Prevalence of Human African Trypanosomiasis And its Associated Risk Factors in Itezhi-Tezhi District of Zambia.

By

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A Dissertation Submitted to The University of Zambia in Fulfilment of the partial Requirements for the award of Degree of Master in Science One Health Laboratory Sciences.

University of Zambia

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DECLARATION

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CERTIFICATION OF APPROVAL

This dissertaion for Christine Musonda has been approved as partial fullfilment of the requirements for the award of the master of science degree in One Health Laboratory Sciences at the University of Zambia.

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ABSTRACT

Human African Trypanosomiasis (HAT) is a debilitating zoonotic disease caused by trypanosomes which are transmitted by tsetse flies of the genus *Glossina.* This study aimed to determine the prevalence and risk factors of HAT in Itezhi-Tezhi (ITT) District of Zambia through active surveillance. Human infective trypanosomes have been demonstrated to be in circulation in cattle and tsetse flies as well as wildlife. Equally, ITT has HAT epidermic and sporadic case history. This was a cross-sectional study that collected 114 blood samples from febrile patients aged 11 years and above with history of tsetse fly bites in ITT district. Samples were subjected to four tests namely packed cell volume (PCV), microscopy, internal transcribed spacer (ITS)/serum resistance antigen (SRA) polymerase chain reaction (PCR) and SRA loop mediated isothermal Amplification (SRA-LAMP). Structured questionnaires collected data on risk factors as well as the district's health delivery system through patients and health worker interviews respectively. Chi-square test was used to check for association between PCV and risk factors. P- values under 0.05 were considered statistically significant.

A total of 22 samples had PCV below the minimum acceptable value of which 13 were males and nine were females. Low PCV was more in individuals in the age range of 21-30 and least observed in age range of 31-40 years. Low PCV was also observed more in farmers and least in unemployed individuals. Low PCV was statistically associated with participants source of water for domestic use and animals kept at home with p=values of 0.028 and 0.012.

There was no discrepancy in results for microscopy, PCR and LAMP tests as all samples tested negative on the three platforms giving zero % prevalence. Risk analysis revealed that people in ITT district were at a higher risk of contracting HAT due to their activities, including proximity to Kafue National Park and availability of tsetse fly vector. There were inadequate health personnel trained in HAT, HAT treatment guidelines, basic tools for HAT diagnoses, HAT education to the community and referrals of patients with ceaseless fever. Health delivery systems present were inadequate to support robust HAT surveillance.

Key words: Human African Trypanosomiasis, surveillance, risk factors, health delivery system, Itezhi-Tezhi.

DEDICATION

I dedicate this research to God almighty for preserving my life till this far in the midst of the COVID-19 pandemic. To my husband Andrew C. Mutalanshi for his immense support during the entire period of the program, my son Andrew M. Mutalanshi and my parents Joseph and Emelia. To all my sisters, friends and relatives for their spiritual support, encouragement and believing in me. To all the health care workers for their time and effort to make this research successful.

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LIST OF ABBREVIATIONS

ACEIDHA	Africa Centre of Excellence for Infectious Diseases of Humans and Animals
AT	African Trypanosomiasis
°C	Degrees Celsius
CDC	Centre for Disease Control
CSF	Cerebral Spinal Fluid
DNA	Deoxyribonucleic acid
DRC	Democratic Republic of Congo
ELISA	Enzyme Linked Immunosorbent Assay
GMA	Game Management Area
HAI	Hospital Affiliated Information Register
HAT	Human African Trypanosomiasis
HCW	Health Care Worker
HF	Health Facility
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ITT	Itezhi-Tezhi
ITS	Internal Transcribed Spacer
KNP	Kafue National Park
LAMP	Loop Mediated Isothermal Amplification
MOH	Ministry of Health
PCR	Polymerase Chain reaction
PCV	Packed Cell Volume
RBC	Red Blood Cell
RDT	Rapid Diagnostic Tests
IBM SPSS	Statistical package for social sciences
SRA	Serum Resistance Antigen
TB	Tuberculosis
UTR	Untranslated Regions
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Human African Trypanosomiasis (HAT) is a zoonotic disease which affects humans. Trypanosomes are hemoflagellate extracellular protozoans that develop efficient escape mechanisms to manipulate the entire host immune response. If trypanosomes are left untreated, they are fatal. Animals serve as reservoirs and humans get infected occasionally (Franco *et al.,* 2014). Human African Trypanosomiasis (HAT) is a neglected tropical disease because of its low incidence and mortality. *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* are responsible for causing HAT or sleeping sickness (Franco *et al.,* 2014). The trypanosomes are transmitted by tsetse flies of the genus *Glossina* (WHO,2013*).* The dispersal of the disease corresponds with the distribution of the tsetse fly vector and is called the tsetse belt or green desert (Shaw *et al.*, 2014). This is because of the fact that about 10 million km² of probable high yield land is made unsuitable for farming (Shaw *et al.*, 2014). Majority of the 39 tsetse infested countries in Africa are poorly developed, impoverished and are heavily indebted. This is attributed to the insufficient presence of high yielding animals (Shaw *et al.*, 2014). Therefore, it is estimated that about five billion US dollar worth of animal economy is lost (Giordani *et al*.,2016, Yora *et al*.,2016) and about 60 million people living in the tsetse belt are at probable risk of HAT infection with mortality rate of 10,000 per year (Simarro *et al.*, 2014). From 2009 to 2013, Zambia contributed 4% of the HAT cases in Africa (WHO 2013, Cecchi *et al.*, 2012).

Transmission of the trypanosomes from the tsetse fly happens when tsetse flies feed on infected humans or animals. The tsetse fly ingests blood stream trypomastigotes which later transform into procyclic trypomastigotes that multiply by binary fission in the tsetse midgut. The procyclic trypomastigotes transform into epimastigotes when they leave the midgut and multiply in the salivary gland into metacyclic trypomastigotes (CDC, 2019). These metacyclic trypomastigotes then get injected into humans or animals through bites where they get to multiply by binary fission in blood, lymph and cerebral spinal fluid (CSF) resulting in neurological complications, cerebral pathology and death (CDC,2019). Both forms present with unspecific signs and symptoms which may vary from person to person and foci. Some of the signs include intermittent fever, headache, splenomegaly, skin problems, pruritus, weakness, anaemia, sleeping disturbances and lymphadenopathies (WHO,2013).

Trypanosoma brucei rhodesiense causes acute and severe infection while *Trypanosoma brucei gambiense* causes chronic and mild infection (Buyst et al., 1974).

Human African Trypanosomiasis being a disease of rural and poorly developed areas makes rapid diagnosis and reporting difficult due to lack of laboratory infrastructure, tools and expertise in the remote rural areas where the disease occurs. In patients with low parasitaemia, demonstration of parasites in blood and lymph can be problematic and therefore it is estimated that 20 to 30 percent of the patients remain undiagnosed (Robays *et al*., 2004), hence the need to use sensitive diagnostic tools such as Polymerase chain reaction (PCR) and Loop mediated isothermal amplification (LAMP).

Itezhi-Tezhi district is classified as rural and approximately 50% of the district is in Kafue National Park (KNP), while the rest is gazzetted as Game Management Area (GMA) or forest (Itezhi-Tezhi District Health Office Action Plan and Budget.,2019-2021). The economic activities in Itezhi-Tezhi district include; fishing, crop farming, cattle rearing and tourism. Apart from livestock, the district is further endowed with wildlife such as *Bubalus bubalis*, *Loxodonta*, *Hippopotamus amphibius*, *Equus quagga*, *Connochaetes* and *Kobus leche*, *Panthera leo*, *Panthera pardus*, *Acinonyx jubatus* and *hyaenidae*. The presence of both the vector and reservoir animals makes the environment conducive for HAT infection. Therefore, an integrated system comprising of aerial spraying, insecticide treated targets and trypanocide drug use are used to manage AT in Itezhi- Tezhi. However, despite the presence of an integrated system of control, the district still continues to report sporadic cases of HAT in new, old and also from areas where control was once achieved (Mwanasakale *et al*., 2011). Therefore, in order to provide information that will enhance the already existing disease control strategies and provide timely diagnosis and treatment of HAT, this study aimed to determine the prevalence of HAT and its risk factors in Itezhi-Tezhi District of Zambia.

1.2 Statement of the problem

Narratives on HAT in Zambian policy, reveals that Zambian government has other diseases of priority other than HAT and does not have funds to keep areas free of tsetse flies (Grant *et al.,*2015). Major focus on AT control is emphasized on cattle and not humans (Grant *et al*, 2015). In Zambia, challenges to diagnose and manage HAT in endemic rural areas have been demonstrated (Mulenga, Likwa and Namangala, 2015). Human infective trypanosomes have been demonstrated to be in circulation in cattle and tsetse flies (Nakamura *et al.*, 2021) as well as wildlife(Squarre *et al.*, 2020). The increased encroachment of people and tourists in KNP increases the risk of contracting HAT due to increased human/livestock/wildlife

interface and conservation strategies currently in place have sustained preservation of tsetse flies and AT (Grant, Anderson and Machila, 2015). History shows that the Kafue ecosystem reported epidemics of HAT in the past (DNPW), 2010). A case study reported by Squarre *et al* (2016) demonstrated Kafue ecosystem to be a re-emerging foci for HAT. Despite this, poor surveillance of HAT in Itezhi-Tezhi gives a false impression that cases do not exist despite the area being conducive for HAT transmission(Squarre *et al.,* 2020).

1.3 Justification of the study

In the recent years the KNP has seen a well-managed growth in a number of safari camps and lodges that operate around it. This new interest has brought with it more visitors and investment to the area notably in infrastructure, roads and airstrips as well as reinforced wildlife conservation that has kept endangered animal species thriving. This development in the area has also brought in improved healthcare facilities, however these facilities are poorly supported and usually lack standard diagnostic microscopes and instead rely on rapid diagnostic tests (RDTs) for malaria and HIV diagnosis leaving other ailments like HAT unchecked. This implies that HAT is rarely diagnosed and as such is heavily under reported. In the same vein, diseases such as malaria tend to be over reported. Furthermore, previous studies in Itezhi- Tezhi district have demonstrated the presence of sporadic cases of HAT in humans, wildlife and domestic animals (Squarre *et al*.,2016, Squarre *et al*.,2020, Nakamura *et al*.,2021). However, these studies did not provide an active surveillance of HAT in humans presenting with fever at any local health facility, thus this study aimed at addressing the limitations of previous studies by attempting to highlight the prevalence of HAT in humans presenting with fever in rural health facilities of Itezhi-Tezhi district. Despite Itezhi-Tezhi being conducive for HAT transmission, it is not listed among the districts mainly reporting HAT cases in Zambia. Furthermore, no study has provided information on health delivery system in the district. Therefore, this study also aimed at assessing HAT health delivery system in order to provide information that will enhance the already existing health systems and contribute positively to control of HAT in Zambia. The information obtained from this study has potential to improve and alleviate challenges faced in rural areas in terms of diagnostics and management of HAT.

1.4 Research questions

1.4.1 What is the prevalence of HAT among febrile patients attending selected rural health facilities in ITT district?

1.4.2 What risk factors are associated with HAT among febrile patients attending selected rural health facilities in ITT district?

1.4.3 Are the health support structures present in selected rural health facilities in ITT district adequate to ensure robust HAT surveillance?

1.5 Study objectives

1.5.1General objective

To determine the prevalence of HAT and its associated risk factors in Itezhi-Tezhi district of Zambia.

1.5.2 Specific objectives

1.5.2.1 To determine the prevalence of HAT among febrile patients presenting at selected rural health facilities in ITT district.

1.5.2.2 To determine factors associated with HAT infection among febrile patients attending selected rural health facilities in ITT district.

1.5.2.3 To assess the district's HAT surveillance support structure present at selected rural health facilities in ITT district.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition and history of HAT

HAT is an infectious debilitating zoonotic disease caused by trypanosomes and transmitted by tsetse flies. Based on genes coding the small subunit ribosomal RNA, phylogenetic reconstruction shows that salivarian trypanosomes isolated from other trypanosomes 300 million years ago (Haag, O'hUigin and Overath, 1998). Salivarian trypanosomes became gut normal commensals of early insects which evolved 380 million years ago (Steverding, 2008). Since the emergence of tsetse flies 35 million years ago, trypanosomes have been transmitted by blood sucking insects (Steverding, 2008).

Modern times have described HAT to be closely linked to slave trade and that the first account of HAT came from ship doctors and medical officers who worked for slave trade companies (Steverding, 2008). Ship doctors were tasked by ship owners to explore the sleeping sickness disease that caused a lot of loses. In 1734, the first accurate medical report was published by the English naval surgeon in which he described neurological late-stage symptoms (Cox, 2004). In 1803, Thomas Winterbottom published a report referring to the characteristic sign of swollen lymph glands along the back of the neck in the early stage of the disease (Cox, 2004). He equally mentioned that this symptom was known long before by Arabian slave traders who held back from importing slaves with this sign (Winkle *et al*., 2005). Even though throughout the 19th century HAT reports increased, no one had any real idea about the nature of the illness (Cox, 2004). David Livingstone first suggested that nagana was caused by tsetse fly bites and in 1852 the existence of the disease in the Limpopo valley and along the Zambezi River, lakes Nyasa and Tanganyika where all cattle had died due to tsetse fly bites was reported (Winkle *et al.*, 2005). Forty to fifty years later, trypanosomes were identified as causative agents of nagana and sleeping sickness (Steverding, 2008). In 1895, David Bruce a Scottish pathologist and microbiologist discovered *T. brucei* as the cause of trypanosomiasis (Bruce,1895).

Africa experienced three severe sleeping sickness epidemics in the 20th century which commenced in 1896 and lasted until 1906 and affected mainly Uganda and Congo (WHO, 2006). In Zambia, it was first recognized during epidemics of *rhodesiense* HAT which were reported from the northern and southern regions of the Luangwa valley and Kafue river

valley in the 1960s and early 1970s (Buyst, 1974). In more recent times, less than 100 cases of HAT are reported in Zambia annually and these are mainly from Chama, Mpika, Chipata, Mambwe and Rufunsa districts (WHO, 2015).

2.2 Aetiology of Trypanosomiasis

Trypanosomiasis is caused by infection with protozoan parasites belonging to the phylum: Euglenozoa, class: Kinetoplastea, order: Trypanosomatida, family: Trypanosomatidae, genus *Trypanosoma* (Figure 2.1). The *Trypanosoma* species responsible for HAT *is T.b. rhodesiense.* They are transmitted to humans by tsetse fly bites which have acquired their infection from humans or from animals harbouring human pathogenic parasites (WHO. 2021). The six trypanosomes species that have been classically characterized in the KNP are all found in animal reservoirs (Squarre *et al.,* 2020). These include *Trypanosoma brucei brucei, T. godfreyi, T. congolense, T. sumiae, T. theileri* including the human infective *T.b. rhodesiense* which was detected in Bubalus bubalis and Hippotragus niger with 9.4% and 12.5% prevalence, respectively (Squarre *et al*., 2020). Another study conducted in the Kafue ecosystem that assessed the diversity of African trypanosomes in tsetse flies and cattle found a prevalence of 26.85% and 12.65%, respectively (Nakamura *et al*., 2021). Prevalence of human infective *Trypanosoma brucei rhodesiense* in cattle was 5.42% (Nakamura *et al*., 2021).

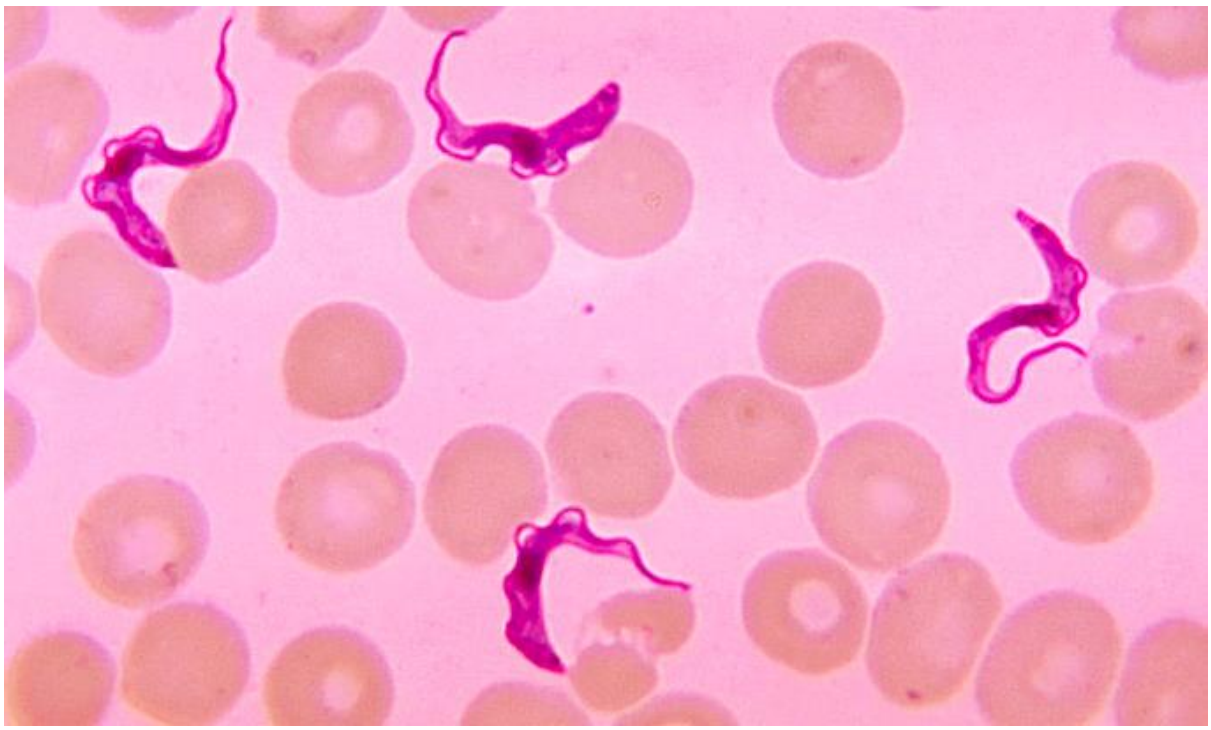

Figure 2.1: Trypanosomes on a blood smear. Source: *Trypanosoma sp*. PHIL 613 lores - African trypanosomiasis - Wikipedia [02/01/2022].

2.3 Transmission of trypanosomiasis to humans

The tsetse flies of the genus *Glossina* are responsible for transmission of Trypanosomes. Four species have been identified in Zambia namely *G. morsitans morsitan, G. brevipalpis, G. morsitans centralis and G. pallidipes* (Laohasinnarong *et al.*, 2015). The transmission cycle (figure 2.3.1) depends on the interactivity among infective parasites, mammalian reservoirs and habitat infested with *Glossina* tsetse flies (Franco *et a*l., 2014). Biological development of the trypanosomes only occurs in 2-5% of the tsetse flies (Franco *et al.*, 2014). On average only 1% of the tsetse flies will carry *T. Brucei* spp mature infection (Molyneux, 1980). However, even with the reduced ingestion of parasites in the blood meal, infection of a fly can result and one bite by the infected tsetse fly is enough to transmit infection to another mammalian host (Maudlin, I. and Welburn, 1989, Thiuta *et al.*,2008). The number of the trypanosomes injected into the mammal is related to the likelihood of HAT transmission (Franco *et al.*, 2014). Certain factors impact the transmission of the disease by the tsetse fly such as infection rates, vulnerability of the vector to infection, bulkiness of tsetse fly populations, survival of the tsetse flies in the vegetation, ease access to other blood meals, intensity and constant contact between host and tsetse fly (Franco *et al.*, 2014). Both male and female tsetse flies are hematophagous with females living longer and being more in numbers. The tsetse fly life span varies between seasons with the longest life span ranging from 3-5 months during rainy season and the shortest ranging from 1-2 months during the dry season (Franco *et al.*, 2014). Humans are mostly bitten by *G. fuscipes* and *G. palpalis* as they are drawn by human odour (Hargrove, 1976; Torr *et al.*, 2012), with *G. palpalis* feeding on many species. *G. morsitans* rarely bites humans due to the fact that human odour acts as a repellent (Rayaisse *et al.*, 2010).

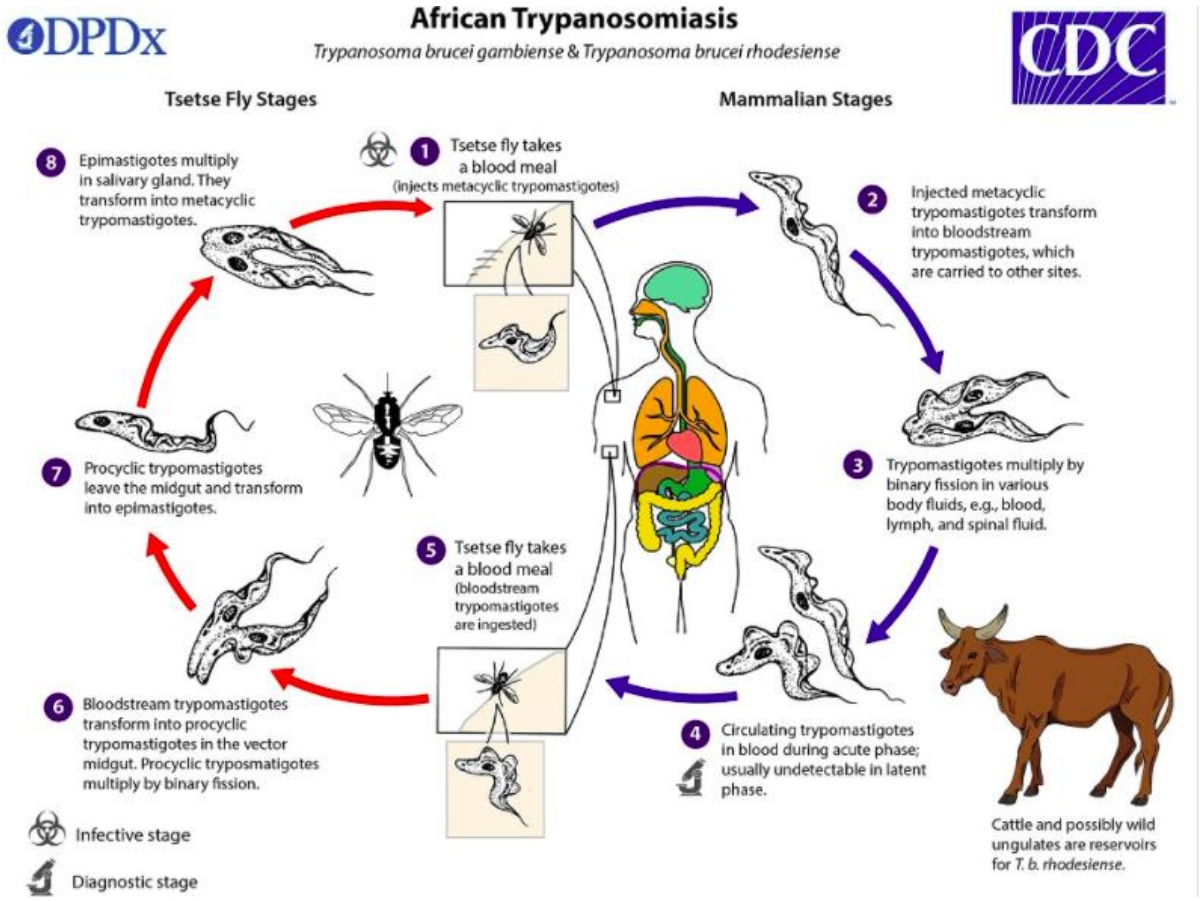

Figure 2.2: Life cycle of *Trypanosoma brucei* spp

Source CDC (2019): https://www.cdc.gov/parasites/sleepingsickness/biology.html [2/01/2022].

2.4 Diagnosis of Human African Trypanosomiasis

Several methods are used to diagnose trypanosomiasis and these are clinical, parasitological, serological and molecular tests. Screening of *T. b rhodesiense* infection in humans still relies on clinical features. Serological tests detect the humoral response in the blood, serum and cerebral spinal fluid (CSF). Microscopy, the standard reference diagnostic tool has high specificity and low sensitivity as compared to molecular tests (Marcotty, Simukoko and Berkvens, 2008). Its lack of sensitivity means that many patients maybe missed which may lead to death. Concentration methods like microhematocrit centrifuge, quantitative buffy coat and mini- anion exchange centrifugation technique improve sensitivity of microscopy but these are absent in resource limited settings (Mugasa *et al.*, 2012). On the other hand, molecular tests such as PCR are highly sensitive and specific for stage 1 HAT diagnosis (Mugasa *et al.*, 2012). Equally, LAMP has a higher sensitivity and specificity than

microscopy and has been described to be a more reliable test in detecting trypanosomes (Lisulo *et al.*, 2014).

Packed cell volume (PCV) which is the volume of blood occupied by red blood cells has been used as an indicator of trypanosomiasis infections (Sidibé, 2017). The existence of infections such as trypanosome infection and malaria have been found to crucially lower the PCV independent of age and sex (Nieuwenhove *et al.*, 2001; Goselle, Onwuliri and Onwuliri, 2009). A study in eastern province of Zambia by Marcotty *et al* (2008) showed that PCV value was a good individual indicator of presence of Trypanosoma infection in cattle. Further, the prevalence of AAT in anaemic cattle has been investigated using PCV as an indicator (Mbewe *et al*., 2015). Despite pathogenic trypanosome being strongly associated with low PCV, it only accounted for 41% of the cases (Mbewe *et al*., 2015). Attainability of a number of diagnostic tests, management of trypanosomiasis through diagnosis and treatment remains difficult (Connor and Van de Bossche, 2004). Simplified parasitological diagnostic tools such as buffy coat method tend to have low sensitivity in cases of low parasitaemia leaving 50% of the cases undiagnosed and untreated (Picozzi *et al*., 2002). Diagnostic pitfalls can be improved upon by utilizing molecular diagnostic tests that are highly specific and sensitive (Geysen, Delespaux and Geerts, 2003). In a meta-analysis study, PCR sensitivity on blood samples was determined to be 99% while specificity was 97.7% (Mugasa *et al.*, 2012). The study concluded that PCR had sufficient accuracy to replace microscopy where facilities allow (Mugasa *et al.*, 2012). In Zambia, a study on the use of LAMP showed that it had consistent results with microscopy which is commonly used for diagnosis of HAT. Therefore, LAMP was described as a prospective tool for HAT detection, staging and could serve as a useful tool for making therapeutic decisions (Namangala *et al*., 2013). Despite LAMP being easy, time efficient and very sensitive, it still has several challenges which limits its use in resource poor laboratories in most developing countries (Hayashida *et al.*, 2015). Another study which was done in Eastern province to determine prevalence of African Trypanosome species in dogs suggested that LAMP can be a reliable test for clinical management of cases as it was able to pick infection that was equally picked by microscopy and many more (Lisulo *et a*l., 2014). Furthermore, LAMP was found to have a high probability of detecting positive cases than microscopy.

2.5 Prevalence of HAT and its co-infections

Estimates of the burden of many tropical diseases including HAT may be inexact because of weak health surveillance infrastructure in impoverished countries. In regions where wildlife is the major reservoir, human cases materialize from time to time even when several years have passed without cases (Simarro *et al.*, 2011), while in regions where livestock is the main reservoir cases are seen more frequently (Odiit, M., Bessell, P.R., Fèvre, E.M., Robinson, T., Kinoti, J., Coleman, P.G., Welburn, S.C., McDermott, J. and Woolhouse, 2006). Human cases can be absent in both scenarios even though the parasite is still present in domestic reservoirs (Franco *et al.*, 2014). The neuropsychological impairment caused by HAT lead to some patients being hidden, discriminated or abandoned (Cordon-obras *et al.*, 2009). In a study which was done in Democratic Republic of Congo (DRC) by Mumba *et al*, (2011) to estimate HAT disease burden, leftover dried blood spots were tested using ELISA test followed by confirmatory trypanolysis and PCR tests. Results from this study revealed that there were more cases of HAT than what was reported. High prevalence of HAT was found in surveillance blind spots in DRC (Mumba *et al.*, 2011).

In Kenya, a study revealed that HAT patients have multiple co- infections which influence the disease pathogenesis and complicate management of HAT (Kagira *et al.*, 2011). Contrary to a study in Tanzania, co-infections with Malaria and HIV did not influence clinical presentation and therefore concluded that a comprehensive understanding of clinical presentation of HAT among Health workers and affected communities was important. Also understanding implications of co-infections could help aid resolution and differential diagnosis (Kuepfer *et al.*, 2011). Another retrospective analysis of clinical data from 258 HAT patients who reported to Lwala hospital in Uganda between 2005-2012 revealed about 93.4% of the cases were diagnosed as late stage and fever was significantly higher in patients below 18 years old (Kato *et al.*, 2015).

Between 2009-2018, Zambia reported a total of 60 cases of *T.b. rhodesiense* (Franco *et al*., 2020). Less than 100 cases are reported annually. These cases are usually reported from Chama, Mpika, Chipata, Mambwe and Rufunsa districts (Mulenga *et al*,.2015).

2.6 Risk factors for Human African Trypanosomiasis

Risk factor for HAT due to *T. rhodesiense* is associated with presence of non-human reservoirs such as wildlife and domestic animals. Increased contact between humans and animals increases the possibility of being in contact with infected tsetse flies that usually

feeds on animals (Franco *et al.*, 2014). Additionally, human activities colonizing animal environments and pressure to utilise new land areas for domestic animal grazing facilitate transmission of HAT. The activities and behaviour made by different age and sex groups predisposes to HAT (Abel *et al*., 2004). Children are reportedly less affected than adults. In some areas such as mangroves, teens present a higher rate of infection and this is related to fishing and leisure activities in water areas (Vanhecke C, Guevart E, Ezzedine K, 2010). In woodland and riverine forest galleries tsetse flies are found near rivers and streams. Transmission is connected to activities that have been developed along the water bodies such as fetching water, gold mining, washing clothing, fishing and leisure activities like boat cruising (Tongue *et al*., 2011, Robay *et al*., 2004). The island of vegetation between the woodland savannah and forest makes a good habitat for tsetse flies as well as a good area for agriculture, thus making farming activity a risk factor. Fishing campground are regions where human-tsetse fly contact can enormously happen (Franco *et al*., 2014). In regions where risk-activities include fishing, hunting and mining, disease burden is more in males. In regions such as transitional vegetation areas, infection is associated with agriculture and domestic activities at water bodies. Similar disease burdens have been reported in both males and females in such areas (Abel *et al*., 2004). In national parks and game management areas, HAT infection is associated with the close interface between humans and wildlife. Exposure to HAT also increases during movements of humans, animals and tsetse flies out or into reserve areas during particular times of the year.

Residents living in the boundaries of GMA and national parks are at highest risks. Movement of livestock for grazing in these areas was found to be another risk factor for herdsmen (Allsopp, 1972) and there is also possibility that the risk can be conveyed to other areas where cattle are moving and grazing making cattle rearing a risk factor. A study in Mbuji mayi in DRC revealed that HAT cases were associated with participation in different activities such as drawing water from wells, walking alongside the river bed and in peat lands, bathing near rivers, diamond mining and trafficking or buying diamond from diggers (Bilonda *et al.*,2015). Another cross-sectional study done by Rutto *et al* (2013) at the international boundary of Kenya and Uganda, explored social-economic and cultural determinants of HAT. It was reported that insufficient understanding on the disease cycle of tsetse fly and its control methods, culture, farming practices, demographic and socio-economic factors played a role in the incidence of HAT. The specific activities involved bathing in the river, herding, fishing and staying in bushy area (Rutto *et al.*, 2013).

Familial clusters also occur in *T.b. rhodesiense* HAT, which are mostly interconnected to similar exposure to vectors particularly since there are shared behavioural and spatial risk factors among household members (Zoller *et al*.,2008). In a candidate gene association study in DRC which used a family-based sample of 106 families belonging to the Yansi ethnic group and had been exposed to infection since birth revealed that the Human Leukocyte Antigen (HLA) G3' Untranslated Region (UTR) haplotypes were significantly associated with HAT (Courtin *et al*., 2013). In Zambia, dogs were found to be potential links for trypanosome exchange between livestock and humans in a study which was done in Eastern province which indicated the risk of contracting HAT (Lisulo *et al*., 2014).

2.7 Health delivery system of Human African Trypanosomiasis

In Uganda, a study by Acup *et al* (2017) on factors influencing passive surveillance for HAT targeting 86 medical personnel using a structured questionnaire revealed that most staff were aware of HAT from radiobroadcasts, newspapers and word of mouth. Essential understanding of the aetiology, clinical signs and HAT treatment drugs was minimal among staff and the interviewee didn't know if HAT was endemic in their districts. Therefore, the study concluded that there was a paramount gap in lower-level facilities in patient referral, diagnosis and reporting as compared to specialist treatment centres who were knowledgeable and optimistic when it came to HAT diagnosis and management (Acup *et al*., 2017). In Zambia, a questionnaire study in Chama and Mambwe districts involving 23 Health centres with 101 health personnel revealed that participants from both districts had basic understanding on how to recognize HAT presumptive cases and indicated that HAT was to be suspected upon ceaseless irregular fever and headache (Mulenga *et al.,* 2015). Various challenges were highlighted including low numbers of qualified personnel, general absence of HAT surveillance and control programs and neither district had LAMP nor PCR diagnostic tools (Mulenga *et al.,* 2015). A similar study in Mpika district, northern Zambia targeting health workers across 10 rural health facilities concluded that 46% of the 28 participants scored more than 50% on general knowledge about HAT and none of the participants had understanding on how to differentiate the acute and chronic clinical stages of HAT (Mwanakasale *et al.,* 2013). Acknowledged challenges were shortage of trained health workers, inadequate diagnostic and treatment centres, lack of more sensitive laboratory diagnostic techniques and shortage of trypanocides (Mwanakasale, Songolo and Daka, 2013).

CHAPTER THREE

3.0: RESEARCH MATERIALS AND METHODS

3.1 Study site- The study was conducted at Itumbi, Mutenda and Ngoma rural health facilities (figure 3.1) in Itezhi-Tezhi district. These rural health facilities are in or very close to the KNP and GMA. Itezhi-Tezhi is part of Southern province and lies approximately 178.6 km north of Mumbwa and 62 km north west of Namwala district across the Kafue River on the north bank. The Kafue river is the largest in the district and it is from this that lake Itezhi-Tezhi emanates (Itezhi-Tezhi District Health Office Action Plan and Budget,2019-2021). Itezhi-Tezhi has swampy plains known as Kafue flats which cover 60% of the district while 40% is highland covered by thick forest. The district has three distinct seasons: the rainy season from November to April; the cold season from May to July: and the hot season from August to October. The temperature ranges between 3 º C in the cold season and 36 ºC in the hot dry season. The average mean rainfall is 40mm. This climate is favourable for agriculture. Most of the people have resolved to settling along the Kafue river and around lake Itezhi-Tezhi (to pursue the fishing industry) and in the Urban area (for formal employment and trading). The rest are scattered all over the plains for purposes of cattle rearing. Itezhi-Tezhi has a surface area of 13,064² km with a population of 86,720 people as of 2019 of which 50.9% are females and 49.1% are males (CSO,2010). Annual growth rate is rated at 4.8% (CSO,2010) due to growing levels of fishing activities and infrastructure development. Itezhi-Tezhi shares borders with Kaoma in the west, Kazungula and Kalomo in the south west, Mumbwa in the north, Choma in the south, Namwala in south east and Mazabuka in the east.

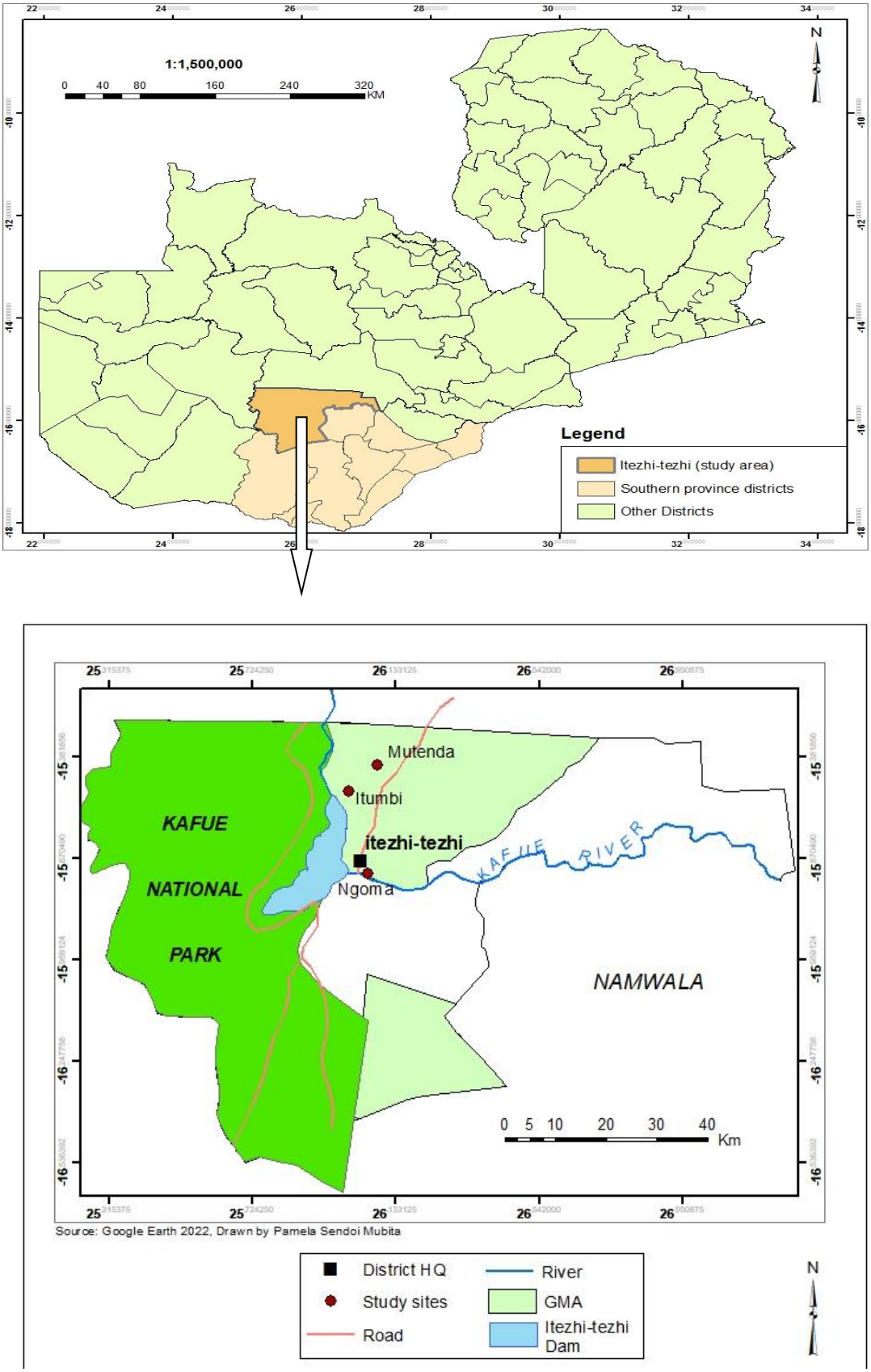

Figure 3.1: Location of study areas in proximity to KNP.

3.2 Study design – This was a cross-sectional study that involved blood sample collection, administration of questionnaires and review of Hospital affiliated information register (HIA) data under notifiable diseases including Trypanosomiasis.

3.3 Study Participants- Individuals aged 11 years and above seeking medical care at Itumbi, Mutenda and Ngoma rural health facilities and surrounding communities were purposively selected and included upon assent/consent.

3.3.1 Case definition- Suspected case was a patient presenting with signs and symptoms (fever or history of fever) similar to HAT with a history of tsetse fly bites.

3.3.2 Confirmed case- All patients whose blood tested positive by Microscopy, PCR or LAMP.

3.3.3 Inclusion criteria- Patients above 11 years with history of fever, tsetse fly bites and signed consent/assent were included in the study.

3.3.4 Exclusion criteria- Patients below 11 years with no fever and those who failed to sign the consent or assent were excluded.

3.4 Sample size- Patients were purposively sampled, but their inclusion in the study was largely based on their willingness to participate. Written informed consent from each patient was required prior to their participation in the study. The Cochran formular (Cochran 1977) was used to ascertain sample size for the study presuming a 50% prevalence so as to obtain sufficient sample size to approximate the population prevalence with a good precision.

$N=Z^2 P(1-P)/d^2$. Where N= required sample size, Z= Confidence level at 95% (Standard value of 1.96), d=level of precision at 5%, P= Proportion (0.5%) =384. Assumptions, Prevalence 50%, Confidence interval at 95%, Precision at 0.05.

Since total number (N) of febrile patients was 147 for the month of August, 2021 in HIA register and study population was less than 10,000 the following formular was used to get final sample size.

$$n = \frac{n_0}{1 + \frac{(n_0 - 1)}{N}}$$

Where;

n =final sample size, n_0 =required sample size, N= number of febrile patients

n= 384/ 1+ [(384-1)/147]

Final sample size (n) =107

3.4.1 Sampling procedure

The sample size of 107 was equally distributed among the 3 rural health facilities. Participants were purposively selected among those who presented with fever and history of tsetse fly bites.

3.5 Determination of prevalence of Human African Trypanosomiasis

3.5.1 Sample collection and Laboratory procedure

A total of 114 blood samples were collected in Ethylenediaminetetraaacetic acid (EDTA) tubes (n=108) via venepuncture as well as capillary tube (n=6) from finger prick. Blood from the capillary tube was spotted onto Whatman FTA Classic Cards (GE Healthcare, Madison, WI, USA) for patients who didn't consent for venepuncture. The FTA cards were air dried, placed in zip lock storage bags containing silica gel and stored at room temperature away from sunlight. Whole blood samples were stored between 4 to 8 degrees Celsius in a temperature monitored cool box or refrigerator after collection and during transportation to the University of Zambia, School of Veterinary Medicine Laboratory.

3.5.2 Determination of Packed Cell Volume

Packed cell volume was measured as an adjunct or indirect indicator of HAT in the absence of other anaemia causing conditions (Marcotty, Simukoko and Berkvens, 2008; Mbewe, 2015). Briefly, blood was placed in capillary tube, sealed at one end and then centrifuged in a microhematocrit centrifuge for 4 minutes at 9000 revolutions per minute. The PCV was then read using a haematocrit reader to determine PCV. A total of 113 blood samples were analysed for PCV in this manner.

3.5.3 Microscopy

Thin blood smears were prepared from 114 samples, stained with 10% giemsa stain and examined for the presence of trypanosomes under the light microscope at ×100 immersion oil.

3.5.4 Extraction of Deoxyribonucleic acid

Deoxyribonucleic acid (DNA) was extracted from 108 whole blood samples using Qiagen kit for mammalian blood following the manufacturer's instructions. Extraction from the 6 FTA cards was done by first punching out the stained blood circles from FTA card and placing each in eppendoffs tubes followed by the addition of 400 microliters of distilled water. The eppendoffs tubes were then vortexed and incubated at 37 °C for an hour. The eppendoffs

were then heated using dry block at 80°C for 10 minutes and vortexed. The eppendoffs were centrifuged at 2000rpm for 5 minutes. Supernatant containing the DNA was transferred into new eppendoffs.

3.5.5 Polymerase chain reaction and gel electrophoresis

Ribosomal RNA Internal transcribed spacer (ITS)-PCR was performed on DNA samples extracted from both whole blood and FTA card according to the standard operating procedure using AITS-F: CGGAAGTTCACCGATATTGC and AITS-R: AGGAAGCCAAGTCATCCATC (Gaithuma *et al*., 2019) and human Serum Resistance Associated gene (SRA) primers as described by Radwanska *et al* (2002). One Taq quick load polymerase 2X Master mix (New England BioLabs, Ipswich, MA, USA), with standard buffer was prepared and each reaction mix contained 3.55 µL nuclease free water, 0.2 µL of 0.2 µM forward primer,0.2 µL of 0.2 µM reverse primer, 0.05 µL one Taq quick load polymerase and 1.0 µL DNA template. For the negative and positive control 1.0 µL of distilled water and 1.0 µL of *T.b. rhodesiense* DNA were used respectively. The reaction mixtures were placed in a programmable thermocycler with the following conditions of denaturation at 95°C for 10 mins, 30 cycles of 30 seconds at 94°C, 1 minute at 60°C and 1 min at 72°C. A final extension step was carried out at 72°C for 5 minutes. Amplicons were visualised under ultraviolet light on 1.5% agarose gel stained with ethidium bromide. Briefly, agarose was prepared as follows, 1.5g of agarose powder was dissolved in 100ml of TAE buffer followed by heating and intermittent mixing to allow the powder to dissolve completely. Afterwards, 4 µL of Ethidium bromide was added and mixed well with the agarose gel. Expected band sizes for *T. brucei* were 415-431 bp and *T. brucei rhodesiense* 284 bp.

3.5.5 Loop mediated isothermal amplification assay

Liquid SRA LAMP protocol was performed as described by Hayashida (2021). A reaction mixture of 25 µL comprising of 9.6µL nuclease free water, 2.5µL 10x buffer, 1.4µL DNTP, 1.5µL MgSo4, 1 µL of CFI and 1 µL of each of the 6 primers, 1 µL of DNA polymerase and 2 µL of purified DNA template. For the negative control, 2 µL of distilled water was used as template while 2 µL of *T.b.rhodesiense* DNA was used as a positive control. The primer sequences were as follows SRA-FIPm 5’CTGCGTTGAGTACGCATCTTGCACAGACCACAGCAACATC3’, SRA-BIP 5’CGCTCTTACAAGTCTTGCGCCCTTCTGAGATGTGCCCACTG3’, SRA-LFm 5’CGGCATAAAGCGCTGAGA3’, SRA-LB 5’GCAGCGACCAACGGAGCC3’, SRA-F3m 5’AACAAGTATCGGCAGCAACC3’, SRA-B3 5’TCTTACCTTGTGACGCCTG3’. The

LAMP reaction was started for 40 minutes at 60 degrees Celsius. Results were assessed based on colour change from yellow to green fluorescence under the transilluminator or via the naked eye. To avoid contamination, 2 independent working spaces were used. A total of 114 purified DNA samples were analysed and interpreted.

3.6 Determination of HAT risk factors among patients attending Mutenda, Itumbi and Ngoma rural health facilities in Itezhi-Tezhi

3.6.1 Questionnaire survey

Data on demographics and possible factors associated with HAT were collected using a structured questionnaire. Demographic information such as name of the patient, age, gender, residence, occupation, religion and highest level of formal learning were collected.

Data on possible risk factors for contracting HAT such as herding animals, game range viewing, fishing, fetching water in natural holes or pools and food staff dealers from farms was also collected. Targeted participants for questionnaires were those who were seeking medical care at the Itumbi, Mutenda and Ngoma rural health facilities and those who had history of tsetse fly bites in the surrounding areas and those working for the Department of National Parks and Wildlife campsites, lodges in GMA and KNP. Participants were purposively selected and interviewed in a face- to- face manner. The interviews were carried out in English for those that were able to communicate in English and in Ila, Tonga, Nyanja and Bemba for those that could not. A total of 128 questionnaires were administered with a breakdown of 60 in Mutenda, 53 in Itumbi and 15 in Ngoma. Participants had the right to either participate in questionnaire answering or have blood sample collected from them, or participate in both at the same time.

3.7 Assessment of Itezhi-Tezhi district's HAT surveillance support structures

Assessment of district support structure was done through interviews of health care workers using structured questionnaires. The surveillance support structures assessed were human resource availability, formal training in HAT, availability of diagnostic tools and commodities, availability of treatment protocols, referral system and HAT community awareness education. Targeted health care workers were those who attended to patients at the rural health facilities i.e., clinical officers, nurses and community health assistants. A total of 21 questionnaires were administered to health care workers (HCW) at the Mutenda, Itumbi

and Ngoma rural health facilities. This was because establishment of HCW was approximately 8 or less per facility.

3.8 Data analysis

Data collected using structured questionnaires was coded and the responses were entered in the IBM statistical package for social sciences (IBM spss). Data was analysed and frequency tables on demographics were generated. Furthermore, cross tabulation and chi-square test was done with PCV as the dependent variable and demographics, clinical characteristics and risk factors as independent variables to ascertain if there was an association. PCV was used as an indirect indicator of HAT and p- values under 0.05 were considered statistically significant at 95% confidence interval.

3.9 Ethical consideration

Ethical approval was sought from University of Zambia Biomedical Research and Ethics committee (UNZABREC) with reference number 1842-2021 before commencement of the sample and data collection. Permission was also sought from the Ministry of Health through the District Health office. Individual participant consent was obtained through verbal explanation and written consent. To ensure confidentiality, only researchers were allowed to handle blood samples and patient meta data.

CHAPTER FOUR

4.0: RESULTS

4.1 Characteristics of study participants

From the total number of 128 answered questionnaires, 17.2%participants were in the age range of 11-20, 32.0% between 21-30 years, 21.1% between 31-40 and 27.3% above 40 years. Missing information was observed in 2.3% study participants. Furthermore, based on gender, the majority of study participants were males 71.9% with females only accounting for 28.1%.

With regards to access to the health facility, 36.7% participants lived within 5km radius to the facility, 18.0% lived 10km away from the facility, 43.0% lived more than 10km away from the facility. However, 2.3% had missing information of residence. Majority of study participants lived more than 10km away from the health facilities. Furthermore, when occupation was assessed occupation 57.8% were farmers ,7.8% were Fishermen, 15.6% were in formal employment, 5.5% were unemployed and others 9.4%. 3.9% did not indicate their occupation. Majority of study subjects were farmers.

The level of education was distributed as follows, 50.1% of the study participants attained primary level education, 33.6% attained secondary education, 8.6% attained tertiary education and 4.7% had not attended school. Missing information was observed in 32.3. Majority of participants attained primary education as their highest level of education.

4.2 Packed cell volume results

A total of 113 samples (Table 4.2.1) were analysed for PCV and 19.5% study participants had PCV below minimum acceptable value, 72.5 % had PCV within normal acceptable range and 8.0% had PCV above the maximum acceptable value. The PCV results were compared to reference ranges for women (35.5%-44.9%) and men (38.3%-48.6%) respectively. Majority of participants had normal PCV. However, more men 11.5% had low PCV as compared to women 8.0% and majority with low PCV 9.0% were in the age range 21-30. Patient age and sex had no statistical significance on PCV ($p = 0.931$ and 0.143 at 95% CI), respectively.

Table 4.2.1 PCV results according to age and sex of participants

			Low pcv	Normal pcv	High pcv	Total	P- value
Patient age	11-20	n	4	15	1	20.0	
		%	3.6	13.5	0.9	18.0	
	21-30	n	10	29	0	39.0	
		%	9.0	26.1	0.0	35.1	
	31-40	n	2	17	5	24.0	0.931
		%	1.8	15.3	4.5	21.6	
	>40	n	5	20	3	28.0	
		%	4.5	18.0	2.7	25.2	
		N	21	81	9	111	
		%	18.9	73.0	8.1	99.9	
Gender of patient	Female	n	9	19	4	32.0	
		%	8.0	16.8	3.5	28.3	
	Male	n	13	63	5	81.0	
		%	11.5	55.8	4.4	71.7	0.143
Total		N	22	82	9	113	
		%	19.5	72.6	8.0	100	

With regard to PCV and distance from the facility, 9.1% participants with low PCV resided within five km radius of the health facility and 8.2% resided more than 10km from the facility. 10.9% participants with low PCV were farmers. However, participants residence and occupation were not significantly associated with PCV, p = 0.242 and 0.263 respectively (Table 4.2.2).

Table 4.2.2 PCV results according to distance of HF to residence and occupation of participants

			Low pcv	Normal pcv	High pcv	Total	P value
Location of residence Village	within 5km	n	10	28	6	44	
		%	9.1	25.5	5.5	40.0	
	10km away	n	3	16	1	20	
		%	2.7	14.5	0.9	18.2	
	> 10km	n	9	36	1	46	0.242
		%	8.2	32.7	0.9	41.8	
Total		N	22	80	8	110	
		%	20.0	72.7	7.3	100.	
Occupation	farming	n	12	47	5	64	
		%	10.9	42.7	4.5	58.2	
	fishing	n	1	7	2	10	
		%	0.9	6.4	1.8	9.1	0.263
	formal employment	n	7	12	0	19	
		%	6.4	10.9	0.0	17.3	
	unemployed	n	0	5	1	6	
		%	0.0	4.5	0.9	5.5	
	others	n	2	9	0	11	
		%	1.8	8.2	0.0	10.0	
Total		N	22	80	8	110	
		%	20	72.7	7.2	100	

When PCV and fever were assessed, 15.9% participants with low and 2.7% with high PCV measured body temperature of 37.5 ºC. 3.5% participants with low PCV measured body temperature of 38.0 ºC. No participant with low PCV tested positive for Malaria while 2.7% participants with normal PCV tested positive for Malaria. Furthermore, majority of participants 25.6% with normal PCV reported having fever for more than five days (Table 4.2.3). However, fever, days with fever and Malaria results were not significantly associated with PCV (P = 0.673, 0.534,0.471).

Table 4.2.3 PCV results according to fever, Malaria results and days with fever

			Low pcv	Normal pcv	High pcv	Total	p-value
Axillary temperature ºC	37.5	n	18	62	3	83	0.673
		%	15.9	54.9	2.7	73.5	
	38.0	n	4	20	6	30	
		%	3.5	17.7	5.4	26.6	
Total		N	22	82	9	113	
		%	19.5	72.6	7.9	100.	
Malaria results	positive	n	0	3	0	3	0.534
		%	0.0	2.7	0.0	2.7	
	negative	n	22	79	9	110	
		%	19.5	69.9	7.9	97.3	
Total		N	22	82	9	113	
		%	20.0	70.8	7.9	100.0	
Days with fever	2days	n	6	10	0	16	0.471
		%	15.4	25.6	0.0	41.0	
	5-7days	n	2	10	2	14	
		%	5.1	25.6	5.1	35.9	
	8-14days	n	1	5	1	7	
		%	2.6	12.8	2.6	17.9	
	>14days	n	0	2	0	2	
		%	0.0	5.1	0.0	5.1	
Total		N	9	27	3	39	
		%	23.1	69.2	7.7	100.0	

According to Table 4.2.4, 6(8.8%) and 4(5.9%) participants with low PCV complained of headache and general body weakness, respectively. Furthermore, 6(9.0%) participants had taken some medication, 8(11.9%) had not taken any medication before coming to the facility. Signs and symptoms of HAT and self -medication were not significantly associated with PCV.

Table4.2.4 PCV results according to signs and symptoms of HAT and self -medication

			Low pcv	Normal pcv	High pcv	Total	P-value
Signs and symptoms of HAT	Headache	n	6	15	3	24	0.66
		%	8.8	22.1	4.4	35.3	
	skin rash	n	0	3	0	3	
		%	.0	4.4	.0	4.4	
	Itching and inflammation of skin	n	0	1	0	1	
		%	.0	1.5	.0	1.5	
	delayed sensation to pain	n	0	1	0	1	
		%	.0	1.5	.0	1.5	
	general body weakness	n	4	10	3	17	
		%	5.9	14.7	4.4	25.0	
	drowsiness	n	1	0	0	1	
		%	1.5	.0	.0	1.5	
	2 or more signs and symptoms	n	2	17	2	21	
		%	2.9	25.0	2.9	30.9	
Total		N	13	47	8	68	
		%	19.1	69.1	11.8	100.0	
Medicine taken before coming to the facility	yes	n	6	17	4	27	0.79
		%	9.0	25.4	6.0	40.3	
	no	n	8	28	4	40	
		%	11.9	41.8	6.0	59.7	
Total		N	14	45	8	67	
		%	20.9	67.2	11.9	100.0	

With regard to PCV and keeping animals, 11/102(10.8%) and 2/102(2.0%) of participants with low PCV kept cattle and dogs at home, respectively. 10/90 (11.1%) participants with low PCV herded animals (Table 4.2.5). Keeping animals was statistically associated with

PCV (p= 0.011). However, herding animals was not statistically associated with PCV (p= 0.280).

Table 4.2.5 PCV results according to animals kept at home and herded.

			Low pcv	Normal pcv	High pcv	Total	p-value
Animals kept at home	cattle	n	11	15	1	27	0.011
		%	10.8	14.7	1.0	26.5	
	sheep	n	0	1	1	2	
		%	.0	1.0	1.0	2.0	
	goats	n	0	4	0	4	
		%	.0	3.9	.0	3.9	
	dogs	n	2	2	1	5	
		%	2.0	2.0	1.0	4.9	
	others	n	0	5	1	6	
		%	.0	4.9	1.0	5.9	
	2 or more types of animals	n	4	34	2	40	
		%	3.9	33.3	2.0	39.2	
	none of the above	n	1	15	2	18	
		%	.0	14.7	2.0	17.6	
Total		N	18	76	8	102	
		%	17.6	74.5	7.8	100.0	
Herd animals mentioned above	Yes	n	10	43	1	54	0.280
		%	11.1	47.8	1.1	60.0	
	No	n	8	25	3	36	
		%	8.9	27.8	3.3	40.0	
Total		N	18	68	4	90	
		%	20.0	75.6	4.4	100.0	

With regard to PCV and participants source of domestic water in table 4.2.6, 33(31.7%) participants got their water for domestic use from the river followed by 20(19.2%) who got their water for use from the stream. Source of water was statistically associated with PCV (p=value 0.028).

Table 4.2.6 PCV results according to participants source of water for domestic use

			Low pcv	Normal pcv	High pcv	Total	P-value
Source of water domestic use	communal tap	n	2	14	0	16	0.028
		%	1.9	13.5	.0	15.4	
	river	n	5	24	4	33	
		%	4.8	23.1	3.8	31.7	
	stream	n	5	14	1	20	
		%	4.8	13.5	1.0	19.2	
	underground water	n	4	13	0	17	
		%	3.8	12.5	.0	16.3	
	others	n	6	9	3	18	
	Total	N	22	74	8	104	
		%	21.2	71.2	7.7	100	

4.3. Microscopy results

The 114 giemsa stained smear samples were examined by microscopy. No trypanosomes were seen in any of the samples. However, malaria parasites were seen in smears from three samples.

4.4. Polymerase chain reaction results

The 114 DNA samples were subjected to ITS/SRA PCR screening. As shown in figure 4.4.1, none of the samples was positive for *T. b.rhodesiense.*

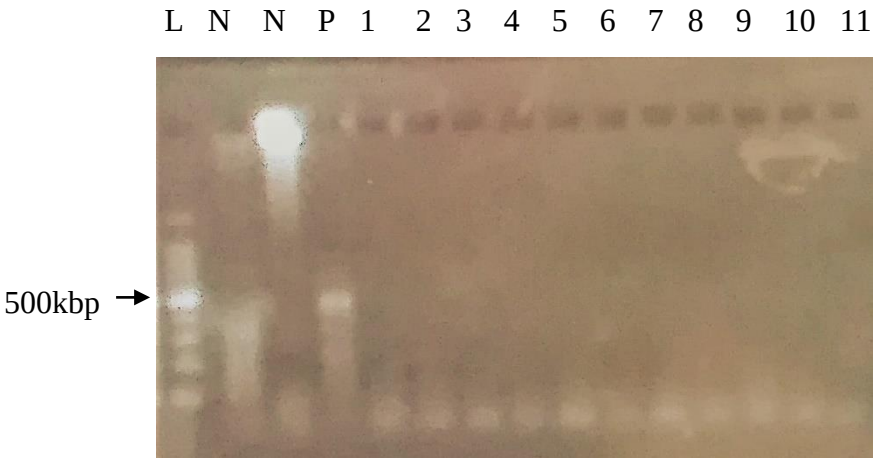

Figure 4.4.1: Representative PCR results. From left to right, first well L-marker, N and N distilled water was added (negative control), fourth well (P) was *T. brucei rhodesiense* DNA sample (positive control). From one to eleven representative DNA template from participants. (Expected band size for *T. brucei* 415-431kbp).

4.5. Loop mediated isothermal amplification results

The 114 samples were also examined for the presence of *T. b. rhodesiense* by SRA-LAMP. As shown in Figure 4.5.1, all the DNA samples tested negative for *T. b. rhodesiense*.

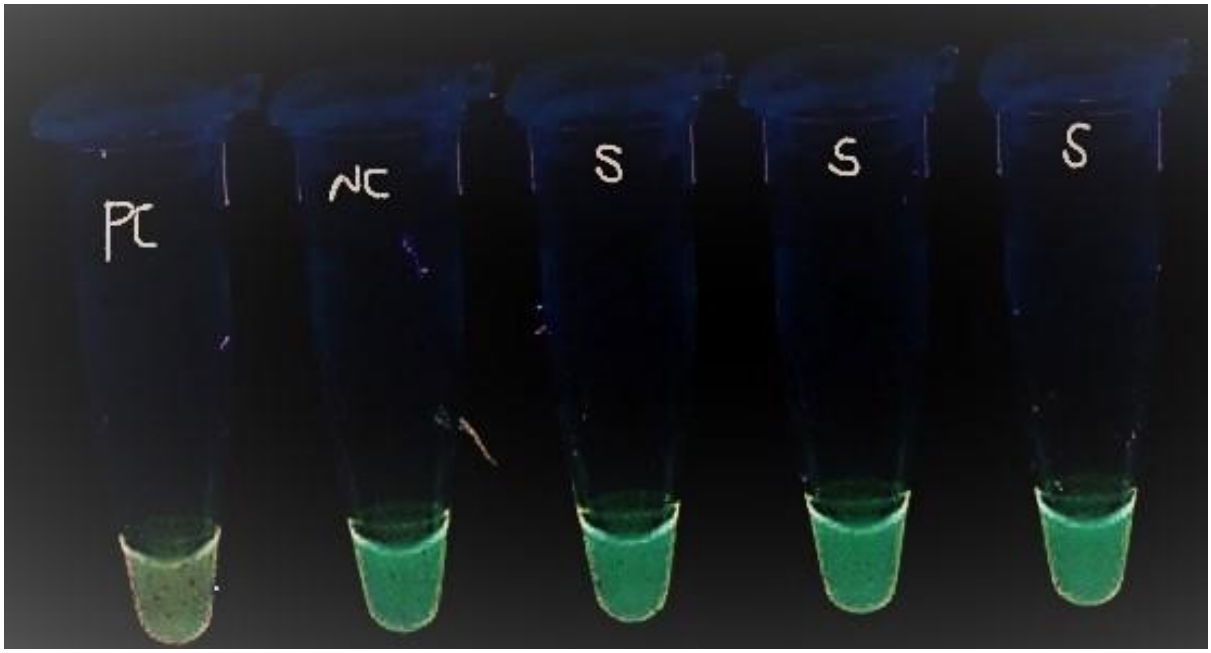

Figure 4.5.1: Representative SRA-LAMP. PC: positive control (*T. b. rhodesiense* DNA); NC: negative control: distilled water was used, S, S, S: representative samples.

4.6 Itezhi-Tezhi district HAT support structure assessment results

The following were the results for the type of Health workers interviewed as shown in figure 4.6.1, 15.8% were clinicians,36.8% were nurses, 26.3% were Environmental health personnel, 21.1% were others for instance community health assistants. Majority of the health care workers were nurses.

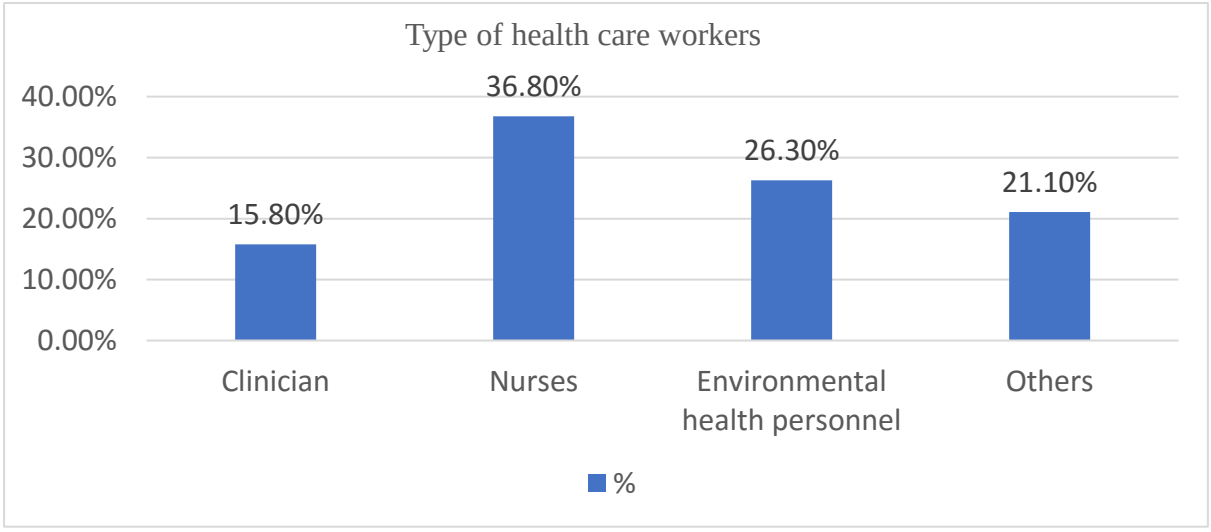

Figure 4.6.1: Type of health workers

Among the study participants, the distribution of years of work experience for the different health care workers was, less than five years (52.6%), six to ten years (31.6%) and more than

ten years (15.8%). Overall, the majority of health care workers had less than five years' working experience. (figure4.6.2)

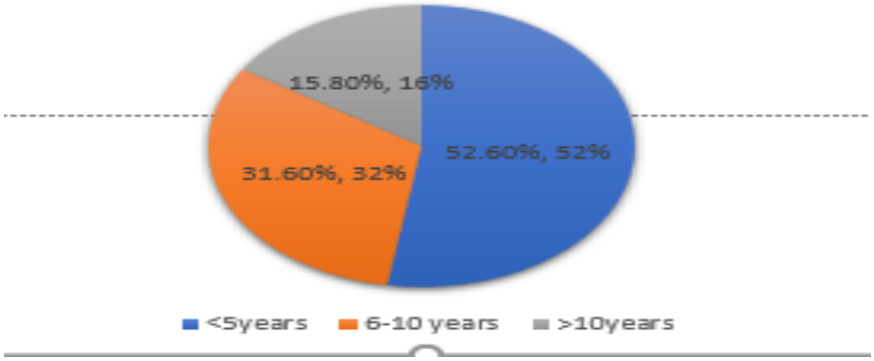

Figure 4.6.2: Years of work

Education level of different interviewed health care workers according to figure 4.6.3 was 47.4% were certificate holders, 52.6% were diploma holders representing the majority.

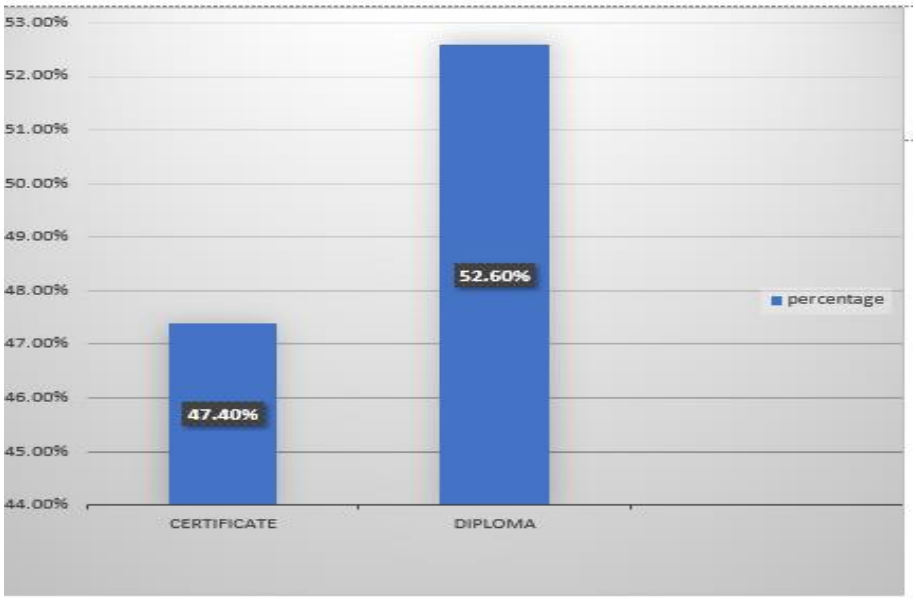

Figure 4.6.3: Education level of health care workers

Majority of health care workers (94.7%) didn't have a formal training or orientation in HAT case detection and management while 5.3% had reported having had some training in HAT (Figure 4.6.4). Health care workers who reported availability of treatment guidelines were 15.8%. Majority of them 84.2% reported guidelines were not available. Furthermore, zero%

reported availability of microscope while 100 % reported microscope was not available. Haematocrit centrifuge was not available at the facility as reported by 100% of HCW. Awareness of HAT education was being conducted by 63.2% of health care workers while 36.8% did not conduct HAT awareness education to the community. With regard to slides and giemsa, 100% reported that slides and giemsa were not available at their facility. Only 5.3% HCW referred patients with ceaseless fever to a higher health facility with diagnostic facilities.

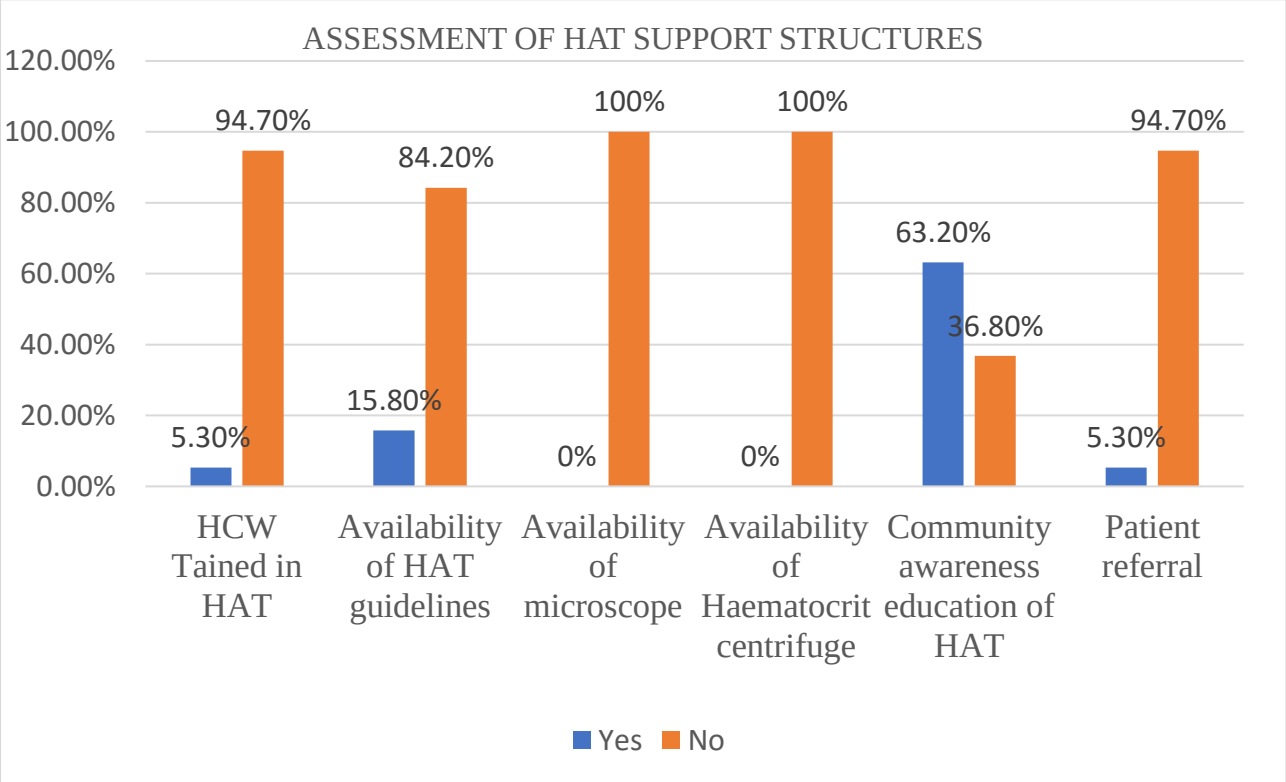

Figure 4.6.4: Assessment of HAT support structure

CHAPTER FIVE

5.0 DISCUSSION

This study aimed to determine the prevalence and risk factors of HAT in Itezhi-Tezhi district. Packed cell volume was used as an indirect indicator for trypanosome infection in the absence of other anaemia causing conditions like Malaria. The presence of *Trypanosoma* infection significantly reduces the PCV irrespective of age and sex (Marcotty, Simukoko and Berkvens, 2008). In this study PCV was also used as an indirect indicator for the presence of trypanosome infection. The study findings revealed 19.5% had PCV below the minimum normal acceptable value for both males and females. It was also noted that none of the participants with low PCV tested positive for Malaria. Low PCV was significantly associated with keeping animals at home and source of domestic water with p-values of 0.01 and 0.028. respectively. This finding suggested that there were other causes of anaemia rather than Malaria and trypanosomes. Malaria positive cases were found among individuals with normal PCV, whose parasitaemia were fairly low. In contrast, a study that investigated prevalence of AAT in anaemic cattle found that even though pathogenic trypanosome was strongly associated with low PCV, it only accounted for 41% of the cases (Mbewe *et al*., 2015) and suggested the need to ascertain other causes of anaemia. The importance of anaemia may well be underestimated because of difficulty in diagnosis especially where parasitaemia may be very low and clinical picture maybe confused with other causes.

The study findings were that there were no positive cases of HAT on all three diagnostic platforms that were used which consistently meant samples were truly negative. This didn't mean HAT cases were absent in Itezhi-Tezhi as evidenced by the HAT cases previously reported in the study site (Squarre *et al*., 2016). The small sample size which was attributed to apathy from the people also lowered the chances of detecting HAT infection. The apathy was alluded to a certain researcher who collected blood samples and promised to send back the results but never did. This left the communities very suspicious and some accusing that their blood was used for rituals. For this reason, not all individuals who presented at the facilities with symptoms were screened despite being sensitized. It has been reported that active and passive surveillance may both underestimate HAT cases and this was evidenced in a study in DRC that found that prevalence of HAT cases were twice more than what was reported (Mumba *et al*., 2011). However, *T.b. rhodesiense* HAT is mainly detected by passive surveillance (Odiit *et al.,* 2004) as opposed to *T.b. gambiense* HAT which both active

and passive surveillance are able to yield positive cases. Because of the fast disease progression of *T.b. rhodesiense* HAT, it was also possible that at the time of sampling, cases were in existence but patients could not come to the facility as they could have been bedridden and some could have died without knowing the cause of their death. Furthermore, because of the stigmatization that comes with neuropsychological impairment, patients tend to be hidden or abandoned (Cordon *et al*., 2009) and some withdraw from healthcare systems to seek help from traditional/spiritual healers because they believe they have been bewitched (Odiit *et al*., 2004). Patients could have also failed to recognise symptoms of HAT and therefore did not present at the healthcare facility to obtain diagnosis and as such were undetected and unreported (Fèvre *et al.*, 2008). In addition, presence of several risk factors to HAT have been evidenced in Itezhi-Tezhi by the detection of *T.b. rhodesiense* in tsetse flies and cattle with a prevalence of 26.85% and 5.42% respectively (Nakamura *et al.*, 2021). Itezhi- Tezhi district is also a region where *T.b. rhodesiense* has been found to be in circulation in wildlife (Squarre *et al.,* 2020). For these reasons, results could not be generalised to mean the district is free of HAT as regions where wildlife is the main reservoir, human cases can happen even after several number of years have passed without recording any case (Simarro *et al*., 2011). Contrary to our findings, human cases are frequently seen in regions where the main reservoir is livestock (Franco *et al.,* 2014). It has also been observed that in instances where the parasite is present in wildlife, livestock and tsetse flies, human cases can be absent (Franco *et al.,* 2014). In 2013, the prevalence of HAT caused by *T.b. rhodesiense* in kenya was 0% which corresponded with our study findings. In the same year, Zambia reported a prevalence of 6 cases while other countries like Malawi which reported prevalence of 35 cases, Tanzania 1 case and Uganda reported the highest with 43 cases (Franco *et al*., 2014). Zambia has been reported to be a country with the largest area at risk covering 32,000km²followed by Malawi with 14,000km²and Tanzania 12,000 km² (Franco *et al.,* 2020). Zambia was equally reported to have had cases of *T.b. rhodesiense* every year between 2009 to 2018 with a total prevalence of 60 cases (Franco *et al.,* 2020).

This study also aimed at determining risk factors associated with HAT. Many studies suggested that *T.b. rhodesiense* HAT is associated with presence of wildlife and domestic animals such as cattle. In this study, keeping of animals such as cattle, goats and sheep was a source of livelihood and was found to be a major activity. Study findings of a study which was done in Mambwe district reported that dogs were a potential link for trypanosome exchange between humans and livestock as SRA gene in trypanosomes was detected in dogs

(Lisulo *et al*., 2014). This study did not check for trypanosomes in dogs. However, the study done by Lisulo *et al*., (2014) indicated that keeping dogs was a possible risk factor to HAT which in this study, some participants kept dogs. The findings also revealed that the age group between 21-30 was predominant among respondents with 35.1%. Though no positive case was detected in our study, findings in Ugandan study revealed that most reported HAT cases were from individuals between the age of 20-29 years (Odiit *et al*., 2004). This age group is the most active population with activities such as fishing, collecting firewood, preparing charcoal and hunting which brings them close to tsetse fly habitats (Odiit *et al*., 2004). Different activities done by different age groups have been known to predispose to HAT. Farming was the most common occupation which is similar to the study findings in Kenya, where almost all inhabitants at risk of HAT were subsistent farmers (Zoller *et al*., 2008). Farming was a risk factor because the vegetation that supports agriculture was also a good habitat for tsetse flies. Fishing was one other occupation done by the respondents which in a study done by (Franco *et al*., 2014) was described as a campground where human-tsetse fly interactivity is high. Transmission is connected to activities done along water bodies (Robay *et al*., 2014) which this study observed. Most activities were done along water bodies such as swimming, drawing water, luxurious procreation such as lodges. Majority of the respondents resided 10km away from the rural health facilities because of similar activities which are being done along the Kafue River. In regions where activities such as fishing, mining and hunting are common, males are at a higher risk (Zoller *et al.*, 2008). Similarly in this study males did similar activities such as fishing and hunting making them prone to HAT. National parks and GMA infection has been associated with encroachment of people into wildlife confined places (Kinung *et al*., 2006) which was similarly observed in this study as most respondents were living in or close to KNP making them more likely to contracting HAT infection.

Furthermore, the study also investigated if the district health support structures present in Itezhi-Tezhi district were adequate for robust HAT surveillance. The systems investigated were in terms of Human resource availability, training, diagnostic facilities, treatment guidelines, referral system and HAT community awareness education. The results obtained showed that majority of healthcare workers at Mutenda, Itumbi and Ngoma rural health facilities were nurses followed by Environmental personnel and clinicians. This was alluded to the fact that majority of healthcare workers are nurses and this was reflective of the national picture. Among the health care workers, majority had less than five years of work

experience. This was similar to study findings by Mulenga *et al*., 2015 which suggested that most healthcare workers prefer to move to urban areas and were not willing to work for long in rural areas. Most of them go to rural areas on first appointment basis and later on leave to urban settings. This suggested that there was high staff attrition with some of them advancing in their studies and moving to higher level health care facilities. Some of the reasons contributing to high attrition is poor road network, difficulties in transportation, poor housing and lack of general social amenities (Mulenga *et al*., 2015) and rare cases of witchcraft. Furthermore, this study revealed that majority of HCW never had a specific training for HAT as it is with other diseases like malaria, TB and HIV. The high suspicion index conditions which were queried during screening of patients by clinicians and nurses were malaria, respiratory infections non pneumonia, diarrhoea and bilharzia. Therefore, this finding proved that HAT was not among the highly suspected diseases and was prone to be missed as a diagnosis by HCW. This study also demonstrated lack of treatment guidelines in the facilities with HCWs affirming absenteeism of guidelines, drugs, diagnostic tools such as microscope, haematocrit centrifuge, slides and giemsa stain as there were only two diagnostic centres in the district. The two diagnostic centres were located on average more than 40 km from the study sites leaving these sites to rely only on rapid tests for diagnosis of ailments like malaria and HIV leaving other conditions unchecked. Therefore, diagnosis was mostly based on clinical signs and symptoms. Furthermore, one of the two diagnostic laboratories in the district lacked qualified laboratory personnel and was being run by classified daily employees who were trained as laboratory assistants in malaria and TB only. The lack of qualified laboratory personnel was also attributed to staff attrition. Most of the HCW conducted HAT awareness education which was fairly good. However, HAT awareness education needed to be heightened in order for the community to be more knowledgeable, aware of the disease transmission cycle and prevention. It could also improve health seeking behaviour and eliminate myths about strange illnesses they are not aware off.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the study findings, no participant tested positive for HAT in the study area. The results did not mean Itezhi-Tezhi district was free of HAT as this study was only conducted at three rural health facilities namely Itumbi, Mutenda and Ngoma. There could have been other causes of anaemia other than HAT and malaria. Additionally, the demographics and activities done in the study area were predisposing factors to HAT. The district support structures present in Itezhi-Tezhi were not adequate to enable a robust HAT surveillance as there were less people trained in basics of HAT. Furthermore, missing treatment guidelines as well as the unavailability of diagnostic tools such as microscope and haematocrit centrifuge contribute to the failure of the district to report possible HAT cases.

6.2 Recommendations

Based on the study findings, the following recommendations are made;

a) Improved continuous passive surveillance for HAT in the district through laboratory diagnosis by microscopy as the area is a high-risk area.
b) Distribution of commodities like slides and giemsa by hospital laboratory to the other facilities and training HCWs in blood smear preparation, staining so that referral of slides for examination can be done.
c) Laboratory staff in the district to also make outreach programs for the purpose of diagnosis from time to time.
d) Health education to the community on HAT transmission cycle and clinical signs and symptoms.
e) Training of health care staff through clinical meetings.
f) The district health office to allocate qualified laboratory staff to the other, lacking qualified laboratory staff.

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ANNEXE 1: PATIENT'S INFORMATION SHEET/CONSENT FORM (ENGLISH)

Title: Prevalence of Human African Trypanosomiasis (HAT) and its associated risk factors in Itezhi -Tezhi district of Zambia.

Part A: Information sheet

Introduction

I, Christine Musonda, a student of Masters of Science in One Health Laboratory Diagnostic sciences at The University of Zambia is requesting for your participation in the research study mentioned above. The essence of the study is to assess the prevalence of and related risk factors associated with HAT among people with fever or a history of fever aged 2 years and above. Before you decide whether or not you should participate in the study, I would like to explain to you the purpose of the study, any risk or benefits and what is expected of you. Your participation in this study is entirely voluntary. You are under no obligation to participate. If you decide that you will not participate, no privilege will be taken away from you. If you agree to participate, you will be asked to sign this consent form.

Purpose of the study

HAT is a draining zoonotic disease caused by trypanosomes which are transmitted by tsetse flies. Transmission happens when taking a blood meal from infected humans or animals. Therefore, we are requesting for your participation/permission in this study whose main objective is to determine the prevalence of HAT and its associated risk factors among patients attending Mutenda, Itumbi and Ngoma rural health facilities.

Expectations of the study

We wish to test patients who have a fever so as to determine whether they could be suffering from HAT. If you agree to take part in the study, we will withdraw about a teaspoonful of blood from a vein in your arm which we shall test for HAT and this will be done by trained research assistants. We shall ask you some questions for about 10 minutes. Your HAT result will be provided to your clinical officer or nurse as soon as possible so that you can be provided with treatment if you need it.

Potential risks and discomforts involved

Except for minor pain, bruising and bleeding that are part of taking blood, there are minimal risks from being in this study. Discomforts may be experienced during responses to some questions and a bit of your time that will be taken as you answer the questions. In addition,

you will not be forced to share information that you will not be comfortable to disclose. In the event that any participant experiences discomfort, the researcher will stop the questions and allow for such information to be omitted. Depending on the circumstances, the researcher will avoid questions that may seem to cause discomfort to any participants and allow for withdrawal of participants where necessary.

Benefits

The direct benefit from this study is that it shall confirm the diagnosis without charging you or taking more blood. Indirectly, information gathered from this study will help the ministry of health (MOH) realise the importance of HAT in this country (specifically in your community) and why there is need to invest in diagnosis, treatment and control. The factors associated with HAT will assist MOH to come up with health awareness messages. This study will also help the clinicians and the community as a whole to think of HAT and other infections other than malaria and other common fevers. No gift or money will be given in exchange for the information obtained. Potential benefits for participating in this study include enhancing the participant's knowledge on HAT. Participants who will be found positive will be treated.

Payment for participation

There are no monetary benefits for participating in this study. However, by participating in the study, you will contribute to information that will assist District Health Office to consider implementing health awareness programs on HAT. Therefore, the time you will spend in discussing the issue is highly appreciated.

Confidentiality

The information collected from you will be strictly private and confidential and intended for research purpose only. Only researchers involved in this study will be allowed to work with your blood and see information. Your name will not be used in any report of this study, presentation or publication but will use only numbers for forwarding your results to your health facility if positive for HAT. Personal information will not be released without your permission except when required by law, The Ministry of Health or the University of Zambia Biomedical Research and Ethics committee may review your records again but this will be done with confidentiality.

Voluntary participation

Participation is voluntary and it is your decision and free will to participate or not. If at any time you wish to withdraw from participating in this study, you can do so freely and it won't affect the care you deserve to get from the facility.

Information and clarification

Please be informed that if you at any time need clarifications over the research study, direct your questions to:

CHRISTINE MUSONDA
UNIVERSITY OF ZAMBIA,
SCHOOL OF VETERINARY MEDICINE,
DEPARTMENT OF BIOMEDICAL SCIENCES,
P.O. BOX 32379,
LUSAKA.
CELL NUMBER: +260976646635
Email address: christinemusonda23@gmail.com

OR

THE SECRETARY,
UNIVERSITY OF ZAMBIA BIOMEDICAL RESEACH AND ETHICS COMMITTE
P.O. BOX 32379,
LUSAKA.
TEL: 260-1-256067
Email address: unzabrec@unza.zm

Rights of research subjects

You may withdraw your consent at any time and discontinue your participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research study.

Part B: Agreement

Please ask any questions or clarification before you sign this form to enrol in the study.

I, Mr/Mrs/Miss……have been explained this study. I have had a chance to ask all questions and I have been answered adequately. I therefore give consent to be included in the study. the risks and benefits have been explained to me. I understand that I can withdraw from the study at any time if I so wish without any consequences. I consent voluntarily to participate in this study.

I agree to join the study

Signature……...

Date…….....

Witness……

Left thumb print (For those who cannot sign)

Thumb print here

ANNEXE 2: PATIENTS'S INFORMATION SHEET/CONSENT FORM (ILA).

KUZYINGUKULUKA KWA BULWAZYI BWALUUKA KU BANTU BABYIYA USYIKONZYA KUZYALWA MWI CILICITI CE ITEZHI-TEZHI MUCISHIYI CA ZAMBIA

KULIPANDULULA

Ume ndi Christine Musonda, ndisiyicikole wakubona buponi bwasyilengwa leza mubusena bwalobona tuzunda mucikolo cikando cacishi ca Zambia ndalomba kusangana amwe ciiyo cambwa awa zeilu. Muzeezo waciiyo ngwakubona beonde bwanulwazyi bobu wayikonzya kuzyolwa umbulwazyi momu bwiluuka bwa mpeyo kuuwa amyaka yobile kuya ezeulu kutana kusala kunjila muciiyo cecu. Ndazanda kumupandulwila ceci ciiyo, umuzeezo waneico. Butongo na impindu ikonzya kuyanwa. Koku nkulyaaba bulyo, kwiina cikusyinicizya kuba momu muciiyo. Na tuzanda kwiina cikulesya. N utazanda, ulyeclele kuwaina cipepa caku vuumina.

CIPANZYA CAKUTANGUNA

CIIYO: Mwiika wa bantu bana Africa bwatu pukatwa luuka ubutongo mbubikonzhyakuleta muchilikiti ca Itezhi-Tezhi mucishi ca Zambia.

MBUBUZYIZA BULWAZYI

Bobu bulwazhi buleenda ukuletwa utu puka na tuzunda tusiyipwa uluuna. Bulwazhibuleenda muli syakulya syabulowa kuuwa kubanyama na kubantu.

MUZEEZO WACIIYO

Tulalomba kusebeza antoomwi muciiyo ceci calo cikwete muzeezo wakubona bweende bwabulwazyhi bwa luuka umbalitu basyiya mu Africa ca nkubona syilwana bantuna bamalwazhi bayanwa kusyipatela sya Mutenda, Itumbi uca-Ngoma.

UCIKONZYA KULETA INTENDA UKUBAKABOTU

Kutateelila kabotu kulaba mukwiingula mi buzyo uciindi ncuukonzya kwiingula mibuzyo kuzunjila tusyinicizyiwa kupabumboni mbuu taleeleli kabotu kupa kubuleya. Muciindi bantu bapupulala mbubataleeli kabotu, mulinguzyi ulazyimika mibuzyo ukullomba twaambototo kwaamba tukusyiwe. Muceeci ciindi, mulinguzyi ulahonzhya kulesya mibuzyo njibatakonzya kuteelela kabotu bantu ukuvuminya babo bakaka kwaamba bamuvuwo muciiyo

SYIYA KUYANWA MUCIIYO

Tulazanda kulungula bantu bayanwa kupya mubili kwamba tubone na bata sata bobu bulwazhyi na mulawumina kuba antoonmwi muciiyo cci tulakusya bulowa buzuzya muungo musyoontomulusyinga lwetasyi lyako bwalo mbutukalingula ku bulwazhi. Tulakubuzya mibuzyo ili yongayana kwachiindi cioyoonto bulyo (10 minutes). Ncitukayana tulakapa mu syilisyi wenu cakufwambaana kwaamba upewe musamu na ulauzanda intenda shiyanwa.

Kunze lyakucisa kusyoonto kwaanduka ukuvwa bulowa akukusyiwa bulowa kuli tumwi tuntenda tukonzhya kuyanwa mu mulimo wezu.

BULUMBU

Impindu yaciiyo ceci kwiipama njabu kuti ilakatendezya ihzhilayacilwazyi kutakubbadalisya na kutakusa bulowa bunji-bunji. Munzhyila imwi twaambo tubwezwa mumulimo wezu tulakayovwa mutabi wamfuulumende ulanganya momi abantu kwamba bazyibe ati ceci cilwazhi nkucili mucisyi(kupama umbuzena mumukala) ukwaamba cileelela kubika lubono mukulingaila, ukubusyilika ukubakwabilila bulwazhi bobu bwaambwa. Tupango twendelelezyanya ubulwazi bobu tulakayovwa mutabi ula nganya momi abantu kwaamba bayane muzeezo na inzhyila syakuya buyiisya bantu kumomi abo. Ceci cilakayovwa basyilisyi ubasyicisyi boonse kwaamba bakubutweluka bobu bulwazhi kunzo lya malaria (intuntumeezhyi) usyimui bulyo syilwazyi syakupya umubili. Kwiina cipo na mali akapewa ku twaambo twaciiyo ceci nkupa lutiibe kubulwazyi bwa luuka mucisyi ubankayaninwa bobu bulwazyi balakasyilikwa.

KUBADDALWA KWABANTU BASANGANA MUCIIYO

Kwina kuliola mali muciiyo ceci. Mukusangana muciiyo ceci mulapa bumboni bukonzhya kuyovwa iyopesyi lilanganya bumi muciliciti kwaamba ibambe twaambo twakupakamika bantu ukuzumya inzyila yabukwabilizyi. Mulalumbwa muciindi ncimwasowa kubamba twaambo twabuluzyi bobu.

MASESECE

Twaambo tunkakabweza tulakuli twa maseseche twakuliiba ntwamuzeezo bulyo wakukapula bobu bulwazhi. Beni bakulau syi umulimo wezu mbubalamuminwe umulimo wezu ukubusebezya bulowa bwako ukubona cilimo. Kusangana umu limo wezu tacikasywa cilaya umatelansyi ako kuba antomwi na peepe na ulazanda kumivwa umulimo wezu wakulugulwa ulakonzya kuleka tacikonzhya kunyonganya bubambe mbuukonzya kuyana kucipatela. Twambo twako tatkonzya kupewa muntu unji cita cazandwa kumulawo wacisyi. Mutabi wamfwulumende ulanga momi na cikolo cikando mucisyi ca Zambia mukulingula momi ubunkutwe ilakonzya kuzubulula kwiita umasesece.

BWEENE BWATWAMBO UKUTUSALAZYA

Na mulazanda kuzyiba twaambo twenu ukusalaliwa, kuciiyi ceci amutume koku:

CHRISTINE MUSONDA,

CIKOLO CIKANDO CA ZAMBIA,

MUTABI WABUSYILISYI BWA SHILENGWA LEZA,

UMUTABI BWATUPANGANO TWABUSYILISYI

IMPOSO 32379,

LUSAKA.

KUTABANA: +260976646635

EMAIL ADDRESS: christinemusonda23@gmail.com

NA

MULEMBI,

CIKOLO CIKANDO CA ZAMBIA ILANGA MALWAZYI ANDEENE UMILAWO YABUSYILISYI

IMPOSO32379,

LUSAKA.

KUTABANA: +260-1-256067

EMAIL ADDRESS: unzabrec@unza.zm

CEELELO CA BULINGAUZYI

Ulakonzya kucileka civuminano cako kufumbwa ciindi ukwiina mulandu-ngupewa. Wima nailumbulwa umulimo wezu waciiyo ceci.

PART B (CIPANZHYA CABILI –CIPANGANO)

Ulakonzya kubuzya kufwimbwa mubuzyo na kusalazyanga kutana kulemba mazyina ako muci pepa camulimo wezu.

Ume ndi……ndapandulilwa mumulimo wezu, ndabuzya yoonse mibuzyo ulimui ndangulwa cakumaninina ubobo ndavuuminakusangana na kunjila wezu bulumbu ubutongo bulimo boonse ndapandulwilwa. Ndazyiba bukuti ndakonzya kumuvwa umulimo na muciiyo cecikutwimbwa ndazanda ukwiina kaambo kacilila peepe. Ndavumina cakulyaaba umulimo wezu. Ndavuwumina kunjila umulimo wezu.

Izyina……..

Mwezi bazuba mwaka……

Kamboni…… Cikumo cikondo cakwitasyi (Babo batalozyakulemba cikumo awa

ANNEXE 3: PATIENT'S QUESTIONNAIRE

Questionnaire No

Patient ID Number ……..

Date of interview……

A. Socio-demographic data

1) Contact number of patient……..

2) Age of patient a) 11-20 b)21-30 c) 31-40 d) 0ver 40

3) Gender of the patient a) Female b) Male

4) location of residence/ village? a) within 5km from the facility b) 10km away from the facility c) more than 10km away from the facility

4)Religion …… a) Christian b) Muslim c) Hindu d) others: specify……

6) Occupation…… a) Farming b) Fishing c) Formal employment d) Un-employed e) Others (Specify)……..

7) Level of highest education attained……. a) Primary school b) Secondary c) Tertiary d) Did not attend school

B. Disease information

1) Axillary temperature of the patient……. a)37.5°C - 38°C b)39°C - 40°C c) above 40°C

2) Malaria RDT screening results: a) Positive b) Negative

3) How many days have you been with fever? a) 2days b)5-7days c) 8-14days d) more than 14 days

4) Have you had any of these signs/ symptoms with the present illness?

a) Head ache b) skin rash c) itching and inflammation of the skin

d) Enlargement of lymph nodes on the back of the neck e) Delayed sensation to pain

f) General body weakness g) Drowsiness

5a) Have you taken or been taking any medicine before coming to the facility? a) Yes b) No

5b) If yes to 5a, which one? a) Antibiotics b) Antimalarials c) Paracetamol d) others

C. Questions on possible risk factors

1. Do you keep these animals in your home? ……a) Cattle b) Sheep c) Goats d) dogs e) others

2a. Do you herd animals? a) Yes b) No

2b.If yes to 2a, what animals? a) Cattle b) Sheep c) Goats d) Dogs e) others (specify)……...

3. Where do your animals graze from? a) In dry grassland b) In wet grass land near a stream/river/ lake

4a. Have your animals been sick recently? a) Yes b) No

4b. If yes to 4a, what animals? a) Cattle b) Sheep c) Goats d) Dogs e) others

5. If yes to 4, have you had your animals treated? a) Yes b) No

6. Where do you get water for domestic use? a) Community tap b) river c) stream d) underground e) others: specify…….

7.Do you swim, bath or wash in a river or stream? A) Yes b) No

8a. Have you had a blood transfusion before? a) Yes b) No

8b If yes to 8a, when was it? a) a week ago b) a month ago c) a year ago d) others; specify…….

ANNEXE 4: HEALTH CARE WORKER INFORMATION SHEET/CONSENT

Part A: Information sheet

Title: Prevalence of Human African Trypanosomiasis (HAT) and its associated risk factors in Itezhi Tezhi district of Zambia.

Introduction

I, Christine Musonda, a student of Masters of Science in One Health Laboratory Diagnostic sciences at The University of Zambia is requesting for your participation in the research study mentioned above. The essence of the study is to assess the prevalence of and related risk factors associated with HAT among people with fever or a history of fever. Before you decide whether or not you should participate in the study, I would like to explain to you the purpose of the study, any risk or benefits and what is expected of you. Your participation in this study is entirely voluntary. You are under no obligation to participate. If you decide that you will not participate, no privilege will be taken away from you. If you agree to participate, you will be asked to sign this consent form.

Purpose of the study

We are requesting for your participation/permission in this study whose main objective is to determine the prevalence of HAT and its associated risk factors among patients attending Mutenda, Itumbi and Ngoma rural health facilities. According to WHO only three districts report HAT cases in Zambia of which Itezhi Tezhi is not among them. Therefore, this study will also assess the district's HAT surveillance support structure present in Itezhi- Tezhi through interviews of health workers.

Risks involved

No risk is involved except a bit of your time that will be taken as you answer the questions

Benefits

Information gathered from this study will help the ministry of health (MOH) realise the importance of HAT in this country (specifically in your community) and why there is need to invest in diagnosis, treatment and control. The factors associated with HAT will assist MOH to come up with health awareness messages. This study will also help the clinicians and the community as a whole to think of HAT and other infections other than malaria and other common fevers. No gift or money will be given in exchange for the information obtained. Potential benefits for participating in this study include enhancing the participant's knowledge on HAT.

Confidentiality

The information collected from you will be strictly private and confidential and intended for research purpose only. Participation is voluntary and it is your decision and free will to participate or not. If at any time you wish to withdraw from participating in this study, you can do so freely and it won't have any consequences. Personal information will not be released without your permission except when required by law, The Ministry of Health or the University of Zambia Biomedical Research and Ethics committee may review your records again but this will be done with confidentiality.

Payment for participation

There are no monetary benefits for participating in this study. However, by participating in the study, you will contribute to information that will assist District Health Office to consider implementing health awareness programs on HAT. Therefore, the time you will spend in answering the questions is highly appreciated.

Information and clarification

Please be informed that if you at any time need clarifications over the research study,

direct your questions to:

CHRISTINE MUSONDA

UNIVERSITY OF ZAMBIA,

SCHOOL OF VETERINARY MEDICINE,

DEPARTMENT OF BIOMEDICAL SCIENCES,

P.O. BOX 32379,

LUSAKA.

CELL NUMBER: +260976646635

Email address: christinemusonda23@gmail.com

OR

THE SECRETARY,

UNIVERSITY OF ZAMBIA BIOMEDICAL RESEACH AND ETHICS COMMITTE

P.O. BOX 32379,

LUSAKA.

TEL: 260-1-256067

Email address: unzabrec@unza.zm

Rights of research subjects

You may withdraw your consent at any time and discontinue your participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research study.

Part B: Agreement

Please ask any questions or clarification before you sign this form to enrol in the study.

I, Mr/Mrs/Miss……have been explained this study. I have had a chance to ask all questions and I have been answered adequately. I therefore give consent to be included in the study. The risks and benefits have been explained to me. I understand that I can withdraw from the study at any time if I so wish without any consequences. I consent voluntarily to participate in this study.

I agree to join the study

Signature…….....

Date…….....

Witness……

ANNEXE 5: HEALTH CARE WORKER QUESTIONNAIRE

Questionnaire ☐

Part A

Demographic and labour data

1. Gender……. a) Female b) Male
2. Age……. a) 18-29 b)30-39 c) >40
3. Occupation…… a) lab personnel b) Clinician c) Nurse d) Environmental health personnel f) Pharmacy personnel e) others; specify……
4. Years of work……. a) less than 5 years b)6-10 years c)> 10 years
5. Education level…… a) Certificate b) Diploma c) Degree

Part B

Health work force

1. Availability of qualified staff? a) yes b) No

2. Are you trained in HAT? a) Yes b) No

Part C

Availability of Equipment, Drugs and Commodities

1. a) Does the facility have a microscope? a) yes b) No

b. If No, are slides prepared and sent to a diagnostic facility? a) yes b) No

2. Does the facility have haematocrit centrifuge? a) yes b) No

3. Does the facility have any PCR platform? a) yes b) No

4. Does the facility have drugs for treating HAT i.e. (suramin, melarsoprol, Pentamidine)? a) Yes b) No

5)Availability of guidelines for treatment? a) Yes b) No

6) Are slides and giemsa stain available? a) Yes b) No

Part D

Community and District services structure

1. Do you conduct community awareness education about HAT? a) Yes b) No
2. Do you refer patients with a ceaseless fever who test negative for malaria? a) Yes b) No

ANNEXE 6: ETHICAL CLEAREANCE

UNIVERSITY OF ZAMBIA
BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
Federal Assurance No. FWA00000338

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia
E-mail: unzarec@unza.zm
IRB00001131 of IORG0000774

15th September 2021

Your REF. No. 1842-2021

Ms. Christine Musonda,
University of Zambia,
School of Veterinary Medicine,
ACEIDA,
P.O Box 32379,
Lusaka.

Dear Ms. Musonda,

RE: PREVALENCE OF HUMAN AFRICAN TRYPANOSOMIASIS AND ITS ASSOCIATED RISK FACTORS IN ITEZHI-TEZHI DISTRICT OF ZAMBIA (REF. NO. 1842-2021)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 13th September, 2021. The proposal is **approved**. The approval is based on the following documents that were submitted for review:

a) **Study proposal**
b) **Questionnaires**
c) **Participant Consent Form**

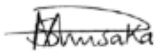
APPROVAL NUMBER : REF. 1842-2021

This number should be used on all correspondence, consent forms and documents as appropriate.

- **APPROVAL DATE : 15th September 2021**
- **TYPE OF APPROVAL : Ordinary**
- **EXPIRATION DATE OF APPROVAL : 14th September 2022**
  After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the UNZABREC Offices should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING**: All SAEs and any other serious challenges/problems having to do with participant welfare, participant safety and study integrity must be reported to UNZABREC within 3 working days using standard forms obtainable from UNZABREC.
- **MODIFICATIONS**: Prior UNZABREC approval using standard forms obtainable from the UNZABREC Offices is required before implementing any changes in the Protocol (including changes in the consent documents).

- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the UNZABREC using standard forms obtainable from the UNZABREC Offices.
- **NHRA:** You are advised to obtain final study clearance and approval to conduct research in Zambia from the National Health Research Authority (NHRA) before commencing the research project.
- **QUESTIONS**: Please contact the UNZABREC on Telephone No.256067 or by e-mail on unzarec@unza.zm.
- **OTHER**: Please be reminded to send in copies of your research findings/results for our records. You are also required to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study. Use the online portal: unza.rhinno.net for further submissions.

Yours sincerely,



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CHAIRPERSON
Tel: +260977925304
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