ASSESSMENT OF PREVALENCE AND KNOWLEDGE OF HUMAN AFRICAN TRYPANOSOMIASIS (HAT) IN DELTA STATE, NIGERIA

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POST GRADUATE SCHOOL

DEPARTMENT OF COMMUNITY MEDICINE

UNIVERSITY OF NIGERIA, ENUGU CAMPUS

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A RESEARCH PROJECT SUBMITTED TO THE POST GRADUATE SCHOOL, UNIVERSITY OF NIGERIA, ENUGU CAMPUS IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTERS DEGREE IN PUBLIC HEALTH (MPH)

DEPARTMENT OF COMMUNITY MEDICINE, COLLEGE OF MEDICINE AND DENTISTRY, UNIVERSITY OF NIGERIA, ENUGU CAMPUS, NIGERIA

NOVEMBER, 2015

DECLARATION

I hereby declare that the research project entitled Assessment of Prevalence andknowledge of Human African Trypanosomiasis (HAT) in Delta State, Nigeria ï 2011was carried out by me in the Department of Community Medicine under the supervision of Dr. E. N. A. Aguwa.

The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of the research project has been previously presented for another degree or diploma at any university.

Uzoma, Emeka Umelo	**----------**	**----------**
Name of Student	**Signature**	**Date**

APPROVAL

THIS PROJECT RESEARCH WORK; ASSESSMENT OF PREVALENCE AND KNOWLEDGE OF HUMAN AFRICAN TRYPANOSOMIASIS (HAT) IN DELTA STATE, NIGERIA `-2011` BY UZOMA,EMEKA UMELO MEETS THE REGULATION GOVERNING THE AWARD OF THE DEGREE OF MASTER OF PUBLIC HEALTH OF THE UNIVERSITY OF NIGERIA, ENUGU CAMPUS AND IS APPROVED FOR ITS CONTRIBUTUION TO SCIENTIFIC KNOWLEDGE AND LITERARY PRESENTATION.

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DR. E. N. AGUWA DATE
SUPERVISOR

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PROF.B.S.C UZOCHUKWU DATE
HEAD, DEPARTMENT OF COMMUNITY MEDICINE

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I glorify God for the gift of life and health without which there will be no me to accomplish this study.

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DEDICATION

This research work is dedicated to the memory of my late father; Mr Bertram .O. Uzoma who slept in the LORD in 1996, but whose immense legacies and solid foundation continue to impact in my life.

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ABBREVIATIONS

AAT	Animal African Trypanosomiasis
AU	African Union
CATT	Card Agglutination Test for Trypanosomiasis
CDC	Centres for Disease Control and Prevention
CDD	Community Directed Distributors of Ivermectin
CI	Confidence Interval
HAT	Human African Trypanosomiasis
IDSR	Integrated Disease Surveillance and Response
NFELTP	Nigerian Field Epidemiology and Laboratory Training Programme
NITR	Nigerian Institute for Trypanosomiasis Research
PATTEC	Pan African Tsetse and Trypanosomiasis Eradication Campaign
WHO	World Health Organization
DALY	Disability Adjusted Life Years
PAAT	Programme Against African Trypanosomiasis
FAO	Food and Agricultral Organization
IAEA	International Atomic Energy Agency
AUIBAR	Arican Union Inter African Bureau for Animal Resources

ABSTRACT

World Health Organization (WHO) earmarked Human African Trypanosomiasis for elimination from Nigeria in 2015, making it one of the Countryôs (Nigeria) priority diseases listed for surveillance through integrated disease surveillance and response. HAT is a major public health problem in sub-Saharan Africa, where it affects mainly the rural poor and is usually fatal when untreated.

The objective of the study was to assess the prevalence of HAT and the level of knowledge exhibited by the people about the disease, its risk factors among the residents of Delta State, Nigeria.

A cross sectional study was conducted in Delta State Nigeria from September to December 2011. Structured questionnaires were used to collect data on demographics, knowledge and attitude about HAT and also exposure to risk. Study population was 1,028 residents, aged five years and above, from seven Local Government Areas (LGAs) in the State. Blood samples of respondents were collected for HAT screening using the card agglutination Test for Trypanosoma brucei gambiense (CATT/T.b. gambiense) reagents and kits. Lymph node aspirates and cerebrospinal fluid of CATT positive respondents were examined for the parasite.

Out of the 672 respondents about the knowledge of HAT, 579 (86.2%) knew at least one correct symptom, 578 (86.0%) knew the cause and mode of transmission of HAT, 562 (83.6%) knew how to seek care while 612 (90.7%) knew how to protect themselves from the vector. 142 (21.1%) with knowledge about HAT got the information from several sources, 114 (17.0%) heard from the media while only 35 (5.2%) heard from health workers.

Out of the 1,028 respondents screened for HAT, 28 were CATT positive, seroprevalence of 2.7%, 19 (67.9%) had knowledge of HAT symptoms, 22 (78.6%) had knowledge of mode of transmission, 12 (42.9%) had knowledge of personal protection from tsetse flies and only 2 (7.1%) had knowledge of how to seek care. Analysis involving two variables (bi-variate analysis) showed that respondents of who know how to seek care were less likely to be sero positive to HAT ($p = 0.02$). Seropositivity to HAT infection was lower among respondents who had knowledge of how to protect themselves from the disease.

All the seven villages from seven Local Government Areas chosen through three stage random sampling in the three senatorial districts were mapped and their coordinates taken using the Global Positioning System (GPS)

HAT is endemic in Delta State, Nigeria. Prevalence rate was low but sero positive persons had high titres for CATT for *T.b. gambiense*. Risk factors for infection included ignorance about where to seek medical care when ill with HAT and knowledge about personal protection against HAT. Mass health education on preventive measures for HAT should be embarked upon and health care providers should be sensitized, for improved suspicion index for HAT diagnosis and management in Delta State and Nigeria.

CHAPTER ONE

1.0 INTRODUCTION:

1.1 Background

Trypanosomiasis (Sleeping sickness) is a parasitic disease of livestock and humans in sub-Saharan Africa. It is also one of the worldôs most neglected tropical diseases that have re-emerged in Africa[1-3]. In the African Continent including Nigeria, trypanosomiasis has severe impact on humans, livestock and other agricultural production systems[4].

Trypasosomiasis are two types; Animal African trypanosomiasis or nagana disease which affects only animals and Human African trypanosomiasis or sleeping sickness which is caused by two subspecies of *T brucei gambiense* and *T bruceirhodiense*.*T. b. gambiense* is responsible for the chronic form of sleeping sickness in West and Central Africa, whereas *T. b. rhodiense* gives rise to the acute form of the disease in East and Southern Africa. There are two distinct stages during the course of sleeping sickness. The first and early stage of the disease also known as the haemolymphatic stage is defined by the restriction of the trypanosome to the blood and the lymph system[5].The symptoms of this stage are fever, headache, joint pains, itching. The second or late stage of the disease, also known as the neurological phase, is characterised by the presence of the parasite in thecerebrospinal fluid. In general this is when the typical signs of the disease occur; confusion, disturbed sleep pattern, extreme lethargy, poor condition and change in mental behaviour. If left untreated, sleeping sickness patients die within months when infected with the T. b. rhodiense or within years when infected with T. b. gambiese. The transmission is from one person to anotheror from animal to man by the bite of an infected tsetse fly. Infectioncould also be through blood transfusion and even laboratory accidents with infected blood[6-7]

In 1985, a hospital based study documented HAT for the first time in Niger Delta area of Southern Nigeria[8]. The hyper endemic status of these areas for HAT was later corroborated by some population based studies[9]. Reports of high catches of the riverine tsetse species (palpalis group) Glossina tachinoides with serious complaints of fly bites nuisance were received from inhabitants of communities of Kaduna, Chamso Bachit, Bakin Kogi, Angware, Durbi and Federe Districts of Mangu, Barkin Ladi and Jos East LGAs of Plateau State respectively. These communities live and work near rivers and streams and were mostly involved in intensive agrarian conditions during the wet and dry seasons[10]. Trypanosomiasis in domestic animals including canine trypanosomiasis have been reported in Nigeria as common occurrence in *T.b. gambiense* belts[11], assymptomatic individuals and some of these domestic animals are suspected to be reservoirs for HAT in endemic areas[12].

1.2 Burden of the disease/Current Scientific Knowledge:

A significant portion of children are affected by the disease and many of them suffer considerable delay in mental development which impacts negatively on school performance and professional advancement[13]. According to the WHO, global burden of the estimates that HAT caused 1.5 million deaths in 2002[14]. This was why at the 25th ISCTRC (International Scientific Council for Trypanosomiasis Research and Control) in Mombassa, Kenya, in October 1999, the idea of anAfrican-wide initiative to controltsetse and trypanosomiasis populations was discussed. During the 36th summit of the African Union in Lome, Togo in July, 2000, a resolution was passed to form the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). The campaign works to eradicate the tsetse vector population levels and subsequently the protozoan disease, by use of insecticide impregnated targets, fly traps, insecticide-treatedcattle, ultra low dose aerial/ground spraying (SAT) of

tsetse resting sites and the sterile insect technique (SIT) [15]. The use of SIT in Zanzibar proved effective in eliminating the entire population of tsetse flies but was expensive and is relatively impractical to use in many of the endemic countries afflicted with African trypanosomiasis[16].

Regular active surveillance, involving detection and prompt treatment of new infections and tsetse fly control is the backbone of the strategy used to control sleeping sickness. Systematic screening of at risk communities is the best approach, because case by case screening is not practical in endemic regions. Systematic screening might be in the form of mobile clinics, or fixed screening centres where teams travel daily to areas of high infection rates. Such screening efforts are important because early symptoms are not evident or serious enough to warrant patients with gambiense disease to seek medical attention, particularly in very remote areas. Also diagnosis of the disease is very difficult and health workers may not associate such general symptoms with trypanosomiasis. Systematic screening allows early stage disease to be detected and treated before the disease progresses, and removes the potential human reservoir[17]. A single case of sexual transmission of West African sleeping sickness has been reported[18].

The current treatment for the first stage disease is intravenous or intramuscular pentamidine (for *T.b gambiense*)[19]. For *T. b. gambiense* second stage, intravenouseflornithine orthe combination of nifurtimox and eflornithine appear to be more effective and easier to give[20]. These threatments may replace melarsoprol when available with the combination being the first line. Intravenous melarsoprol was previously the standard treatment for the second stage (neurological phase) disease and is effective for both types of

Trypanosomiasis.u It is the only treatment for second stage *T. b. rodesiense*, however can cause death in 5% of people who take it. Resistance to melarsoprol can occur[21].

1.3 Statement of the Problem:

Human African Trypanosomiasis (HAT)is one of the re-emerging neglected tropical diseases in Nigeria[22]. HAT is also one of the diseases earmarked by W.H.O for global elimination by 2015. The type present in Nigeria is the chronic form, Trypanosoma *brucei gambiense* which can be fatal if left untreated. The disease is one of Nigeriaôs priority diseases for integrated disease surveillance and response yet little or no information is available on its prevalence and/or endemicity foci in the Country[23].

In addition, required data on HAT in Nigeria are not available as reports received from various parts of the country especially the Niger Delta region were not properly verified and accurate surveys that could delineate the areas are also not conducted due to insecurity in most parts of the country[24].Meanwhile people have continued to die of unknown causes but with signs and symptoms of HAT as the suspicion index for HAT here is low or poor. Therefore, it is rarely diagnosed in most health facilities in Nigeria.Poverty is being generated everyday especially in the rural areas where knowledge about HAT is minimal.

1.4 Justification for the study:

The epidemiological data of trypanosomiasis indicates that it is widespread in 37 countries all in sub-Saharan Africa[25-27]. The World Health Organization (WHO) estimated that globally 60,000,000 people are at risk of infection with a total number of reported cases standing at 500 thousand infections and an annual incidence of 66,000 cases. Among these reported cases, fewer than 15% are diagnosed and treated, while about 100,000 deaths due to Human African

Trypanosomiasis (HAT) are reported annually[28, 29]. An estimated global annual loss of 4.5 million US Dollars is also attributed to trypanosomiasis. HAT is one of Africaôs greatest constraints to socio-economic development severely affecting humans and livestock health, limiting land use, causing poverty and perpetrating under development on the African continent. HAT affects the rural poor and if left untreated may result in high fatality rates[30, 31].

In the year 2000, the WHO health report on improving performance of health systems, noted that the disability adjusted life years (DALYs) loss due to sleeping sickness was 2.05 million[32]. A similar study was carried out between 2000 ï 2002 in Buma community in the Democratic Republic of Congo to estimate HAT related household costs and DALYs using retrospective questionnaire surveys among 57 patients of affected 47 households. Results show that the cost to each household was equivalent to 5 months income for that household. The total number of HAT- related DALYs was 2,145 and interventions to control HAT averted 1,408 DALYs for a cost of 17 US dollars per DALY averted[33].

In 2010, the number of new cases of HAT reported to WHO dropped to less than 10,000 for the first time in 50 years[34]. According to the same report, there were 9,877 reported cases of HAT in 2009 compared to more than 17,600 in 2004 and almost 38,000 in 1998. According to WHO[35] the reduction in the number of reported case of HAT was attributed to strengthened control and surveillance effort by national sleeping sickness programmes in endemic countries over the past 10 years.

In Nigeria, HAT surveillance is conducted through active and passive surveillance systems[36]. HAT is one of the priority diseases for integrated disease surveillance and response (IDSR) and it is reported on a monthly basis by the Federal Ministry of Health[36]. The HAT surveillance system in Nigeria is poor, making it pertinent to identify the endemicity foci

andprevalence of the disease in the country in order to establish a sustained systematic HAT surveillance and elimination mechanism in Nigeria[37].

This study was conducted in Delta State because of the sporadic reports about suspected HAT cases originating from Delta State. Data generated from this study will contribute in providing baseline information, give first hand indications about the extent of HAT problem in the study area, the general knowledge about HAT and its prevention in Delta State. Findings from the study will also establish possible risk factors for HAT and contribute to policy decisions on the development of strategies for prevention, control and subsequent elimination of HAT in Nigeria.

1.5 Objectives of the study:

1.5.1 General study objective:

The general objective of the study is to provide baseline data for the assessment of HAT epidemiological status in Delta State, Nigeria.

1.5.2 Specific objectives:

a. To obtain the demographic variables of the seropositiverespondents from the survey

b. To determine the prevalence of HAT in Delta State.

c. To identify risk factors associated with HAT among the affected persons

d. Toobtain the knowledge of respondents about HAT in Delta State.

e. To generate the geographic coordinates of the villages visited in Delta State.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 *Background*

HAT caused by protozoan of the Trypanosoma *brucei* group is endemic in 36 countries of sub-Saharan Africa, where over 60 million people are exposed and Nigeria is one of these countries[38, 39]. In endemic areas, control of HAT is highly dependent on mass screening and treatment of identified cases to reduce parasite reservoir and lower or disrupt transmission by the vectors, tsetse flies (Glossina species). Studies have shown that asymptomatic carriers and animals play a very important role in HAT transmission. HAT is fatal if untreated.

HAT is the only vector ï borne parasitic disease with a geographical distribution limited to the African continent. In Nigeria, HAT is caused by *Trypanosoma brucei gambiense* and HATdue to *Trypanosoma brucei gambiense* is the chronic form that progresses through two stages[43]

The World Health Organization (WHO) and Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) resolved to strengthen the capacity of the affected countries in order to eliminate the disease by 2015 and or achieve a prevalence rate of less than 1 case per 1,000 people at risk[44].

2.2 *Epidemiology*

Nigeriaôs true endemicity status for HAT is unknown, as less than 10% of the population at risk is currently under surveillance[45], populations in the age group 15-45 years living in remote rural areas are mostly affected and this has led to economic loss and social misery[46].

In 1960, Nigeria reported 5,094 cases in known endemic foci of Kano, Bauchi, Kaduna, Niger, Plateau, Taraba and Benue States. The number of reported cases declined to 944 cases in

1968 and 132 cases in 1974[47]. The situation of HAT in Nigeria is under reported due to the absence of a sustainable systematic surveillance system and insecurity in Nigeria[48]. A detailed retrospective study of surveillance data from some Northern States (Adamawa, Benue, Bornu, Kwara, Plateau, Niger and Taraba) showed the following number of reported HAT cases; 6,879 in 1975; 7,263 in 1976; 1,501 in 1977; 6,964 in 1978; 3,813 in 1981; 938 in 1983; 2 in 1985; 13 in 1987 and 1 in1989[49]respectively.

In Abraka focus of Delta State between 1989 and 1996, 359 people were serologically-positive while 104 were parasitologically positive out of a sample of 3,583 examined. Only 127 of these cases were treated with 7 fatalities recorded[50]. In arelated study, out of 4,966 persons screened, 497 (10%) were seropositive and 298 (6%) of the seropositive had the disease[51]. Between 2002 and 2005, 67 cases of stage two HAT were treated at Eku Baptist Medical centre Eku, Delta State. In 2009, 4 and 44 parasitologically and serologically positive cases were diagnosed respectively from 474 volunteers screened in Abraka, Delta State. In 2010, out of 2,050 persons screened in Delta State by a Federal Ministry of Health team, 2 were microscopically confirmed positive while 11 were serologically positive[53].

In 2010, the World Health Organization reported that *T. b. gambiense* accounted for over 95% of reported cases of HAT globally. Also according to WHO*, T.b. rhodesiense*, found in eastern and southern Africa, represented about 5% of reported cases in acute infections in 2010[54]. HAT is distributed in pockets throughout sub-Saharan Africa, placing an estimated 60 million people at risk of the infection[55]. HAT is the only vector-borne disease with a geographical distribution of its vector, the tsetse fly, which is exclusively found in sub-Saharan Africa between 14^0N and 20^0S[57].

The importance of various components of the epidemiology of trypanosomiasis (human, animal, vector control, agricultural activity and livestock production) and their impact on the development of rural Africa led WHO to promote an inter-sectoral initiative that ultimately became the ProgrammeAgainst African Trypanosomiasis (PAAT) in 1977. This was in collaboration with the Food and Agricultural Organization (FAO), the International Atomic Energy Agency (IAEA) and the African Union Inter African Bureau for Animal Resources (AUIBAR) [58]. In the same vein, African Heads of States and Governments during the African Union Summit in Lome, Togo in the year 2000 established the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) with the aim of making Africa a tsetse and trypanosomiasis free continent[58].

2.3 Mode of transmission and life cycle

HAT, a completely fatal parasitic disease if untreated is transmitted by the bite of an infected tsetse fly (genus Glossina). Causative organisms for the disease in humans in Africa are *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense.* HAT caused by *Trypanosoma bruceigambiense* is the chronic form which occurs in West Africa and Central Africa, including Nigeria[59]. The tsetse fly is infected by ingesting blood containing trypanosomes from an infected human or animal. The parasite multiplies in the tsetse fly for12-30 days. Finally, infective forms develop in the salivary glands and are transferred to a susceptible host during a blood meal.

An infected person can be infected for several months or years without visible major symptoms or signs of the disease[60]. When symptoms emerge, the patient is often already in the advanced state and the central nervous system is affected. The first signs of the *T.b. rhodiense* disease are observed a few months or weeks after infection. Disease develops rapidly and

invades the central nervous system faster than infection by *T.bgambiense*. HAT due to *T.b. gambiense* progressesthrough two stages following asymptomatic period of several weeks or months. The early stage is usually characterized by symptoms comprising general malaise, fatigue, headache, recurrent fever and swollen lymph nodes, cervicallymphadenopathy referred to as Winterbottomôs sign. In the advanced stage of the infection, the disease affects the central nervous system, causing several neurological, physical and mental disorders including insomnia, somnolence and even coma rendering the individual incapacitated. Infected individual are weakened often for many years causing socio-economic losses and poverty.

2.4 Re-emergence of HAT

The re- emergence of sleeping sickness presents a major public health problem. Human African Trypanosomiasis or sleeping sickness is one of the most important but equally most neglected tropical diseases[61, 62]. In 2007, a study on conflict and health was carried out in Uganda to identify the dominant impacts and vulnerabilities associated with conflict, their effects on the transmission determinants of sleeping sickness and the resulting impact on sleeping sickness. The four key impact categories identified were Economic and global effects, decline of health systems and services, forced migration and internal displacement of populations, Regional insecurity and restricted access to external humanitarian support[63]. Results showed that in the conflict areas, decline of health services led to increased duration of human infection. In *T. b*. rhodesiense affected regions, civil war led to reduce cattle treatment, thus increasing the duration of cattle infection andthe reservoir of human infectious parasites for transmission to the human populations. Displacement of people and animals into marginal, bushy or swampy areas further promoted increased human, fly and cattle contact. This showed that conflict had contributed to the risk of contracting sleeping sickness[63]. World Health Organization (WHO) and Centre for

Disease Control (CDC) have also reported at different times that animal reservoir hosts are suspected to be contributing to the resurgence and spread of *Trypanosoma brucei gambiense*[64]. Altogether, there have been three severe global pandemics of HAT, one at the end of the nineteenth century, the second during the 1920s and the third from the 1970s leading to the re-emergence of the disease to the present status[65].

2.5 Demography and HAT

If HAT must be controlled or eliminated from the tropical Africa, demography of survey participants has to be taken seriously, especially seropositive cases. This is important when thisgroup of participants are to be taken for parasitological test. It is also important for staging of the diseases for easy identification of the specimens. Most importantantly, it is very necessary for follow up after survey. If the soropositive persons are confirmed parasitologically positive, the demographic information will be very handy in case control and incidence studies.

2.6 Prevalence of HAT

All cases of African trypanosomiasis are imported from Africa by travellers to any other endemic areas in the world. Infections among travellers are rare, with less than 1 case per year reported among US travellers. Most of these infections are caused by *T. b. rhodesiense* and are acquired in East African games parks.

African trypanosomiasis is confined to tropical Africa between latitudes 15^0N and20^0S or from north of South Africa to south of Algeria, Libya and Egypt.The prevalence of African trypanosomiasis varies from country and region. In 2005 major outbreaks were observed in Angola, the Democratic Republic of Congo and Sudan.Fewer than 50 new cases per year are reported in countries such as Burkina Faso, Cameroun, Equatorial Guinea, Gabon, Kenya, Mozambique, Nigeria, Rwanda, Zambia and Zimbabwe.T. b. gambiense seems to have stopped

and no new cases of African trypanosomiasis have been reported for several decades in countries such as Benin, Botswana, Burundi, Ethiopia, Gambia, Ghana, Guinea Bissau, Liberia, Mali, Namibia, Niger, Senegal, Sierra Leone, Swaziland and Togo.

In 1986, a panel of experts convened by the World Health Organization (WHO) estimated that 70 million people lived in areas where transmission of African trypanosomiasis is possible. In 1998 almost 40,000 cases of the disease were reported, but this number did not reflect the true situation given the remoteness of the affected regions and the focal nature of the disease. Between 300,000 and 500,000 more cases were estimated as remaining undiagnosed and therefore untreated.

During recent epidemic periods, the prevalence of sleeping sickness has reached 50% in several villages in Democratic Republic of Congo, Angola and Southern Sudan. Sleeping sickness was considered the first or second greatest cause of mortality in those communities, even ahead of HIV infection and AIDS. By 2005, surveillance had been reinforced and the number of new cases reported throughout the continent had substantially reduced; between 1998 and 2004, the figures for both forms of Human African Trypanosomiasis together fell from 37,991 to 17,616. African trypanosomiasis has no racial predilection, sexual predilection nor age preference. Exposure can occur at any time. Congenital African trypanosomiasis occurs in children, causing psychomotor retardation and seizure disorders[66]. The WHO HAT surveillance and control programme in 2006, reported that estimated populations of Nigerians at risk of sleeping sickness was 13,000,000 with transmission occurring in one state, Delta where cases were regularly reported from the Abraka focus. It noted that it was difficult to assess whether transmission took place elsewhere in the country owing to the lack of active surveillance and insecurity in some parts of the country[67].

The World Health Organization (WHO) Expert committee on HAT Control and Surveillance met in 1995, regarding the uncertainties between reported cases and actual field situation. The Committee estimated the true number of cases to be at least 10 times more than what was reported. Hence 30,000 cases reported annually, was estimated to represent 3000,000 infected individuals that remained ignored in the field[68].

In 2005, WHO regional Committee for Africa during its fifty fifth sessions in Maputo, Mozambique reported that HAT is endemic in 35 countries in the African region with different levels of endemicity[48]. Countries were classified as i) non endemic with no cases reported in five or more years (ii) unknown endemicity (0-25 new cases per year); (iii) low endemicity (26-100 new cases per year); (iv) moderate endemicity (101-500 new cases per year); (v) highly endemic or epidemic (more than 500 new cases per year)[70].

Unpublished records and data on HAT surveillance in Nigeria, 1931 to 1990, from NITR[71] show that the peak of HAT epidemic in Nigeria was between 1931 ï 1940 when more than 370,000 cases were reported. At the end of 1950, the number of HAT reported cases dropped to about 40,000 and subsequently to about 30,000 at the beginning of 1951. A slight increase in the number of reported cases occurred afterwards and remained same until late 1970ôs. A sharp increase in reported cases was observed during the period, 1981-1990 with infection rates suggesting that the disease may be on the increase unnoticed[71].

A similar study was carried out in 2010 by Simarro et al for the period 2000 -2009 on mapping new HAT cases reported during the period at village level. The HAT status for 23 out of 25 sub-Saharan African countries that reported HAT cases during the period was documented and Nigeria was documented as having reported HAT cases from Edo and Delta States, where

security constraints prevented more accurate surveys to delineate the exact extent of the focus. During the 10 year period, 2000 to 2009, Nigeria reported 119 new HAT cases[69].

In a related study in 2007, the National Trypanosomiasis Control Programme of the Democratic Republic of Congo (DRC) reported 8.162 cases of HAT[72]. To address the problem of over and under estimating the prevalence of HAT, a study was conducted in the DRC in an attempt to obtain accurate assessment of HAT prevalence using the demographic and Health Surveys (DHS). Samples from 2007 DRC DHS were used to estimate the burden of HAT. ELISA tests were conducted on 7,769 leftover dried blood spots followed by confirmatory trypanolysis and PCR tests. Of these samples, 26 specimens in 23 sites were found to be positive. The overall prevalence of HAT in Democratic Republic of Congo (DRC) was calculated using standard sampling weights and found to be 29.7 cases per 100,000 persons. It was estimated that in 2007, atotal of 18,592 persons had HAT in DRC. The results suggested that 56% of the actual HAT cases were not detected and therefore not reported, very close to estimates of 65-75% used by WHO and considered to be under reporting[72].

Another study [73]conducted to determine the situation analysis of HAT in Zambia from January 2000 to April 2007 in three districts of HAT foci in North Eastern Zambia showed that HAT was endemic in the area of Study. During the period under review, 24 HAT cases were reported from the three districts. Review of literature on the occurrence of HAT in Zambia from early 1960s to mid 1990s revealed that HAT transmission foci were widespread in Western and North Western, Lusaka, Eastern Luapula and Northern Provinces of Zambia. The results showed that the distribution of HAT transmission foci was found to be different in the period before and after 2000 when there was no active national tsetse fly and trypanosomiasis control programme in Zambia[73.]

In Abraka focus of Delta State between 1989 and 1996, 359 people were serologically-positive while 104 were parasitologically positive out of a sample of 3,583 examined. Only 127 of these cases were treated with 7 fatalities recorded[50]. In a related study, out of 4,966 persons screened, 497 (10%) were seropositive and 298 (6%) of the seropositive had the disease[51]. Between 2002 and 2005, 67 cases of stage two HAT were treated at Eku Baptist Medical centre Eku, Delta State. In 2009, 4 and 44 parasitologically and serologically positive cases were diagnosed respectively from 474 volunteers screened in Abraka, Delta State. In 2010, out of 2,050 persons screened in Delta State by a Federal Ministry of Health team, 2 were microscopically confirmed positive while 11 were serologically positive[53]. This shows aremarkable reduction in prevalence rate.

Other Countries that have documented high incidence of HAT include Sudan, Angola, Sudan, Uganda and Tanzania. The resurgence of HAT in the last few years was facilitated by civil war, migration, economic crisis, reduced health financing, break down of HAT surveillance systems and reduction in human capacity for HAT control in these areas.

2.7 HAT- parasite classification and biology

Scientific classification for Trypanosoma species is as follows:

The Kingdom it belongs is Excavata, PhylumEuglenozoa, Class Kinetoplastea, Order Trypanomastida, Genus*Trypanosoma*, Species*Trypanosoma brucei,* Sub species is*Trypanosoma brucei gambiense.*

`The genus Trypanosoma is divided into several subgenera based on morphological differences. However the most important subdivision is dividing into groups Salivaria and Stercoraria, the latter involving species that have their development terminated in the rear part of the digestive

tract of the vector. Examples are the triatamine bugs which transmit the American Trypanosomes caused by T. cruzi. The salivary group was derived from ósaliva` since the development in the vector is terminated in the salivary glands. Transmission is innoculative by the injection of infectious metacyclic trypanosomes during blood meals by the vector. Trypanosomes multiply in the hostôs blood in form of trypanomastigotes[74].

Salivarian trypanosomes apparently originated from the area where tse-tse flies (Glossina Spp.) occur in the Tse tse belt of Africa[74]. The most important species of the Salivarian trypanosomes (usually placed in the suborder *Trypanozoon*) is T. *brucei*, subdivided into the subspecies *T. brucei gambiense, T. b. rhodesiense*, *T b. equiperdium* and *T. b. evansi*. These subspecies are sometimes recognized as separate species depending on their habitats. They are transmitted by anumber of Glossina species, where they undergo a complex mode of development. *T. brucei brucei*is unable to infect humans, as it is lyzed by human serum that contains the trypanosome lytic factor, which is a toxic type of high density lipoprotein. Other than man, this flagellate can be found in virtually every warm blooded vertebrate animals, usually antelops, zebras, lions, beasts of burden, goats, pigs etc, where it causes disease called nagana or Nôgana[75].

2.8 Risk factors of HAT

A case control study was designed to analyze the characteristics of HAT cases in Kinshasa and compare them with those of healthy controls[76]. A total of 1,764 people (588 case-patients and 1,176 controls) were interviewed using structured questionnaire. Cases were people infected with HAT and enrolled in the National Human African Trypanosomiasis Control Programme (PNLTHA-DRC) from January 2004 to December 2005. Controls were matched for sex, age, and residence to the corresponding cases, but had negative results from the Card Agglutination Trypanosomiasis Test (CATT) whole blood serologic analysis. Results showed

that cases though identified in the 24 districts of Kinshasa, were concentrated in the outskirts and rural areas. Overall, 25% of the cases live in the urban areas. People in the labour market, age 20 ï 49 years) were most affected. HAT affected men and women equally but had affected people who moved around a lot and those who worked in rural or domestic activities at higher rates, especially those in close contact with water courses. Sleep disorders were the primary clinical sign (85%), cervical adenopathy (66%) and fever (68%) were reported in the cases. Most (75%) were diagnosed at a very advanced stage of infection, the meningo-encephalitic or neurological stage. This showed that interventions targeted at the modifiable characteristics associated with HAT could reduce the morbidity and mortality rates associated with HAT and prevent further spread of this disease[76].

2.9 Clinical manifestation of Human African Trypanosomiasis

Any bite from tsetse fly, whether the fly is infected or not, produces a local reaction. Patients usually notice the exposure because the bite is painful. When the bite is infected, a small local wound typically appears approximately after 5-15 days. A well circumscribed painful papule, with a central blister or ulcer usually develops[77].

Both *T. b. gambiense* and *T. b. rhodesiense* infections are characterised by an early stage, the preliminary haematolymphatic stage during which trypanosomes are found in the blood and nymph nodes and a second (late) stage with symptoms of meningoencephalitis. The differentiation between these stages is made by finding the parasite in the cerebrospinal fluid, increase in white blood cell counts and protein levels in the cerebrospinal fluid. The distinction is important because of consequences for treatment.[78].

Clinical signs and symptoms include oedema, posterior cervical lymphadenopathy (Winter bottomôs sign), rashes, headache, malaise and fever which in most cases progress to the second

stage signs and symptoms of neurological involvement such as insomnia, somnolence and coma[78].

The T. b. *gambiense* first stage, the haematolymphatic stage lasts 6 to 12 months or longer. It is characterized by irregular headaches, general malaise and intermittent peaks of pyrexia (fever) corresponding with successive waves of parasitaemia and antibody production. Trypanosomes travel from the skin to the regional lymphatic, where they cause lymph node swelling (lymphadenopathy). Signs and symptoms of the first stage generally reported in *T. b. gambiense* infection are chronic intermittent fever, headache, severe itching, skin lesions, oedema of the face and other extremities[78].

In *T. b. gambiense*, lyphadenopathy is typically seen in the posterior cervical nodes. The nodes are soft, mobile, and not painful and are classically referred to as winter bottomôs sign. An English doctor, Thomas Master manwinter bottom, working in Sierra lone who first described the disease in1803 was struck by the abundance of swollen cervical lymph nodes in sick persons. Slave traders at the time also knew about this sign and avoided buying people with cervical lymphadenopathy[79]. Other non specific symptoms might be present including anaemia, weight loss, facial swelling and occasionally pericarditis develops leading to heart failure. There can also be endocrine involvement, leading to impotence in men and amenorrhea in women[80].

In the late stage of *T. b. gambiense* infection, which lasts three to sixmonths, progressive diffuse meningo-encephalitis and parenchyma oedema of the brain develop ultimately resulting in coma and death. Symptoms include headache, difficulty concentrating, personality changes, psychosis and sensory disorders. Basal ganglia involvement in the brain results in ataxia and tremors which are similar to those seen in Parkinsonôs disease. Damage to the hypothalamus leads to alteration of the circadian sleep/wake cycle. Consequently patients develop day time somnolence and night

time insomnia, hence the name sleeping sickness. The patient can be woken up but usually quickly falls asleep again. Ultimately the patientôs deterioration progresses to a state of stupor (coma)[80, 81].

In T. b. rhodesiense infection, the same sequence of events are seen as in T. b. gambiense infection, but the presentation and the progression are very fast developing signs of Central Nervous System infection within weeks. The clinical signs of the second stage HAT are neuropsychiatric and endocrinal disorders. Sleep disorder accounts for deregulation of thecircadian rhythm of the sleepwake cycle and a fragmentation of the sleeping pattern rather than the frequently reported inversion of sleep[81]. The neurological symptoms include confusion, tremor, fasciculation, general motor weakness, sensory disturbances, abnormal movements and speech disorders. Psychiatric symptoms might dominate the clinical picture and may constitute the first manifestation of the disease[82]. It also results to fatality if untreated.

2.10 Case Detection of HAT

Regular active surveillance involving detection and prompt treatment of new infections through systematic screening of at risk communities is the best approach since screening everyone is not practical in endemic regions. Systematic screening may be in the form of mobile clinics or fixed screening centres where teams regularly visit areas of high infection rates[56]. Such screening effort is important since early symptoms are not evident or serious enough to make patients with the gambiense disease to seek medical attention, particularly in the very rural areas. Diagnosis of HAT is also difficult and health workers may not associate the general symptoms with trypanosomiasis. Systematic screening allows for the detection of the early stage disease before its progression and removes the potential human reservoir[56].

2.11 Surveillance for Human African Trypanosomiasis(HAT)

Active and passive surveillance are recommended for HAT case detection in Nigeria since HAT is one of the priority diseases earmarked for elimination by WHO and listed for integrated Disease Surveillance and Response by the Federal Ministry of Health. According to studies conducted on HAT surveillance activities in 2008, active screening was stated to be necessary for diseases with extended asymptomatic pre-clinical period[83].

A study was carried out in the Negage focus of the province of Uige in Northern Angola, to assess the operational feasibility of detecting Human African Trypanosomiasis by active and passive case finding using the card agglutination test among 359 patients presenting themselves to health centres with symptoms (passive case finding) and 14,446 people actively screened in villages. Results showed that active case finding identified 251people with positive and agglutination test results, 10 of whom had confirmed parasites. In those presenting for investigation, 34 of 51 with positive card agglutination test results at the dilution of 1:8 or more were used to guide treatment, had parasites in blood, lymph node fluid or cerebrospinal fluid, compared with 10 of the 76 in those detected by active case finding: positive predictive values were for passive case detection and 13% for active case detection. It was only at a cut off dilution of 1:32 or more that the positive predictive value in active case detection was reasonable (46%) and at this dilution, 40% of microscopically proven cases were missed. It was noted that the card agglutination test though useful for initial screening of in active detection of cases with HAT, classically HAT diagnosis is based on direct visualization of parasites in the blood, lymph node aspirate and cerebrospinal fluid. Sensitivity of microscope is often limited owing to low or fluctuating parasitamia[84].

In the same study, focus group discussions held in the villages before administration of questionnaires to collect qualitative data on general knowledge about the disease and a collection of historical information on HAT in the study area were found to add impetus to data collection[84,85,86].

2.12 Mapping of HAT

Following the World Health resolutions 50.36 in 1997 and 56.7 in 2003, the World Health Organization (WHO) committed itself to supporting human African trypanosomiasis endemic countries in their efforts to remove the disease as a public health problem. Mapping the distribution of HAT in time and space has a pivotal role to play if this objective is to be met. For this reason WHO launched the HAT Atlas initiative, jointly implemented with the Food and Agriculture Organization of the United Nations, in the framework of the Programme Against African Trypanosomiasis[87].

Diseases maps are required for planning, managing and monitoring interventions across the whole spectrum of neglected tropical diseases. Strategies to control HAT hinges on early diagnosis and treatment, as well as on vector control. Accurate explicit information on the geographical distribution of HAT is therefore essential to target, monitor and evaluate interventions against the disease.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 General Background of Study Area

The study was conducted in Delta State, Nigeria. Based on the 2006 census figures, Delta State had a population of 4,112,445[235]. With an annual growth rate of 9.3%, the projected population for 2011 is 6,024,732. Delta State has a land area of 17,698 square kilometres with more than 60% on land. The State lies between Longitude 5^{0}00 and 6^{0}45ô East and Latitude 5^{0}·00 and 6^{0}.30ô north. It is bounded in the north by Edo State, in the east by Anambra State, in the south east by Bayelsa State and on the southern flank is the Bight of Benin which covers about 160 kilometres of the States coastline.

Delta State is cosmopolitan with majority of the inhabitants being farmers, fishermen and traders. The study is predominantly agrarian. However there are communities in the State inhabited by herdsmen who move around with their animals in search of pasture. Delta State has 25 Local Government Areas located in three Senatorial wards of Delta Central, Delta North and Delta South. The major ethnic groups in Delta State are Urhobo, Igbo (IKA), Aniocha, Ukwuani, Ezon (Ijaw), Isoko and Itsekiri.

Putting into consideration the three senatorial districts and ethnic groups in Delta State, villages and Local Government Areas (LGAs) were randomly selected for the survey. Consideration was also given to villages and Local Government Areas located around River Ethiope. These villages are characterised by stretched wetlands along the course of River Ethiope. Delta State is generally low ï lying without remarkable hills and has a wide coastal belt that is inter-laced with streams and canals that form part of the Niger Delta. The area has a very high annual rainfall of 2280mm and a temperature of over 27^{o}C. The River Ethiope and the other

smaller rivers provide typical habitats for riverine tsetse flies. The occurrence of *Glossina palpalis palpalis* was documented by the Nigerian Institute for Trypanosomiasis Research (NITR) in the area in 1995[236]. Recently *G. tachinoides* was caught during a study in the area[236]. The study sites were seven out of 25 LGAs in Delta State selected from the three senatorial wards and different ethnic groups in Delta State (Figures 1 and 2).

3.2 Study Design

The study was a cross sectional one, carried out to assess the prevalence and knowledge of Human African Trypanosomiasis (HAT) caused by *Trypanosoma brucei gambiense*in Delta State.

3.3 Sample size determination

Sample size was determined by taking the population of Delta State into consideration and using the formula $n = \frac{(Z)^2 P(1-P)}{d^2}$ ----------[93-96]

Where n = required sample size

Z = 1.96 degree of confidence (statistic for a level of confidence)

P = expected prevalence or estimate of prevalence = 10% i.e. 0.1

1-P = Probability of those without disease = 1- 0.1 = 0.9

If the width of the confidence interval is 10% (0.1) and so d should be half of it (0.05)

d = 0.05 the width of confidence interval

20% of the calculated value was added to the calculated sample size n in order to make up for the anticipated non response, non compliance and lost data during the survey.

$$\frac{1.96 \times 1.96 \times 0.1 \times 0.9}{0.05 \times 0.05} = \frac{0.3457}{0.0025} = 138 \text{ plus } 20\% = 166$$

This is the minimum sample size but a total 1028 was used for the study.

3.4 Sampling technique

A multi stage cluster sampling using senatorial districts, ethnic groups and LGAs was conducted. Simple random sample was used to select villages, households and persons that were sampled for HAT in the clusters and this was carried out by balloting.

The first stage was the selection of the seven LGAs from the senatorial districts, followed by the second stage which was the selection of one village from each of the seven LGAs and subsequently the third stage, selection of the households from each of the villages.

The sampling frame was the villages randomly selected for sampling in Delta state, the sampling units were households randomly selected for sampling. All together seven villages were selected from seven LGAs from the seven ethnic groups in Delta State, taking the three senatorial districts in the state into consideration.

Sampling frames for the different groups were collected from the state Epidemiologist and Community Directed Distributors of Ivermectin in the villages and from each frame, individuals were selected by simple random sample using the ballot method. One person per household was selected and this comprised heads of households or persons selected by members of the household to represent the household.

The study area was identified and delineated by randomly selecting seven LGAs from the three senatorial districts. This was followed by randomly selecting one village from each of the seven LGAs. Using village household lists, 150 households per village were also randomly selected

and the heads of households or their nominees were recruited for the survey. Figure1 and 2 show Delta State LGAs and the study area respectively. Appendices 4 to 9 provide more details about Delta State.

There are three senatorial districts in Delta State namely; Delta Central, Delta North and Delta South. The Ijaws, Itsekiris and Isokos are ethnic groups in Delta Central. Ethnic groups in Delta North are Aniocha, Ika and Ukwuani while Delta South has the Urhobos. LGAs and villages in these areas are shown in(Appendix 1). Also shown is the distribution of the study recruits by village.

The studywas conducted from September 2011 to December 2011.

3.5 Study population

3.5.1 ***Inclusion criteria:*** Children more than five years and adults resident in selected villages and LGAs in Delta State or study area and willing to be sampled were included in the project.

3.5.2 ***Exclusion criteria:*** Children less than five years of age and people not resident in the selected villages or present at the time of the screening exercise were excluded.

3.6 Data collection instruments:

The study was conducted from September to December 2011 and the data collection methods were:

a. Questionnaire administration

b. Focus group discussions (FGD)

c. HAT screening

d. Use of Global positioning Systems (GPS) to collect coordinates of HAT endemic villages.

Some of the study instruments comprising of the under listed are shown on Appendices 1-3 and 8 - 9.

a. Guide documents for focus group discussions, social mobilization activities and assessment of the general public knowledge about HAT and its control.

b. Structured questionnaire for pre- testing and main survey

c. Forms and checklists for data collection in HAT screening for prevalence survey.

d. Algorithm (a set of rules that must be followed when solving a particular problem) for HAT case detection and management

e. Procedure for HAT diagnosis

f. Manufacturers directions for use of CATT reagents and kits

Social mobilization activities and focus group discussions were carried out to ensure compliance and cooperation by the recruits and villagers during the study period.

All participants that met the eligibility criteria were interviewed, bled and examined for HAT. Interviews were conducted on the eligible participants at the point of blood sample collection. The questionnaire was pre tested in Umutu village, Ukwuani LGA Delta State. Data for the studywere collected using pre tested interviewer administered structured questionnaires by well trained research assistants.

Research assistants who worked as local guides were engaged in the study area. The research assistants were trained to enable them administers the questionnaire effectively, assist in the field activities and sample collection.

3.7 Data collection method

Sensitization and social mobilization of policy makers and community members (Government officials, leaders, influential persons in the communities and the general public) was done. The sensitization was about the project and its benefits to the communities, Delta State and Nigeria. Health facility personnel in the study area were also sensitized on the use of some of their resources for bench work and collection of cerebro spinal fluid from lumbar puncture where necessary. The details of the methods used are:

3.7.1 Questionnaire administration

The structured questionnaire had questions on the under listed variables:

a. Demographic variables - age, gender, level of education, occupation, duration of stay in village, residence, marital status and ethnicity. These information were gotten from the respondents and documented.

b. Knowledge of HAT and its symptoms, preventive measures against HAT, where to seek help when ill (health seeking behaviour) vector, control and treatment.

c. Exposure (risk) factors for HAT infection and transmission

d. Sources of information about HAT in the communities

Some of the data for the study were collected through interviewer-administered questionnaires by well trained field assistants to 1028 recruits. Pre- tested questionnaires (see Appendix 7) were administered to the project recruits and other volunteers. Information gathered from the questionnaires included but were not limited to the peoples knowledge about HAT, its prevention, control and risk factors about the disease. Informed consent was obtained from the study participantsbefore they were recruited and controlled for the study.

3.7.2 Focus group discussion

Focus group discussions were conducted in the State and selected communities for enquires about available information on HAT in the communities and by the villagers. The qualitative approach was conducted to further acquire more in dept information about the disease in the state. From the conversations more information about the disease in the communities, possible vectors, affected persons and areas affected, the communitiesô perception and beliefs about the disease, control measures for the disease and socio cultural peculiarities about the communities were gathered.Focus group discussions were held in the villages before administration of questionnaires to collect qualitative data on general knowledge about the disease and screening for HAT in Delta State. Historical information about the disease in the communities and methods of control in these communities were sought. Additional information was sought regarding the villagers opinion about the impact of the disease and its vector on production, productivity, land use, demography and health of communities. The discussions were held with women groups and village development communities. The Focus Group Discussion Guide (Appendix 1) was used to guide the discussions. Field notes were made during the discussions and recordings with tapes were also made.

3.7.3 Geographical data

Geographical coordinates of villages selected for the project were collected with the GPS (Global Positioning System) equipment and documented for subsequent mapping of the villages and data management. Other relevant information about study area was gathered and documented.

3.7.4 HAT Prevalence study

In diagnosing *T. b. gambiense* HAT (Human African Trypanosomiasis), a three step pathway was involved namely screening, diagnostic confirmation and staging**.** The screening for HAT was conducted in seven villages of seven LGAs namely; Burutu, Ethiope East, Ika North East, Isoko South, Ndokwa East and Oshimili South using the procedure for HAT diagnosis.

Procedures for HAT diagnosis comprised the following:

a. Examination of clinical signs and symptoms -Recruits were physically examined for symptoms like winter bottomôs sign, bloating, rashes and nervous systeminvolvement. Enquiries were also made from them about Somnolence and Insomnia**.**

b. Indirect diagnostic evidence -These were serological tests using the CATT *T. b. gambiense* kits and reagents.

The principle is that infection with *Trypanosoma brucei gambiense* results in the production of circulating antibodies against several surface antigens of the parasites. 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 *Trypanosoma brucei gambiense.*

The test was performed on a plastic or plasticized card. One drop of undiluted blood (screening test) or 25µl of diluted plasma/ serum (confirmation test) are mixed with one drop of reconstituted antigen. When antibodies are present in the test sample, trypanosomes agglutinate within 5 minutes rotation at 60 rpm (rotation per minute).

i. The CATT controls were reconstituted according to the manufacturerôs instructions.

With the aid of a syringe, 0.5ml of CATT buffer was added to a vial of the positive and negative control.

Quality of the antigen was checked and tested using one drop each of the positive and negative controls of the antigen after reconstitution of each vial of CATR antigen.

ii. Preparation of test samples (Undiluted blood was used for screening)

Finger tip was disinfected using mentholated spirit and pierced with a micro lancet.

Initial blood was wiped off.

A heparinised capillary tube was filled for about $^{3}/_{4}$ of its length avoiding air bubbles with the blood.

The capillary tube was tilted several times thereby mixing the blood with the heparin and placed horizontally on a capillary tube holder.

Then a suction bulb was used to drop the undiluted blood in a marked area on the card.

During the field surveys, blood samples of 5 ï 10 persons were collected and tested at a time.

Blood sample was mixed again before testing and when sedimentation of red blood cells was observed in the capillary tube and also when coagulation was observed, a new sample was collected.

Any sample that agglutinated was tagged a CATT positive sample and so further tests werecarried out, if not they were discharged.

However, for the CATT positive samples, further tests were carried out.

Quantitative analysis (titration) of the blood plasma/serum was carried out on the CATT positive samples.

Two fold dilutions of ¼, 1/8, 1/16, 1/32, and 1/64 in CATT buffer were prepared and using a micro pipette, 25µl of each dilution was added in attest area on the card.

1 drop (45µl) of well homogenized CATT antigen was added in each test area.

To obtain drops of constant volume, the dropper was held vertically and drops allowed falling freely without touching the card. A stirrer was used to mix the reaction mixture. The stirring rod was wiped after each use. The test card was rotated on a flat bed orbital rotator for 5 minutes at 60 rpm.

The results were read after 5 minutes rotation, before removing the card from the rotator. Results were read as follows and for the titration, read off at the point of end dilution.

+++ = Strongly positive (very strong agglutination),

++ = Positive (strong agglutination),

+ = Positive (moderate agglutination),

± = weakly positive (weak agglutination),

- = Negative (No agglutination). Note that the CATT reagents were kept in cool boxes to preserve their potency. The different antigens and buffers of different stock were not mixed.

c. Direct diagnostic evidence-These aremicroscopic/parasitological techniques and concentration methods. Wet blood film and buffy coat examination (Haematocrit centrifugation technique) were carried out only on sample population that tested positive serologically for confirmation of results. Format for the direct diagnostic test are provided in appendix 10. Lymph node aspirate was also collected from those that had signs of cervical lymphoadenopathy and examined for trypanosomes using the same x 100 microscopic magnification.

d. Staging of the disease - The participants that tested positive for the direct diagnostic evidence (trypanosomes were detected in their blood under a microscope with x100 magnification were further subjected to another procedure known as lumber puncture. Cerebrospinal fluid was collected and examined for the presence or absence of trypanosomes. This procedure was carried out only on participants with CATT titration of 1:8 and above who were willing (informed consent). White blood cell count in cerebrospinal fluid of participants with positive CATT results was carried outusing Neubauer counting chamber with a depth of 1mm). White blood cell counts higher than 5ml $x10^6$/L were indicative of infection (Appendix 10). All data generated were documented appropriately using recording forms (Appendix 10)

3.7.5. Laboratory analysis and source of reagents

Laboratory analysis was conducted in laboratories at the health facilities in LGAs were study was conducted. The CATT/*T. b. gambiense* CATT (Card agglutination Test for Trypanosomiasis) rapid test kit was procured from the Tropical Institute of Medicine Antwerp Belgium, the only manufacturers of CATT kit at the moment.A seropositive individual was a

person tested with the CATT reagents and on screening had a positive *T. b, gambiense* serological result with symptoms for HAT. Trypanosomiasis was confirmed when *T. b. gambiense* was demonstrated in the body fluids while a sero negative individual was anyperson tested with the CATT reagents who had a negative T. b. gambiense serological result and no symptoms for HAT were observed during screening exercise.

3.8 Data Management

Completed questionnaires were reviewed in the field before the electronic data entry to ensure that they were correctly administered by the interviewers. Data collected were entered and analysed using Microsoft excels 2007 version and Epidemiological-Info 6 version 3.5.3 software. Descriptive and analytical statistics were used to summarize the data obtained (percentage, 95% confidence interval, means, standard deviation). The differences in sero negative and sero positive participants were compared across the investigated variables using odds ratio and chi-square tests. The analytical data analysis was conducted using the chi- square test to compare categorical variables such as age, sex and occupation. Test result s was considered as significant if P value was Ò0.05. Only univariate and bivariate analysis were carried out on the data. Tables and figures were used for a holistic presentation of the result. Maps were drawn with the aid of Health Mapper Version 4.2 using coordinates generated in the field. Data discussions and recordings from the focus group discussions were also reviewed, analysed and documented appropriately.

3.9 Ethical Considerations

Approval for this study was obtained from the Delta State Ministry of Health/Primary Health Care Development Agency Ethics Committee. Permission was also obtained from the

Community leaders of the villages where the study was carried out. Informed consent was obtained from each eligible recruit for the study before questionnaire administration and sample collection. Confidentiality of information obtained was maintained (Appendix 24).

3.10. Limitations

The study could only cover seven LGAs because of several limitations which included the following:

a. Inadequate funding for the project

b. Insecurity in most parts of Delta State

c. Unfriendly topography

d. Lack of cooperation from some of the study recruits owing to fear of HAT stigmatization.

e. Insufficient capacity for HAT case detection, confirmation procedures and management.

CHAPTER FOUR

4.0 RESULTS

Reports show that a total of 1.028 questionnaires were administered with all the study participants responding appropriately in a response rate of 100%.

4.1 Socio-demographic data of respondents

The respondents comprised 518(50.4%) male, most of them (33.3%) were between 25 ï 34 years old and mean/average age of the respondents was 33.5 years. Most of the respondents were married (65.5%) and were educated to the secondary (28.1%) and tertiary (31.1%) education (Table 2 below). The female respondents were 510 (49.6%) meaning that more men responded that women.The age group that had more frequency was 25 ï 34 by 342 with a percentage of 33.3%. Conversly the age group that recorded the least frequency was 5-14 with 36 (3.5%). Married people turne out more than divorced or separated people 704 (68.5%) by 6 (0.6%).

As for the demographic variables of the serological positives, they were 28 in number. The details were shown in the table 2b below.

Table 2: Socio – demographic characteristics of the respondents, Delta State, 2011

Characteristics		**FREQUENCY (N = 1028)**	**Percentage (%)**
Gender	Female	510	49.6
	Male	518	50.4
Age group (years)	5-14	36	3.5
	15-24	158	15.4
	25-34	342	33.3
	35-44	191	18.6
	45-54	183	17.8
	55 &above	118	11.5
Mean Age ± SD	33.5± 6.1		
Marital Status	Single	284	27.6
	Married	704	68.5
	Widowed	34	3.3
	Divorced/Separated	6	0.6
Educational Level	No formal Education	174	16.9
	Primary	209	20.3
	Quoranic16	16	1.6
	Secondary	289	28.1
	Tertiary	320	31.1
	Others	20	1.9
Occupation	Artisan	118	11.5
	Others	210	20.4
	Farming/Fishermen	215	20.9
	Petty trading	151	14.7
	Retiree	22	2.1
	Student	233	22.7
	Health worker	79	7.7
Religion	Christianity	930	90.5
	Islam	35	3.4
	Traditional	48	4.7
	Others	15	1.5
Domiciled in village	Equal to 5 years	134	13.0
	5-10 years	200	19.5
	More than 10 years	694	67.5

Table 2b:Details of demographical information of the sero positive participants by village.

VILLAGE	AGE	SEX X	OCCUPATIONO	CATT		Wet blood film	CSF		
				Positive	Titre		Tryps +ve	WBC Count	HAT Symptoms
Aballa Uno	8	M	Child	+ve	1/128	-ve	-		Swollen stomach
	34	F	Teacher (Worker)	+ve	1/8	-ve	-		
	6	M	Child	+ve	1/8	-ve	-		
Tuomo	59	M	Worker	+ve	1/64	-ve	-		Rashes and swollen face
Urhuoka	11	F	Student	+ve	1/64	-ve	-		Rashes and swollen face
	7	M	Child	+ve	1/8	-ve	-t-ve	>5xl0^6/L	Dead had swollen face, stomach, lyphadenopathy
	80	F	Farmer	+ve	1/8	-ve	-ve	1.4xl0^6/L	Nil
	6	M	Child	*l +ve*	1:8	' -ve	-		*Nil*
	11	F	Student	+ve	1:128	-ve	-ve	1.4xl0^6/L	Fever
	60	F	Farmer	+ve	1:16	-ve	-		Nil
	6	M	Child	+ve	1:8	-ve	-ve	1.4xl0^6/L	Nil
	75	F	Farmer	+ve	1:32	-ve	-		Nil
		/ M	/ *Businessma* n	/ +ve	*{* 1:64	/ -ve	*I*	'	Fever
	25	M	Student	+ve	1:128	-ve	-ve	5.4xl0^6/L	fever, rashes
	14	M	Student	+ve	1:32	-ve	-		Nil
	9	M	Child	+ve	1:128	-ve	-		Nil
	25	M	Student	+ve	1:8	-ve	-ve	2.6xl0^6/L	Nil
	60	F	Farmer	+ve	1:128	-ve	-ve	5xl0^6/L	Nil
	12	F	Student	-s-ve	1:128	-ve	-		Nil
	31	F	Trader	+ve	1:128	-ve	-ve		Nil
	26	F	Student	+ve	1:128	-ve	+ve	6.6xl0^6/L	Comatose but treated and recovered, titre now '1:16
Owhelogbo	35	F	Student	+ve	1:8	-ve	-ve	1.4xl0^6/L	Fever
	6	M	Child	+ve	1:8	-ve	-		Fever
	22	F	Business	+ve	1:8	-ve	-		Fever
Ugbolu	40	M	Farmer	+ve	1:8	-ve	-ve	5xl0^6/L	Nil
	7	M	Child	+ve	1:8	-ve	-ve	1.6xl0^6/L	Nil
	70	F	Farmer	+ve	1:8	-ve	-		Nil
	35 35	F	Trader	+ve	1:64	-ve . 1	-ve	SxlO'/L	Fever, rashes

4.2 HAT prevalence

Twenty eight (2.7%) of the 1,028 respondents were HAT sero positive and out of the 28, two (7-1%) were confirmed to be microscopically HAT positive. Both confirmed cases had HAT stage two and one of them died before the study was completed. Therefore Prevalence was 2 cases per1000.

Seventeen(1.7%) sero positive cases were identified in Urhuorka during the survey and among these 17 cases were the 2 cases that were microscopically confirmed HAT cases. This was followed by Ugbolu village (0.4%) , Aballa Uno and Owhelogbo with (0.3%) respectively. Only 1 case (0.1%) was identified in Tuomo (Table 3). Sero positive cases were identified in five out of the seven villages sampled.

Table 3: Distribution of HAT sero positive cases in study area, Delta State, 2011.

LGA	Village	Number sampled	Number sero negative	Number sero positive (%)	Percentage	Number of microscopically confirmed cases(%)
Burutu	Tuomo	131	130	1(1.9)	0.1	0
Warri South	Pessu	94	94	0(0.0)	0	0
Oshimili South	Ugbolu	190	186	4(2.1)	0.4	0
Ethiope East	Urhoka	224	207	17(7.6)	1.7	2
Isoko North	Owhelogbo	100	97	3(3.0)	0.3	0
Ika South	Agbo Nta	127	0	0(0.0)	0	0
Ndokwa East	Aballa Uno	162	159	3(1.9)	0.3	0
Total	**Total**	**1028**	**1000**	**28**	**2.7**	**2(0.2)**

Table 4 shows details of the sero positive cases identified during the study by age and sex. The ages of 28 sero positive cases ranged from 6 to 75 years. Out of the 28 sero positive cases, 14(50%) were female meaning that male to female ratio is 1:1. The sero positive participants were mostlychildren (32.14%), students (25%) and farmers (21.4%). Altogether, the children and students made up more than half (57.14%) of the sero positive cases (Table 5). No Trypanosomes were seen in the blood samples and lymph node aspirates of all the 28 sero positive cases but were seen in the cerebro spinal fluid of the two confirmed cases.All 28 cases had high CATT titres; 8(28.6%) had 1:128, 4(14.3%) had 1:64, 1(3.6%) each had 1:32 and 1:16 respectively while 13(46.4%) had titres of 1:8. Only 12(42.8%) out of the 28 sero positive participants agreed for the collection of their cerebrospinalfluid.3 (25%) out of the 12 that had their cerebrospinal fluid examined for trypanosomes had white blood cell count that were more than 5x 10^6/L. 7(25%) of the 28 sero positive cases had visible symptoms of HAT.

Table 4:Distribution of sero positive cases by age and sex

Age range (years)	**Seropositive**	**Sex**	
		Male	**Female**
5-14	12(42.9%)	9(32.1)	3(10.7)
15-24	1(3.6)	0.0	1(3.6)
25-34	5(17.9)	2(7.2)	3(10.7)
35-44	3(10.7)	1(3.6)	2(7.2)
45-54	1(3.6)	1(3.6)	0.0
55 & above	6(21.4)	1(3.6)	5(17.9)
Total	**28**	**14(50)**	**14(50)**

Table 5: Distribution of sero positive cases by occupational groups

Occupational group	**Number sero positive (%)**
Students and children	16(57.1)
Teachers/public servants	2(7.2)
Farmers	6(21.4)
Traders	4(14.3)
Total	**28(100)**

4.3 Knowledge about HAT and risk factors

Out of the 1028 respondents across the various LGAs in Delta State, 672(65.4%) reported that they had heard of Human African Trypanosomiasis (HAT) at various times in the past. Out of these, 578(86.0%) knew the correct cause and mode of transmission of the disease, 562(83.6%) knew how to seek care while 612(91.9%) knew how to protect themselves from the vector, tsetse fly, which transmits the disease. Figure 3 shows distribution of or according to knowledge of HAT by respondents.

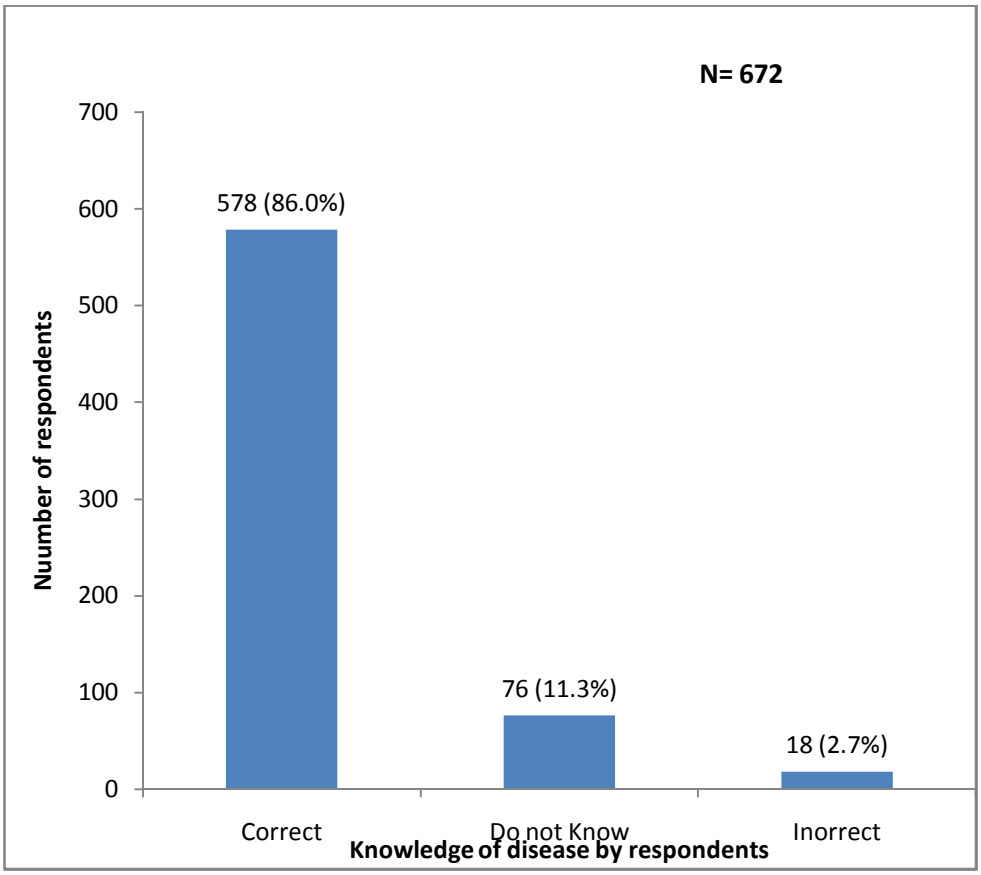

Figure 1: Distribution of knowledge of HAT by respondents, Delta 2011

4.4 Knowledge about HAT transmission

Of the 672 respondents who reported that they were knowledgeable about the cause and mode of transmission of Human African Trypanosomiasis, only 578(86.0%) of them actually knew the correct mode of transmission. Out of the 578 respondents, 126(18.8%) heard about HAT from media workshops, family and friends, town announcers, posters and in school. Respondents that heard from the media were 122(18.2%), 109 heard from family and friends. Workshops only appeared to be the source of information people least benefitted from regarding information and knowledge about HAT. More details are provided in figure 4 below.

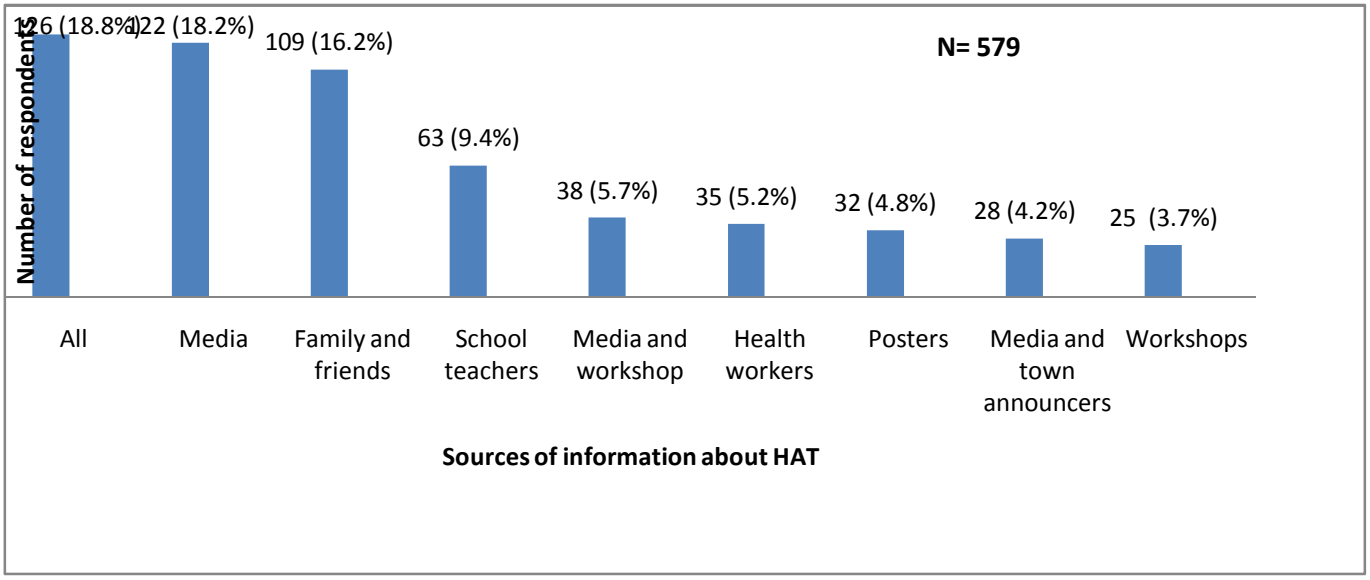

Figure 2: Sources of information about HAT, Delta 2011

4.5 Knowledge of Symptoms of HAT

Of the 579 respondents who knew at least one correct symptom of HAT, 522(90.2%) of them knew about somnolence alone being the most common symptom of HAT. Others listed somnolence and insomnia(4.8%), somnolence and dizziness (3.8%) and taxia (1.2%). Details of the symptoms as described by the respondents are shown in figure 5 below.

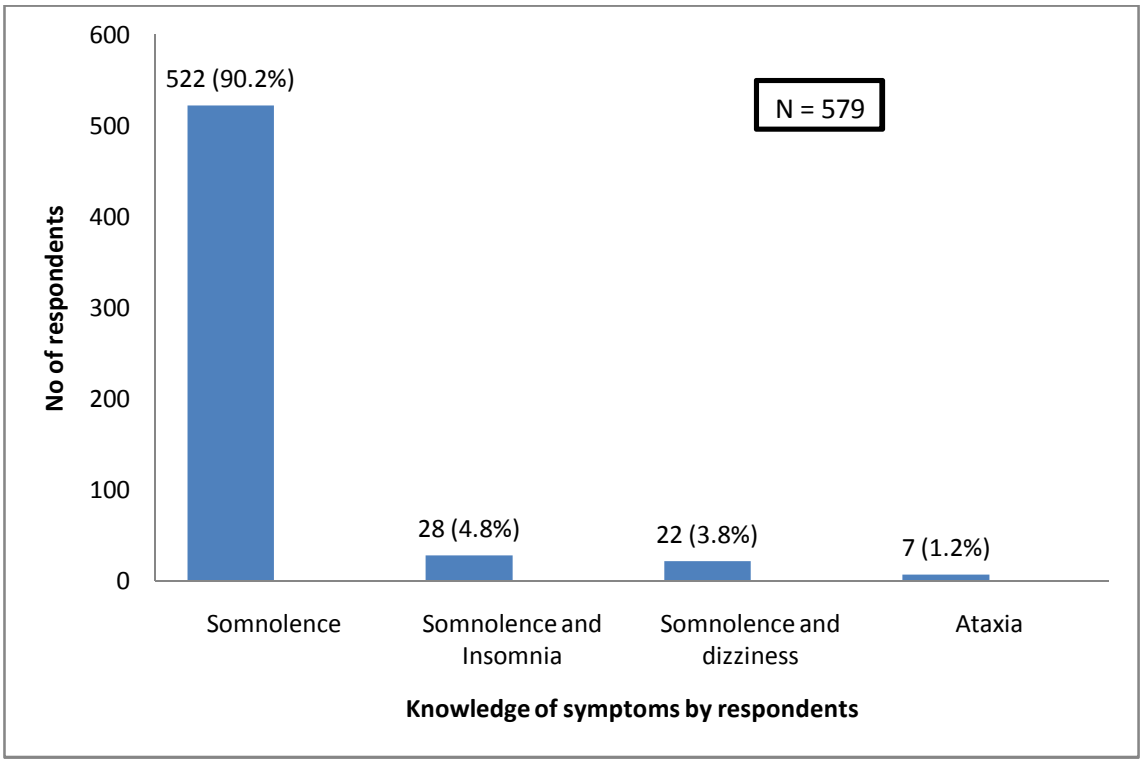

Figure 3: Knowledge of HAT symptoms by respondents, Delta 2011

4.6 *Knowledge about HAT occupational groups*

Out of the 578 respondents that had knowledge of the cause and mode of transmission of HAT 162 (28.0%) were students, 154 (26.6%) were farmers and fishermen, 86 (14.9%) were public and private workers including business men and 53 (9.2%) were health workers. Figure 6 shows the distribution of knowledge of the mode of transmission of the disease as reported by various occupational groups.

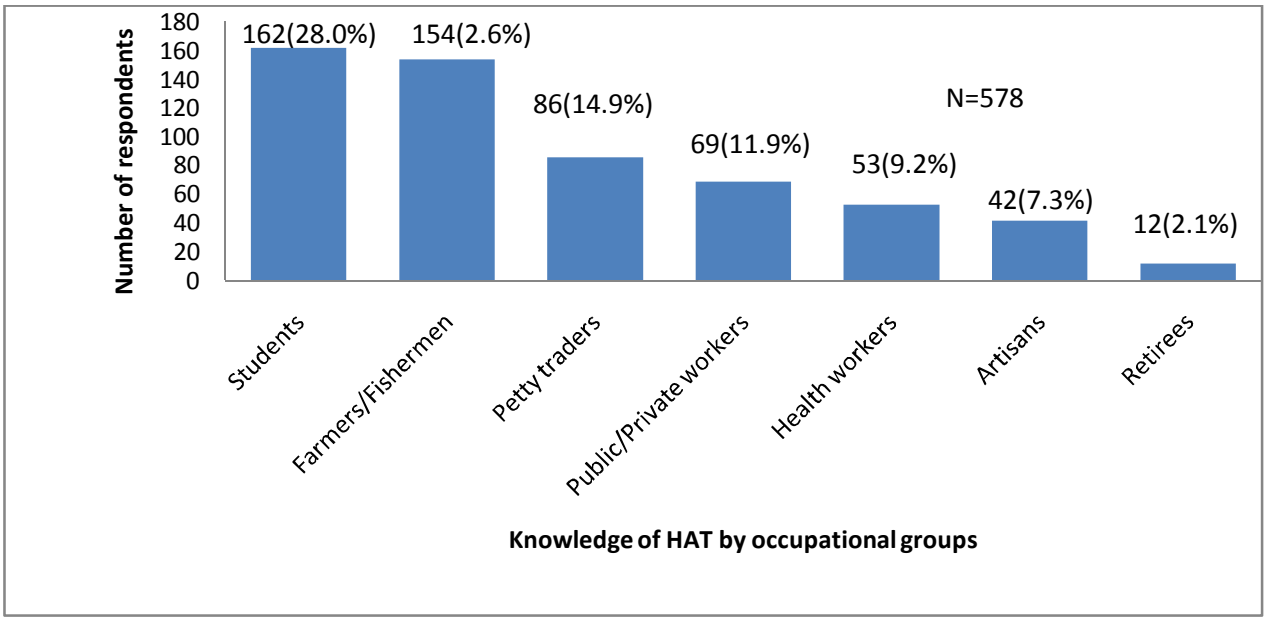

Figure 4: Knowledge of HAT mode of transmission as exhibited by respondents among various occupational groups, Delta State 2011.

Out of the 79 (7.7%) respondents who were health workers as shown in figure 6, only 55 (69.6%) correctly knew how to seek care, 53 (67.1%) knew the correct cause of the disease and mode of transmission, 52 (65.8%) knew the correct symptoms for HAT and 37 (46.8%) knew how to protect themselves from the bites of the vector, Tsetse flies (Table 5).

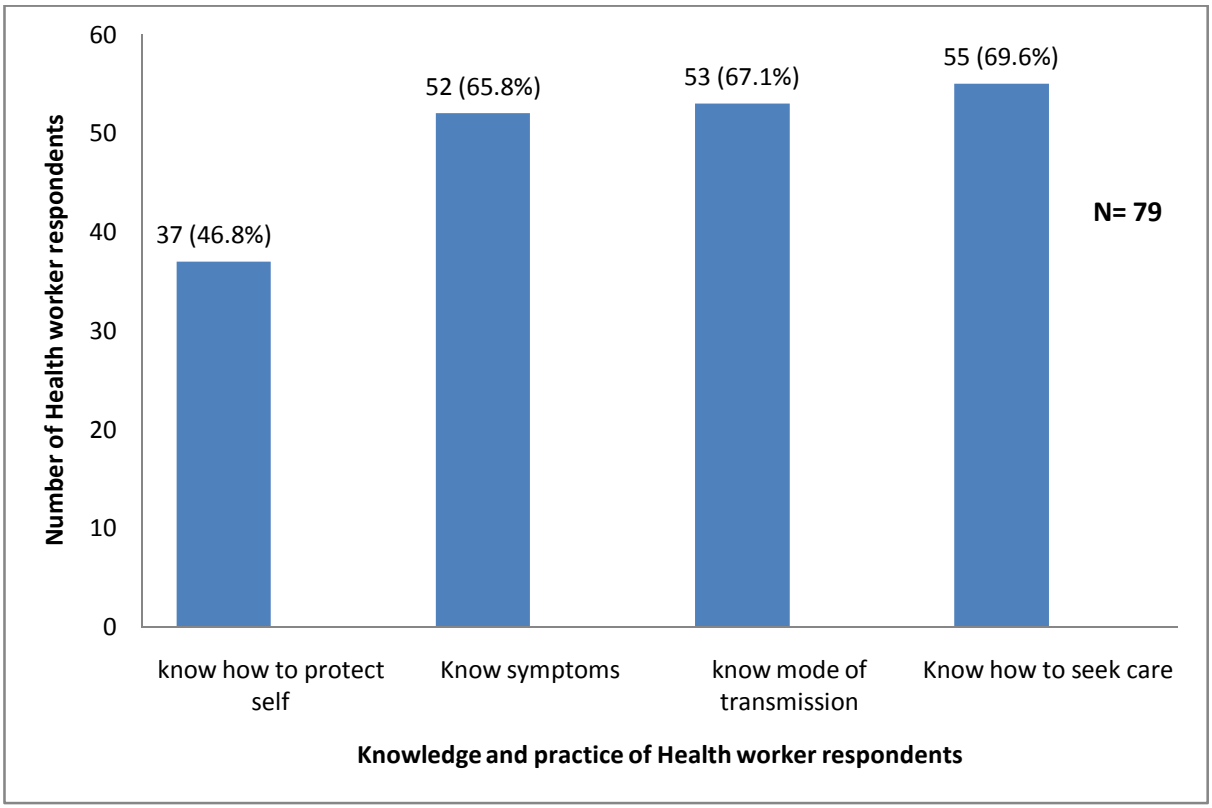

Figure 5: Knowledge and practice of 79 health worker respondents against HAT risk factors

4.7 Knowledge about personal protection against HAT transmission

Figure 8 shows the distribution of knowledge of personal protection against HAT by the respondents who claimed to have the knowledge of personal protection against the HAT vector, Tsetse fly. Of the 612 respondents, 203 (33.2%) stated that wearing protective clothing was protective, sleeping under insecticide treated nets (ITNs) was mentioned by 171 (27.9%) respondents, clearing bushes around residents and water domestically used mentioned by 117 (19.1%), use of insect repellents including burning fire around dwellings stated by 79 (12.9%) and killing Tsetse flies including use of synthetic insecticide in residences mentioned by 42 (6.9%) respondents were also protective.

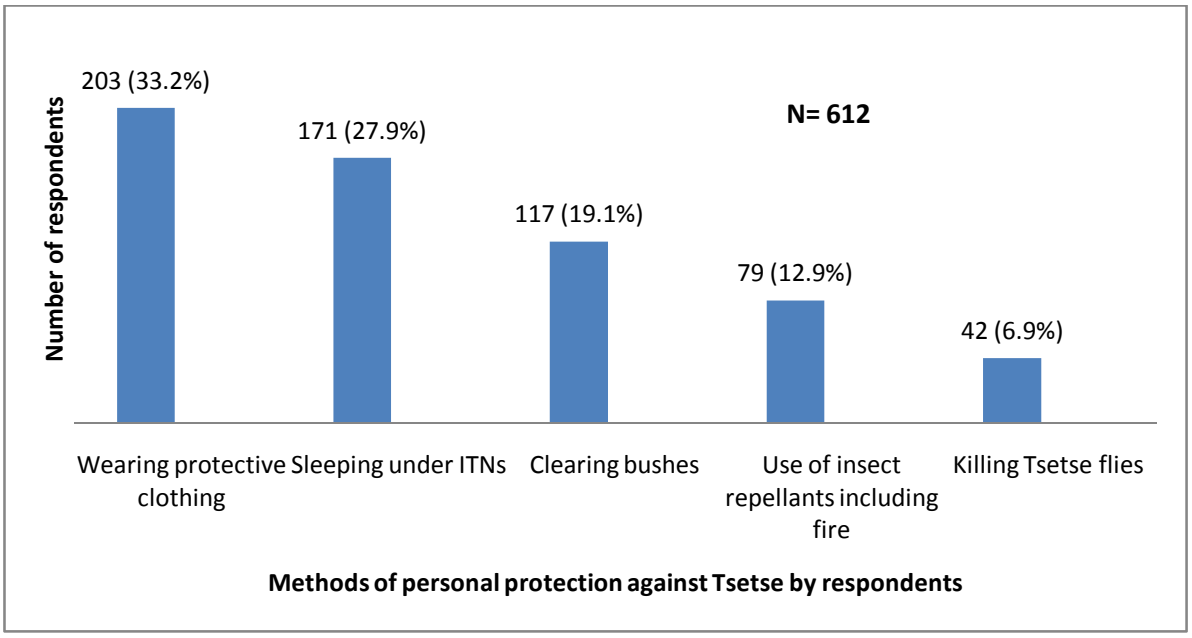

Figure 6: Knowledge of personal protection from vector transmitting HAT asexhibited by the respondents

4.8 Knowledge of HAT among those who were seropositive

Of the 1028 respondents screened for HAT, only 28 92.7%) were sero positive. Of these 28 respondents who tested positive for HAT, 17 (60%) were from Ethiope East, 4 (14.22%) from Oshimili South, 3 (10.7%) each from Isoko North and Ndokwa East and 1 (3.6%) from Burutu LGAs of Delta State.

Fifteen(53.6%) out of the 28 respondents who were sero positive had knowledge of symptoms of HAT, 22 (78.6%) had knowledge of personal protection against the vector transmitting HAT, 23(82.1%) had knowledge of the cause and mode of transmission of HAT and only three (10.7%) and knowledge of how to seek care when ill with the disease (Table 6).

Table 6: Knowledge of factors that can protect one from contacting HAT by sero positive cases

Factors	Number (%)
Knowledge of cause of HAT and mode of transmission	23 (82.1)
Knowledge of personal protection	22 (78.6)
Knowledge of symptoms of HAT	15 (53.6)
Knowledge of how to seek care when ill with HAT	3 (10.7)

As shown on table 7, factors associated to seropositivity to HAT varied significantly among respondents in Delta State. Knowledge about HAT symptoms was associated with sero positivity of HAT and was found to be significant from the bivariate analysis, or (95% CI) was 1.04 (0.49 ï 2.22). The bivariate analysis indicated that knowledge of the symptoms of the disease was not significant to seroposotivity. However, knowledge of where to seek help when ill with the disease, personal protection and cause of disease were significant to sero positivity.

This means that the two main risk factors of Hat in the study area are; ignorance of where to seek medical help and lack of knowledge about personal protection.

Table 7: Bivariate analysis of factors responsible for HAT among Sero positive respondents in Delta State, Nigeria 2011.

Variable	Seropositive N%	Seronegative N%	OR 95% CI	P value
Knowledge of Symptoms	15(2.6)	564(97.4)	1.04(0.49ï 2.22)	0.94
Knowledge of cause of HAT	23(4.0)	555(96.0)	4.37(1.65-11.58)	0.02
Knowledge of how to seek care	3(0.5)	559(99.5)	0.095(0.03-0.32)	<0.01
Knowledge of personal protection	22(3.6)	590(96.4)	4.69(1.88-11.66	<0.01
Length of stay in the village	27(3.1)	848(96.9)	4.84(0.65-35.88)	0.06

Values sValues significant at pÒ0.05, Values for mid-P exact and values for Fisher exact test

Alsoto calculate final knowledge score where 672 respondents (65.4%) reported having heard about

HAT, 578(86.0%) knew the correct cause and mode of transmission, 562(83.6%) knew how to seek

Care, 612(91.9%) knew how to protect themselves from the vector where each correct question is sc

Ored 1 correct or 0 for wrong,;

Poor Knowledge score = Total score< 50%

Average knowledge score = Total score 50% to <75%

Good knowledge score = 75% and above

Therefore $\frac{\text{Total correct answer}}{\text{Total possible correct answer}} \times \frac{100}{1}$ = Final Knowledge score

$\frac{672+578+562+612}{1028+1028+1028+1028}$ = 58.9% (A good knowledge score)

4.9 Geographical data from study area

Table 8 shows geographic coordinates of the study area, seven villages in seven LGAs of Delta State Nigeria and figure 8 shows spatial representation of these villages on the map of Delta State.

. **Table 8: Geographic coordinates of villages in study area**

LGA	Village	Coordinates	
		Latitude	Longitude
Burutu	Tuomo	5.12719	6.56909
Ika South	Agbo Nta	5.01576	7.51312
Isoko North	Owhelogbo	5.53102	6.12904
Ethiope East	Urhuorka	5.68624	6.00310
Ndokwa West	Aballa Uno	5.82820	6.56909
Oshimili South	Ugbolu	6.14368	6.73116
Warri South	Pessu	5.53489	5.63842

4.10 Analysis of Focus Group Discussion

Table 9 shows data from field notes and excerpts from transcripts generated during the seven sessions of focus group discussions held in the study sites. The discussions were held with women groups in the villages in Delta North, a village development committee in Urhuorka village in Delta South senatorial district and youths in villages in Delta Central senatorial districts. The Focus Group Discussion Guide (Appendix 6) was used during the discussions.

Most participants in the focus group discussion had good knowledge of HAT and the vector.

Participants knew symptoms of HAT and mentioned villages they claimed had reported HAT cases. During the discussion, it was observed that almost all the ethnic groups had a name for HAT and the vector, Tsetse. Discussants stated that they did not seek medical attention when ill or advice people that had HAT to do so because they believed HAT may not be cured with orthodox medicine, Medicare was expensive for them and they werealso avoiding stigmatization**.** Case reporting to appropriate places was poor. Most of the discussants believed they could be healed by the oracles. Discussants stated that their best means of having access to health information is through the radio (electronic media) transmitted in their local languages and dialects.

Table 9: Hand coded Fieldnotes from Focus Group Discussion on HAT, September 2011

QUESTIONS:	RESPONSE:
Knowledge, attitude and Community practice	Common diseaseses in our community are malaria, HIV/AIDs, childhood diseases and slee ping sickness. Yes we have HAT and I have seen people with it. It has been long since we had it in my place but l learnt that people from neighbouring villages still suffer from it. Local names of HAT given were Ure, Amo-ula, Aisan orun sisun, Buru don, Emu ula, kuda chio mbachi, Emu oran, Iba ura, Iberibe, Idumeje, Odo fever, Sapele fever, Kobo ejurun, Ula elo/Mbele hule, Umuokoro lowa,Oga ovwerhe, Yeshi, Sienden, Ula anu isi, Ula eezo, Utade uno, Sien bunudon. People get disease by the bite of Tsetse fly. Local names of Tsetse were given as Agbangba, Adagbrassa, Agbanyigba Ebu, Odudu, Odudu Oji, Odudu enyi. I saw the disease in Aballa Uno, Akoko Uno, Ashaka `The disease is in Aviara, Bomadi,Kweale, koko, Umunede, Ika, Patani, Ugono, Oko Amakom Ogbareke still suffer the disease, person die there sefô They get the disease in Urhuorka, Beach koko, for my village, Ayara town, Ashaka, Aleibiri, Agbon Olomu/Etefe village, Aboh, Dunflox hospital Abraka, Opuama, Mile 5ô We have seen person with disease last year but he is dead nowô We see people 3 months ago, last week, 2weeks ago, recently.ô
Case Detection and reporting behaviour	I dey affect women and young men, small children tooõõ If people are sick, we pray for them. Asked what they would do if someone has HAT, a man replied ask him to see oracle because he cannot be treated in hospitalõõ The groups mentioned the symptoms when asked but said they will not report to health services and cost makes me not to report or advice so.
Access to health information	``Make you send message through radio in different languages; ijaw, ika, .urhobo, pidgin English, isoko and others.

CHAPTER FIVE

5.0 DISCUSSION

From the study above, 28 (2.7%) out of 1028 persons sampled were sero positive for HAT using the CATT/*Trypanosoma brucei gambiense* and in 2 samples out of the 28 trypanosomes were sighted under the microscope. This is an indication that Human African Trypanosomiasis, characterised by non specific clinical presentation and no consistent pathogenic manifestation in Nigeria. The prevalence rate is lower but leans towards findings from previous studies that reported HAT outbreaks in Nigeria resulting in death in some endemic foci yet the status of the disease is still not well understood in Nigeria. In the present study, only 2 cases were confirmed by definitive diagnosis but all the 28 sero positive cases had had high CATT/*T. b. gambiense* titres. Fifteen (53.6%) of the 28 seropositive cases had CATT/*T. b. gambiense* titres of 1:16 to 1:128. Four of the seropositive cases which included the 2 confirmed cases had almost same symptoms and high white blood cell count ($>5x10^6$L) in the cerebrospinal fluid analysis indicative of infection.

Between 1989 and 1996, out of the 3,583 people examined in the Abraka focus of Delta State, 104(2.9%) were microscopically confirmed positive and 359 (10%) were sero positive. Only 127 of these cases could be treated and seven fatalities were recorded. In a related study, out of the 4, 966 persons screened, 497 (10%) were sero positive, and 298 (6%) of the seropositive had the disease. Sixty seven of these HAT cases, second stage, were treated at the Eku Baptist Medical Centre Eku Delta State between 2002 and 2005.

A prospective cohort study conducted in Kajo-keji County of Southern Sudan 2002[235] where socio-demographic and laboratory data of a cohort of 2,274 serologically suspected individuals were collected and analysed. Among 33% (n=749) that attended at least one follow

up visit, HAT was confirmed in 64 (9%) cases. Individuals with lower initial CATT plasma (CATT P) end dilution titres had lowest risks (10.4 and 13.8/100 person-years for 1:4 and 1:8 titres respectively, that significantly increased for higher dilutions. The cumulative yearly risk was also high (76%) in individuals found with high (11-20) cells in the cerebrospinal fluid, but this involved only 8 patients. In conclusion, it was recommended that treatment with pentamidine (first line medication for HAT stage 1) should be considered for all serologically suspected individuals with a CATT-P end-dilution titer 1:16 and above in areas of a moderate to high prevalence of HAT.

In the present study, length of stay in the villages where study was conducted was not significantly related and same was the case with knowledge of the disease. These results were not in conformity with those from a study carried out on sleeping sickness and tsetse awareness in Zambia in !988, a total of 1940 Tambo and Lambya men and women that live in Luangwa Valley of Isoko District in the Northern province of Zambia were interviewed to determine their knowledge of HAT and tsetse flies. Result from the study show that 98.4% of the respondents who had lived in the area for at least one month knew that tsetse fly bites transmitted trypanosomiasis, only among the people who lived in the area for less than one month believed that witchcraft and bad water transmitted sleeping sickness (0.9 and 0.1 respectively). In the same study, 94.2% of the people who lived in the area for more than one month also knew the cause and method of transmission of the disease.

However, like in the Zambian study, 3 (0.01%) people in the present study stated that the disease was caused by ancestral spirits and living near bad water. In all, there was a reasonably high level of awareness among all the respondents, 86% (n=672) regardless of their length of duration of residence in the villages and their age groups.

In the present study, 126 (18.8%) of the 578 respondents that had their source of information about HAT from a combination of all the sources of information appeared to support advocacy and social mobilization approach which according to WHO demonstrates that the correct problem identification synergized with community engagement can potentially reduce transmission, spread and morbidity.

Unfortunately, out of the 79 (7.7%) respondents who were health workers, only 55 (69.6%) knew how to seek care, 53 (67.1%) knew the course of the disease and its mode of transmission, 52 (65.8%) knew the correct symptoms of HAT and 37 (46.8%) knew how to protect themselves from the bites of the vector, tsetse fly. In situations like this, the health care personnel will be unable to provide the necessary information and care to the seekers of health and the general public.

In another study in Democratic republic of Congo, the level of Knowledge, behaviour, practice and local beliefs about sleeping sickness among residents of the endemic zone of Kinshasa were assessed. A total of 437 cases and 874 controls were included in thecase control study using structured questionnaire and focus group discussion. The level of knowledge of elementary concepts about trypanosomiasis was low about case- patients (44%). That trypanosomiasis originated supernaturally was the belief and these were considered to be divine, sorcery and transgression of rules. Several respondents (31.4 %) called on churches for help and some (87%) of participants were in favour of passive detection. Factors associated with the acquisition of knowledge of Human African Trypanosomiasis included education level (elevated :81%, Low:19%; $p<0.0001$), age> or = 20years old; 89.9%, <20 years old: 10.1%; $p <0.0001$, sex (57.2% of patients were males and 42.8%) were females; $p <0.001$, birth place (51.4%) were

not native of Kinshasa and 48.6%) were indigenous or born in Kinshasa ; $p<0.05$) and travel or stay in endemic areas (yes: 56.3%, no:43.7%; $p<0.0001$[236].

Socio demographic factors were not found to affect acquisition of knowledge about HAT in the present study. Data from the present study show risk factors that are sero positive for HAT to be lack of knowledge, about how to seek care when ill, lack of knowledge about personal protection against the vectors ($p<0.01$ respectively) and lack of knowledge about the cause of the disease ($p=0.002$). On the other hand in Uganda, a case controlled study conducted in 17 villages to assess risk factors for HAT showed having a family member with history of HAT , presence of cattle around the homestead, wetland buffer<500m ($p<0.05$) and male (63%). These data support the fact that HAT outbreaks in previously unaffected northern districts in Uganda have been linked to cattle migration and human proximity to the animal reservoir. Risk factors identified in the present study were not similar to those identified in the case control study carried out in Uganda. This is because T. b. gambiense, the species found in Nigeria is not zoonotic unlike T. b. rhodesiense found in Uganda.

Studies on mapping using geographic coordinates have added impetus to sparcial epidemiology and contributed immensely to disease surveillance, control and management. World Health Organization (WHO) aims at mapping HAT endemicity foci[237]. Collection and use of the geographical coordinates contribute to the efficient development of the village level mapping of HAT endemicity foci. According to previous work done on the generationof geographic coordinates and mapping for HAT[238], data generated in this study will provide detailed and reliable representation of the known geographic and special distribution of the disease.. It will contribute to the promotion of research and targeted disease control activities. It will also reduce the number of undetected and unreported cases and provide more reliable

evidence that will be crucial tools for making informed decisions to plan and monitor HAT elimination activities[238].

Data from focus group discussion were similar to data from the qualitative analysis.Level of knowledge about the disease and its mode of transmission were high. Discussants had knowledge of the symptoms of the disease and to some extent how to protect themselves from the vectors. Case detection and reporting, also seeking care when infected with HAT was poor. Focus group discussions are particularly useful for exploring peopleôs knowledge and experiences and could be used to examine not only what people think but how they think and why they think that way. They are considered to be data collection techniques that are sensitive to cultural variables and are often used in cross cultural research work with ethnic minorities such as those in this study[239]. Tapping into such personal communication was important to highlight sub cultural values or group norms. Analysis of the result developed from the discussions provided information on shared and common knowledge about HAT in Delta State. Control of HAT needs a firm and sustained commitment of political institutions, healthcare providers, donor agencies and other stake holders over a long time, as well as stable socioeconomic environment. Due to the recent resurgence of sleeping sickness, an effective control and surveillance initiative is imperative.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study has confirmed that Human African Trypanosomiasis (HAT) exists in Nigeria with a sero prevalence of 2.7% occurring in areas previously unknown to be endemic. HAT is a public health problem in Nigeria that needs to be addressed immediately considering the cumbersome method of diagnosis and the potential risk factors sero positive cases may constitute as disease reservoirs in the community.Distribution of the disease was found to affect most age groups and especially the age group, 5 to 14 years, children and students. The study established the fact that poor knowledge about how to seek care by the villagers protect themselves from the vectors of the disease and not know the cause of the disease was risk factors of HAT transmission in the study area. Length of habitation in the community did not contribute to people that were sero positive. Transmission was ongoing in the communities as most sero positive cases had very high CATT/T. b. *gambiense* titres, >1:16. Many health workers in Delta State did not know how to seek care, protect themselves and even recognize symptoms of HAT, therefore would be unable to give adequate care and information to the HAT cases.

Apart from the urgent need for better tools for the field diagnosis of this neglected tropical disease, improved access to diagnosis and treatment for the population at risk remains the greatest challenge for the coming years. Tapping into interpersonal communication through focus group discussions was important to highlight indept cultural values and group norms. Review and analysis of results derived from these discussions provided information on shared and common knowledge about the disease in the state.

Serological tests such as CATT/*T. b. gambiense* tests are useful for population screening to identify suspected cases but may not be sufficiently reliable for definitive diagnosis since variations in sensitivity and specificity of the serological tests have been observed in various places and disease conditions. Therefore the CATT/T. b. gambiense is followed normally by a parasitological test (microscopic examination) of the sero positive samples for confirmation of infection in most endemic areas but unfortunately, field parasitological methods lack sensitivity and so the management of sero positive individuals and especially the persons that are positive with CATT result but negative parasitological remains controversial and disturbing.

6.2 Recommendations

Following the present study, the following recommendations may be necessary if we are to successfully control and ultimately eliminate Human African Trypanosomiasis from Nigeria and the sub region in line with the PATTEC initiative:

1. The National/ Delta State Government should invest more in the control and prevention of Human African Trypanosomiasis.
2. Massive publicity campaigns in the different Delta languages should be embarked upon at all levels on HAT symptoms, cause of disease, protection from the disease vectors and how to seek care when they are ill.
3. Sensitization, training and retraining activities on HAT control, prevention and management should be conducted regularly for health care providers to build capacity adequate enough for HAT control, prevention and management in the state.
4. Systematic, efficient and sustainable HAT surveillance systems should be established and maintained in Delta State.

5. Adequate awareness about control and prevention of HAT should inculcated into the school curriculum for Delta State students/pupils since students and people within the age group 5 to 14 years were mostly affected by the disease.
6. Delta State Ministry of Health should sensitize physicians on proper medical diagnosis of illnesses. That is to say that physicians and laboratory scientists should have a high suspicion index for HAT infection especially for patients living across the tsetse infested areas. Governments on their part should make health care services affordable and accessible to enable the rural poor who are most afflicted by this disease to have the boldness to seek Medicare when they are ill.
7. Lessons manifesting from this study could and should be used to strengthen the national HAT control and elimination programme.

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APPENDICES

Appendix 1: Showing study area and distribution of respondents, Delta State 2011.

Senatorial districts	*Ethnic group*	*LGA*	*Village*	*Number of respondents*
Delta Central	Ijaw	Burutu	Tuomo	131
	Itsekiri	Warri South	Pessu	94
	Isoko	Isoko North	Owhelogbo Edemoko community	100
Delta North	Aniocha	Oshimili South	Ugbolu	190
	Ika	Ika South	Agbo Nta	127
	Ukwuani	Ndokwa East	Aballa Uno	162
Delta South	Urhobo	Ethiope East	Urhuorka	224
Total	**Total**	**7 LGAs**	**7 Villages**	**1028**

APPENDIX 2: HUMAN AFRICAN TRYPANOSOMIASIS OR SLEEPING SICKNESS BASELINE SURVEY- Focus Group Discussion Guide

- **GENERAL**

Preliminaries

A. **Introduction**

Welcome participants

Describe what FGD is- a group discussion that allows you to discuss the topic among yourself rather than talking to us.

B. **Purpose/Module Operandi**:

We will be discussing diseases that affect you now and or affected you in the past, and in particular, Human African Trypanosomiasis (HAT) otherwise called sleeping sickness.

We are interest in all your ideas, comments and suggestions.

All comments both positive and negative are welcome.

Please feel free to disagree with one another. We would like to have many points of view.

(WE WOULD WANT YOU TO DISCUSS ALL THE ISSUES AMONG YOURSELVES)

(Explain of audio tape)

All comments are confidential and for research purposes only.

We would also want you to speak one at a time so that the rape recorder can pick your voice appropriately and clearly, and we heat and understand each other properly.

KNOWLEDGE, ATTITUDE AND COMMUNITY PRACTICES OF HAT

What are the common diseases that affect people in this community now or in the recent past (5-15 years back?) Probe for HAT is not mentioned, and the common name or name in vernacular.

Have you ever seen a person with HAT? If yes, probe for decryption: symptoms and signs if possible ï winter bottomôs sign, sleep and nervous disorders, cachexia (body/muscle wasting).

What do you think should be done to someone that has HAT in your community?

Have you ever had HAT in this community? If Yes, probe for:

Trend over time

Who is most affected in the community; men, women or children

Why does having HAT worry them, Probe by:

Individual level
Family level
Community level

- When someone has HAT who do you send him/her to for treatment? Probe by:
  Children
  Adult males
  Adult females
- How does a person get HAT?
- What are the things people in the community should do to avoid getting HAT? Probe for a listing of the actions required.

Reporting behavior

- If your brother or sister or other family member has symptoms of HAT- general and continued feeling of ill health, sleeping and nervous disorders, cachexia, winter bottomôs sign-, what will you do? Probe for IMMEDIATE reporting to the:
  i. Village based health volunteer, or ii. Village head/leader or
  ii. Nearest health facility iv. Traditional medicine doctor v. nothing is done
- Insist that village based health volunteer and village head/leader are away from the village at the time of suspecting the case and ask what they will do in the circumstance.
- What are the means available to you for reporting to the health facility staff? Probe for:
  Adult males
  Adult females
- If you see a suspected case of HAT what circumstances may stop you from immediately reporting to the nearest health facility? Probe for:
  Adult males
  Adult females
  Distance of Heath facility to community
  Sacrificing farm work time to report to health facility
  How frequent absenteeism by PHC facility health workers may affect his/her zeal to report.

- In this community, if a child notices they may have HAT, who will the child reportto?
- In this community, if a woman notices she may have HAT, who will she report to?

- In this community, if a man notices he may have HAT, who will he report to? Some people may have HAT and decide not to let anybody know. Why is that so?

- Have you heard announcements about reporting suspected cases of HAT?

- If on two separate occasions you treck to the health facility to report suspected casesbut fail to get attention will you report any more suspected cases that you see?

Access to information

- If government were to constantly give you information on HAT, what are the available means for the information to reach you in this community?

Thank respondents and play a bit of the tape for them to hear. Also talk to them about the project and seek their cooperation and support during the exercise.

Appendix 3:Study Participants' Information Sheet

Title of the Study:

PREVALENCE AND KNOWLEDGE OF HUMAN AFRICAN TRYPANOSOMIASIS (HAT) IN DELTA STATE, NIGERIA - 2011

Brief Explanation of the Purpose of Research:

Recent data on the burden of disease and endemicity foci of Human African Trypanosomiasis (HAT) or Sleeping Sickness in Nigeria are not available. The data generated by this study will add impetus to the few available data on risk factors and extent of the problem in the study area. This study focuses on residents of Delta State. This is because suspected HAT cases have been reported from different parts of the country and especially Delta State and the form present in Nigeria is chronic, presenting like several other common ailments in Nigeria and may be fatal if undetected and untreated. Due to poor index of suspicion for the disease, it is normally rarely diagnosed in most health facilities in the country and when it is suspected it is usually in the second stage of the disease which in most cases ends up in death.

The main purpose of this research is to provide base line data for the assessment of HAT epidemiological status in order to determine the prevalence and awareness of HAT in Delta State. Nigeria. The specific objectives are;

a) To determine the prevalence of HAT in Delta State

b) To assess the risk factors associated with HAT among the affected persons.

c) To assess the general awareness about HAT in Delta State

d) To generate geographic coordinates and map identified HAT endemic villages in study area;

e) To suggest ways for control and prevention of HAT in Delta State and Nigeria.

Expected benefits for Participants in the Study:

Free medical examination and management of HAT where necessary will be provided for participants by qualified health personnel. The results of the test will be communicated to participants so that they are aware of their health status and seek medical care on time where necessary. Multivitamins and beverages will be given to each participant. Capacity will also be developed in the State, especially at the community levels, to improve early case detection, reporting, seeking medical assistance early and management of HAT.

Responsibility of Participants and what is expected of them in the Study:

Each participant is expected to voluntarily participate by signing the consent form. Each participant is also expected to willingly allow the research team finger prick them and collect a drop of blood each for serological tests and where necessary, an additional 5mls of blood to be from his or her arm for further examination (CATT *T. b. gambiense* titration), and a lumbar puncture for the collection of cerebro spinal fluid for further examination (staging of the disease) where necessary. The processes stated above will avail the research team the opportunity to detect the disease where it exists and manage the case effectively. Each participant is also expected to willingly answer the questions read to him or her by the interviewer,

Risks for participating in the Study:

Drawing blood from the arm is usually very safe and only causes discomfort for a short time. It is a routine procedure done in most health facilities and should not cause any harm especially

When done by qualified health care providers. The lumbar puncture is also safe if done by skilled health providers. It may cause some discomfort and head ache afterwards but analgesics and anti inflammatory medication are provided after the procedure to avoid these effects. Participants are also followed up for a week to monitor for any other effects. Other less common risks are slight swelling around the puncture sites.

Privacy and Confidentiality:
All participants will be assured that privacy and confidentiality of all information provided as well as the test results will be maintained.

Voluntary Withdrawal from Study:
All participants will be assured that they can voluntarily withdraw from the study if they are no longer interested at any point in time when they wish to withdraw or do not want some of thes carried out on them.

Appendix 4

GENERAL QUESTIONNAIRE ON HUMAN AFRICAN TRYPANOSOMIASIS KNOWLEDGE ATTITUDE BEHAVIOUR AND PRACTICE (KABP) SURVEY

Introduction:

Until recently, Nigeria was considered to have eliminated Human African Trypanosomiasis (HAT) or Sleeping Sickness, a Neglected Tropical Disease that can be fatal if untreated. Contrary to that view, the disease is re-emerging and has been detected in some parts of the country where hither to, it never occurred. Nigeria established the HAT elimination Programme in 2007.

HAT that is prevalent in Nigeria is the chronic form which is often mistaken for other common diseases prevalent in our country and is therefore not detected early. As a result, the disease gets to the second or terminal stage before it is detected and at this stage management becomes more difficult. With the present survey, the endemicity foci for HAT and your thoughts about the disease will be identified. This information will help government plan better health care services for the people of Nigeria and put necessary interventions for the elimination of HAT in place.

My name is .....and we are from the Federal Ministry of Health toask you a few questions about HAT and also take samples for analyses for HAT. Your frank views and opinions are going to help us map out how we will eliminate HAT from Nigeria. I will appreciate it if you can spend about 30 minutes talking with me. All information given by you will be strictly treated as confidential. I will write down your name if you consent to it, otherwise I will not. Writing your name will enable me to identify you and manage the condition effectively if you are found to be affected by HAT.

We would also be collecting very little quantity of blood from your arm which will be tested for the presence of *Trypanosoma brucei gambiense,* the causative organism for HAT and where necessary further test will be carried out but this will be explained to you if the need arises. Drawing blood from the arm is usually very safe and only causes discomfort for a short time. Otherless common risks are bruising and swelling around the puncture site. You will be given theresult of this test later.

Your participation is voluntary and you are not obliged to answer any questions you do not wantto answer or allow any procedure you do not want to be carried out on you. Your participation and any information you may give will not be used against you.

Do we have your permission to continue?
If yes - continue with the interview and tests.
If No - stop the interview, tests and/or procedure; thank the respondent, note 'one refusal' on the Non-respondent form; move to the next respondent's house or position.
Thank you.

HAT 011/001 **House No._____**

A. Village Information

State: Delta----------
LGA_____Village_____

Village Coordinates: Latitude _____Longitude_____

Village population estimate_____

Other related information about village _____

B. Socio-demographic information

1. Name _____(Chief, Elder, Mr. Mrs. Miss, Other)

   First Initial Surname

2. Sex (Male Female)

3. Age (years) approximate age of respondent

   a. Less than 5

   b. 5-14 years

   b. 15 -24years

   c. 25 - 34 years

   d. 35-44 years

   e. 45 - 54 years

f. 55 years and over _____

4. *What is your marital status?*

a. Single

b. Married

c. Widowed

d. Divorced/Separated

d. Other (specify)

5. *What is your religion?*

a. *Christianity*

b. Islam

c. Traditional religion

d. Other (specify)

6. Which ethnic group do you belong to? Specify) _____

7. What is your highest level of education?

a. No formal education

b. Quranic

c. Primary

d. Secondary

e. Tertiary

f. Others (specify)

8. How long have you livedin this village?

a. Less than 5 years
b. 5- 10 years
c. More than 10 years

9. What is your residential Address/Location (Ward etc)?

10. Occupation (What work do you do to earn a living)?
   a. Student

   b. Petty trading

   c. Farming/Fisherman

d. Retiree

e. Artisan (builder, bricklayer, mechanic, tailoring, shoe maker, blacksmith, carpenter,electrician etc. Indicate what the person does)

f. Health worker

g. Other formal employment, private or government (specify)

C. Knowledge about HAT

1. Have you heard about HAT before? (Yes/No)

2. If yes, what is your source of information? (Can tick more than one option)

a. Village volunteer health worker

b. Works hop/seminar

*c.*TV/Radio

d. Health worker

e. Newspapers/magazines

f. School teacher

g. Family/friends

h. Internet

i. Posters

j. Others (specify)

3. Describe the symptoms - correct incorrect do not know_____(write **correct** if respondent Mention any of the symptoms e.g. somnolence, insomnia, ataxia etc)

4. What is the local name in the village?_____

5. What do you think causes the disease? - Correct incorrect do not know _____ write **correct** if respondent mentions Tsetse flies or other related terms)

D. Prevention, Control and Management of HAT

1. In your opinion, how can you protect yourself from the disease? _____

2. What do you do when you see someone ill with the disease?-

3. Do you think adequate care and management are provided for HAT in the clinics or Communities?

E. Other

1. Have you seen anyone with the disease in recent times? Yes/No/Not sure

2. If yes, when did you see the person (month, year)?_____

3. If yes, where did you see the person (State village name)_____

4. If government wants to regularly give members of your community information on prevention of HAT and other diseases, suggest the best three information channels that can be used.

a. ----------

b. ----------

c. ----------

4. Do you live near forests or thick bushes?Yes/No

5. Do you live near flowing rivers and streams?Yes/No

6. Do you have domestic animals in your compound? Yes/No

7. If yes, which ones?

a. Goats

b. Cows

c. Dogs

d. Pigs

e. Ducks

f. Chickens

g. Other (specify)

Thank the Respondent

Interviewerôs **Name**, Signature and Date ----------

Supervisor'sName, Signature and Date ----------

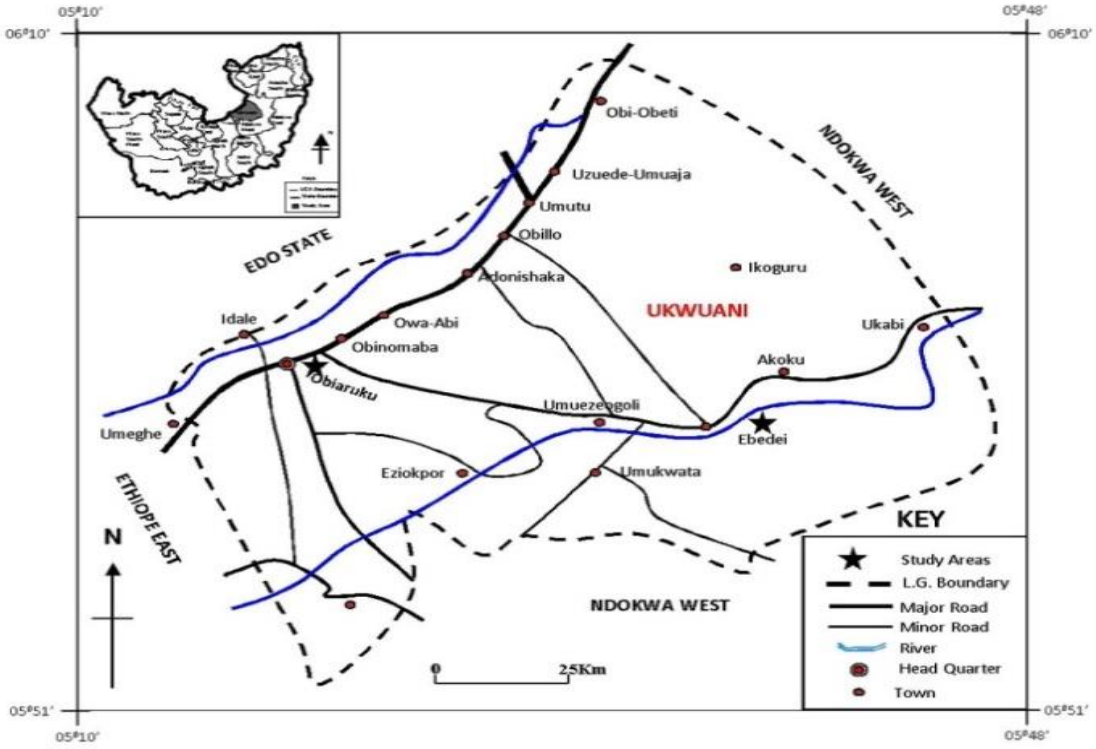
Appendix 5: **Delta State 3Senatorialwards and 25 LGAs.**

Delta South	Headquarters (LGAs)	Delta Central	Headquarters (LGAs)	Delta North	Headquarters (LGAs)
Ethiope East	Isiokolo	Bomadi	Bomadi	Aniocha South	Ogwashi Uku
Ethiope west	Oghara	Burutu	Burutu	Aniocha north	Isele uku
Okpe	Orerokpe	Warri south	Warri	Ika south	Agbor
Sapele	Sapele	Isoko south	Oleh	Ika north east	Owa Oyibu
Udu	Otor Udu	Isoko north	Ozoro	Ndokwa east	Aboh
Uvwie	Effurun	Patani	Patani	Ndokwa west	Kwale
Ughelli south	Otu Jeremi	Warri south west	Ogbe-ijaw	Oshimili south	Oshimili
Ughelli north	Ughelli	Warri north	Koko	Oshimili north	Akwukwu
				Ukwuani	Obiaruku

APPENDIX 6:

Delta State 2006 Census figures (Source: National Population Commission, Nigeria)

s/No	LGA	Males	Females	Total
1	ANIOCHA NORTH	52634	52077	104711
2	ANIOCHA SOUTH	69632	70972	140604
3	BOMADI	43083	43561	86644
4	BURUTU	110416	99250	209666
5	ETHIOPE EAST	100257	100535	200792
6	ETHIOPE WEST	102445	101147	203592
7	IKA NORTH EAST	91414	92243	183657
8	IKA SOUTH	79628	82966	162594
9	ISOKO NORTH	71820	72335	144155
10	ISOKO SOUTH	114391	113321	227712
11	NDOKWA EAST	52350	50821	103171
12	NDOKWA WEST	79018	70307	149365
13	OKPE	67995	62034	130029
14	OSHIMILI NORTH	56405	58911	115316
15	OSHIMILI SOUTH	80274	69329	149603
16	PATANI	34046	33661	67707
17	SAPELE	85305	86583	171888
18	UDU	71242	72119	143361
19	UGHELI NORTH	159192	161836	321028
20	UCHELLI SOUTH	109379	104197	213576
21	UKWANI	59162	61228	120390
22	UVWIE	95051	96421	191472
23	WARRI NORTH	69754	67546	137300
24	WARRI SOUTH	156098	147319	303417
25	WARRI SOUTH WEST	63315	53366	116681



Appendix 7: showing map of villages in Delta State along River Ethiope and a pictorial showing the ecosystem (Source: WHO)

Appendix 8: PROCEDURES FOR DIAGNOSIS OF HUMAN AFRICAN TRYPANOSOMIASIS (SLEEPING SICKNESS)

For the diagnosis of sleeping sickness we use the following procedures

1. Serological tests - to look for antibodies against *T. b. gambiense*.
2. Parasitological test of blood and lymph nodes aspirates - To look for the trypanosomes in the body fluids.
3. Involvement of the central nervous system.

1. SEROLOGICAL TESTS.

Introduction.

SERUM/PLASMA - Liquid part of blood having antibodies.

ANTIBODIES - Chemicals produced by the human or animal body to fight foreign organisms in the body.

When a foreign organism enters our bodies our system produces chemicals (antibodies) to fight it.

The chemicals (antibodies) continue being produced as long as the organism is there. When the organism is no longer in the body the chemicals stop being produced in large quantities, but some remain in our system in case the foreign organism comes back.

Sometimes we test if the chemicals against certain organism in our bodies to determine its presence. This test is known as serological test.

CATT stands for Card Agglutination Test for Trypanosomiasis. This is a diagnostic test for the detection of human trypanosomiasis caused by *Trypanosoma brucei gambiense.* The test detects the circulating antibodies that are produced following infection by *T. gambiense.*

PRINCIPLE

Infection with *T. gambiense* results in production of antibodies against several antigens of the parasite. These antibodies can be demonstrated in whole blood, serum or plasma by agglutination tests. The CATT reagent is a freeze-dried suspension of fixed, stained and stabilized blood from trypomastigotes of well refined serotypes (i.e. this is the antigen).

The test is carried out on plastic cards. One drop of whole blood or one drop of diluted plasma or serum from the patient is mixed with one drop of reconstituted CATT reagent. If antibodies are present, the antigen in the CATT reagent agglutinates within 5 minutes. This can be seen macroscopically.

REAGENTS

1. CATT Reagent - Stabilized freeze-dried suspension of fixed, stained trypanosomes.
2. CATT Buffer - For reconstitution of CATT reagent, control sera and for dilution of test samples. Preservative used is 0.1% sodium azide.
3. Positive and Negative controls.
4. CATT accessories i.e. CATT cards, applicator sticks, droppers, capillary tubes, rubber teats syringe and needle

Reconstitution of reagents - *CATT/Trypanosoma brucei gambiense*

Using the syringe provided, take 2.5 ml of the buffer and add to one vial of reagent.

Mix by turning the vials gently,

Let it stand for a few minutes.

The reconstituted reagent must be kept in the refrigerator and used for a maximum of 7 days.

Controls.

Using separate syringes take 0.5 ml of the buffer and add to the control vials.

Mix by turning the vials gently. Let them stand for a few minutes.

Reagent quality control.

All newly reconstituted reagents must be tested against known positive and negative control for quality control.

Do quality control for the reagents as follows ï

- Remove the rubber caps of the vials of the CATT reagents and controls and fit the droppers to each.
- On the CATT card label one test area as positive (+ve) and one negative (-ve)
- Add one drop of the well-mixed CATT reagent to each of the test areas.
- Add one drop of the positive control to the test area marked +ve and mix with the CATT
- Reagent using the applicator sticks provided spread the mixture over the whole test area as marked on the card.

Add one drop of the negative control to test area marked -ve and mix with the CATT reagent

Using the applicator sticks provided again spread the mixture on the whole test area as marked.

- Place the CATT card with the positive and negative control specimen on the rotator
- and
  Rotate for 5 minutes at 60 rpm. (Revolutions per minute).

- Observe macroscopically for agglutination.
  Positive control - Agglutination within 5 minutes

  Negative control - No Agglutination after 5 minutes.

If the results of the control specimen are correct then the reagents is good and may be used. If

the results are not ok, reconstitute a new reagent and repeat.

If the second reagent results are not in good condition, a new one should be opened and the

quality control procedure repeated. Record all results in the quality control sheet

BLOOD COLLECTION PROCEDURE.

Blood is collected in the laboratory for different reasons.

The type of sample to be collected is determined by the reason the blood is needed.

Blood can be used to determine the presence or absence of disease in the body.

The 2 main types of blood samples are ð

- Capillary blood. ,
- Venous blood.

Capillary blood is collected from the capillaries - the smallest blood vessels in the body. It is normally collected from the fingers, toes, earlobes etc. This is collected when only a small quantity of blood is needed e.g. blood film, capillary tube blood sample.

Venous blood is collected from the veins - the blood vessels taking blood from the body organs to the heart. This is collected when large quantities of blood are needed e.g. when we need to analyse the plasma or serum.

Warning

Blood can have a lot of organisms including HIV the causative agent for AIDS.

COLLECTION OF CAPILLARY BLOOD.

Areas to collect the sample.

1. Fingers and toes.
2. Heel.

Items needed for blood collection.

- Non-sterile gloves.
- Disinfectant.
- Cotton wool.
- Sterile lancet.

Method.

- Explain to the patient about the procedure.
- Make sure all the items needed are within reach.
- Put on the non - sterile gloves.
- Identify the area to collect the sample the best area for the finger is on the side of the ballof the finger, for the heel in the middle.
- Clean the area with a cotton swab with disinfectant.
- Using the sterile lancet, prick the disinfected once and fast.
- Dispose the used lancet into a sharps container.
- Wipe the first drop of blood using a dry cotton swab.
- Press again to get a fresh drop of blood.
- Place the drop in the appropriate collection vessel (Slide, capillary tube etc).

- Give the patient a dry cotton swab to press the puncture site.

COL.LECTION OF VENOUS BLOOD.

Areas to collect sample.

1. At the front junction of the upper arm and lower arm.
2. Back of the hand.

Items needed.

- Non ï sterile gloves
- Disinfectant
- Cotton wool
- Syringe (vacutainer tube)
- Needle (vacutainer needle and holder)
- Adhesive tape

Procedure

Using vacutainer system.

Principle.

1. Explain to the patient about the procedure.
2. Make sure all the items needed are within reach.
3. Put on the non - sterile gloves.
4. Identify the area and the vein to collect the sample.
5. Clean the area with a cotton swab with disinfectant.
6. Connect the needle to the needle holder.
7. Gently insert the needle into the vein.
8. When inside the vein connect the vacutainer tube to the other end of the needle.

a. If the needle is in the vein the blood will flow into the tube.

b. If no blood flows into the needle, the needle is not in the vein so disconnect the tube from the needle then removes the needle from the skin. (NB: do not remove the needle before disconnecting the tube because air will enter the vacutainer tube and cannot be used again).

9. After about 2 ml of blood has flowed to the tube (the tube is set for a specific amount of blood), disconnect the tube from the needle then put a cotton swab over the puncture site and remove the needle from the vein.

10. Remove the needle from the holder and dispose into a sharps container - do not recap the needle.

11. Put an adhesive tape on the cotton swab over the puncture site.

Using needle and syringe.

Followsteps 1 to 5 above.

6. Connect the needle to the syringe.

7. Gently insert the needle into the vein.

8. If the needle is in the vein a small drop of blood will appear at the junction of the syringe and needle.

9. Pull the plunger of the syringe for blood to flow into the syring

10 After enough blood has flowed to the syringe, put a cotton swab over the puncture site and remove the needle from the vein.

11. Remove the needle from the holder and dispose into a sharps container - do not recap the needle.

12. Put the sample in the appropriate container.

13. Put an adhesive tape on the cotton swab over the puncture site.

NB: If at any time when the needle is in the patientôs body should the skin start swelling, remove the needle because blood is flowing into the tissues from a punctured vein. This is very painful to the patient. Do not try to manipulate the needle inside the patient's body than necessary.

PROCEDURE FOR CATT TEST

Method:

Following the capillary blood procedure, collect blood into the capillary tube and place the tube

on the capillary tube holder.

Requirements

- Examination gloves
- Cotton wool.
- Capillary tube holder
- CATT reagent and accessories
- Rotator with power supply

CATT whole blood.

TEST PROCEDURE

- At the bottom right side corner of the CATT card write down the patient numbers to be performed on the card (e.g. 1 - 10, 11 - 20, 21 - 30 etc). Collect capillary blood into the capillary tubes provided.
- Place them on the capillary tube holder.
- Add one drop of the CATT reagents onto each of the test areas as per the number of specimen to be tested.
- Fit the rubber teats on the capillary tubes and dispense the blood on the appropriate test area next to the drop of reagent.
- Mix the blood and the reagent using the applicator sticks and spread the reaction mixture on the whole test area. Thoroughly wipe the applicator stick using dry cotton wool or gauze between each specimen mixed.
- Place the CATT card with the reaction mixture on the rotator and rotate for 5 minutes at 60 rpm.
- Observe for agglutination Record the results in the registration card.

Discard the used CATT cards in the waste container and the capillary tubes in the sharps container .

RESULTS

Agglutination - Positive
No Agglutination - Negative

CATT Dilution for titration

•Requirements.

- Venous blood (Preferably EDTA anti - coagulated).
- Cotton.
- Micro titration plate
- Automatic pipette (10 - 100).
- Pipette tips
- CATT reagents and accessories.
- Rotator.

a) Centrifuge the venous blood tube in order to get either plasma or serum.

b) Arrange all the requirements on a bench

c) Adjust the micropipette to measure 50 ul

d) Using a marker divide the micro titration *plate wells into* groups of four (each patient's Sample will require 4 wells) according to the number of samples. Label clearly.

(e) Using the micropipette, pipette 50 uL of the buffer and put in each of the wells required.

Discard the micropipette tip after putting the buffer in all the required wells

f) Pipette 50 uL of the serum or plasma and add to the first well of the corresponding patient. Mix well and then draw 50 uL from the first well and add to the second well.

Repeat the same to the third and fourth wells. Discard the pipette tips in the waste container. Do the same procedure to all the other patient samples.

The dilutions in the wells will be as follows;

- 1st Well- 1:2(1/2)
- 2nd Well =1:4(1/4)
- 3rd Well =1:8(1/8)

g) For each patient take 25 uL the samples from the 2nd, and 3rd, place them on a CATT card

(Separate circles) and perform a CATT test as described above. A new yellow tip should be used

for each dilution.

h) Record the results clearly indicating the dilutions e.g. CATT 1/4 - Positive, CATT

1/8 ï Positive

i) Dispose properly all the used items.

2. PARASITOLOGICAL TESTS ON BLOOD AND LYMPH NODES.

In the early stages of the disease, the parasites are found in the lymph nodes and later in the blood. After the serological tests indicate production of antibodies in large amounts a Configuration of the parasites in the body is necessary.

BLOOD TEST.

Micro-haematocrit capillary tube centrifugation technique (CTC OR WOO TEST)

Requirements

Capillary tubes (Heparinized/anti-coagulated)

Viewing chamber (commercial or improvised)

Blood collection items

Micro-haematocrit centrifuge

Sealing clay (crystal seal)

Procedure

- Collect venous blood from the patient into an anti-coagulated tube or collect capillary blood into an anti-coagulated capillary tube. If capillary blood, collect in 6 tubes.
- Fill 6 capillary tubes % full with the anti-coagulated blood if venous blood was collected.
- Seal one end of the tubes with sealing/modelling clay.
- Place the capillary tubes in the centrifuge with the sealed ends facing outside.
- Ensure they are well balanced.
- Centrifuge at high speed (about 11,000 rpm) for 5 minutes.
- Let the centrifuge stop completely before removing the capillary tubes.

- Lay the capillary tubes on the viewing chamber, add a drop of water on the viewing (area used for examination) to improve the contrast.
- Place the chamber on the microscope and examine at the Buffy coat (between the blood cells and the plasma) using XI0 objective with reduced condenser aperture. Examine through all the tubes, one by one, by turning them slowly over 360° to ensure the whole circumference of the tube is examined thoroughly.
- Record the results.

Results

Trypanosomes if present are seen as tiny moving organisms in the plasma just above the layer of leukocytes (in the Buffy coat). Be careful of microfilaria can also be seen in the WOO test.

GLANDS

Collection and examination of gland aspirates

Lymph nodes are found in the neck, in the armpit and the groin.

The nodes found along the neck are known as cervical glands, in the armpit are axillary glands and in the groin are the inguinal glands.

Infected lymph glands are swollen and form a lump.

Infection with *T. gambiense* commonly leads to the swelling of the cervical glands.

If the nodes are swollen due to T. Gambiense infection, the parasites can be seen when we observe the lymph fluid under the microscope.

Collection of gland juice (lymph node fluid).

Method

Item required:

Sterile needle (26G)

Syringe (5 ml)

Slides and cover slips

Chlorohexidine (disinfectant) or sterile cotton swabs

Cotton wool

Procedure

- Make sure all items are available.
- Prepare the syringe by pulling the piston back as far as possible.
- Allow the patient to sit comfortably.
- Explain the procedure to the patient,
- Feel both sides of the neck from the base of the neck to the ear.
- Disinfect the chosen site with swollen glands using a piece of cotton wool soaked in iodine or suitable disinfectant.
- Take the gland between the thumb and index finger of the left hand. Hold it steady, at the same time making it stand out.
- Block the opening in the hub of the needle and introduce it at right angle into the centre of the gland in two stages -

  a) First pierce the skin

  b) Second penetrate the gland

- Remove your finger from the hub and with your left hand, gently knead the gland. With your right hand, revolve the needle in both directions. The glandular fluid will ooze into the needle.

  Block the opening in the hub of the needle then withdraw the needle in one rapid movement.

- Å Then apply a swab dipped in iodine to the point of entry.
- Å Attach the syringe (piston pulled back) to the needle.
- Å Place the needle on the slide. Push the piston gently down the barrel to discharge the glandular fluid contained in the needle onto the slide
- Å Cover the sample with a cover slip.

NB: The fluid is cream coloured with no blood. If blood stained and no lymph fluid evident, repeat collection.

Examination

- Examine the slide immediately under the microscope using x40 objective.
- Record the results.

Results

Trypanosomes are seen moving rapidly with the help of the undulating membrane and flagella

between the white blood cells.

Be careful of false positives due to red cells movement under the WBCs

CENTRAL NERVOUS SYSTEM (CNS) INVOLVEMENT(Collection of cerebro spinal HAT screening used for staging for reasons of case management).

At the late stages of the disease we find the parasites in the central nervous system. In the central nervous system there are no organisms and when an organism enters here the body responds by producing white blood cells to fight against the organisms. The normal white blood cell count in the CNS is less than 5 cells per mm of CSF. An increase of the WBC's in the CSF indicates a foreign body present. Since the patients we are dealing with have already shown evidence of having sleeping sickness parasites in their bodies, an increase in the WBC is most probably due to presence of the parasites in the brain even if we do not see the parasite itself.

To establish if the parasites are in the CNS, we collect the cerebral spinal fluid (CSF) to check for trypanosomes or white blood cell count.

Collection of the cerebral spinal fluid.(Lumbar puncture).

As stated above, the central nervous system does not contain organisms so introduction of anorganism will result to infection in the brain which is dangerous.

Collection of the CSF must be done in sterile procedure.

After CSF is collected the pressure in the CNS drops and a patient needs to rest for about 2 hours. Sometimes a patient may experience headache so analgesic drugs/ tablets are given in case.

Requirements

- Sterile gauze.
- Iodine.
- LP needle.
- Adhesive plaster.
- Sterile gloves.
- Galipot.
- Paracetamol tablets.

Procedure (This is one method. The most important factor in the procedure is to be sterile).

- Make sure all items are ready and within your reach.
- Open the sterile gloves packet.
- Open the sterile gauze packets and let the gauze drop on the open glove packet (Do not touch the gauze with bare hands.
- Let the patient sit on a bench.
- Explain the procedure to the patient and make the patient at ease.
- Tell the patient to cross his hand on the chest and look down to his lap.
- Hold the pelvic bone with both of your hands and using your thumb make an imaginary line along the spinal column and when the thumb is at the same level with pelvic bone, get the intra disc gaps and make a mark at this region.

Put on a sterile glove on the left hand (right hand if you are left handed).

- With sterile gauze soaked in iodine sterilize the region starting from the centre outward do this twice.
- If need be confirm the mark made using this hand
- Sterilize the area again - hold the sterile gauze with the gloved hand.
- Open the sterile LP needle (Your glove is no longer sterile!!)
- Put on the other sterile glove on your other hand.

- Take the needle with this hand and insert it at the mark made on the spinal column the needle should go in smoothly.
- Open the stylet of the needle for the CSF to flow (if the needle is in the right place the CSF will flow drop by drop), if no fluid flows push the needle slightly and check again (If no fluid flows out remove the needle and try again - Do not try more than twice, ask for assistance).
- Discard the first 3 drops of CSF.
- After 15 drops of CSF have flowed in to the tube reinsert the stylet and put sterile gauze at the entry point then pull the needle out in one move.
- Put a piece of adhesive gauze at the position and give the patient paracetamol and direct the patient to the resting area.
- The patience should rest at least for 1 hour but preferably 2 hours

[Blood stained CSF is not suitable for cell count]

Analysis of CSF

The tests done on CSF include:

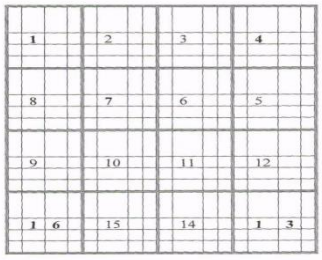
Total white blood cells count

Examination for trypanosomes

1. Total White cells count (WCC).

(a) WCC count using the standard Fuchs Rosenthal counting chamber **(This is the standard chamber).**

The standard Fuchs Rosenthal counting chamber has a depth of 0.2mm.



Requirements

- Counting chamber (Standard Fuchs Rosenthal)
- Cover glasses
- Tally counter
- Pasteur pipette
- Sample (undiluted)
- Petri dish containing wet tissue or filter paper (moist chamber)

Procedure

1. Take a clean chamber and fix the cover glass by pressing it on to the counting chamber until rainbow colours (Newton rings) appear. The cover glass should stick on the chamber.

2. Gently shake the CSF sample to mix. Fill the counting chamber with the CSF using the Pasteur pipette (charge the chamber). Avoid air bubbles and do not overfill the chamber. If the fluid overflows, remove the cover glass, rinse the counting chamber, dry and start again.

3. Place the chamber in a Petri dish containing wet tissue or filter paper (moist chamber) for 2 - 3 minutes for the cells to settle. This prevents the fluids drying while the cells are settling.

4. Place the chamber on the microscope stage. Swing the X10 objective into position and reduce the iris diaphragm. Bring the cells and the rulings of the chamber into focus.

5. Count in the four corner squares of the chamber and any one square in the middle.

6. No calculation is required therefore the number of cells counted is the number of cells per mm^3.

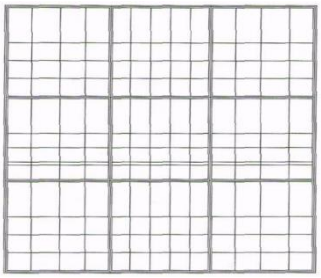
7. If the number of WCCs is high, repeat the test using a dilution with Turk's solution

   (50ul of CSF and 50ul of Turk's solution count the number of cells as described above

   and multiply the result by 2)

(b) WCC count using the improved Neubauer counting chamber.

The new improved Neubauer chamber has a depth of 0.1 mm



Procedure

1. Take a clean chamber and gently press the cover glass until rainbow colours (Newton rings) Appear.

2 Using the Pasteur pipette take some sample of CSF from the tube and fill the counting Chamber with the CSF (charge the chamber). Do not overfill the chamber.

3. Put the chamber on the moist chamber for 2 - 3 minutes for the cells to settle.

4. Place the chamber on the microscope stage. Focus using X10 objective and count the cells in all the 9 squares of the chamber. To better differentiate WCC from impurities or RBC use X40 objective if you need. Count also the cells overlapping the margins of the upper and right hand sides of the squares. Do not count the cells overlapping the margins of the lower and left hand side of the squares.

5. Calculate the number of cells in 1 ul (per mm) as follows:
Multiply the number of cells counted by 10 (1/0.1) then divide by the number of squares counted (9)
Example: {f 9 cells are counted in all the squares, then the number of cells per mm^3 (ul) is 9 x 10/9= 10 cells/mm^3, i.e. 9x10/9-10 cells/mm^3

6. Record the results after calculation per mm

7. Clean the chamber and cover glass and dry with gauze or absorbent cotton wool.

2. Examination for trypanosomes: Double centrifugation

- Put the CSF in a centrifuge tube and centrifuge for 10 minutes at 2000 rpm (or use the deposit in the procedure above).
- Pour out the supernatant in to another tube.
- Mix the sediment.
- Collect the sediment in 2 capillary tubes.

- Seal the capillary tubes and centrifuge as for WOO test for 2 minutes.
- Mount the tubes on a chamber (WOO) and examine for trypanosomes at the base of thetubes.

INTERPRETATION OF THE CSF RESULTS

If trypanosomes are seen in the CSF then the result is positive if no trypanosomes seen the result is negative.

STAGING

CASES (parasitological cases')

STAGE 1 (SSD

This is a patient who has been found to have trypanosomes either in blood or gland or but the CNS has not been infected.

Summary:

- ❖ WOO, mAECT or GP: positive
- ❖ There is **NO** trypanosomes in the CSF and
- ❖ The total White cells count in CSF (WCC) is between 0 -5 cells/mm^3

STAGE 2 (SS2

This is the late stage and occurs when the CNS is infected.

Summary

- ❖ WOO, mAECT or GP: positive
- ❖ Trypanosomes are found in the CSF and/or
- ❖ WCC count is greater the 5 cells/mm

Or

- ❖ WOO, mAECT and GP negative
- ❖ CATT dilutions positive up to 1/8
- ❖ WCC greater than 20 cells/mm3
- ❖ No tryps seen in CSF

SUSPECT CASES (serological cases)

- ❖ WOO, mAECT and GP negative
- ❖ CATT dilutions positive up to 1/8

- ❖ No tryps seen in CSF.
- ❖ WCC lower or equal to 20 cells/mm3

Appendix 9:

CATT/ *T.b.gambiense* - Serodiagnosis of Human African Trypanosomiasis

(Sleeping Sickness} due to T.b. gambiense (Manufacturer's notes)

i. PRINCIPLE

Infection with *Trypanosoma brucei gambiense* results in production of circulating antibodies against several surface antigens of the parasites. 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 *trypanosome brucei gambiense.*

The test is performed on a plastified card. One drop of undiluted blood {screening test} or 25 µl of diluted plasma/serum {confirmation test} are mixed with one drop of reconstituted antigen. When antibodies are present in the test sample, trypanosomes agglutinate within 5 minutes rotation at 60 rpm.

(*) CATT= Card Agglutination Test for Trypanosomiasis

ii. REAGENTS

1. **CATT-ANTIGEN** (2.5 ml / vial)

   . Freeze dried suspension of purified, fixed and stained trypanosomes

   . Preservative: sodium azide (0.1%)

   . Storage: refrigerator (+2°C / +8°C) or freezer (-20°C).

2. **CATT-BUFFER** (30ml/vial)

- o Phosphate Buffered Saline (pH 7.2).
- o Use for reconstitution of CATT-antigen, positive and negative controls + preparation of sample dilutions.
- o Preservatives: sodium azide (0.1%).
- o **Storage**: refrigerator (+2°C / +8°C). DO NOT FREEZE!

3. **POSITIVE CONTROL** (0.5 ml/vial)

   - Freeze dried goat antiserum
   - Preservatives: sodium azide (0.1%).
   - **Storage**: refrigerator (+2°C / +8°C) or freezer (-20°C)

4. NEGATIVE CONTROL (0.5 ml/vail)
   - Freeze solution of bovine albumin
   - Preservatives: sodium azide (0.1%)
   - **Storage**: refrigerator (+2°C / +8°C) or freezer (-20°C).

iii. EXECUTION OF THE TEST

1. Reconstitution of the CATT-ANTIGEN

- Using the syringe, add 2.5 ml of CATT buffer to a vial of freeze dried CATT antigen.
- Immediately shake the vial for a few seconds so as to obtain a homogeneous suspension.

❖ Put a dropper on the vial

❖ The antigen suspension is ready for use

Notes: a. before each use, shake the vial for a few seconds.

b. Keep the CATT antigen out of the sun and dust.

2. Reconstitution of the Controls

Å Using the syringe, add 0.5 ml of CATT buffer to a vial of the positive and the negative control.

Å Put a dropper on each vial.

Note: After reconstitution of each vial of CATT antigen, test one drop of the positive control and one drop of the negative control to check the quality of the antigen.

3. Preparation of Test samples

3.1 Screening test on undiluted blood

Å Disinfected finger tip and prick with a micro lancet.

Å Wipe off the first drop of blood.

Å Fill a heparinized capillary tube for about % of its length avoiding air bubbles.

Å Incline the capillary tube several times in order to mix the blood with the heparin.

Å Place the capillary tubes horizontally on a capillary tube holder.

Using the suction bulb, put 1 drop of undiluted blood in a test area on the card.

During field surveys:

Å Collect blood samples of 5 to 10 persons.

Å Proceed immediately afterwards to execution of the test.

Å Should sedimentation of red blood cells occur in the capillary tube, mix the blood again before testing.

Å Should coagulation of the blood occur, take a new sample.

Å In order to avoid drying out of the blood at the extremities of the capillary tube, put the holder in a box containing a wet cotton plug.

3.2 Confirmation test on diluted blood, serum or plasma

Å Prepare a serum or plasma sample by any suitable method.

Å Dilute blood (1/2) or serum/plasma (1/4) in CATT buffer.

Using a micropipette, put 25 ul of diluted blood, serum or plasma in a test area on the card.

3.3 Quantitative method (titration) on serum/plasma

Å Prepare two fold dilutions $^{1}/4$,1/8, 1/16, 1/32 and 1/64 in CATT buffer.

Using a micropipette, put 25 ul of each dilution in a test area on the card.

4. Agglutination reaction

1) On a test area of the card, put;

- 1 drop of undiluted blood

OR

25µL of diluted blood, serum or plasma

Or

25µL of the twofolio dilutions

(2)Then add:

1 drop (about 45 ul) of the well homogenized CATT antigen in each test area.

Note: In order to obtain drops of constant volume, hold the dropper vertically and allow the drops to fall freely without touching the card.

3) Using a stirring rod, mix and spread out the reaction mixture to about 1mm from the edge of the test area. Wipe off the stirring rod after each use.

4) Rotate the test card on a flat bed orbital rotator for 5 **minutes** at **60rpm.**

Notes:
1. In order to prevent the reaction mixtures from drying out, always close the lid of the rotator and put a wet cotton plug beneath.

2. If no **electrical rotator** is available, rock the card so as to impart to the reaction mixture a constant circular movement by slowly tilting it, always in the same director, from left-back to right-forth.

IV. READING AND INTERPRETATION

Å After 5 minutes rotation, read the results **before removing the card from the rotator,**

Å When working manually, read the results while tilting the card gently,

Å Read the results

++ = **STRONGLY POSITIVE** (very strong agglutination)
++ - **POSITIVE** (strong agglutination)
+ = **POSITIVE** (moderate agglutination)
± = **WEAKLY POSITIVE** (weak agglutination)
- = **NEGATIVE** (absence of agglutination)

1. V. STABILITY, STORAGE AND EXPIRY DATEStability

- Å The freeze dried reagents (antigen, positive and negative controls) and the CATT buffer remain stable for 1 year when stored in a refrigerator between +2°C and +8°C. At higher peratures, i.e. +45oC, the freeze dried reagents retain their activity for at least 1 week.

- Å After reconstitution, the reagents can be used during 1 week when stored between +2°C and +8°C, or up to 8 hours at 37°C.

Notes:

- These values are only an indication on the stability of the reagents but are not recommendations for prolonged storage!

- DO NOT freeze the reconstituted antigen suspension!

2. Recommendations for storage and shipment

- Å Freeze dried! reagents (antigen, control's): in me refrigerator *(+2^{0}C* to *+8^{0}C)* or in the freezer (-20°C).

- Å CATT buffer: in the refrigerator (+2°C a +8°C) - **Do not freeze!**

- Å During transport, storage and handling: avoid exposure to heat and director sunlight.

- Å It is recommended to dispatch the reagents from a central storage centre to the field under refrigerated conditions (cold chain)

3. Shelf life/Expiry date

When stored under the prescribed storage conditions, all the reagents will retain their activity until the expiry date mentioned on the "Reagent" boxes and on the packing list.

VII. PRESENTATION

1. KIT REAGENTS (Packing size: 250 tests)

Contents:
- 5 vials CATT-Antigen
- 1 vial Positive Control
- 1 vial Negative Control
- 1 vial CATT-Buffer
- 1 direction for use

2. KIT REAGENTS (Packing size: 500 tests)

Contents: - 11 vials CATT-Antigen

- 1 vial Positive Control
- 1 vial Negative Control
- 1 vial CATT-Buffer
- 1 directions for use

3. KIT ACCESSORIES (Packing size: 250 tests)

Contents: - 250 heparinized capillary tubes

- 26 plastified test cards
- 3 stirring rods
- Suction bulb
- 1 syringe (2.5 ml)
- 7 droppers

4. **CARD TEST ROTATOR**

A 12VDC-CARD TEST ROTATOR for performing the CATT in the laboratory (connected to a 220VAC power source by means of an AC/DC adaptor) or in the field (connected to a 12V car battery) is also available. (Not included in the kit, should be ordered separately).

Notes: Microlancets are not included in the Accessory Kits and should be ordered separately.

IMPORTANT: Never mix up reagents (antigen, controls, buffer) of different stock and deliveries!

Appendix 10: HUMAN AFRICAN TRYPANOSOMIASIS (HAT) SCREENING FORM

S/No	LGA	Village	Coordinates		Names	Age	Sex	Occupation	CATT		Wet blood film	CSF			Remarks/ Contact Tet. No
				Long					Positive e	litre		Tryps +ve	WBC Count	HAT Symptom	

Appendix 11: Focus Group Discussion and mobilization session, Aballa Uno, 2011

Appendix 13: HAT Screening session during study, Delta State 2011

Appendix 14: Details of demographical information of the sero positive participants by village.

VILLAGE	AGE	SEX	OCCUPATION	CATT		Wet blood film	CSF		
				Positive	Titre		Tryp +ve	WBC Count	HAT Symptoms
Aballa Uno	8	M	Child	+ve	1/128	-ve	-		Swollen stomach
	34	F	Teacher (Worker)	+ve	1/8	-ve	-		
	6	M	Child	+ve	1/8	-ve	-		
Tuomo	59	M	Worker	+ve	1/64	-ve	-		Rashes and swollen face
Urhuoka	11	F	Student	+ve	1/64	-ve	-		Rashes and swollen face
	7	M	Child	+ve	1/8	-ve	-t-ve	>5xl0^6/L	Dead had swollen face, stomach, lyphadenopathy
	80	F	Farmer	+ve	1/8	-ve	-ve	1.4xl0^6/L	Nil
	6	M	Child	*l +ve*	1:8	' -ve	_		*Nil*
	11	F	Student	+ve	1:128	-ve	-ve	1.4xl0^6/L	Fever
	60	F	Farmer	+ve	1:16	-ve	-		Nil
	6	M	Child	+ve	1:8	-ve	-ve	1.4xl0^6/L	Nil
	75	F	Farmer	+ve	1:32	-ve	-		Nil
	45	/ M	/ *Businessma* n	/ +ve	*{* 1:64	/ -ve	*I*	'	Fever
	25	M	Student	+ve	1:128	-ve	-ve	5.4xl0^6/L	fever, rashes
	14	M	Student	+ve	1:32	-ve	-		Nil
	9	M	Child	+ve	1:128	-ve	-		Nil
	25	M	Student	+ve	1:8	-ve	-ve	2.6xl0^6/L	Nil
	60	F	Farmer	+ve	1:128	-ve	-ve	5xl0^6/L	Nil
	12	F	Student	-s-ve	1:128	-ve	-		Nil
	31	F	Trader	+ve	1:128	-ve	-ve		Nil
	26	F	Student	+ve	1:128	-ve	+ve	6.6xl0^6/L	Comatose but treated and recovered, titre now '1:16
Owhelogbo	35	F	Student	+ve	1:8	-ve	-ve	1.4xl0^6/L	Fever
	6	M	Child	+ve	1:8	-ve	-		Fever
	22	F	Business	+ve	1:8	-ve	-		Fever
Ugbolu	40	M	Farmer	+ve	1:8	-ve	-ve	5xl0^6/L	Nil
	7	M	Child	+ve	1:8	-ve	-ve	1.6xl0^6/L	Nil
	70	F	Farmer	+ve	1:8	-ve	-		Nil
	35	F	Trader	+ve	1:64	-ve	-ve	SxlO'/L	Fever, rashes

Appendix 21: Copy of Ethical clearance for the research protect

DELTA STATE

DELTA STATE PRIMARY HEALTH CARE DEVELOPMENT AGENCY

Tel: 056282424
Fax: 056282424
Email deltasphcda@yahoo.com

No. 1 Onyeka Close
Off DBS Road,
P.M.B. 95114
Asaba.

Your Ref:
Our Ref: PHA/CS.79/T1/17

3rd August, 2010

NIGEP /HAT UNIT
Public Health Department
Federal Ministry of Health
Abuja.

Sir,

RE: APPLICATION FOR ETHICAL PERMISSION TO SCREEN FOR HUMAN AFRICAN TRYPANOSOMIASIS (SLEEPING SICKNESS – HAT) IN ETHIOPE EAST AND UKWUANI LGAs

I am directed to refer to your letter dated 26th June, 2010, on the above subject matter and to inform you that the Honourable Commissioner, Ministry of Health, Delta State has granted the approval sought by your office to enable you carry out the programme of activities.

2. Accept my best wishes.

3. Thanks.

[redacted]ha Ndudi (Mr)
For: Honourable Commissioner.