the parasite products, yet a significant proportion of the antibodies is either polyspecific or auto-reactive (Hudson *et al*., 1976; Williams *et al*., 1996; Kobayakawa *et al*., 1979; Askonas *et al*., 1979; Ellis *et al*., 1987). Moreover, later in the infection, the B-cells

become suppressed or exhausted, resulting in a total absence of IgG responses and a strongly reduced IgM response (Hudson and Terry, 1979).

Using B-cell (μMT) and IgM-deficient mice, it has been shown that in the murine experimental *T. brucei* trypanosomiasis, B-cells were crucial for periodic peak parasitemia clearance, whereas the IgM antibody played a limited role (Magez *et al*., 2008). However, in the *T. evansi* infection model, the IgM has been shown to play an important role in the control of the disease (Baral *et al*., 2007) suggesting the role of different antibodies can vary with different trypanosome strains.

2.6.4. Immunosuppression

One of the striking features of the trypanosome infections is the dramatic suppression of the immune responses, which might result in a high susceptibility to opportunistic infections. The generalized immune suppression has been reported to affect a large variety of both the humoral (B cell) and the cellular (T-cell and macrophage) immune functions (Taylor, 1998), consequently leading to occurrence of the trypanosome-induced immunopathology. (Darji *et al.,* 1992; Flynn and Sileghem, 1991; Sileghem *et al*., 1991). Although the existence of the immunosuppression has been known for long time, the unresolved question was whether the immunosuppression was mediated by the macrophages or the T cells. There are suggestions that both cells might be involved (Tabel *et al*., 2008).

Suppressive macrophages elicited by the *T. brucei* infection play a central role in the immunosuppression observed in this infection (Borowy *et al*., 1990; Sileghem *et al*., 1989; Schleifer and Mansfield, 1993). The immunosuppression is characterized by an inhibition of the

T cell proliferation due to down regulation of both IL-2 production and expression of IL-2 receptor (Darji *et al.,* 1992; Sileghem *et al*., 1989). Prostaglandins and nitric oxide (NO) impair mitogen-induced T-cell proliferation in the spleen, peritoneal cavity, and lymph nodes of *T.brucei* infected mice but only during the early stage of infection (Schleifer and Mansfield, 1993; Beschin *et al*., 1998; Mabbot *et al*., 1998) At the early stage of the infection, the involvement of TNF and IFN-γ in the inhibition of T-cell proliferation seems to be involved in an up-regulation of prostaglandins and NO synthesis (Magez *et al*., 1999; Darji *et al*., 1996). In addition, TNF promotes the development of suppressive cells by inducing IFN-γ production in the lymph nodes of *T. brucei* infected mice (Darji *et al*., 1996). Moreover, *T. brucei* infection also impairs the MHC class II antigen presenting capacity of the classically activated macrophages (Namangala *et al*., 2000) resulting in a reduced T-cell activation. But at the late stage of infection, inhibition of T-cell proliferation in the lymph nodes occurs through NO/prostaglandin independent pathway, whereby IFN-γ released by CD8+ T-cell plays a crucial role (Beschin *et al*., 1998; Darji *et al*., 1996). There are reports showing at the late stage of infection, macrophages displaying an anti-inflammatory cytokine production, which might modulate in several aspect of the immune system as the infection progresses (Raes *et al*., 2002; Namangala *et al*., 2001). Factors like IL-10 secreted by the macrophages of the infected animals are shown to inhibit antigen presentation (Ding *et al.,* 1993) and contributing to the impairment of T-cell activation.

However, the mechanisms of suppression by the alternatively activated macrophages elicited at later stage of the African trypanosome infections are not fully understood. Regulatory T cells (Tregs) have also been shown to limit the production of IFN-γ by CD4+ and CD8+ T cells and

also down regulate the activation of macrophages (Guilliams *et al*., 2007; Guilliams *et al*., 2008). Furthermore, these Tregs are suggested to suppress the NKT cell (Tabel *et al*., 2008). Presentation of glycolipids to the NKT cells in the context of CD1d have been suggested in the trypanosome infection (Tabel *et al*., 2008) or a GPI treatment (Stijlemans *et al*., 2007)

2.6.5. Immunopathology

As mentioned earlier, uncontrolled type I immune reaction of the host leads to a pathological condition. The major pathological complication associated with the human trypanosomiasis is the neurological disorder which is finally manifested as 'sleeping sickness'. However the pathological symptoms observed in experimental trypanosomiasis are mainly loss of body weight, fever, reduced locomotory activity, splenomegaly, and liver damages. One of the common pathological features observed in human, bovine as well as the experimental murine trypanosomiasis is the loss of RBC count, that is, anemia. Here, the degree of anemia might be considered as an indicator of the disease severity (D'leteren *et al*., 1998). At least in case of the bovine trypanosomiasis, one aspect of the trypanotolerance is the measurement of the ability to control the infection associated anemia and subsequently the loss of productivityof the host (Naessens, 2006). Anemia during trypanosomiasis might be due to either loss of RBC, for example, the cytokine-activated macrophages (M1 cells) are suggested to be responsible for the enhanced phagocytosis of parasites as well as the RBCs (Stijlemans *et al*., 2008) or due to inability to mount a vigorous compensatory erythropoietic response (Stijlemans *et al*., 2008; Akinbamijo *et al*., 1998).

During trypanosome infections, TNF are involved both in parasitemia control and infection associated pathology such as anemia, neurological disorders, fever and cachexia during both

human and animal trypanosomiasis (Taylor, 1998; Taylor and Mertens, 1999) In view of this dual role, lots of works reveal how trypanosomal components induce TNF. In this regard, VSG was identified as major TNF inducing component in trypanosome-soluble extract. Both sVSG and mfVSG were shown to manifest similar TNF inducing capacities but by a detailed analysis it was indicated that they are working in a different way. The GIP moiety of the VSG via the GIP associated galactose side chain is responsible for direct induction of TNF. Yet, the mfVSG, but not the sVSG, stimulates macrophages toward IL-1 secretion and acquisition of the LPS-responsiveness and is, as such, involved in indirect TNF production. Thus, the VSG has two distinct macrophage activating components (Magez *et al*., 1998). TNF can signal for cellular activities through 2 different receptors; TNFR1 (CD120a) and TNF-R2 (CD120b). It is suggested, that TNF-R2 signaling in trypanosomiasis mediates infection associated pathology, whereas TNF-R1 signaling has no impact on infection (Magez *et al*., 2004).

It was also shown that serum TNF levels correlate with the severity of neuropathological symptoms in the human sleeping sickness (Okomo-Assoumou *et al*., 1995), however, some studies found no correlation between the TNF serum level and pathology of the HAT (Lejon *et al*., 2002; MacLean *et al*., 2001). In the same line of research, there are reports that demonstrate the enhanced expression of TNF mRNA in the brain of *T. brucei*-infected mice (Hunter *et al*., 2001; Hunter *et al*., 1992) and the correlation in trypanosome-infected cattle between TNF production by monocytes and the severity of diseases associated anemia (Sileghem *et al*., 1994). Hence, the accumulated knowledge about trypanosome-elicited production of TNF indicates that while this cytokine might be beneficial during the early stage of infection through its role in parasite clearance, the overall pathology-inducing aspect overrules in a negative way. As outlined before, due to highly sophisticated antigenic variation, an antiparasitic vaccination

seems to be very difficult. However, the knowledge of the immunopathology gives a basis for a design of an anti disease vaccination (Toya, 2009).

2.6.6. Chancre

The local response in the skin corresponds to the first protection developed by the host. Following inoculation of *T. brucei* into mammalian hosts, by the tsetse fly, a local skin reaction is induced by trypanosome proliferation and appears a few days after inoculation. In efferent lymphatic vessels, trypanosomes have been detected in lymph 1-2 days before the chancre. Their number declined during development of the chancre (6 days) and later increased. They are detected in the blood 5 days after inoculation. In *T. congolense*-infected sheep, neutrophils predominate in the early days and then T and B lymphocytes infiltrate the chancre. Later, T lymphocytes predominate, especially CD8+ T cells (Mwangi *et al*., 1990). An early response due to an increase in CD4+ and CD8+ T cells was revealed by flow cytometry in the afferent lymph draining the chancre. As the chancres regressed there was an increase in lymphoblast and surface immunoglobulin bearing cells (Mwangi *et al*., 1996). During this first stage, trypanosomes expressed Variable Antigen Types (VATs) found characteristically in the tsetse fly, which changed after few days. An antibody response specific to these VATs appeared in the lymph and then in the plasma (Barry and Emergy, 1984).

2.6.7. Complement

Both in humans and animals, complement activation by two pathways is detected in HAT. The alternative pathway, independent of specific antibodies, was studied by the induction of trypanosome lysis (*T. congolense* and *T.b. brucei*) observed after the addition of fresh serum. Serum could induce trypanosome lysis only on uncoated VSG trypanosomes, as observed during the cycle of this parasite (procyclic forms). However, the appearance of VSG on parasites prevents trypanosome lysis by this alternative pathway (Ferrante and Allison, 1983). For another strain of *T.b. gambiense*, it was demonstrated that the alternative pathway was incompletely activated without generation of the terminal complex (C5-C9) able to induce membrane lysis (Devine *et al*., 1986). The classical pathway, mediated by specific antibodies against trypanosomes, was also described and could be involved in parasite clearance by antibody-mediated lysis and/or opsonisation. The coated stages of *T.b.brucei* are lysed by antibodies with activation of complement by the classical pathway. Nevertheless, during these complement activations, the appearance of soluble fragments, including C3a and C5a anaphylatoxins and the C567 complex, could induce, on the one hand, the chemotactism of neutrophils and monocytes and, on the other hand, the release of amines involved in vasoconstriction and an increase in vascular permeability participating in the initial inflammatory response in the chancre. Immune complexes can also activate the complement. These immune complexes are constituted by antibodies specific to trypanosomes (e.g. anti-VSG antibody) leading to a rapid elimination of complement-fixing immune complexes (Russo *et al*., 1994) or by auto antibodies , such as rheumatoid factor or anti-nucleic acid antibodies. These immune complexes with complement activation are also involved in some adverse effects, especially in tissue damage mediated by

immune complex deposits (Nielsen, 1985), such as thrombosis and glomerular involvement (Bruijn *et al*., 1988; Van Velthuysen *et al*., 1994).

2.6.8. Natural Killer Cells

Natural killer (NK) cells have been identified as an important defense mechanism against tumor cells and intracellular pathogens, especially viruses. They are considered to belong to the lymphocyte lineage and have functions in both innate and acquired immune responses. NK cells lyse extracellular parasites. NK cells from *T. cruzi*-infected mice have been shown to exhibit significant activity against trypomastigotes of *T. cruzi* (Hatcher and Kuhn, 1982). NK cells secrete cytokines and especially IFN-γ and TNF-α, which play major roles in trypanosomiasis and are regulated by cytokines which can activate or inhibit NK cell functions. NK cells also participate in the initiation of the inflammatory response, through the synthesis of chemokines. In *T. brucei*-infected mice, NK activity was not modified in the early stages of infection, but was severely reduced from day 9 onwards (Askonas and Bancroft, 1984). By contrast, NK cells were activated in mice infected with a natural extracellular trypanosome (*T. musculi*) and their critical role was demonstrated by the effects of their depletion by antiserum against asialo GM1 or their activation by polycytidylic co polymer (Albright *et al*., 1997).

2.6.9. T Cells

Initial studies have evidenced alterations in T cell functions in trypanosomiasis, both *in vivo* and *in vitro* (Mansfield and Wallace, 1974). Histological examination revealed a massive B cell expansion in the lymph nodes and spleen, which replaced the thymus-dependant area in *T.b. brucei* TREU 667-infected mice. These changes were seen within 7 days post-infection and

persisted for at least 70 days. Moreover the role of T cells in controlling infection was not clear (Askonas and Bancroft, 1984).

Trypanosome antigen-specific T cell response was difficult to identify. In several studies, a transient proliferative T cell response to trypanosome antigens was noted in the first days of the infection followed by an absence of response (Gasbarre *et al.,* 1980). The kinetoplastid membrane protein of African trypanosomes is a potent stimulator of T lymphocyte proliferation (Tolson *et al*., 1994). In *T. b. brucei* - infected mice, an increased proliferation of T cells was noted in the first days of infection in spleen and bone marrow, T blasts disappeared very rapidly. In *T. congolense*-infected cattle, antigen-specific proliferation of T cells was obtained with more or less difficulty according to the antigen, the T cell population and the time used. However, a strong trypanosome-specific T cell proliferation occurred in infected cattle following treatment (Emery *et al*., 1980; Lutje *et al*., 1995). Most T cells in humans and mice bear Tαβ antigen receptors. These cells possess surface markers, which allow the discrimination of CD4+ T cells (helper T cells) and CD8+ T cells (cytotoxic T cells). The knowledge of T cell subsets has been deeply modified by the discovery of two subsets of T helper cells, Th1 and Th2 cells. Th1 cells expressing a functional T cell response directed to VSG are generated in *T.b. rhodesiense*-infected mice. VSG specific T cells were found predominantly in the peritoneum. These cells did not proliferate but made a substantial IFN-γ and IL-2 cytokine response (Schleifer *et al*., 1993). The cellular phenotype of VSG-responsive T cells (CD4+ CD3+) indicates that the VSG appear to preferentially stimulate a Th1 cell subset during infection. Analysis of lymphocyte subsets in regional lymph nodes of *T. congolense*-infected N'Dama (trypanotolerant) and Boran (trypanosusceptible) were performed by flow cytometry. In both breeds, a significant decrease in the percentage of CD2+ and CD4+ T cells was observed, associated with an increase in the

percentage of CD8+ T cells, B cells and γ δT cells. VSG and two invariant antigens (33 kDa cysteine protease and 66 kDa antigen homologous to immunoglobulin heavy chain binding protein hsp70/Bip) induced *in vitro* proliferation and synthesis of IL-2 and IFN-γ (Authié *et al*., 1992; Boulangé and Authié, 1994; Lutje *et al*., 1995). No significant differences in the *in vitro* proliferation of lymph node cells to VSG, Concanavalin A (Con A) or hsp 70/Bip were observed between the two breeds. However, IFN-γ production in response to ConA was higher in Boran at 35 days post infection.

Human and mouse immune systems contain few γ δT cells, in marked contrast to those of ruminants (Hein and MacKay, 1991). Functions of γ δT cells remain largely unknown. Involvement of γ δT cells in malaria and leishmaniasis has been observed (Rosat *et al*., 1995; Rzepczyk *et al*., 1997). A proliferative response of CD8+ T cells and γ δT cells from trypanotolerant N'Dama to an antigen complex containing immunodominant epitopes was observed whereas a quasi absence of response was observed in trypano susceptible Boran. The role of this γ δT cell response in parasite resistance remains unclear. So, γ δT cells, as CD4+ or CD8+, do not proliferate when stimulated with soluble VSG *in vitro* (Flynn and Sileghem, 1994).

2.6.10. B Cells

In African trypanosomiasis, the main feature is a dramatic increase in immunoglobulin (Ig) levels (especially IgM), including trypanosome-specific antibodies and non-specific Ig production induced by cytokine activation of B cells. Some of these antibodies are also raised against auto antigens, corresponding to non-specific polyclonal activation of B-cells producing natural auto antibodies and also to antigen-driven antibodies induced by molecular mimicry.

DNA from *T.b. brucei* stimulated B cell proliferation (Shoda *et al*., 2001). In *T.b. brucei*-infected mice, B lymphocytes display an aberrant activation phenotype (Sacco *et al*., 1994). Antibodies specific to trypanosomes are induced by several parasite antigens, including variant and invariant VSG epitopes, as well as membrane, cytoplasmic and nuclear antigens, through T-dependent and T-independent pathways (Reinitz and Mansfield, 1990). In contrast, specific trypanosome B cell response, depending on T cell regulation, was depressed. Several factors may contribute to this immunosuppression. Macrophages may become unable to present antigens to T cells (by defects in antigen processing and association of epitopes with MHC Class II) and produce immunosuppressive factors as nitric oxide (NO), prostaglandins (PG), and cytokines. An increase in immunosuppressive cytokines, such as INF-γ and transforming growth factor (TGF)-β, was also detected during infection. However, TGF-β is known to inhibit the production of IL-4, IL-5, IL-6, the major cytokines implied in B cell proliferation and differentiation (Fargeas *et al*., 1992). Several autoantibodies are detected during African trypanosomiasis. High levels of polyclonal Igs were a marked feature of HAT. The specificity of these IgS is frequently characterized against a large range of auto antigens. Auto antibodies were directed against red blood cells (Kobayakawa *et al*., 1979), liver and cardiolipids (MacKenzie and Boreham, 1974), nucleic acids: DNA and RNA (Kobayakawa *et al*., 1979; Hunter *et al*., 1992b), intermediate filaments (Anthoons *et al*., 1986) and rheumatoid factors (Kazyumba *et al*., 1986). Auto antibodies directed against components of CNS myelin have also been reported. They are specific for the major glycosphingolipids of myelin, the galactocerebrosides, and were detected in sera from both experimentally infected animals (Jauberteau *et al*., 1991) and patients from the Ivory Coast (Amevigbe *et al*., 1992). Other auto antibodies directed against not yet characterized

proteins have been described in HAT patients (Asonganyi *et al*., 1989) as well as antibodies directed at myelin basic protein in experimentally infected animals (Hunter *et al*., 1992a).

Other antibodies were raised against an epitope containing L-tryptophan, a precursor to the neuro transmitter serotonin, (Okomo-Assoumou *et al*., 1995b). In some cases, these auto antibodies (anti-galactocerebrosides and anti-neurofilaments) are associated with the neurological stage of the disease and their detection in sera and CSF could contribute towards defining the neurological involvement of HAT (Courtioux *et al*., 2005). *In vivo* demyelinisation has been produced by purified antibodies to galactocerebroside (Saida *et al*., 1979). There are several hypotheses for the origin of these antibodies. They may be induced by a non-specific stimulation of B cells producing natural autoantibodies (Arneborn *et al*., 1983; Mortazavi- Milani *et al*., 1984). In other cases, antigen-driven autoantibodies are specific to epitopes of the causative infecting agent with molecular mimicry to self antigens, inducing a cross-reactivity to intermediate filaments (Dales *et al*., 1983; Fujinami *et al*., 1983; Davies, 1997) as demonstrated for anti-neurofilament and anti-galactocerebroside antibodies which recognised respectively a flagellar component and a proteolipidic epitope of trypanosomes, and epitopes expressed by neurones (Ayed *et al*., 1997; Girard *et al*., 2000). A subpopulation of B cells identified by the expression of high levels of surface Igs and of CD5 in humans and Ly-1 in mice is responsible for most serum IgM (Kipps, 1990). These CD5 cells produce auto antibodies, and antibodies to thymus-independent antigens. In cattle infected with *T. congolense*, a dramatic increase in these cells (more than four times the control value in blood) was measured and correlated with increases in serum Igs and in the absolute number of B cells (Naessens and Williams, 1992). An induction of these CD5 B cells (directly by parasite products or indirectly through the cytokine

network) could account for the alteration in immunoglobulin synthesis and antibody production observed in trypanosomiasis.

2.6.11. Macrophages

Mononuclear phagocytes play a key role in all steps of immune response in the inflammatory phase, as antigen presenting cells, in specific immunity, in synergy with antibodies and cytokines. They also can be involved in immunosuppressive and immunopathological phenomena. Quantitative, biochemical and functional changes of mononuclear phagocytes are observed in trypanosomiasis. In *T. brucei*- infected mice, histological examination showed a marked expansion in macrophages of the liver, spleen and bone marrow. The Kupffer cells in the Liver increased in number and were often found in mitosis. The cells contained abundant phagolysosomes (vacuolated cytoplasm) (Takayanagi *et al*., 1992).

Macrophages are highly sensitive to environmental factors, especially microorganisms, microorganism-derived products and cytokines.

The immunological clearance of [75Se]-methionine-labelled *T.brucei* in mice has been conducted to investigate the respective roles of antibodies, macrophage activation and complement in the removal of circulating parasites. The clearance was largely accomplished by antibody-mediated hepatic phagocytosis. C3 is necessary for the full opsonic activity present in murine clearance in passively immunized mice (MacAskill *et al*., 1980). These *in vivo* studies extend previous studies on the *in vitro* phagocytic function of macrophages in the presence of immune serum (Takayanagi *et al*., 1992). Trypanosomes are highly sensitive to the cytostatic / cytotoxic effects of these compounds (Vincendeau and Daulouède, 1991; Vincendeau *et al*., 1992). They are highly reactive radicals with short half-lives, which can react together to form

potent and more stable effector molecules able to act on distant targets such as extracellular parasites. It has been shown that *T.b. gambiense* are highly sensitive to S nitroso-compounds, which are new effector molecules synthesized by activated human macrophages *in vitro* (Mnaimneh *et al*., 1997). Nitrosylated compounds could represent new effectors molecules with a potent effect on targets distant from macrophages. In a recent study DNA from *T.b. brucei* have increased macrophage production of IL-12, TNF-α and NO (Shoda *et al*., 2001).

Macrophages are also active in secreting PGs which modulate lymphocyte and macrophage functions. During a *T.b. brucei* infection, the ratio of PGE2/PGF1a is reversed, with an overproduction of PGE2 (Fierer *et al.,* 1984). Macrophages are involved in immunosuppressive mechanism, and VSG can also inhibit macrophage functions (Flynn and Sileghem, 1991; Schleifer and Mansfield, 1993; Coller *et al*., 2003).

Macrophages respond to, and synthesise, a large number of cytokines. The production of IL-1 is increased in *T.b. brucei*-infected mice, but this increase may be due to release rather than synthesis (Sileghem *et al*., 1989). In murine macrophages, VSG induces IL-1 and TNF-α synthesis. Human monocytes can also be induced by trypanosomes and secreted factors from trypanosomes to express TNF-α RNA transcript and secrete TNF-α in culture supernatants (Daulouède *et al*., 2001). Classical and alternative states of macrophage activation are observed in trypanosomiasis. Classical activation precedes alternative activation in murine trypanosomiasis. However, both activation states are expressed in these mice. By inducing alternative macrophage activation, trypanosomes induce host arginase which both decrease trypanocidal nitrosylated compound synthesis and increases L-ornithine production

(Gobert *et al*., 2000). L-ornithine is the first step of polyamine synthesis, essential for parasite growth and trypanothione synthesis (Vincendeau *et al*., 2003).

2.6.12. Cytokines and Chemokines

A profound dysregulation of the cytokine network is observed in trypanosomiasis. The first evidence of overproduction of TNF-α/cachectin was shown in *T.b. brucei*-infected rabbits. (Rouzer and Cerami, 1980). TNF-α is known to induce fever, asthenia, cachexia and hypertriglyceridemia. High levels of TNF-α are associated with the presence of patent inflammatory signs in the early phase of human trypanosomiasis and of major neurologic signs in the late phase (Okomo-Assoumou *et al*., 1995a). A persistently increased serum TNF-α level could contribute to the hypergammaglobulinemia observed in trypanosomiasis because the role of TNF-α on activation, proliferation and differentiation of B cells has already been shown (Roldan *et al*., 1992). Nevertheless, TNF-α participates in the mechanisms leading to trypanosome elimination: TNF-α acts indirectly in a cascade of events leading to cell activation or directly on parasites due to its cytotoxic properties (Lucas *et al*., 1994). Initial control of parasitemia in *T.b. brucei*-infected mice was diminished by the injection of anti-TNF-α antibodies (Lucas *et al*., 1993). VSG can trigger TNF-α production by macrophages, which are the cells, which produce the most of this molecule. Moreover, TNF- α production can be stimulated by IFN-γ . IFN-γ and TGF-β can be produced by CD8 T cells activated by TLTF released by *T.b. brucei* (Vaidya *et al*., 1997). TGF-β has immunosuppressive effects. An interesting fact is that IFN-γ stimulates parasite growth (Olsson *et al*., 1991). The binding of epidermal growth factor (EGF) on *T.b. brucei* receptors favored parasite growth and was one of the first cytokine-parasite interactions noted (Hide *et al*., 1989). All these data show that by interfering with the cytokine network and by using cytokines as growth factor, trypanosomes can completely modify the effectors functions of the immune system. The effects of cytokines could also be completely different according to the presence of co-stimulators and the time period

during which they are produced in trypanosome infected animals. Chemokines also play essential roles in infectious disease control. They induce cell recruitment and activation. They induce adhesion molecules on cells of the immune system, which can bind to various cells, mainly endothelial cells, which express adapted ligands (Hickey, 1999). Cytokines and chemokines can also be involved in neurological disorders (Sorensen *et al*., 1999). So, TNF-α has been reported to contribute to the pathophysiology of cerebral malaria. Mice chronically infected with *T.b. brucei* develop inflammatory lesions of the CNS after treatment with subcurative doses of a trypanocidal agent (Hunter *et al*., 1991). Chemokines favour macrophage and lymphocyte recruitment in CNS of *T.b. brucei*-infected animals. The activity of these cells in precise and selective areas of CNS might induce alterations leading to various disorders, such as sleep and endocrine disorders (Lundkvist *et al*., 2004; Buguet *et al*., 1993; 2001).

The presence of TNF-α RNA transcript in the CNS of these mice suggests that TNF-α production could play a role in these lesions. Also, TNF-α and other cytokines contribute to the generation of somnogenic molecules such as IL-1 (Pentreath, 1994). In a recent study an intra cerebral infusion of soluble type I TNF-α receptor reduced trypanosome-induced neurodegeneration (Ning *et al*., 2003). High levels of plasmatic IL-10 are also found in human trypanosomiasis.

2.6.13 Nitric oxide (NO)

Nitric oxide is a short-lived diatomic free radical synthesised from L-arginine byNOsynthase (NOS). Calcium-dependent constitutive NOS (cNOS) release small amounts (picomoles) of NO within a short time, whereas calcium-independent inducible NOS (iNOS) release high levels (nanomoles) of NO for a long time. Expression of iNOS in macrophages, neutrophils, hepatocytes, endothelial cells and epithelial cells is regulated at transcription level by a number

of agents, including microbial products and cytokines. NO produced by cytokine-activated macrophages is important in host defense and plays a crucial role in controlling infections *in vivo*. The role of NO and cytokines has been studied in detail in mice infected by intracellular parasites as *Leishmania*.

NO or other nitrogen intermediates can also react with the oxygen intermediates and form peroxynitrite and hydrogen radicals. Moreover, NO can form nitrosylated compounds which is able to transport and liberate NO on targets distant from NO producing cells. Nitrosylated compounds can not only act on extracellular parasites, but also modify parasite antigens and host cell function. These compounds may have various effects (parasite killing, alteration of tissue functions such as neurotransmission, etc.) according to their localization (spleen, liver, peritoneum, CNS, etc.). Nitrotyrosine, a marker of peroxynitrite formation, and iNOS are immunodetected in the brains of *T.b. brucei*-infected mice. Nitrotyrosine staining is associated with the appearance of neurological signs (Keita *et al*., 2000).

In HAT, nitrite production is increased at first. NO can also be stored as nitroso compounds. This NO-adducts is indirectly detected, as they induce the appearance of antibodies directed to nitrosylated antigens (Semballa *et al*., 2004). Arginase induction by parasites might be considered as a new strategy elaborated by parasites to escape host defence and benefit growth factors.

It is recommended that investigation on appropriate immunological means, associated with chemotherapeutic agents, might be useful in curing chemotherapy-resistant trypanosomiasis. (Philippe and Bernard, 2006)

2.7. ANIMAL RESERVOIR

The great significance of domestic and wild animal hosts for Rhodesian sleeping sickness is undisputed. As a result of the close proximity of livestock to humans and the relative ease with which they can be treated, Welburn *et al.* (2006) propose that sleeping sickness would most effectively be controlled locally by fighting the parasite in livestock as part of farming practices. Ng'ayo *et al*. (2005) show that domestic animals (here a sheep, a goat and a pig) harbor *T. b. rhodesiense* even in areas with few recent cases of sleeping sickness. The role of animal reservoirs is much more controversial in Gambian sleeping sickness. An unbiased observer would be astonished how strongly workers in this field seem willing to dismiss the important role of animals in *T. b. gambiense* epidemiology without much proper data from systematic surveys. Clearly, this mindset has not helped us gain a realistic view of sleeping sickness epidemiology in West Africa. Pigs have been shown repeatedly to harbour *T. b gambiense* (Simo *et al*., 2005). Even though pigs can clear infections in less than 6 months, as Penchenier *et al*. (2005) demonstrate, they are still capable of maintaining parasite populations outside the human cycle. Njiokou *et al*. (2006) now show evidence that eight wild animal species (out of 36 species sampled) belonging to four orders (primates, artiodactyls, rodents, carnivores), host *T. b. gambiense* group 1 parasites. Even if these animal species play minor roles during epidemics, they probably hold the key to where the parasites are maintained between epidemics and where new epidemics start. Hopefully, the accumulated evidence on animals carrying *T. b. gambiense* group 1 parasites will finally lead to the thorough rethinking of the role of animal hosts in Gambian sleeping sickness. Van den Bossche *et al*. (2005) further highlighted the potential impact of animal reservoirs by showing that the transmissibility of *T. b. rhodesiense* to tsetse flies is independent of parasitemia and is efficient even at very low parasitemias. This means that

each tsetse bite on an infected host has a similar probability of leading to a mature infection, and that even animals with very low parasite loads play an important epidemiological role, a result that is also of great importance for disease modeling (Van den Bossche *et al*., 2005)

A 34.4% prevalence of *T.b.gambiense* was reported in pigs from the Fontem Sleeping Sickness focus of the Republic of Cameroon using PCR (Simo *et al*., 2006).

2.8. PREVENTION, CONTROL AND RESEARCH PRIORITY

2.8.1 Prevention and Control

There is no vaccine against trypanosome infection, and chemoprophylaxis is not recommended because of the toxicity of the drugs and the low risk of infection. The only preventive measure is reduction of tsetse fly bites. The flies are attracted to dark colors, in particular blue and black, and to the motion of vehicles. They can bite through thin clothes, and insect repellents provide only part protection. Travelers can take some preventive measures, such as avoidance of areas where tsetse flies are known to be present, travelling in cars with screened or closed windows in endemic foci, use of insect repellents, and clothes of wrist and ankle length. After a person is bitten by a tsetse fly, they should be monitored, although the risk of an infection is low. If a chancre, fever, or other symptom develops, an aspirate of the chancre, the blood, and possibly a lymph node aspirate should be examined for the presence of trypanosomes. In *T. b. gambiense* areas the CATT could be used. The most important control measure for *T. b. gambiense* disease is active case-finding followed by treatment of the identified patients. Infected people can remain asymptomatic for long periods before they develop signs of sleeping sickness, but they always act as a reservoir. Although animals have a less important role as reservoirs for *T. b. gambiense*, for *T. b. rhodesiense,* domestic (cattle, dogs) and wild animals (mainly antelopes) provide a vast

reservoir that should be taken into consideration for control (Kabasa, 2007; Welburn *et al*., 2006).

Vector control by use of tsetse fly traps or screens, in combination with odors that attract the flies, or insecticides, helps to reduce the fly density. Chemoprophylaxis is no longer in use because of the poor risk-benefit ratio caused by the adverse effects of the drugs. The biological cycle of human African trypanosomiasis is very fragile because transmissions through the tsetse fly is complex and slow (WHO, 2002). As a result, even in countries where the disease is endemic, less than 0.1% of flies carry mature parasites. The prevalence in man is also low apart from in epidemic foci. If the density of flies and infected individuals is lower than crucial limits, transmission can break down. This situation is more likely to occur for disease due to *T. b. gambiense* (small animal reservoir) than for that caused by *T. b. rhodesiense* (substantial reservoir of domestic and wild animals). In 2002, the WHO HAT control and surveillance programme established a worldwide alliance to eliminate sleeping sickness (WHO, 2002) Even earlier, the Pan-African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) was founded as a taskforce uniting African countries affected by African trypanosomiasis to fight and eventually eliminate the disease (AU, 2002). The main strategy of PATTEC is elimination of the tsetse fly in isolated foci through an integrated approach that combines insecticide spraying, traps, and the sterile insect technique. The use of sterile male flies resulted in the eradication of tsetse flies on Unguja Island in Zanzibar (Vreysen *et al.,* 2000) but the enormous cost of this method is likely to restrict its use, especially in areas where several vector species cause transmission. Many tsetse control experts do not share the PATTEC view, but rather advocate the use of simple technologies such as traps in combination with insecticides and attractants (Maudlin, 2006).

For the region endemic for *T. b. rhodesiense* a restricted application of insecticides to cattle (especially belly and legs) represents a cost-effective method of tsetse control (Torr *et al*., 2007) Elimination of African trypanosomiasis is thought to be feasible by WHO (Barrett, 2006) However, a prerequisite is to have new reliable methods for diagnosis and staging, and new, safe, effective, and easy-to-apply drugs for both stages of disease. Furthermore, participation of the national programmes of all affected African countries is paramount. To guarantee sustainability, additional partners are needed, international organisations, nongovernmental organisations, and philanthropic organizations to maintain the effort over decades. If this strategy can be realized, efforts to eliminate the disease will have a real chance of success (Barrett, 2006).

2.8.2. Research Priorities

Recent efforts have focused on finding optimum therapeutic regimens and on development of combination therapy with drugs already registered or those used to treat related diseases. For example, the oral drug nifurtimox, registered for treatment of Chagas disease, was considered for compassionate treatment, in combination with other trypanocidal drugs, of patients who did not respond to melarsoprol (Priotto *et al*., 2006). Gastrointestinal disturbances with nausea, abdominal pains, and vomiting are very frequent, and neurological adverse reactions with general convulsions, tremor, or agitation can occur (Jordan, 1993). The frequency of adverse reactions increases with treatment duration and all are rapidly reversible after discontinuation of the drug (Pepin *et al*., 1992). Various combinations of eflornithine, melarsoprol, and nifurtimox have been tested and in all trials the efficacy was better than with monotherapy. However, combinations containing melarsoprol resulted in very high rates of severe adverse drug reactions (Priotto *et al*., 2006; Bisser *et al*., 2007). As a result of those investigations, a multi country trial of nifurtimox-eflornithine combination therapy was undertaken to compare the standard

eflornithine therapy with an abridged regimen consisting of 200 mg/kg of eflornithine given as a short intravenous infusion every 12 h for 7 days, combined with nifurtimox 5 mg/kg given orally every 8 h for 10 days. This regimen reduces the number of infusions from 56 to 14 and the treatment duration from 14 to 10 days (Chapppuis, 2007; Priotto *et al*., 2009). Some data reported so far suggest the combination is rather well tolerated and has a good intermediary effectiveness (Priotto *et al*., 2009). The development of new medicines against human African trypanosomiasis underwent a serious setback when the new oral first-stage diamidine drug, pafuramidine maleate (DB289), failed almost at the end of the development programme because of nephrotoxicity (Pohlig *et al.,* 2008; 2005). However, a back-up programme identified new diamidines that could cure a CNS mouse model of infection (J.E. Hall and R. Brun, unpublished results). Currently, no molecules are at the stage of clinical development for treatment of African trypanosomiasis. However, one molecule, the nitroimidazole fexinidazole, has been advanced from discovery to the preclinical stage, and profiling is in progress. If this process is successful, the first phase 1 studies in man should begin in 2009 (dndi.org)

2.9. ADVANCES IN VECTOR CONTROL

Current vector control interventions involve the use of insecticides (through the sequential aerosol spraying technique, insecticide-treated targets (Allsopp, 2001; Allsopp and Hursey, 2004) or insecticide-treated animals (Vale *et al.,* 1999), the use of traps (Leak, 1999) and the sterile insect technique (SIT) (Molyneux, 2001). The sequential aerosol technique, which uses extremely low concentration of insecticide through several consecutive aerial sprayings, can effectively clear large areas of tsetse flies in a relatively short time, but it is expensive and requires substantial economic and infrastructure support. Pour-ons or selective spraying

application of insecticides to animals on which tsetse feed are another effective means of vector control. (Molyneux, 2001).

Odour-baited targets or traps have been used in many countries to effectively suppress tsetse population. The relative low cost and simplicity of the traps or targets recommends them for use by local communities, but they are applied on a scale so small that control efforts are bound to be frustrated by re-invasion. While effective baits have been developed for savannah tsetse, to date no such baits exist for riverine tsetse, which are major vectors of HAT. However, research continues in an attempt to develop effective baits for the latter species (Molyneux, 2001).

The SIT, which involves the release of laboratory-reared and sterilised males to compete with wild males so that females inseminated by them produce no offspring, has been effectively used for eradication of tsetse (*G. austeni*), for example, in Unguja Island in Zanzibar (Vreysen *et al*., 2000). The cost of SIT is, however, exorbitant. The feasibility of this costly approach in areas where multiple species are present remains doubtful (Enserink, 2007).

Two bacteria have been implicated in modifying vector competence of their host, and a third symbiont can confer mating sterility. However, further research is needed to turn such new knowledge into practical use for disease control. Despite the considerable progress made in controlling the vector, an ideal methodology easily accessible to the population at risk still does not exist (Enserink, 2007).

2.10 DIAGNOSIS OF HAT

Diagnosis of HAT depends on identification of either *T. b.gambiense* or *T. b. rhodesiense* (Buscher and Lejon, 2004). Across West Africa *T. b.gambiense* is anthroponotic, giving rise to a chronic disease, normally fatal within three years of infection (Checci *et al*., 2008). In East Africa, zoonotic *T. b. rhodesiense* circulates between people, wildlife and domestic livestock

(Welburn *et al.,* 2001) and is normally fatal within six months of infection (Odiit *et al.,* 1997). Clinical evidence of HAT infection still depends on visualization of parasites in blood, lymph or CSF. Clinical signs and serological or molecular methods can provide indirect evidence of infection but, because treatments are toxic, the presence of parasites must be confirmed before treatment. HAT symptoms are nonspecific, variable and inconsistent, and alone are insufficient for diagnosis (Chappuis *et al*., 2005). Moreover, HAT symptoms can be confused with those of malaria, enteric fever, tubercular meningitis and HIV (Chappuis *et al*., 2005). Treatment is determined by assessing the stage of disease progression.

2.10.1. Parasitological Detection

Evidence for infection is provided by microscopy of lymph, blood and/or CSF. *T. b. rhodesiense* usually achieves a higher density in blood than *T. b.gambiense* where parasite densities range from 10 000 ml-1 to less than 100 ml-1 (below the threshold of microscopy) (Brun *et al*., 2010). Examination of blood from a finger prick is the simplest but least sensitive method (detection limit 10 000 trypanosomes ml-1); thick blood films (in which the corner of a clean slide is used to spread a drop of blood in a circle of diameter 1–2 cm) can improve sensitivity to 5000 trypanosomes ml-1 (Buscher and Lejon, 2004). Concentrating the parasite can also improve sensitivity: haematocrit centrifugation (HCT) was reported in 1902 (De Raadt, 2005) and a triple centrifugation technique in 1938 (Yorke, 1938); HCT methods were revived in 1970 (Woo, 1970) and improved using dark-ground/phase contrast microscopy (Murray *et al*., 1977). Quantitative buffy coat uses tubes developed for malaria diagnosis and combines HCT centrifugation with fluorescent detection (acridinde orange) of trypanosomes (Bailey and Smith, 1992). The mini anion exchange centrifugation technique (mAECT) can separate trypanosomes

from red cells before centrifugation (Lumsden *et al.,* 1979), enabling detection of less than 100 trypanosomes ml-1. The most sensitive techniques for parasite detection in lymph and blood are direct examination, and mAECT, when used in parallel, can further improve sensitivity (Miezan *et al*., 1994); an improved mAECT has been developed (Buscher *et al,* 2009). An in vitro isolation technique requires examination over several weeks (Aerts *et al*., 1992). It is not possible to discriminate *T. b. gambiense* and *T. b. rhodesiense* by microscopy.

2.10.2. Serological Diagnosis

Several techniques have been developed to detect humoral responses in blood, serum and CSF, the most practical being the card agglutination test for trypanosomiasis (CATT) for *T. b. gambiense* based on the variable surface antigen type LiTat 1.3 (Magnus *et al.,* 1978). CATT testing uses heparinised whole blood, followed by further testing of blood, plasma and serum dilutions for individuals that initially tested positive. CATT has limitations: false positives and false negatives, it cannot be used to indicate cure because antibodies can persist for up to three years following treatment (Lejon *et al*., 2010; Paquet *et al.,* 1992). CATT tests are not packaged for individual tests, antigen production is complex (Chappuis *et al*., 2005) and CATT screening requires trained personnel and follow-up can be onerous, especially if CATT-positive aparasitaemic individuals are identified. CATT false negatives have been observed in parasitologically positive cases in Cameroon and Uganda, and were attributed to the absence of LiTat 1.3 (Dukes *et al*., 1992; Kanmogne *et al*., 1996) although CATT false negatives have also been observed in strains positive by PCR for the gene encoding LiTat 1.3 (Enyaru *et al*., 1998). False negatives can result from complement-mediated inhibition (Pansaerts *et al.,* 1998) that has been addressed by a new version of CATT (Magnus *et al.,* 2002). CATT false positives can

occur in patients with malaria that might have had transient infection with non-human infective trypanosomes such as *Trypanosoma brucei brucei* (Magnus *et al*., 1978). Sera from Cameroonian CATT positives failed to lyse Li Tat 1.3 VAT trypanosomes, suggesting cross reactivity (Dukes *et al*., 1984). Aparasitaemic serological suspects are observed, and this could reflect lack of CATT specificity or failure of parasitological tests in cases of weak or fluctuating parasitaemia (Dukes *et al.,* 1984; Truc *et al*., 1994); alternatively a patient could be controlling the parasitaemia (Garcia *et al.,* 2000). Attempts to validate CATT against molecular PCR tests have generated more questions than answers (Koffi *et al.,* 2006; Wastling *et al.,* 2011)). CATT has been modified for blood samples stored on filter paper (e.g. the Testryp micro method) (Miezan *et al.,* 1991) and CATTFP (Chappuis *et al*., 2002), and the latest format, CATT-D10, uses thermostable lyophilised medium produced in 10 unit vials suitable for use in remote healthcare facilities with insufficient cases to justify opening 50 unit vials (Hasker *et al*., 2010). The LATEX/ *T. b. gambiense* test developed as an alternative to CATT (Buscher *et al*., 1999) uses a combination of three surface antigens (Li Tat 1.3, 1.5 and 1.6) and is equally sensitive but more specific than CATT (Chappuis *et al.,* 2005). Immunofluorescence assays, ELISA and immune trypanolysis methods for anti-trypanosome antibody detection can be useful where there are good laboratory facilities (Chappuis *et al.,* 2005). There is no equivalent to CATT for serological screening of *T. b. rhodesiense* (Brun *et al.,* 2010)

2.10.3. Staging

On parasite detection in blood or lymph, progression of HAT is assessed by examination of CSF. A patient is in stage 1 if the CSF white cell count is _5 ml-1 and trypanosomes are not observed (Weisser and Hall, 2009). Stage 2 is characterized by the presence of trypanosomes and/or a white blood cell count of _20 ml-1. There is an intermediate stage when trypanosomes are not

observed, and the cell count falls between these values; patients in this category might or might not require stage 2 treatments. Staging is complicated and national programmes can use different cell count thresholds and might not consider an intermediate category. Since the early 1900s, concentration techniques have improved the sensitivity of CSF examination (WHO, 2007); double centrifugation (DC) was introduced in 1988 (Laveran and Mesnil, 1912) but is no longer practiced (Cattand *et al*., 1988). In 2000, a modified single centrifugation technique (MSC) was introduced (Miezan *et al*., 2000): CSF is centrifuged and examined by light microscopy in a mAECT viewing chamber. The MSC method has been further improved by the use of mAECT tubes that can hold 4 ml of CSF (Buscher *et al*, 2009). MSC is as sensitive as DC and easier to perform.

2.10.4. Molecular Diagnostics

PCR is presented as a sensitive and specific method for the detection and identification of human-infective trypanosomes. For all *T. brucei s.l*., a PCR assay targeting a 177 bp satellite repeat has been available for twenty years (Moser *et al.,* 1989). Following the discovery of the SRA gene for human serum resistance in *T. b. rhodesiense* (Xong *et al*., 1998), PCR assays were developed (Welburn *et al*., 2001) with the DNA equivalent of a single trypanosome sufficient for amplification. A multiplex reaction includes an internal control to confirm sufficient DNA in a reaction (Picozzi *et al.,* 2008). SRA is a 378 bp deletion within a variable surface glycoprotein (VSG) gene; specific primers must therefore span this deletion site (Degreef and Hamers, 1994; Campillo and Harrington, 2003). PCR identification of *T. b. gambiense* uses primers amplifying the single copy glycoprotein (TgsGP) gene that does not confer human serum resistance. Single-round and nested PCRs have been developed with a detection limit of 10 trypanosomes per ml-1

(Rdwanska *et al*., 2002). PCR can be applied to blood samples collected and stored preferably on Whatman FTA1 cards that permitaccurate species-specific trypanosome identification for large-scale surveys (Cox *et al.,* 2010). This approach has been used to quantify domestic livestock carrying *T. b. rhodesiense* to monitor and evaluate the impact of the public–private partnership engaged in the campaign to eliminate Rhodesian sleeping sickness from Uganda.

2.10.5. Isothermal Amplification-based Techniques

PCR has limitations in resource-poor settings, including the need for a thermo cycler and a reliable electricity supply. Isothermal DNA amplification offers some solutions: the first isothermal, the one-step transcription-based nucleic acid amplification system (Guatelli *et al.,* 1990), evolved into transcription mediated amplification (TMA) and nucleic acid sequence based amplification (NASBA) (Chan and Fox, 1999; Compton, 1991; Walker *et al.,* 1992). Small single- stranded circular DNAs were shown to behave as catalytic templates for DNA synthesis under isothermal conditions for sequence-specific amplification (Fire and Xu, 1995). Loop-mediated isothermal amplification (LAMP) uses a strand-displacement polymerase and requires 1 h incubation (at 58–65 8C) followed by a short termination step at 80 8C, after which several methods for end-point detection can be applied (Wastling *et al*., 2010). Subsequent developments were nicking enzyme amplification technology (NEAR) (Lizardi *et al.,* 1998) and helicase dependent amplification (HDA) (Zhang *et al*., 2001). Finally, recombinase polymerase amplification (RPA) was developed (Van Ness *et al*., 2003).

2.10.5.1. *LAMP for HAT*

LAMP was first applied to HAT as a pan-Trypanozoon assay targeting paraflagellar rod protein A (PfrA) (Kuboki *et al*., 2003). The assay detects all *T. b. brucei sl*. and *Trypanosoma evansi* and

can use FTA cards spotted with infected blood. PfrA was assumed to code for major components of the paraflagellar rod, PFR1 and PFR2 (Schlaeppi *et al*., 1989; Berriman *et al.,* 2005). A *T. b. gambiense* specific LAMP assay targets the 5.8S rRNA-ITS2. Two further LAMP assays were developed for all trypanozoon species targeting the ribosomal mobile element (RIME) (Njiru *et al*., 2008) and for *T. b. rhodesiense*, targeting the serum resistance-associated gene (SRA) (Njiru *et al.,* 2008). RIME, present at 400 copies per genome, was used for strain typing *T. brucei s.l*. (Tilley *et al.,* 2003; Simo *et al.,* 2005). LAMP RIME primers used RIME sequences from *T. b. brucei, T. b. rhodesiense* and *T.evansi* (Matovu *et al.,* 2010), and LAMP RIME and SRA have been used to identify trypanosomes in patient blood spotted on Whatman FTA cards (Mugasa *et al*., 2008).

2.10.5.2. *NASBA for HAT*

NASBA (Mugasa *et al.,* 2009) targets 18S rRNA for *T. brucei s.l*., but does not discriminate between subspecies. Assays have a detection limit of 10 parasites ml-1, comparable to that of LAMP SRA assays, and when tested on blood from confirmed HAT cases NASBA detected *T. brucei s.l.* in significantly more patient samples than microscopy alone. NABSA has been coupled to an oligochromatographic dipstick detection format (NASBA-OC) for read-out within 5–10 min (Matovu *et al.,* 2010). NASBA-OC was compared to a composite reference standard (microscopy, microhaematocrit centrifugation with microscopy, or mAECT with microscopy) on blood and CSF samples from Uganda (*T. b. rhodesiense*) and the Democratic Republic of the Congo (*T. b. gambiense*), and was found to be more sensitive than microscopy for blood samples but not for CSF. NASBA-OC was more sensitive and specific than PCR-OC for the same gene but NASBAOC was not compared to *T. b. rhodesiense* PCR (Matovu *et al*., 2010).

2.10.6. FIND and Sleeping Sickness Diagnostics

HAT diagnostic tests have not usually been manufactured with full registration approved by a regulatory agency. The Foundation for Innovative New Diagnostics (FIND) was launched in 2003 as a non-profit foundation supporting 'the development of diagnostic tests for diseases of poverty, including TB, HAT and malaria'. FIND adopted a 'piggyback' approach – developing technology platforms applicable to multiple diseases (Ndung'u *et al*., 2010; Boehme *et al*., 2007). FIND working with partners in academia developed the LAMP RIME and LAMP SRA assays for Trypanozoon and *T. b. rhodesiense*. LAMP (Notomi *et al.,* 2000) is being developed by FIND as an isothermal DNA amplification technique for sleeping sickness.

2.10.7. Non-Molecular Diagnostics for Sleeping Sickness

Improved microscopy and serology-based diagnostics and staging tools are needed for HAT. FIND and partners have improved test components for mAECT and developed a simple light-emitting diode (LED)-based fluorescence microscope using acridine orange (Buscher *et al,* 2009). However, nanobody based parasite antigen detection systems also show promise; nanobodies are small (15 kDa) antibody fragments, derived from camelid heavy chain antibodies, which bind antigen via one single-chain variable domain. Parasite specific nanobodies are generated by immunization of camelids with antigen and are purified using bacterial, yeast or plant expression systems. They bind with high affinity, are small and stable with a long shelf-life, and can be humanized by altering specific amino acid sequences to reduce the risk of inducing anti-nanobody antibodies (Muyldermans *et al*., 2009). A nanobody targeting the conserved asparagine-linked carbohydrate of trypanosome VSGs detects parasites by

fluorescence microscopy (Stijlemans *et al.,* 2004; Saerens *et al.,* 2008). Two antigen detection formats are in development: VSG specific dipstick format assay (detection limit 10 parasites ml-1), and nanobody-coated magnetic bead capture for antigen 'fishing' plus detection with a second complementary nanobody (Magez and Radwanska, 2009). Single-chain variable fragment (scFv) antibodies also offer potential, can be produced economically, and have been engineered into larger, multivalent, bi-specific and conjugated forms (Magez and Radwanska, 2009). Because lumbar puncture is painful and risky, there is an urgent need for the identification of blood-based biomarkers to distinguish between early and late stage disease. A panel of three brain damage-marker proteins could be used to discriminate stage 2 (Hainard *et al.,* 2009). The WHO (WHO, 2009) has emphasized the need to develop simple diagnostic tools for the neglected tropical diseases and for sleeping sickness. The holy grail remains a dipstick test comparable to the paper strips currently used to diagnose diabetes – that can be simply dipped into blood and provide bedside diagnosis (Deborggraeve *et al.,* 2006).

2.10.8. No Gold Standard

New diagnostics are generally evaluated by comparison to a validated reference test, but there is no gold standard for HAT. To overcome this, latent class analysis methods have been developed to estimate the sensitivity and specificity of two or more index tests (Rutjes *et al.,* 2007; Espeland and Handelman, 1989). Frequentist (Hui and Zhou, 1998; Enoe *et al.,* 2000) and Bayesian (Branscum *et al.,* 2005) approaches have been developed for latent class analysis in which individuals that are not detected by any of the available tests are assumed to be uninfected. It is important to use a range of tests covering the spectrum of biological responses to infection, otherwise a group of infections could be missed, the sensitivity of other tests will be

overestimated, and true prevalence underestimated (Toft *et al.,* 2005). A Bayesian formulation of the Hui–Walter latent class model has been used to compare two PCR-based molecular detection assays for *T. brucei s.l.* (Bronsvoort *et al.,* 2010).

2.11. TREATMENT

2.11.1 Pentamidine

Few drugs are available to treat human African trypanosomiasis and selection is based mainly on the disease stage and causative pathogen. At present, all drugs are donated to W.H.O. by the producers (ifpma.org; Bayer.co). Pentamidine is the drug of choice for treatment of first stage disease caused by *T. b. gambiense*. It is given intramuscularly for a week, unless it can be given as an intravenous infusion in saline over 2 h. There is pharmacokinetic evidence that three injections might be equally effective (Bronner, 1994; Bronner *et al*., 1991) and a comparative clinical trial is in progress to test this strategy (trial registration ISRCTN55042030). By contrast, the use of pentamidine in intermediate-stage disease (that is, up to ten or 20 white blood cells per μL in CSF) (Doua *et al*., 1996) has produced equivocal outcomes 9,799,100 and should not be generally recommended. Attention has been drawn to an unintended modification of the dose calculation from the base to the salt (Dorlo and Kager, 2008) but this change should lead to a clarification of the label rather than a change in practice, because pentamidine at the currently used dose is still very effective. Pentamidine is generally well tolerated. When given by intramuscular injection, site pain and transient swelling, abdominal pain and gastrointestinal problems, and hypoglycaemia (5–40%) are the most frequently reported thrombocytopenia, hyperkalaemia, and QT-prolongation, which are seen in treatment of other diseases (eg, *Pneumocystis jirovecii* infection), (Anon, 2008) are rarely reported, probably because of the scarcity of adequate methods for patient monitoring.

2.11.2. Suramin

Suramin is used for first-stage *T. b. rhodesiense* disease, but is generally avoided against *T. b. gambiense* disease in western and central Africa because where *Onchocerca* spp. are also present, its high activity against these parasites can expose patients to the risk of severe allergic reactions. The recommended dose regimens for suramin are complex and last up to 30 days (WHO, 1986). The compound deteriorates rapidly in air and should be injected immediately after dilution in distilled water (Gustaffson, 1987). Adverse drug reactions are frequent but usually mild and reversible, including nephrotoxicity, peripheral neuropathy, and bone marrow toxicity with agranulocytosis and thrombocytopenia. Rare acute and late hypersensitivity reactions can occur (Jordan, 1993) of which the acute reaction is the reason for the low test dose generally applied before treatment initiation.

2.11.3. Melarsoprol

The organoarsenic compound melarsoprol remains the most widely used drug for treatment of second-stage disease caused by *T. b. gambiense* in resource-poor countries where the new drug eflornithine is not available or affordable, and it is the only choice for second-stage *T. b. rhodesiense*. For *T. b. gambiense* an abridged treatment schedule of ten injections on consecutive days was recommended by the International Scientific Council for Trypanosomiasis Research and Control (Schmid *et al*., 2005). For treatment of *T. b. rhodesiense*, various lengthy and complex treatment schedules are still used; work on the abridged schedule is continuing. Adverse reactions to melarsoprol are frequent and can be severe or even life-threatening. The most important reaction is an encephalopathic syndrome, which occurs with very variable frequency in an average 4.7% of *T. b. gambiense* and 8.0% of *T. b. rhodesiense* patients, with a case fatality rate of 44% and 57%, respectively (Seixas, 2004) For the management of encephalopathic

syndrome, dexamethasone and diazepam are recommended (Medecins Sans Frontieres, 2007) Careful monitoring of the patient during treatment is crucial and although not indicative, the appearance of fever or fever combined with headaches can be regarded as warning signs (Blum *et al*., 2001). Skin reactions such as pruritus and maculopapular eruptions are fairly common, but severe complications such as bullous eruptions occur in less than 1% of cases (WHO, 1998; Schmid *et al*., 2005) Peripheral motor (palsy) or sensorial (paraesthesia) neuropathies have been reported. A good injection technique is mandatory to mitigate the irritating and painful effects of the injections, often leading to thrombophlebitis. In several foci, treatment failures have reached 30% of those treated. These failures suggest the emergence of resistance to melarsoprol, in which a P2 adenosine transporter might be implicated (Barret *et al*., 2007). So far, however, difficulties in retrieval of *T. b. gambiense* isolates from patients have hampered demonstration of parasite resistance.

2.11.4. Eflornithine

Eflornithine is the only new molecule for the treatment of human African trypanosomiasis that has been registered in the past 50 years. Several studies comparing melarsoprol with eflornithine have shown a clearly reduced mortality with eflornithine, which is therefore recommended as the first-line treatment for second-stage *T. b. gambiense* disease, (Chappuis *et al*., 2005; Balasegaram *et al*., 2006; Checci *et al*., 2007) but the use of eflornithine against *T. b. rhodesiense* is not advised, because this organism is innately less susceptible to the drug than is *T. b. gambiense* (Iten *et al*., 1997). Treatment of *T. b. gambiense* infection with eflornithine lasts for 2 weeks, and because of the short half-life of the drug, four short infusions per day are necessary. This regimen hampers the replacement of melarsoprol by eflornithine in rural public

treatment facilities. The provision of kits by W.H.O., including all the necessary ancillary materials, could mitigate some of the difficulties (Priotto *et al*., 2008). Attempts were made to reduce the difficulty of drug administration by use of an oral formulation, but were abandoned after a pharmacokinetic trial gave discouraging results (Na-Bangchang *et al*., 2004; Jansson *et al*., 2008). Adverse drug reactions are similar to those of other cytostatic drugs, and include bone marrow toxicity leading to anaemia, leucopenia, and thrombocytopenia (25–50%), gastrointestinal symptoms (10–39%), and convulsions (7%) (Buri and Brun, 2003). Superimposed bacterial infection at the catheter site can lead to life-threatening sepsis, but this event can be prevented by adequate nursing care (Chappuis *et al.,* 2004).

`2.11.5. Nifurtimox

Nifurtimox (Lampite; manufactured by Bayer) is a 5-nitrofuran administered orally and is currently registered in some Latin American countries for the treatment of Chagas disease. Side-effects are extremely common and 50% of patients with Chagas disease are unable to complete a full course of treatment. Nevertheless, nifurtimox has been used on compassionate grounds to treat late-stage HAT where eflornithine or melarsoprol are ineffective. Small-scale clinical trials have shown contradictory results and an optimal treatment schedule needs to be developed.

The mode of action of nifurtimox is not fully elucidated, but probably involves cyclical reduction and oxidation of the nitro group producing superoxide (O22), hydrogen peroxide and free radicals (Fries and Fairlamb, 2003; Docampo, 1990) The oxidative stress induced by this futile redox cycling is thought to damage cellular components such as DNA, membrane lipids and proteins. Because trypanothione metabolism is central to these antioxidant functions, there is a biochemical rationale for combination chemotherapy with either melarsoprol or eflornithine (Jennings, 1993).

CHAPTER 3

MATERIALS AND METHODS

3.1. STUDY AREA:

This study was carried out in Ankpa General Hospital in Ankpa L.G.A. of Kogi State. The Ankpa General Hospital is the reference hospital for HIV diagnosis, counseling and treatment in the local government. Ankpa Local Government's headquarter is Ankpa on the A233 highway in the west of the area between latitude 7°22′14″ and 7.37056°N and longitude 7°37′31″ and 7.62528°E. It has an area of 1,200 km² and a population of 267,353 (NPC, 2006). The Local Government is home to the Kogi State College of Education and is about 40km from Kogi State University, Ayingba. Igala is the major dialect spoken, the remaining ethnic groups include the Yoruba, Igbo, Hausa, Fulani,Edo, Calabar, Idoma, Tiv and others (NPC, 2006). Predominant occupation of the people include; farming, lumbering, civil service, hunting and fruit gathering. To The North, the study area shares boundary with Abejukolo, to the East, it shares boundary with Benue state, to the South, it shares boundary with Okpo and to the West it shares boundary with Dekina and Ugwalawo. vegetation is mainly forest-savannah.

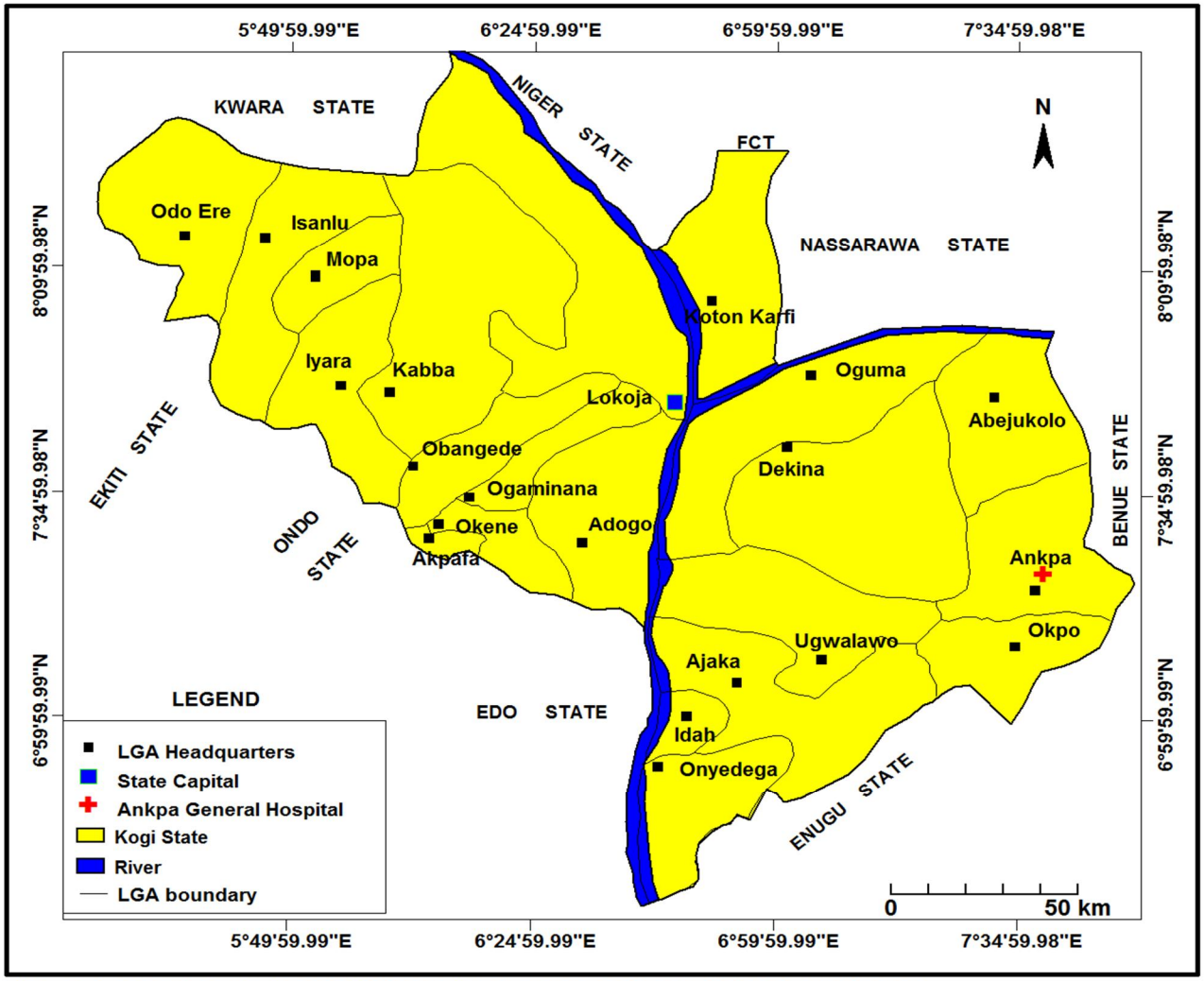

FIG. 3.1: KOGI STATE SHOWING THE LOCATION OF ANKPA GENERAL

Source : Modified from Kogi State Administrative Map

3.2. SAMPLE SIZE DETERMINATION

A pilot study was carried out to calculate the expected prevalence rates. One hundred samples were collected and 9.0% prevalence was obtained. Sample size was then determined based on the expected prevalence, using the formula of Thrusfield (1997).

$$n = \frac{z^2pq}{d^2}$$

$$n=\frac{1.96^2Pexp(1-Pexp)}{d^2}$$

Where

$q = 1-p$

z = appropriate value for the standard normal deviate for desired confidence = 1.96

n= Sample size

$P\ exp$ = Expected prevalence (9.0% pilot study)

d = Desired absolute precision of 5% (0.05)

$$n \text{ for humans} = \frac{1.96^2 \times 0.09\ (1-0.09)}{(0.05)^2}$$

= 125.85

A total of 465 human blood samples were collected

3.3. SAMPLING PROCEDURE

Ethical clearance was obtained from the State Ministry of Health. Blood samples were collected from HIV patients in Ankpa General Hospital in the local government. The Ankpa General Hospital is the reference hospital for HIV diagnosis, counseling and treatment in the local government. Blood samples were collected with the help of the laboratory staff from the subjects after their informed consent. Subjects to be sampled were selected using convenient sampling and the sex, age and occupation of the subjects were recorded.

3.4. SAMPLE COLLECTION

Blood samples were collected between August and December. Blood samples were collected on Tuesdays and Thursdays as these days represent clinic days of the HIV patients. Two mls of whole blood was aseptically collected via the median cubicle vein using 5ml syringe and 21G needle and was immediately transferred into clean labeled sample bottles containing ethylene diamine tetra-acetic acid (EDTA) and gently shaken until the blood was properly mixed with the anti coagulant. Samples were then divided in two parts. One part was immediately examined parasitologically and the other part, serologically.

3.5. SEROLOGICAL DIAGNOSIS

A Card agglutination Test for *Trypanosoma brucei gambiense* (CATT) test kit was used for the diagnosis and was obtained from the Institute of Tropical Medicine, Antwerp, Belgium.

3.5.1. CATT/*T.b. gambiense*

The test was performed according to the manufacturer's instruction and based on the following principles and methodology. *Trypanosoma brucei gambiense* infection produces circulating antibodies against several surface antigens of the parasite. Such antibodies can be demonstrated in the blood, plasma or serum of the infected host by direct agglutination. The CATT-antigen is a freeze dried suspension of purified, fixed and stained bloodstream form trypanosomes expressing a predominant variable antigen type of *T. b. gambiense.* The test was performed on a plastified card. One drop of undiluted blood was mixed with one drop of reconstituted antigen. When antibodies were present in the test sample, trypanosomes agglutinated within 5 minutes rotation at 60 rotations per minute. In the present study, the test card was rotated manually.

3.6. PARASITOLOGICAL DIAGNOSIS

Detection of trypanosomes was based on microscopic examination of blood sample using wet mount preparation as described in the Protozoology Laboratory manual of The Department of Veterinary Parasitology and Entomology, Ahmadu Bello University Zaria and Haematocrit Centrifugation Technique (HCT) as described by Woo (1971). Blood samples were immediately examined to prevent lysis of the parasites.

3.6.1. Wet mount

A flat glass slide was used to prepare the wet mount. The slide was cleaned and freed of dust or other fine particles. A few drops of the blood were sucked into a medicine dropper. The flat glass slide was picked up with one hand, grasping it by the outer edges. One drop of the blood was

then placed in the medicine dropper on top of the slide, making sure the drop goes on the center portion of the slide. The cover slip was carefully picked using the free hand and was grasped by the outer edges. The cover slip was then placed on top of the slide, making sure the edges of the cover slip match up with the edges of the slide. It was kept as horizontal and steady as possible, and was placed on the viewing tray of the microscope and viewed under an x40 objective.

3.6.2. Haematocrit centrifugation technique

Two-thirds to three-quarters of the venous blood was filled in the capillary tube and was well mixed. One end of the tube was then sealed with clay. The filled tube was placed in the microhematocrit centrifuge, with the plugged end away from the center of the centrifuge. It was then centrifuged at a preset speed of 10,000 to 12,000 rpm for 5 minutes. It was centrifuged for an additional 3 minutes if the hematocrit exceeds 50%. The tube was then placed in the microhematocrit reader and was read following the manufacturer's instructions on the microhematocrit reading device.

3.7. QUESTIONNAIRE SURVEY

Four hundred and sixty five close ended structured questionnaires were administered and information on the knowledge and awareness of the HIV patients to HAT were obtained. Questionnaires were administered after blood samples were collected.

3.8. DATA AND STATISTICAL ANALYSIS

Data collected at the end of the study was analyzed using the statistical package for social sciences 17.0 (SPSS Inc. Chicago, Illinois, United State of America).

The prevalence of HAT was calculated by dividing the number of infected individuals by the total number of individuals examined and expressed as a percentage. Chi square test was used to test for significant differences in the age, occupation and sex-specific prevalence rates of HAT and value of $p<0.05$ were considered significant.

CHAPTER 4

RESULTS

4.1 OVERALL SERO PREVALENCE OF HAT IN ANKPA GENERAL HOSPITAL

Sero-prevalence of HAT in Ankpa Local government among HIV patients was 3.01%. There was no parasite detected using the wet mount and HCT.

4.2 SEX – BASED SERO PREVALENCE OF HAT IN ANKPA GENERAL HOSPITAL

Prevalences among females and males were 3.60% and 1.52% respectively. There was no significant association ($p>0.05$) between the infection and sex (Table 1).

4.3 AGE – BASED SERO PREVALENCE OF HAT IN ANKPA GENERAL HOSPITAL

A prevalence of 0%, 3.63% and 2.04% was observed among the age groups <18 years, 18-45 years and above 45 years respectively which was also not significant ($p>0.05$) (Table 2).

4.4 OCCUPATIONAL – BASED SERO PREVALENCE OF HAT IN ANKPA GENERAL HOSPITAL

There was no significant association ($p>0.05$) between the infection and occupation. Farmers had the highest sero prevalence of 5.00%, while tailors and civil servants had a prevalence of 4.17% and 3.57% respectively (Table 3).

Table 1: Sex-based prevalence of *T.b.gambiense* Li Tat 1.3 surface antibodies as evaluated by CATT

Sex	Number examined	Number and (%) positive
Male	132	2(1.52)
Female	333	12(3.60)
Total	465	14(3.01)

p value = 0.3677

Table 2: Age-based prevalence of *T.b.gambiense* Li Tat 1.3 surface antibodies as evaluated by CATT

Age	Number examined	Number and (%) positive
<18	37	0(0)
18-45	330	12(3.63)
>45	98	2(2.04)
Total	465	14(3.01)

p value = 0.3855

Table 3: Occupational-based prevalence of *T.b.gambiense* li tat 1.3 surface antibodies as evaluated by CATT

Occupation	Number examined	Number and (%) positive
Farming	200	10(5.00)
Tailoring	48	2(4.17)
Civil Service	56	2(3.57)
Commercial driving	13	0(0)
Teaching	12	0(0)
Business	36	0(0)
Artisan	100	0(0)

p value = 0.6703

4.5 DEMOGRAPHIC FEATURES OF THE RESPONDENTS

Demographic features of age, sex, occupation and education for 465 respondents are given in Table 4. There was significant association ($p<0.05$) between age and level of awareness to Human African Trypanosomosis (HAT). Respondents within the age range of 18-45 years were more aware of HAT followed by those above 45 years. Respondents less than 18 years of age were the least aware. There was no significant association ($p>0.05$) between awareness and sex, although females were more aware than males. There was however significant association ($p<0.05$) between awareness and occupation. There was also a significant association ($p<0.05$) between awareness to HAT and education.

Table 4: Demographic feature and awareness to human African trypanosomosis

Demographic Features	Total number and (%) sampled	Number and (%) aware	*p value*
SEX			0.1623
Male	132 (28.39)	99 (21.29)	
Female	333 (71.61)	270 (58.06)	
AGE			0.0001
<18	37 (7.96)	10 (2.15)	
18-45	330 (70.97)	290 (62.37)	
>45	98 (21.08)	69 (14.84)	
OCCUPATION			0.0001
Farming	200 (42.01)	180 (38.71)	
Tailoring	48 (10.32)	30 (6.45)	
Civil service	56 (12.04)	40 (8.60)	
Commercial driving	13(2.80)	10 (2.15)	
Teaching	12 (2.58)	10 (2.15)	
Business	36 (7.74)	20 (4.30)	
Artisan	100 (21.51)	79 (16.99)	
EDUCATION			0.0001
Primary	200 (43.01)	150 (32.26)	
Secondary	128 (27.53)	100 (21.51)	
Tertiary	109 (23.44)	105 (22.58)	
None	28 (6.02)	14 (3.01)	

4.6 AWARENESS TO HUMAN AFRICAN TRYPANOSOMOSIS

Out of the 465 respondents, 369/465 (79.35%) heard about HAT and 96/465 (20.64%) never did (Table 5). Table 5 also shows the various media of awareness to HAT which included school 119/369 (32.25%) and stories/myth 250/369 (67.75%). Of the 465 respondents, 289/465 (62.15%) live in the village while 176/465 (37.85%) live in the town (Table 5). The source of water in the study area also varied based on the questionnaire analysis, 100/465 (21.51%) had boreholes while 289/465 (62.15%) and 76/465 (16.34%) relied on streams and water tanks respectively. Exactly 390/465 (83.87%) of the respondents have heard of tsetse fly. None however, according to the respondents had been bitten by tsetse fly and none had also been screened for HAT (Table 5).

Table 5: Awareness to Human African Trypanosomosis

QUESTION	RESPONSE	NUMBER (%)
Have you ever heard of Human African Trypanosomosis?	Yes	369 (79.35)
	No	96 (20.64)
What is the medium through which you heard about Human African Trypanosomosis?	School	119 (32.25)
	Television	0 (0)
	Radio	0 (0)
	Print media	0 (0)
	Medical personnel	0 (0)
	Myth/Stories	250 (67.75)
Where do you live?	Town	176 (37.85)
	Village	289 (62.15)
What is your source of water	Well/ Borehole	100 (21.51)
	River/ Stream	289 (62.15)
	Water tank	76 (16.34)
Have you ever heard of tsetse fly?	Yes	75 (16.13)
	No	390 (83.87)

Have you ever been bitten by tsetse fly?	Yes	0 (0)
	No	465 (100)
Have you ever been screened for Human African Trypanosomosis?	Yes	0 (0)
	No	465 (100)

4.7 KNOWLEDGE OF RESPONDENTS TO HUMAN AFRICAN TRYPANOSOMOSIS

There were variations as regards the respondents understanding of the cause, transmission, prevention and cure of human African trypanosomosis in the study area. While 50/369 (13.55%) of the respondents thought it was caused by tsetse bite, 150/369 (40.65%), 60/369 (16.26%), 35/369 (9.49%) thought it was caused by farm work, poor nourishment, hereditary respectively. (Table 6). On the transmission, 200/369 (54.20%) thought it is transmitted from person to person, 100/369 (27.10%) refuted it and 69/369 (18.70%) claimed to have no idea (Table 6). A high proportion of the respondents 81.30% thought it could be prevented or cured therapeutically in the ratio of 81.30% and 82.66% respectively (Table 6).

Table 6: Knowledge of respondents to human African trypanosomosis

QUESTIONS	RESPONSE	NUMBER (%)
What do you think is the cause of human African trypanosomosis?	Black fly bites	0 (0)
	Mosquitoe bites	0 (0)
	Tsetse fly bites	50 (13.55)
	Farm work	150 (40.65)
	Heredity	35 (9.49)
	Poor nourishment	60 (16.26)
	Witchcraft	0 (0)
	Others	0 (0)
	Cannot say	74 (20.05)
Do you think it can be transmitted from person to person?	Yes	200 (54.20)
	No	100 (27.10)
	No idea	69 (18.70)
Do you think it can be prevented?	Yes	300 (81.30)
	No	19 (3.52)
	No idea	50 (13.55)
How do you think it can be prevented?	Isolating the sick	0 (0)
	Use of drugs	171 (57.00)
	Killing the tsetse fly	119 (39.67)
	Avoid going to the river/stream	0 (0)
	Wearing protective clothes	0 (0)

	No idea	10 (3.33)
Do you think it can be cured?	Yes	305 (82.66)
	No	60 (16.26)
	No idea	4 (1.08)

CHAPTER 5

DISCUSSION

This study has established serological evidence of Human African Trypanosomosis among Human Immunodeficiency Virus outpatients in Ankpa General Hospital. The sharing of boundary between Ankpa Local Government and Benue State might be responsible for the existence of HAT antibody. This is because Benue State has a reported HAT endemic area (Aiyedun and Amodu, 1974) and these patients might interact and travel to the endemic area where they get bitten by infected tsetse flies. Being a general hospital, there is every possibility that patients come from different parts of the country to seek treatment. These patients might have come from an area with infected tsetse fly where they might have been bitten which might contribute to the existence of the antibody.

The presence of several wildlife species (Herder *et al*., 2002; Njiokou *et al*., 2002; Simo *et al*., 2006), and domestic animals (Nkinin *et al*., 2002; Simo *et al*., 2006) in the Local Government area acting as reservoir might also contribute to the existence of the antibody as the tsetse fly might feed on these animals acting as reservoir and become infected, they in turn bite these patients who also becomes infected.

The overall sero prevalence in this study was higher (3.01%) than that of Karshima (2010) in Taraba state. This might be as a result of sample being collected from immunocompromised individuals. Another probable reason might be as a result of cross reaction with other infection like malaria (Diallo *et al.,* 1996). The prevalence in this study was lower than that observed by Airauhi *et al*, (2006) and Osue *et al*, (2008) in Abraka, Delta state with a prevalence of 17.0%

and 9.8% respectively. The reason might be that these authors used a highly sensitive Polymerase Chain Reaction technique resulting to a higher prevalence.

Though not statistically significant (p = 0.3677), the higher prevalence in female (3.60%) as compared to males (1.52%) may be due to the activities of females such as fetching of water, firewood, washing of clothes in streams and picking of fruits, which put them at higher risk of exposure to infection through contact with the infected tsetse vector (Pepin *et al*., 2002).

It has been observed that there are substantial variations between men and women in the type and duration of their daily activities within the local tsetse biotopes where the disease might be transmitted, possibly resulting in profound differences in exposure between men and women and the children who, usually follow their mothers. The adult men are mainly traders, artisans and junior or senior workers in government offices thus they have lower intensity of fly-contact compared with women. Recently, there has been considerable interest towards the influence of sex or gender on tropical diseases, sex referring to a biological characteristic while gender denotes socially constructed behaviour, expectations and roles that derive from, but may not depend on sex (Vlassoff and Manderson, 1998).

Similarity in incidence of African trypanosomosis between genders was reported in many countries. For instance in Fankana-Kalakitini, Kwamouth and Nioki foci of the Congo women had higher incidence of trypanosomosis than men (Henry *et al.,* 1982; Pepin *et al*., 2002). This result is in contrast with that observed by Karshima (2010), where males had higher prevalence than females.

Though age at diagnosis has to be considered as complex variable (Garcia *et al*., 2002), the prevalence of the antibody was much higher in the age group 18-45 years (3.63%) and above 45

years (2.04%). Despite being immunocompromised, this is the active age where activities such as farming, hunting, results to increased contact with the tsetse vector. Reeder *et al*.,(1997) and Baird (1998) stated that although children and adults can be considered as uniformly susceptible to infection early during exposure, protection later increases in age dependent manner related to possible constitutional differences between children's and adults' immune system. High sero prevalence was recorded in adults rather than in children during *T.b. gambiense* sleeping sickness surveillance in the Ivory Coast (Stanghellini and Duvallat, 1981) and also in Southern Sudan (Muhammed *et al*., 2010). The high sero-prevalence of the disease in the age group 18-45 years and above 45 years might be attributed to the community social behaviour and daily activities which take them into closer and more frequent contact with the vector. In general terms, the frequency of human/fly contact decides the disease incidence rate (Gouteux, 1985). No sero prevalence was recorded in the age group 0-17 years as sleeping sickness appears more often in adult than in children (Frezil *et al*., 1981; Moore *et al*., 1999). It is also statistically not significant (p=0.3855).

Although not statistically significant (p=0.6703), the prevalence was highest among farmers (5.00%) than any other occupation. According to Moore *et al*. (1999), daily activities such as farming, fetching of water and hunting described as important risk factors for HAT in forest area are responsible for higher prevalence rates. Parasitological diagnosis was however negative.

The questionnaire analysis showed a high level of awareness to HAT among the respondents. When asked about sleeping sickness (Oga olu as HAT is locally named in Igala), the respondents were quick to acknowledge that they heard about it. Interviewing one of the respondents, a 50 year old female trader, she said "when an adult or child sleeps all the time, we ask the individual

if he or she has sleeping sickness (Oga olu). Most times, this is said in other to make fun of the individual. Our ancestors told us such disease exist".

The medium of awareness were mainly through stories told and through schools. None of the respondents were however aware through medical personnel, radio or television. This might be because HAT in Ankpa Local Government is not a prioritise disease and as such, there is no awareness campaign of the disease. Based on the medium of awareness, the respondents in this survey had no knowledge of the risk factors involved in the transmission of HAT and also were never screened for HAT which is evident in the prevalence obtained in this study.

Majority of the respondents from this survey were females. There was however no statistical association between sex of the respondents and awareness to Human African Trypanosomosis. Furthermore, there was a significant association between the level of education, occupation and age of the respondents and awareness to the infection. This indicates that more educational exposure may increase awareness to HAT. Moreso, awareness to HAT from the survey seems to increase with an increase in age. The occupation of the respondents from the survey showed that farmers were most aware since their activities regularly takes them to tsetse fly habitat were they are likely to get bitten by an infected tsetse fly. This might be responsible for the prevalence of HAT obtained in this study.

The survey also revealed that the respondents' major source of water was from stream. This indicates that the respondents usually have frequent and more contact with the tsetse fly vector as the frequency of human/fly contact decides the disease incidence rate (Gouteux, 1985). This might also explain the prevalence of HAT obtained in this study.

The survey also revealed that though majority of the respondents were aware of HAT, the respondents knowledge on the cause, transmission, prevention and control of HAT was however poor. The respondents' level of education, occupation and age could play a major role in improving the level of knowledge of the respondents as this survey showed a significant association between education, occupation, age and awareness to HAT. This however might also explain the prevalence level obtained in this study.

The level of awareness reported in this work is lower than that reported by Karshima (2010). This might probably be due to the fact that the disease has been reported in the state and as such people became aware. It might also be due to the fact that questionnaires were only administered to the subject sampled unlike in Karshima (2010) in Taraba states where they were administered to the general populace.

To the best of our knowledge, this study is the first to serologically detect *T.b.gambiense* li tat 1.3 surface antibodies among HIV patients in Ankpa General Hospital and Kogi State.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

Human African Trypanosomosis is a serious disease of man and animal. The present study was aimed at investigating the occurrence of Human African Trypanosomosis among Human Immunodeficiency Virus patients in Ankpa General Hospital, Kogi State. *Trypanosoma brucei gambiense* infection decreases the specificities of antibody detection test for HIV diagnosis. Human African Trypanosomosis symptoms are nonspecific, variable and inconsistent, and alone are insufficient for diagnosis. Moreover, HAT symptoms can be confused with those of malaria, enteric fever, tubercular meningitis and HIV. Ethical clearance was obtained from the State Ministry of Health. Blood samples were collected from HIV patients at the Ankpa General Hospital after their consent was sought. Subjects to be sampled were selected using convenience sampling with the sex, age and occupation of the HIV patients recorded. Four hundred and sixty five blood samples were collected from HIV patients. They were screened serologically using Card agglutination test for *T.b gambiense* (CATT) and parasitologically using the wet mount and haematocrit centrifugation technique (HCT). The overall sero-prevalence of HAT in Ankpa General Hospital among HIV patients was 3.01% (14/465). Sero-prevalence among the females and males was 3.60% (12/333) and 1.52% (2/132) respectively. There was no significant association ($p>0.05$) between the infection and sex. There was a sero-prevalence of 3.63% (12/330) and 2.04% (2/98) among the age groups of 18-45 years and above 45 years respectively which were not statistically significant ($p>0.05$). There was no significant association ($p>0.05$) between the infection and occupation, farmers had the highest sero-prevalence of 5.00%

(10/200). No parasites were however detected in the blood samples. The questionnaire survey showed that 79.35% (369/465) of the respondents were aware of HAT and 20.64% (96/465) were not. There was a significant association ($p<0.05$) between education, age, occupation and awareness to HAT. There was no significant association between sex and awareness to HAT ($p>0.05$). This study has established a serological evidence of HAT among HIV patients in Ankpa General Hospital, Kogi State.

6.2. CONCLUSION

This study has established a sero-prevalence of 3.01% *T.b.gambiense* li Tat 1.3 surface antibody among HIV patients in Ankpa General Hospital, Kogi State. No parasites were detected.

The major limitation to this work was that parasitological diagnosis gave a negative result. This would have given us a true picture of the infection.

6.3 RECOMMENDATIONS

Since it is stipulated that once sleeping sickness is established in an area, it is unlikely to disappear unless drastic control measure is instituted (Maudlin *et al.,* 2004), it is recommended that tsetse fly control measure should be put in place and it is also recommended that HAT is prioritize in the State. There is also the need to investigate the role of wildlife and domestic animals in the transmission of the disease.

Since HAT is shown to decrease the specificities of antibody detection test for HIV diagnosis (Lejon *et al.,* 2010), it is recommended that specific validation on three HIV test is carried out to increase specificity.

It is also recommended that public campaign on HAT should be carried out to increase both the awareness and knowledge of the populace to HAT in order to alert the people on the impact of the disease and how best to avoid it.

Finally, it is recommended that further studies should be carried out to serologically, parasitologically and biologically diagnose HAT, not just among immunocompromised individual but the population of Ankpa and Kogi State as a whole. Serological diagnosis would give a clue of HAT, parasitological diagnosis would give a true picture of the disease and biological diagnosis (Cerebrospinal fluid examination) of the seropositive subjects would help determine the stage of the disease.

Some strain of *T. b gambiense* lack the specific *gene* coding for CATT-antigen (Penchenier *et al.*, 2003), For this reason, the use of other diagnostic devices and procedures that might help to detect the true infection with *T. b. gambiense* are demanded.

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APPENDIX A: QUESTIONNAIRE ON AWARENESS TO HUMAN AFRICAN TRYPANOSOMOSIS.

Please kindly fill and tick where appropriate.

1. IDENTIFICATION NUMBER:
2. DATE

DEMOGRAPHIC INFORMATION

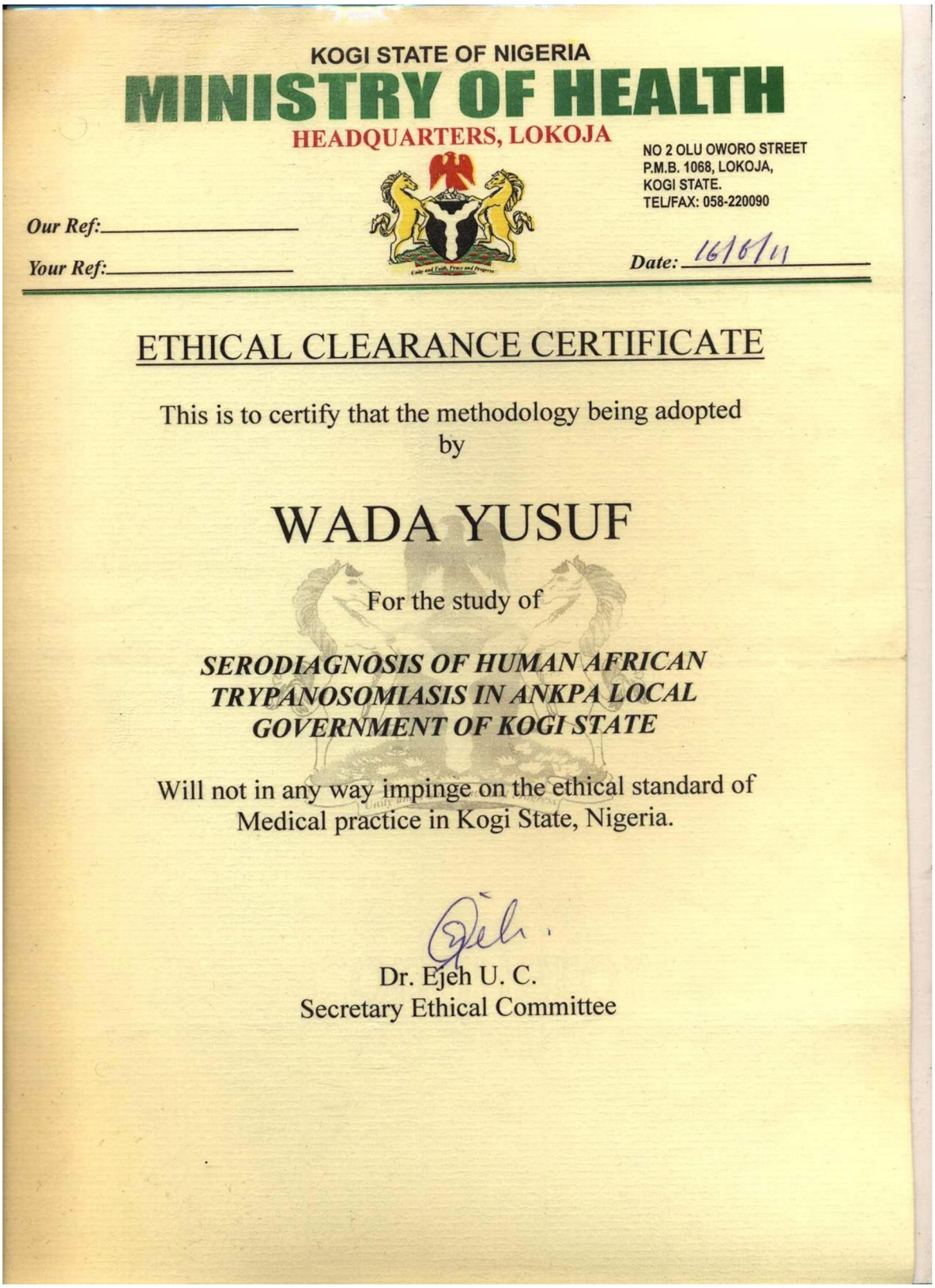
3. SEX: Male { } Female { }
4. AGE 10-20{ } 20-30{ } 30-40{ } 40-50{ } 50-60{ }
5. EDUCATIONAL QUALIFICATION: None { } Primary { } Secondary { } Tertiary { }Others {specify}
6. OCCUPATION: Farmer { } Daily laborer { } Fisherman { } Civil servant { } Hunter { } Lumberman { } Others {specify}

INFORMATION ON THE DISEASE

7. Have you ever heard of sleeping sickness? Yes { } No { }
8. If yes, through which means? Television { } Radio { } Medical personnel { } Others {specify}
9. Where do you live? Township { } Village { }
10. If in the village, what is your source of water? Well/borehole { } Rivers { } Streams { } Others {specify}
11. Is your house located near a forest? Yes { } No { }
12. Are you aware of tsetse flies? Yes { } No { }
13. Have you ever been bitten by a tsetse fly? Yes { } No { }
14. Have you ever been screened for sleeping sickness? Yes { } No { }

15. What do you think is the cause of sleeping sickness? Blackfly bites { } Mosquitoe bites { } tsetse fly bites { } Farm work { } Heredity { } Poor nourishment { } Witchcraft { } Others { } Cannot say { }
16. Do you think it can be transmitted from person to person? Yes { } No { } No idea
17. Do you think it can be prevented? Yes { } No { } No idea { }
18. Do you think it can be cured? Yes { } No { } No idea { }
19. How do you think it can be prevented? Isolation of sick person { } Use of drugs{ } Killing the tsetse flies { } Avoid going to the river/stream { } Wearing protective clothes { } Others { } Uncertain { }

APPENDIX B: ETHICAL CLEARANCE CERTIFICATE



KOGI STATE OF NIGERIA

MINISTRY OF HEALTH

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NO 2 OLU OWORO STREET
P.M.B. 1068, LOKOJA,
KOGI STATE.
TEL/FAX: 058-220090

*Our Ref:*_____

*Your Ref:*_____

Date: 16/6/11

ETHICAL CLEARANCE CERTIFICATE

This is to certify that the methodology being adopted by

WADA YUSUF

For the study of

SERODIAGNOSIS OF HUMAN AFRICAN TRYPANOSOMIASIS IN ANKPA LOCAL GOVERNMENT OF KOGI STATE

Will not in any way impinge on the ethical standard of Medical practice in Kogi State, Nigeria.

Ejeh.

Dr. Ejeh U. C.
Secretary Ethical Committee