TSETSE AND TRYPANOSOMOSIS IN MEATU DISTRICT: SOCIOECONOMIC ASSESSMENT, PARASITOLOGICAL AND MOLECULAR CHARACTERIZATION

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ABSTRACT

This study sought to evaluate tsetse and trypanosomes and to investigate the socio economic importance of trypanosomosis in Meatu district where sleeping sickness cases have been reported in villages that border state-protected wildlife parks. Socio economic data indicated that 77.5% of respondents in the study area were agro-pastoralists. Tsetse flies were well known to be associated with both African Animal Trypanosomosis (AAT), which was well known (90%) and Human African Trypanosomosis (HAT) which was fairly known (40%). Abusive use of anti trypanocidal drug in cattle treatment was observed (48.4%) threatening not only the economy but also public health. Significant difference in the occurrence of tsetse species was observed with *G. pallidipes* contributing 50% compared to *G. swynnertoni* and *G. morsitans.* Overall occurrence of animal trypanosomes in cattle was 2.4% with *Trypanosoma congolense* and *T. vivax* being the main trypanosome species identified microscopically. The packed cell volume (PCV) measurements revealed that prevalence of anaemia was 8.3% but anaemia was not correlated with trypanosomosis. Molecular characterization by PCR revealed *T. congolense* in cattle and *T. simiae* as well as *T. godfreyi* in tsetse. Moreover, phylogenetic tree showed monophyletic nature of the salivaria trypanosomes. However, closely related to other species from different countries the identified species formed distinct clusters themselves with higher significant support. Neither parasitological nor molecular technique identified human-infective trypanosomes. Since the trypanosomosis risk is permanent; its control remains a significant approach in protecting public and animal health against both diseases. Moreover, findings of this study clearly indicate that awareness of HAT is poor whereas current tsetse control practices being not sustainable. Therefore, strategic and integrated control approach involving community should be advocated.

DECLARATION

I, **Deusdedit John** do hereby declare to the Senate of Sokoine University of Agriculture that the work presented here is my original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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DEDICATION

This Thesis is dedicated to my mother Magdalena and wife Theodora.

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LIST OF ABBREVIATIONS

AAT	African Animal Trypanosomosis
BLAST	Basic Local Alignment Search Tool
CI	Confidence Interval
COSTECH	Commission for Science and Technology
DED	District Executive Director
DLFDO	District Livestock and Fisheries Development Officer
DNA	Deoxy- ribose Nucleic Acid
FTA	Flinders Technology Associate
FTD	Flies per Trap per Day
HAT	Human African Trypanosomosis
IRA	Institute of Rural Assessment
ITS	Internal Transcribed Spacer
KAP	Knowledge Attitude and Practice
LAMP	Loop-Mediated Isothermal Amplification
NIMR	National Institute for Medical Research
PCV	Packed Cell Volume
RIME	Random Insertion Mobile Element
RNA	Ribose Nucleic Acid
RPM	Rotation per minute
SRA	Serum Resistance Associated Gene
TVLA	Tanzania Veterinary Laboratory Agency
VSG	Variant Surface Glycoprotein
VVBD	Vector and Vector Borne Diseases Institute

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

There are 11 species, subspecies and subgroups of tsetse-transmitted trypanosomes that cause devastation of the economy and threaten public health in sub-Saharan African countries. *Trypanosoma congolense, T. vivax, T. brucei brucei* and *T. simiae* are the major pathogenic trypanosomes of livestock (Isaac *et al.*, 2016). In humans, *Trypanosoma brucei rhodesiense* is the main pathogen of acute disease in eastern and southern Africa, which accounts for 2% of all reported sleeping sickness cases. *Trypanosoma brucei gambiense* is the major pathogen causing chronic form of the disease in western Africa, where it accounts for 98% of all reported sleeping sickness cases (WHO, 1998). Despite the fact that the two diseases occur in specific foci they are also geographically separated by the Great Rift Valley (Fèvre *et al.*, 2006). At the present time occurrence of the two diseases has been reported in Uganda (Fèvre *et al.*, 2001) and there are possibilities of introduction of *T. brucei gambiense* in western Tanzania through refugees from neighbouring countries where the pathogen is prevalent.

Tanzania has 4 million people and 4.4 million livestock exposed to the risk of contracting tsetse-borne trypanosomosis particularly in remote areas where health services are inaccessible. Thirty two percent of its 945 203 km² is estimated to be infested with seven species of tsetse flies (Daffa *et al.*, 2013). From Shaw *et al.* (2014) computations, it can be estimated that Tanzania shillings 4.5 billion (US$2.3 million) are lost annually due to animal trypanosomosis. Simarro *et al.* (2010) reports less than 100 Rhodesian sleeping sickness cases are being reported annually, most of them being from western regions of Tabora, Kigoma and Rukwa and Katavi (Kibona *et al.*, 2002) which constitute active

sleeping sickness foci. Other foci include the occasionally active in north-western zone that include Mara, Arusha, Shinyanga and Manyara regions (Kaare *et al.*, 2007) and relatively silent foci in the southern zone (Mtwara, Lindi and Ruvuma).

Constant monitoring of these foci by screening of people and treatment of active cases, screening animals and tsetse, is considered a significant approach in combating the disease and preventing epidemics (Aksoy, 2011). Inadequate resources in most of sub-Saharan countries hinder satisfactory monitoring and control of Human African Trypanosomosis (HAT) due to difficult and expensive diagnosis and treatment of the disease (Kennedy, 2013). The use of molecular tools in monitoring and diagnosis of the disease is recommendable as the techniques are highly sensitive and specific when compared to conventional parasitological and serological methods. On the other hand, most of the molecular techniques require well-equipped laboratory with water, electricity, machines which are expensive, chemical reagents as well as skilled personnel to operate. The molecular techniques include DNA probes and species specific PCRs (Masake *et al.*, 1997; Masiga *et al.*, 1992; Moser *et al.*, 1989; Welburn *et al.*, 2001). It has been indicated that PCRs targeting Internal Transcribed Spacer (ITS) identifies multiple trypanosome in a single PCR using a single set of primers (Cox *et al.*, 2005; Njiru *et al.*, 2005). The methods stand as the best choices for screening large number of samples for trypanosomosis and provide opportunities for identification of mixed infection in the sample (Desquesnes and Dávila, 2002). Additionally, Loop mediated Isothermal Amplification (LAMP) technique (Notomi *et al.*, 2000) is a revolutionary technique in epidemiology which provides amplification of DNA under isothermal conditions.

Sleeping sickness reports from Serengeti National Park (Kaare *et al.*, 2007) which comprises the Serengeti ecosystem (Maswa game reserve, Serengeti and Ngorongoro

Conservation Area) and earlier study by Malele *et al.* (2007) in the ecosystem that identified *T. b. rhodesiense* in tsetse, necessitated need of conducting research in Meatu district, where largest part of Maswa game reserve is found.

1.2 Problem Statement and Justification

The threat of contracting infective trypanosomes among livestock and humans in Meatu district is high due to some of the villages being located at the vicinity of wildlife conserved areas. The district borders Maswa Game reserve to the north, and Ngorongoro Conservation Area (NCA) on the north eastern part. These protected parks are reported to be active source of tsetse and infections to the neighbouring areas since ecological and environmental conditions favours their existence. There is therefore a need to generate the information about tsetse and trypanosomes in Meatu district, particularly in the villages that border Maswa Game reserve and Makao Wildlife Management Area. The information generated will eventually assist in planning strategies for tsetse control, to improve human health and food security in the area.

1.3 Hypothesis

It is hypothesised that community understanding of the tsetse fly and its diseases is affected by different demographic factors and the occurrence of the tsetse species as well as human and animal infective trypanosomes in tsetse and cattle is widespread in villages of Meatu district that are close to state protected parks.

1.4 Research Questions

i. What is the importance of trypanosomosis in Meatu district?

ii. What are the tsetse species and their population density in Meatu district?

iii. Do human infective trypanosomes occur in tsetse species and cattle of Meatu district?

1.5 Objectives

1.5.1 Overall objective

Assessment of socioeconomic impact of tsetse and trypanosomes as well as occurrence of tsetse and trypanosomes in Meatu district.

1.5.2 Specific objectives

i. To assess socio-economic impact of tsetse and trypanosomosis in Meatu district.

ii. To identify tsetse species and establish their density in Meatu district.

iii. To identify trypanosomes in tsetse and cattle by molecular techniques.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Human Activities and Trypanosomes

The increase of human population and associated demand for basic needs has influenced the global climate change which in turn have changed disease occurrence patterns and distribution (Githeko *et al.*, 2000). There has been an increase in food demand resulting in increased crop cultivation, necessitating massive clearing of vegetation which favour wild animals and tsetse flies. This practice has reduced the tsetse belt, in turn the trypanosome infections have concentrated in tsetse loci (Malele *et al.*, 2011b). The management of state controlled National Parks (NPs), Wildlife management areas (WMAs) and Game reserves (GRs) precludes tsetse control making protected areas rich sources of tsetse species. The vector frequently re-infest village lands bordering the conservation areas posing trypanosomosis threat to both human and livestock (Kinung'hi *et al.*, 2006).

Though selective poaching and hunting can influence wildlife ecological imbalance, by some animal species being more affected and therefore narrowing the host preference for the flies to feed, this habit does not threaten tsetse fly extinction. The flies feeding preference to a particular animal is not permanent as they switch and adapt to the available hosts. This in turn has negative role in trypanosomes transmission and host range diversification (Muturi *et al.*, 2011). Human activities including hunting, timbering, beekeeping, charcoal burning, cultivation and pastoralism influence both tsetse-human contacts and hence increasing risk of acquiring HAT (Munang'andu *et al.*, 2012).

Knowledge, attitude and practices (KAP) of people on tsetse and trypanosomosis have direct impact on tsetse and trypanosome occurrence and distribution while incorrect KAP

being linked to emergency of drug resistant strains, environmental destruction and biodiversity loss (Tsegaye *et al.*, 2015).

2.2 Trypanosome and Trypanosomosis

Trypanosomes are extracellular protozoan parasites of the genus *Trypanosoma*. Taking into consideration the mode by which they are transmitted to the vertebrate host, two major groups of trypanosomes are distinct; salivaria group transmitted via saliva and stercoraria the group transmitted via feaces of the biting flies during feeding on host. The two groups are well known to cause African trypanosomosis and American trypanosomosis respectively (Hoare, 1972). Principally salivarian trypanosomes are transmitted cyclically by tsetse species. Different maturation sites within the vector have led into four sub genera description (Table 2.1).

Table 2.1: Description of different species of trypanosome subgenus

Subgenus	Species	Description
Trypanozoon	*T. brucei*	*T. b. rhodesiense* and *T. b. gambiense* cause HAT. *T. b. brucei* causes mild disease in cattle (Fèvre *et al.*, 2006)
	T. evansi	Causes disease in camels, horses, dogs, bovid and is transmitted mechanically by tsetse and other blood sucking flies (Hoare, 1972)
	T. equiperdum	Causes diseases in horse and is transmitted via copulation (Brun *et al.*, 1998)
Pycnomonas	*T. suis*	Causes disease to pigs, camels and dogs and is transmitted cyclically by tsetse (Hutchinson and Gibson, 2015)
Duttonella	*T. vivax*	Causes disease in cattle and is transmitted by tsetse and mechanically and other blood sucking insects (Adams *et al.*, 2009)
Nannomonas	*T. congolense*	Most important as a pathogen of cattle but can also cause disease in other species. Three groups – savannah, forest and Kilifi (Bengaly *et al.*, 2002)
	T. simiae	Causes acute, fatal disease in pigs subspecies *T. simiae* Tsavo (Isoun, 1968)
	T. godfreyi	Causes chronic, occasionally fatal disease in pigs (Masiga *et al.*, 1996)

Trypanozoon and *Pycnomonas* trypanosomes are found in the salivary gland, *Duttonella* develop in the proboscis and often transmitted mechanically by other biting insects, whereby *Nannomonas* trypanosome develop in the mid-gut and mature in mouthparts. (Lloyd and Johnson, 1924).

The African trypanosomes lead into two distinct diseases; African Animal Trypanosomosis (AAT), caused by at least single species in all three sub genera while Human African Trypanosomosis (HAT) caused by species of *Trypanozoon* sub genera only. The pathogens responsible for HAT belong to *T. brucei* complex comprised of species that are morphologically indistinguishable. Through the use of molecular tools two HAT forms are distinguished by detecting the Serum Resistance Associated (SRA) gene (Welburn *et al.*, 2001) and *T. b. gambiense* specific glycoprotein (TbgSG) gene for Rhodesian and Gambian sleeping sickness respectively (Fèvre *et al.*, 2006). The two traits make them to overcome the human immunity and lead to disease. This character is absent in AAT pathogens *T. b. brucei* inclusive and is considered to be a reason for unsuccessful establishment of the animal trypanosomes in human (Aksoy, 2011).

2.3 Trypanosome and Host

When the infective trypomastigotes are injected in host body, the animal triggers immune system to synthesize antibodies. Trypanosomes, through Variable Surface Glycoprotein (VSG) (Myler, 1993) escape the host immune system to establish and progress into the disease by changing their variant surface glycoproteins (MacGregor *et al.*, 2012). Nagana and sleeping sickness are the diseases classified based on the host specificity of trypanosomes, while those infecting animals cannot infect humans, those which infect human can infect animals (WHO, 1998). The most important trypanosomes species infecting livestock are *T. congolense*, *T. vivax*, *T. b. brucei*, *T. simiae* and *T. godfreyi*.

Trypanosoma congolense is the most prevalent and widespread pathogenic species for livestock in tropical Africa. Its sub types *T. congolense* savannah is the most pathogenic and is responsible for acute infections in animals (Bengaly *et al.*, 2002), while the other two; *T. congolense* forest and Kilifi types cause mild infections. Other trypanosomes including *T. simiae, T. godfreyi* and *T. evansi* infect only domestic pigs and camels respectively. *Trypanosoma brucei rhodesiense* and *T. brucei gambiense* are the species causing sleeping sickness (WHO, 1998).

Wild animals are main source of blood meal for tsetse and reservoirs of acute human infective trypanosomes in the wild (FAO, 1982). However findings have shown that domestic animals particularly cattle harbour the infective trypanosome (Welburn *et al.*, 2001), pigs (Hamill *et al.*, 2013) as well as goats and sheep (Ruiz *et al.*, 2015). Mixed infections where two *Trypanosoma* species infect the same host are common (Malele *et al.*, 2011a; Ruiz *et al.*, 2015). Humans are well known as reservoirs of chronic form of sleeping sickness.

2.4 Trypanosome and Vector

Tsetse flies (Genus: *Glossina*) are obligate haematophagus insect vectors responsible for transmitting trypanosomosis. Tsetse are grouped into three major subgenera/groups Figure 2.1); *fusca, morsitans* and *palpalis* strictly distributed in thickly forests, savannah plains and water bodies (lakes and rivers) respectively (FAO, 1982). Refractoriness of the vector to the parasite determines parasite establishment and subsequent transmission to the host. Welburn and Maudlin (1999) reported that a small proportion infected blood meal fed by tsetse establish into metacyclic trypanosome and stipulated that *Sodalis glossinidius* symbiotic micro-organisms present in tsetse favour establishment of trypanosomes in mid-gut through complex biochemical mechanism. Hao *et al.* (2001) suggested that midgut

lectins and the insect immune system, affect the success or failure of the parasite's development in vector. Trypanosome infections in tsetse reduce its flight activity, longevity and reproductive fitness (Bursell, 1981).

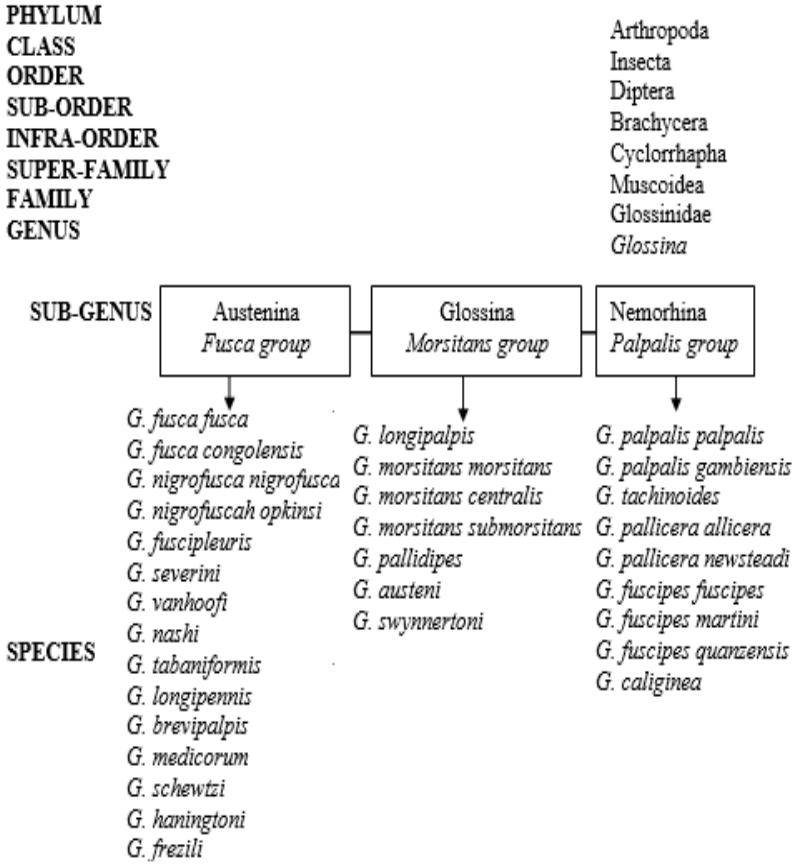

Figure 2.1: Classification of tsetse species Bouyer (2006) as cited by Sow (2013)

Trypanosomes develop and mature differently within tsetse (Figure 2.2) which forms a basis for sub genera description.

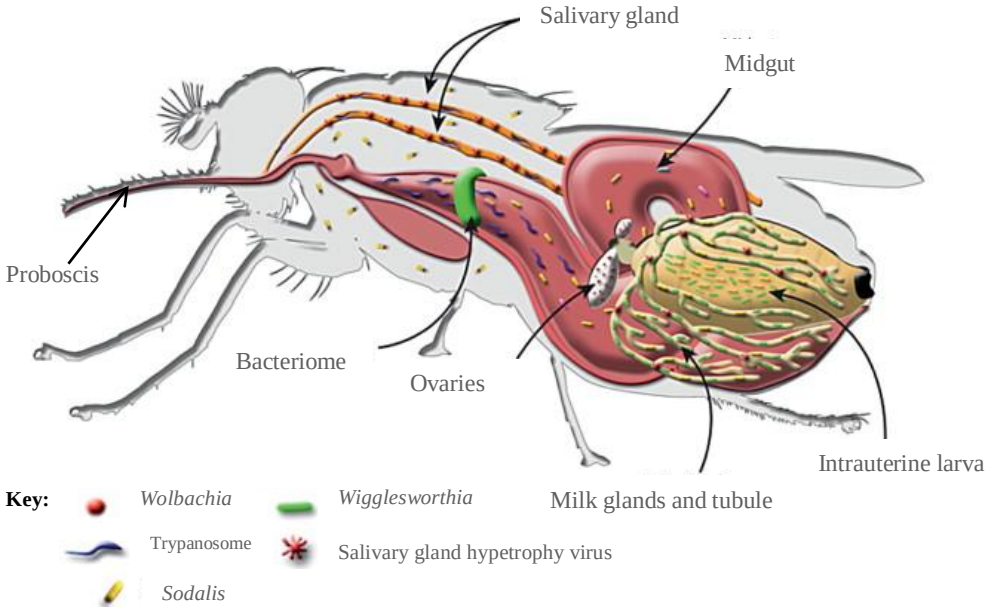

Figure 2.2: Internal organs of female tsetse fly (Aksoy *et al.*, 2013)

2.5 Life cycle of Trypanosome in Tsetse Vector and Mammalian Host

Trypanosomes develop and behave differently depending on whether in host or vector. Generally, all African trypanosomes are transmitted cyclically through tsetse flies (Vickerman, 1985). Conversely, evidence suggests that *T. vivax* and *T. congolense* can be transmitted mechanically by other blood sucking insects (Desquesnes and Dia, 2003). The vector acquires infections or transmits them when feeding on host via saliva (Figure 2.3). Bloodstream forms (slender/stumpy) are acquired once infected blood meal is ingested. The susceptibility to infection acquisition is age dependent, where newly emerged flies are susceptible (Welburn and Maudlin, 1992) in their first meal than old flies, whereas the infected flies feed more often than non- infected (Kubi *et al.*, 2006). Successful trypanosomes establishment in tsetse entails two stages of differentiation and maturation (Vickerman, 1985).

With exception to bloodstream forms of *T. brucei* complex, other metacyclic trypomatigotes forms have to change (morphology and metabolism) immediately into procyclic.

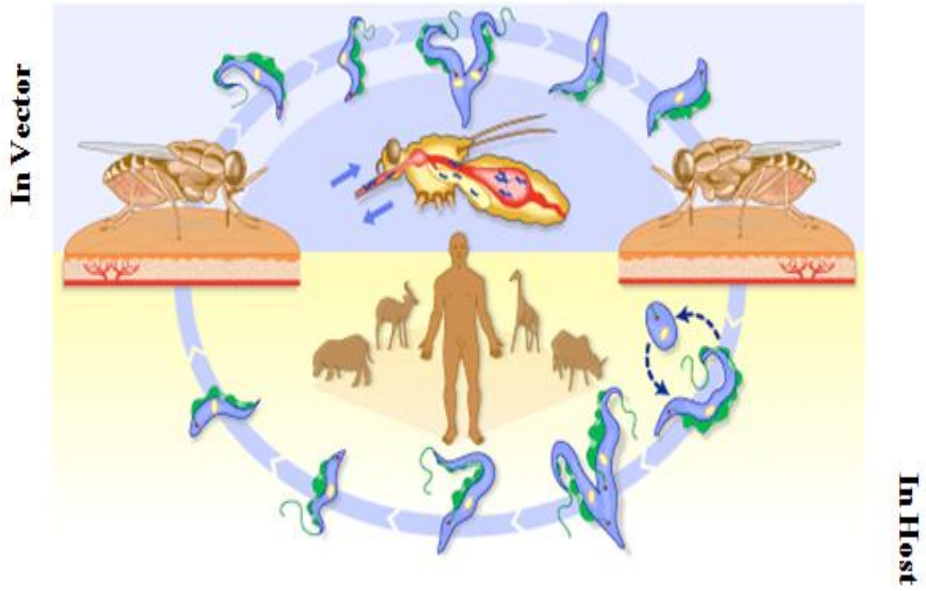

Figure 2.3: Life cycle of trypanosomes (WHO, 2016)

This form enables them to survive in the tsetse midgut. Thereafter, replications occur forming epimastigotes and once establishment is attained the epimatigotes move to different species maturation sites mouthparts (*Nannomonas*) and salivary gland (*Trypanozoon* and *Pycnomonas*) where they mature by differentiating from epimastigotes to metacyclic trypomatigote forms, that are infective to mammals (Figure 2.2).

The development of trypanosomes in mammalian host commences when the metacyclic trypomatigotes are inoculated into the host during feeding. The trypanosomes become localized at the bite site where they replicate and differentiate into slender forms

(trypanosome infective to tsetse) which later they become released into blood stream via the lymphatic system colonising tissues and nervous system.

2.6 Conventional Trypanosomes Diagnostic Techniques

Traditionally, trypanosome infections in tsetse flies are identified through dissection and microscopic observation of the species maturation sites; proboscis, salivary gland and midgut (Lloyd and Johnson, 1924).

In contrast, trypanosome identification in vertebrate hosts is done microscopically through observation of trypanosomes through either wet blood smear, thick or thin Giemsa stained blood smears. The use of Buffy Coat Technique (BCT) and PCV readings curbs the low sensitivities of thick and thin blood smears (Murray *et al.*, 1977). These techniques are more sensitive than thick and thin smears and are used as individual or in combination with other techniques to detect trypanosomes in vertebrates (Marcotty *et al.*, 2008).

However, being used as reference methods and useful as in remote settings where the tsetse and trypanosomes are prevalent and laboratory infrastructures are limited, it is arguably true that these techniques are laborious requiring expertise in dissection and in microscopy, can have low sensitivity, cannot identify mixed and distinguish immature infections. Trypanosomes description beyond the subgenus level is not possible (Enyaru *et al.*, 2010).

Serological assays such as CATT (Card Agglutination Test for Trypanosomes) used for detecting *T. b. gambiense* (Magnus *et al.*, 1978*)* identify trypanosomes indirectly by detecting antibodies in mammalian host whereas others like dot-ELISA (Enzyme-Linked Immune Sorbent Assay) can be used in detecting antigens in tsetse by the use of known

antibodies (Ouma *et al.*, 2000). These techniques as for parasitological techniques cannot identify new species, distinguish active and cured infections and neither distinguish mixed infections (Gichuki *et al.*, 1994).

2.7 Molecular techniques and trypanosomes

The development of molecular techniques has provided solution to the low sensitivity of parasitological and serological techniques. Their uses have increased upon the discovery of Polymerase Chain Reaction (PCR). As discussed above, trypanosomes were detected directly through microscopy and further differentiated into respective subgenera according to developmental site in tsetse (also shown in Figure 2.2) and morphology in mammalian host (Hoare, 1972). The molecular techniques such as DNA probes and PCRs are more sensitive than serological and parasitological techniques.

2.7.1 DNA probes

The use of DNA probes in trypanosome detection has been possible upon discovery of large amount of satellite DNA in the nucleus that are highly repeated with a different nucleotide frequency than bulk DNA, thus allowing separation of species basing on density (Gibson, 2002). Principally these unique sequences are used as probes to unambiguously identify species. The use probes have enabled detection of mixed infections and subspecies description (Mugittu *et al.*, 2001), although the techniques does not identify new species.

2.7.2 Species specific primer techniques

PCR is amongst several DNA amplification techniques including nucleic acid sequence based amplification (NASBA), self-sustained sequence replication (3SR), strand displacement amplification (SDA) and loop mediated isothermal amplification (LAMP)

(Fakruddin *et al.*, 2013). Contrary to the others, PCR uses heat in initiation of the synthesis (in the denaturation step of DNA), prior to polymerase use for amplification (Mullis, 1990). Its use in trypanosome detection has increased in many ways, the knowledge of the epidemiology of trypanosomosis (Malele *et al.*, 2011a; Welburn *et al.*, 2001). The species- specific PCR techniques developed target satellite DNA and serves in identification of specific trypanosomes in a single reaction through the use of specific primers. Satellite based *Trypanosoma brucei* subspecies PCR- developed by (Moser *et al.*, 1989) identifies *T. brucei* subspecies from the *Trypanozoon* subgenus at 116 bp; whereas; *T. brucei rhodesiense* PCR identifies the pathogen of the acute form of sleeping sickness by targeting the SRA gene that is absent from other *T. brucei* subspecies at 667 bp (Welburn *et al.*, 2001).

Furthermore, Masake *et al.* (1997) developed assay that identifies *T. vivax*. In addition, there exists a PCR assay that detects *T. congolense* sub groups; *T. congolense* savannah, *T. congolense* forest, *T. congolens*e Kilifi, and *T. simiae* (Masiga *et al.*, 1992). Restriction Fragment Length Polymorphism (RFLP) PCR, however, being specific, is laborious and expensive and due to the utilisation of specific endonuclease and pre requisite of undamaged DNA (Geysen *et al.*, 2003). However, being sensitive, these techniques require multiple PCR tests to reveal trypanosomes in host which increases cost and requires time (Thumbi *et al.*, 2008).

The LAMP assay amplifies trypanosome DNA efficiently and rapidly under isothermal conditions using simple heating block. Furthermore, SRA-LAMP and RIME-LAMP techniques, have unambiguously enabled understanding of epidemiology of HAT. The techniques identify *T. b. rhodesiense* from others in the *Trypanozoon* subgenus and *Trypanozoon* subgenus from *Duttonella* and *Nannomonas* subgenus (Njiru *et al.*, 2008a,b).

2.7.3 Pan trypanosome PCR

The drive to develop a comprehensive technique to differentiate trypanosome species in single PCR, using single pair of primers, was due to shortcomings of species specific assays. The goal has been achieved upon discovery of highly conserved sequences in the ITS regions of ribosomal DNA; ITS 1 and ITS2 (Figure 2.4) (Hillis and Dixon, 1991). For amplification of the ITS1 region, the primers anneal to 18S position and 5.8S, for forward and backward primers respectively.

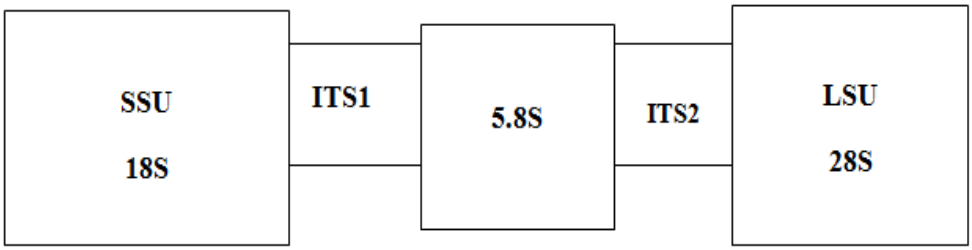

Figure 2.4: ITS regions of trypanosome (Cox *et al.*, 2005)

High sequence variation in these regions, have resulted in PCR products of differing lengths therefore providing a basis of separating trypanosome into species, subspecies or group Table 2.2 (Geysen *et al.*, 2003; Njiru *et al.*, 2005).

Table 2.2: Trypanosome band sizes from nested and conventional PCR

Species	**Band sizes (bp)**	
	Nested PCR (Cox *et al.*, 2005)	**Conventional PCR (Njiru *et al.*, 2005)**
T. congolense savannah	1413	700
T. congolense forest	1513	710
T. congolense Kilifi/Kenya coast	1422	620
T. simiae Tsavo	951	370
T. brucei subspecies	1207 – 1224	480
T. simiae	847	400
T. vivax	611	250
T. godfreyi		300
T. theileri	998	-

The subsequent development of primers (Table 2.3) for ITS1 PCR (Njiru *et al.*, 2005) and nested ITS PCR (Cox *et al.*, 2005), has enabled identification of trypanosome infections in a single PCR and has paved way into discovery of new trypanosome species, and mixed infections (Malele *et al.*, 2011a).

Table 2.3: ITS primers for nested and conventional PCR

PCR	Primer Sequence	Reference
Nested ITS	ITS 1: 5' - GAT TAC GTC CCT GCC ATT TG - 3'	Cox *et al.* (2005)
	ITS 2: 5' - TTG TTC GCT ATC GGT CTT CC - 3'	
	ITS 3: 5' - GGA AGC AAA AGT CGT AAC AAG G -3'	
	ITS 4: 5' - TGT TTT CTT TTC CTC CGC TG - 3'	
Conventional ITS1	CF: 5'-CCGGAAGTTCACCGATATTG-3'	Njiru *et al.* (2005)
	BR:5'-TTGCTGCGTTCTTCAACGAA-3'	

ITS1 PCR has been used in trypanosome characterization in various studies in tsetse and vertebrate hosts, Manangwa *et al.* (2016) investigated the role of *G. fuscipes* in transmission of trypanosomosis in lake Victoria basin, Salekwa *et al.* (2014) investigated trypanosomes in tsetse flies in Simanjiro, while Ruiz *et al.* (2015) investigated the role of domestic animals in transmission of HAT in the NCA. The technique has been used to identify HAT pathogens in domestic pigs (Hamill *et al.*, 2013) and characterizing trypanosomes in wildlife and tsetse species (Auty *et al.*, 2012b).

It is arguably true that ITS based PCRs have lower sensitivity than species specific assays (Auty, 2009). However, the techniques are still useful in identifying large field samples, and ideally suitable for monitoring purposes, as it gives a rough picture of what is happening in the population (Cox *et al.*, 2005). The highly conserved sequences of the 18S gene in the ITS1 region across trypanosome species justify its use in phylogeny analysis.

The technique was used in this study to assess the prevalence of sleeping sickness in tsetse and cattle, as well as in phylogeny analysis.

2.7.4 Phylogenetic analysis

Phylogenetic analysis is the investigation that seeks to explain the evolutionary relationship among biological units that share common ancestor (Harrison and Langdale, 2006). The biological units include genes, species, and genomes. The phylogenetic analysis is distinct from Linnean morphological evolutionary classification, from the fact that it involves the use of molecular traits (DNA and protein sequences), other than morphological traits in inferring evolutionary relationship.

The choice of gene of interest is the essential step, depending mostly on how far the biological unit is to be inferred, whether recent or ancient evolutionary relationship. In the phylogenetic analysis of trypanosomes the use of satellite DNA has been limited by the difficultness in assembling shortgun DNA segments composed of repetitive elements, rapid and non-uniform evolution of satellite DNA (Plohl, 2005). Furthermore, protein coding genes for example Glyceraldehyde 3- phosphate dehydrogenase (GAPDH) gene (which specifies production of the glycolytic enzyme that catalyse breakdown of glucose into energy and carbon dioxide) (Adams *et al.*, 2010) are not widely used in phylogenetic analysis due few reference sequences available.

Additionally, the protein coding genes are highly conserved and thus more suitable in depicting ancient evolution and evolutionary relationship between distant related taxa. The small subunit of the ribosome (SSU) in turn have been widely used to infer genetic evolutionary on wide range of organisms and the fact that it is highly conserved across species and numerous reference sequences available make it suitable for evolutionary

studies. The gene was selected not only due to the fact that occurrence of trypanosomes in tsetse and cattle can be determined but also phylogenetic analysis can be inferred.

2.7.4.1 Sequence alignment

This can be referred to as method for arranging the sequences of DNA, RNA, or protein for the purpose of identifying homologous positions (Xia, 2007). Each sequence is assigned to a separate row and homologous positions in different sequences are lined up in columns. Two types of sequence alignment are distinct; Pair wise sequence alignment (PSA) which utilise the entire sequences in search for homologous sequence between two sequences. Multiple sequence alignment (MSA) in turn compares two and more than three sequences (Morgenstern *et al.*, 1996).

Programs have been designed to sort the complex calculations involved in the sequence alignment. Most of alignment algorithm present aligns sequences locally and they are designed based on specified assumptions. Commonly used programs includes; BLAST (Basic Local Alignment Search Tool), usually used in database searches like Gene Bank. It is used to search the query sequence for similarity match with known sequence and gives score to indicate the similarity. Another one is Clustal W which assumes constant evolutionary rate and thus it widely used in alignment of similar sequences (Thompson *et al.*, 1994). In contrast to Clustal W, MUSCLE (Multiple Sequence Comparison by Log Expectation) algorithm designed by Edgar (2004) is a high accuracy and high throughput method that assumes different rate of evolution and thus is suitable for heterogeneous sequences alignment. In addition to depicting similarity between sequences, alignment is used as the data for phylogenetic analysis.

2.7.4.2 Phylogenetic tree construction methods

There are several methods for tree construction, but most of them can be classified into two main categories; Distance based and character based methods. The two methods are distinguished, based on principle that distance-based methods calculate distance between sequences and uses it in construction of phylogenetic tree whereas character-based methods in turn consider all characters in inferring phylogenetic relationship between taxa. Neighbor- joining (NJ) (Saitou and Nei, 1987) and Unweighed Pair Group Method with Arithmetic Mean (UPGMA) (Sneath and Sokal, 1973) encompass distance methods. The UPGMA strictly adheres to molecular clock whereas NJ adheres less to the constant molecular evolution and thus allows inference of distant related taxa. In these methods the optimal tree is obtained by calculating evolutionary distance between sequences, and these distances are used to construct the tree, while the branch length and the patterns do represent the distance matrix and thus closely related taxa are grouped together (Hall, 2008).

Moreover, Maximum Parsimony (MP) (Nei and Kumar, 2000), Maximum likelihood (ML), and Bayesian methods constitute the character based methods. The methods are also commonly referred to as tree searching methods because they generate several optimal trees and choose the best one. The ML and the Bayesian methods however being most accurate they involve time consuming statistical calculations that find the probability of the tree that likely present the observed data.

Correct phylogenetic inference requires the determination of the tree root/ out group, as this allows full assessment of direction of change. The phylogenetic tree of this study was strongly rooted by selected species of Euglenida and stramenopiles to infer the evolutionary relationship of the salivaria trypanosomes. These are free living flagellates.

According to Hughes and Piontkivska (2003), the organisms from the two families constitute unquestionably out-groups to the genus *Trypanosoma* and urged that genus *Leishmania, Endotrypanum, Bodo* and *Crithidia* are not proper out groups, since they are closely related to trypanosomatidae family.

The MP method was chosen to infer the molecular evolution of trypanosomes in Meatu because of; i) The heterogeneity of the sequence alignment data which suggested higher evolutionary changes, thus distance methods would not give correct inference and ii) the consideration of all informative characters in given data set (which is the assumption of character based methods) in tree construction would be more informative and provide a reasonable phylogenetic tree.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

Meatu district is located at Longitude 34°8'34.49"E and Latitude 2°57'4.9"S. It is amongst the five districts of the recently formed Simiyu region (carved from former Shinyanga region district) (Figure 3.1). It has the size of about 8 871 km^2. The population size is estimated at 296 616 people according to the 2012 Tanzania National Census (URT, 2012). It has uni-modal rainfall pattern, which usually starts from October and ends in May. The average annual rainfall ranges from 400 mm to 900 mm. Its vegetation is mainly open bush savannah, dominated by Acacia species. As indicated in the map presented in Figure 3.1 three species of tsetse fly; *G. swynnertoni, G. pallidipes* and *G. morsitans* occur (IRA unpublished, 2012).

3.2 Experimental Design

Cross-sectional study was conducted during September 2015 in Meatu district. The study targeted Mwangudo and Mwanyahina wards specifically to their four villages (Mwangudo, Buganza, Mwanyahina and Makao). Selection of the study areas was based on vicinity to the protected parks, relative cattle population and history of trypanosomosis endemicity. In each village, socio-economic, entomological and parasitological data were collected. Molecular techniques were applied to ascertain trypanosome species diversity.

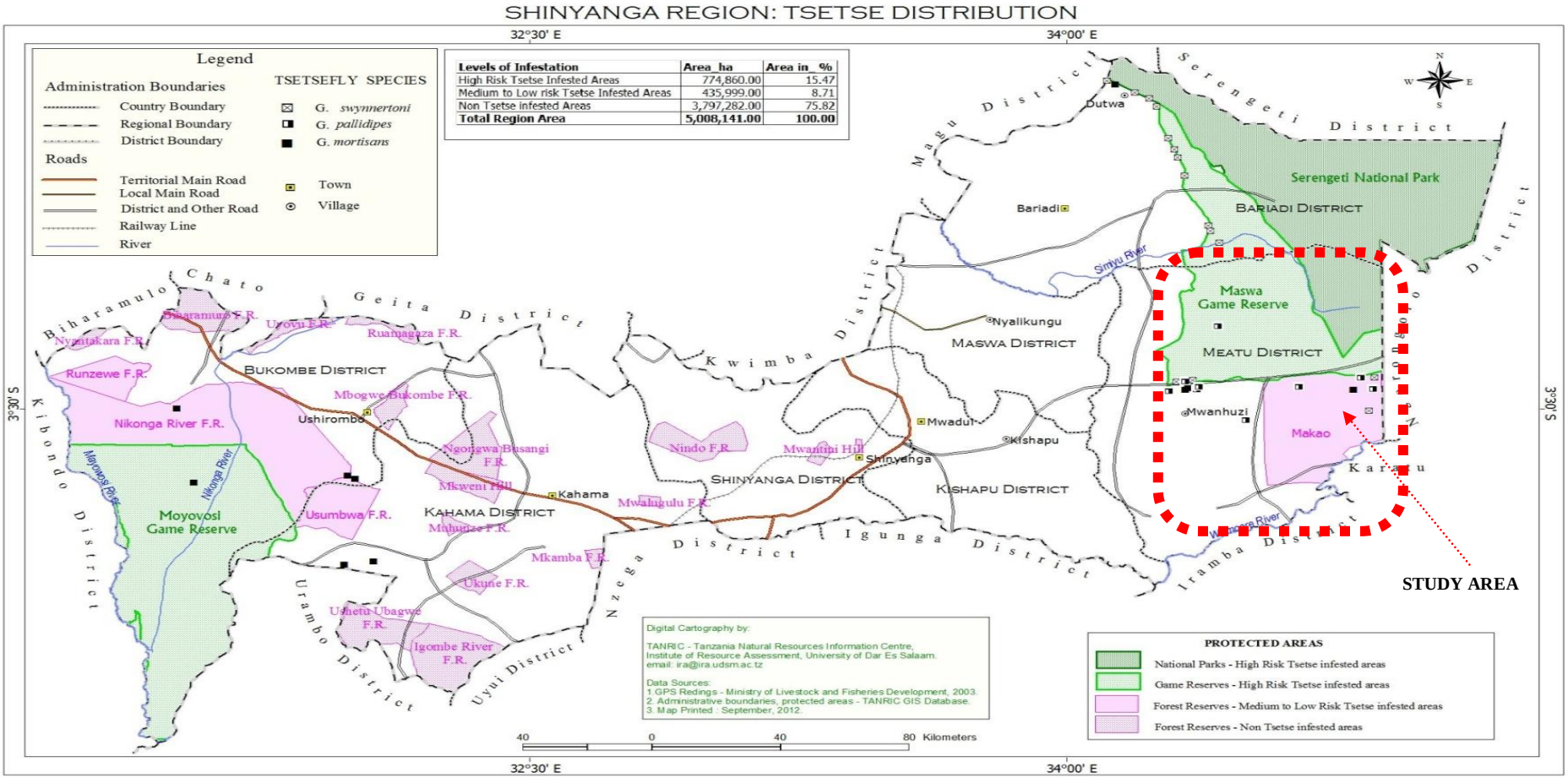

Figure 3.1: Map showing the study area and distribution of tsetse (IRA, 2012)

3.3 Sample size and Sampling Procedure

A precision of 5% and 95% confidence interval (CI) was used to determine sample size for KAP survey, tsetse flies and cattle for parasite identification. The formula indicated in Equation 3.1 was used. Proportion of 50% of the population was assumed to have appropriate knowledge on tsetse and trypanosomosis (Naing *et al.*, 2006) therefore sample size of 385 respondents was obtained. For consideration in the study respondents had to be 18 years of age and above. In order to obtain number of tsetse flies for molecular studies, prevalence of *T. b. rhodesiense* in wild caught tsetse was estimated at 0.01% (Auty *et al.*, 2012b) giving total of 15.21 non-teneral tsetse cluster samples. Nevertheless, in order to obtain number of cattle to be included in the study prevalence of trypanosomosis in cattle was estimated to be 28% (Haji *et al.*, 2015) therefore total of 310 cattle to be examined was obtained of which were randomly selected for blood sampling from the herd kraals.

Equation 3.1: Calculation of sample size

Sample size= Z^2 p (1-p) / d^2

Where;

Z= 1.96 (95% CI factor)

p= proportion and

d= precision

3.4 Ethical Consideration and Permission to Conduct the Study

The current research was approved by the Research and Postgraduate Studies committee of the College of Veterinary Medicine and Biomedical Sciences. Furthermore, the permission to conduct the study in the district was granted by the office of District Executive Director (DED) after the study objectives were introduced. The District Agriculture and Livestock and Fisheries office (DLFDO), through the village extension

officers and village executive officers, were responsible for the supervision of the project activities at the village. Oral consent by all eligible participants (of 18 years of age and above) was requested before being interviewed. Nevertheless, collection of blood sample from cattle was possible only after consent was granted by household head. The blood was collected from jugular vein of cattle by a qualified veterinarian.

3.5 Data Collection

3.5.1 Socio-economic data

Socio economic data of the community KAP on tsetse flies and trypanosomosis were collected through a semi-structured questionnaire (Appendix 1) Kiswahili translated version of the questionnaire was available to respondents.

3.5.2 Tsetse collection

Sixteen baited traps of four types; NZI (Mihok, 2002), NGU (Brightwell *et al.*, 1987), S3 (Ndegwa and Mihok, 2007) and Biconical (Challier *et al.*, 1977) were used (Figure 3.2). The traps were deployed in the identified vegetation and at a place that was visible. About 100ml of acetone was poured into an empty plastic bottle (500ml) and a small hole was pierced on the cap to allow slow escape of vapour and set below the trap. Additionally, a nylon sachet (5cm×5cm) containing phenol was placed below the trap, and both attractants were set towards the direction of the wind from which the flies will be attracted to the trap against the wind. An empty plastic bottle of 1000 ml was adopted as cage and used in collecting trapped flies. Trap rotation and milking of the traps was done in the morning (about 9.00-10.00am) by replacing with an empty cage to a trap for three consecutive days.

Figure 3.2: Traps used in the study

Non-teneral tsetse flies from each village surveyed were sorted according species and sex and preserved in sterile, absolute ethanol contained in 1500µl Eppendorf tubes. Flies were packed into pools of five flies per tube for further molecular determination of parasites in the laboratory.

3.5.3 Parasitological screening for trypanosomes

Blood samples from cattle were collected from jugular vein using Ethylene Diamine Tetra Acetic Acid (EDTA) vacutainer tubes, labelled and stored in ice packed cool box. Collected blood samples were examined for Packed Cell Volume (PCV) (Murray *et al.*, 1977; Woo, 1970). Briefly, a proportion of blood sample was drawn from vacutainer tubes using capillary tubes and crystal-sealed. The samples were centrifuged for 5 minutes at 12 000rpm (rotations per minute) (Hawksley Micro haematocrit centrifuge), then PCV readings were taken on a PCV reader (Hawksley Micro haematocrit reader) and later the capillary tubes were examined under light microscope (Olympus CX2IFS1) for observation of trypanosomes at the blood-plasma junction.

Microscopically trypanosomes were further determined by thick blood smears Giemsa stained. Buffy coat samples (including positive and those whose PCV was ≤24%) were extruded on Whatman FTA® cards matrices, air dried before being stored in nylon sheets containing silica gel for further molecular analysis in the laboratory.

3.5.4 Molecular studies

3.5.4.1 DNA extraction

DNA was extracted from pooled samples of tsetse flies by ammonium acetate precipitation (Bruford *et al.*, 1988). Before extraction of DNA, absolute ethanol was poured off from the sample and the specimens left to dry overnight thereafter ground and homogenised using sterile pestles, newly sterilised for each sample. Five hundred microliters, phosphate buffered saline (PBS) was added and vortexed for three minutes then 100µl of supernatant was pipetted off to the new labelled Eppendorf tubes. Into the tubes 250µl of digisol buffer and 10µl of (proteinase K stored at -20^{0}C) were added and vortexed for 30 seconds followed by incubation at 55^{0}C for 1 hour. Three hundred microliters of ammonium acetate were added to each Eppendorf tubes and vortexed for 30 seconds repeatedly for 5 minutes. The mixture was centrifuged (Hettich D-78532) at 14 000rpm for 15 minutes and then supernatant transferred to new clean and sterile 1500µl Eppendorf tubes. One millilitre, 100% ethanol was added to the supernatant, vortexed and inverted gently 10-20 times for DNA precipitation followed by centrifugation at 14 000rpm for 15 minutes. The supernatant was pipetted off.

Nine hundred microliters of 70% cold ethanol (stored at -20^{0}C) was added and the Eppendorf tubes inverted gently 10 times to rinse the DNA pellet and ethanol was gently poured off. Thereafter the Eppendorf tubes were opened and inverted and left to dry

overnight. One hundred microliters of Tris EDTA buffer were added to each Eppendorf tubes and stored at -20^{0}C until used for PCR.

DNA was extracted from Buffy coat samples preserved on FTA cards by 20% Chelex® resin which has been described as the best technique for DNA isolation from samples stored on FTA cards (Ahmed *et al.*, 2011). Briefly, 10 hole punches (1.2mm) were taken from the FTA card using Harris® Micro-punch and added into 1500 µl sterile Eppendorf tubes, 300µl of 20% Chelex was added and vortexed for 20 seconds to ensure the beads were distributed evenly and incubated at 55^{0}C for 1 hour. Thereafter, samples were agitated and centrifuged at 14 000rpm for 10 seconds followed by boiling for 10 minutes. One hundred microliters of supernatant were transferred to new sterile Eppendorf tubes and stored at -20^{0}C until used for PCR.

3.5.4.2 Amplification of trypanosome DNA and sequencing

ITS1 amplification was carried out in 25µl reaction mixture containing 2µl DNA template, 9.5µl distilled water, 12.5µl *Taq*1× Master mix (New England Biolabs MO270L) and 0.5µl of each ITS1 primers (Table 2.3). Primers and PCR conditions used were as described by Njiru *et al.* (2005). Briefly an initial denaturation was done at 94^{0}C for 30 seconds, followed by 35 cycles of denaturation at 94^{0}C for 30 seconds, annealing step at 58^{0}C for 45 seconds, elongation and extension steps each at 68^{0}C for 5 minutes.

The PCR products in 1.5% agarose gel were stained with Ethidium bromide and visualized through the ultra Violet-Trans-illuminator (Uvitec DOC-CF08.XD). DNA extracted from *T. evansi* isolated and characterised from Sudan (kindly donated by Dr. Hamid Ibrahim Noor from Khartoum University, North Sudan) was used as positive control and PCR water as negative control. Samples were classified into respective species as shown in

Table 2.2. Seven positive trypanosome PCR products were sent to Bioneer, Korea for purification and sequencing of 18S gene.

3.6 Data Analysis

3.6.1 Analysis of socio economic data

Socio-economic data were summarized, coded and entered in Microsoft Excel (2007). The coded data were analysed using Epi info® 7 (CDC, 2014) computer software. Qualitative and quantitative analysis which included both descriptive and inferential statistics were conducted to describe and compare different responses on the investigated parameters.

3.6.2 Analysis of parasitological and entomological data

Parasitological as well as entomological data collected from animals and tsetse from the four villages were entered in Microsoft Excel (2007) and apparent density (FTD), occurrence of nagana and anaemia calculated. A cut off point of ≤ 24% PCV (Marcotty *et al.*, 2008) was used for considering cattle anaemic. Furthermore, the records were transferred to Epi Info® 7. Chi-square (χ^2) test was used to compare occurrence of trypanosomes and anaemia between villages and cattle groups. One-way Analysis of variance (ANOVA) was used to analyse the variations of tsetse catches and cattle PCV in the villages. Tsetse fly counts were used as dependent variable and trap type, tsetse species and village/ward as grouping variables. The overall comparison of tsetse species and the traps regardless of the tsetse flies' species was done using generalized linear model analysis. PCV of cattle was used as dependent variable whereas nagana, anaemia, were independent variables. Separation of means was done at 95% Confidence Interval (CI) and significance level of 5% in all statistical tests.

3.6.3 Analysis of molecular data

3.6.3.1 PCR based prevalence of trypanosomes

The PCR results from buffy coats and pooled tsetse samples were analysed for computation of occurrence of trypanosomes in cattle and tsetse. Chi-square (χ^2) test was used to compare infections between cattle groups and between villages, and between tsetse species and village for cattle and tsetse respectively.

3.6.3.2 Phylogenetic analysis

Twelve sequences of *Trypanosoma* species containing 18S gene were downloaded from Gene Bank using mega BLAST algorithm (Table 3.1). (The sequences were selected based on E-value and sequence similarity with the seven new sequences).

Table 3.1: Gene Bank reference sequences used in the phylogenetic analysis

Accession number	Source	Species	Percent identity
U22315.1	Brown rat	*T. congolense*	86%
DQ317416.1	Unknown	*T. vivax*	70%
JN673389.1	Lion	*T. congolense* savannah	85%
AB301937	Mouse	*T. congolense*	76%
JX910374.1	Unknown	*T. brucei*	80%
KR028191	Cattle	*T. congolense* savannah	70%
FJ712718.1	Mouse	*T. congolense*	85%
JX853185.1	Cattle	*Trypanosoma theileri*	82%
U22317.1	Brown rat	*T. congolense* Kilifi	98%
AB301941	Deer	*T. congolense*	84%
U22319	Brown rat	*T. congolense* forest	93%
AB742531	Tsetse fly	*T. congolense*	76%

The seven new 18S sequences (Appendix 2.2), 12 sequences from Gene bank and out group 18S sequences of *Prymnesium parvum* and *Nannochloropsis gaditana* free living flagellates (members of Chrysophyceae and Eustigmatophyceae families) were aligned by

MUSCLE (Multiple Sequence Comparison by Log Expectation) in alignment (Edgar, 2004) method and subsequent tree was inferred using Maximum Parsimony (MP) at 100 bootstrap in both methods, default settings as incorporated in MEGA (Molecular Evolutionary Genetic Analysis) software version 6 were used (Tamura *et al.*, 2013).

CHAPTER FOUR

4.0 RESULTS

This chapter describes the results from socio-economic, tsetse fly collection, parasitological as well as molecular studies collected through a cross sectional study. These results are from Mwanyahina and Mwangudo wards of Meatu district in their respective four villages (Mwanyahina, Buganza, Mwangudo and Makao). Figure 4.1 shows the villages surveyed.

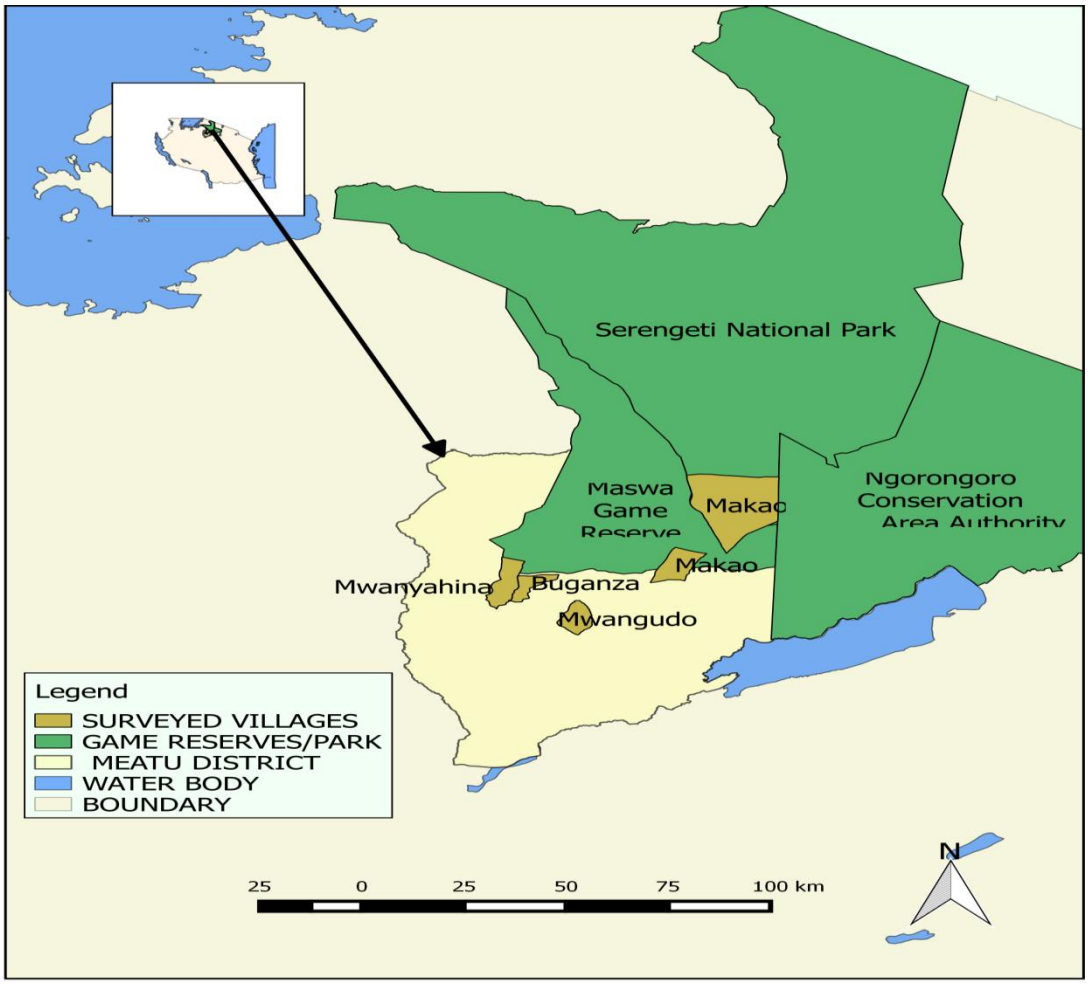

Figure 4.1: Map of Meatu district showing the villages surveyed

4.1 Socio Economic Studies

4.1.1 Socio-demographic characteristics

Information on Knowledge, Attitude and Practices (KAP) was collected from 306 respondents in the selected two wards covering four villages. (Table 4.1) summarizes socio-demographic information on the respondents. Makao village contributed 46.4% of all respondents followed by Mwanyahina, Buganza and Mwangudo. Among respondents 52.3% were females and males constituted 47.7%. The largest (32.7%) group of the respondents were aged 18-29 years while the least (21.6%) group had 40-39 years. Seventy percent of the respondents were literate whereby 60.1% had attained primary education, 8.17% secondary education while 2.61% were college graduates.

Table 4.1: Socio-demographic characteristics

	Wards					
	Mwanyahina		**Mwangudo**		**TOTAL**	
	N	%	N	%	N	%
Age (In years)						
18-29	46	36.0	54	30.3	100	32.7
30-39	24	18.8	46	25.8	70	22.8
40-49	28	21.9	38	21.4	66	21.6
>50	30	23.4	40	22.5	70	22.8
Gender						
Male	78	60.9	68	38.2	146	47.7
Female	50	39.1	110	61.8	160	52.3
Education level						
None	39	30.5	50	28.1	89	29.1
Primary	78	60.9	106	59.6	184	60.1
Secondary	9	7.0	16	8.99	25	8.17
College	2	1.56	6	3.57	8	2.61
Occupation						
Agro pastoral	95	74.2	132	74.2	227	74.2
Pastoralists	16	12.5	20	11.2	36	11.8
Civil Servant	9	7.03	14	7.87	23	7.52
Other activities	8	6.25	12	6.74	20	6.54

Three major means of livelihood in descending order were agro-pastoralism, pastoralism and other activities (of bee-keeping, retail shops) corresponding to 74.2%, 11.8% and 6.54% respectively. Women contributed a fairly large proportion of agro-pastoral. Pastoralism in contrast was a male's activity.

4.1.2 Knowledge about tsetse fly

Table 4.2 illustrates tsetse fly awareness and risk perception of the respondents in the four villages. The results show that 88.2% knew tsetse flies, and respondents aged 18-29 years were more aware of tsetse flies than the other age groups (P=0.005). Also males were more aware of the vector than females when compared by chi square test (P=0.001). About 92.6% of the respondents experienced tsetse fly biting in recent times, males being more affected than females (P=0.012). Bush/forest, forest and bush roads grazing areas, and near home/office were regarded most risky areas where one could encounter tsetse fly corresponding to 42.7%, 28.2%, 19.5% and 9.5% respectively. No significant difference was observed between age group, education level, gender, occupation and village (P>0.05) on regard to response on tsetse fly risky places. Pain/swelling at the site of tsetse bite (62.1%) and fever (6.5%) were observed after a tsetse bite by respondents while 26.6% responded that nothing happened. Few respondents (9.5%) reported visiting hospital upon tsetse fly bites whereas majority (86.7%) did nothing once bitten by the tsetse flies. Dry season was reported to be associated with more flies (57.0%) compared to wet season (28.4%) while 14.6% did not know. Despite the fact that no statistically significant difference (P>0.05) was observed among independent variables studied with regard to perceived risks associated with tsetse bite, 39.6% of the respondents scored disease/fever as the most important threat associated with tsetse bite, while 22.0%, 21.0% and 16.4% respondents scored disturbance, pain/injury and anaemia respectively.

Table 4.2: Tsetse awareness and risk perception

	Villages									
	Buganza		Mwanyahina		Makao		Mwangudo		TOTAL	
	N	%	N	%	N	%	N	%	N	%
Tsetse awareness										
Yes	41	73.2	61	87.1	130	91.6	37	97.4	269	88.2
No	15	26.8	9	12.9	11	7.75	1	2.6	36	11.8
Encountered tsetse bite recently										
Yes	40	97.6	53	86.9	120	92.3	36	97.3	249	92.6
No	1	2.4	8	13.1	10	7.6	1	2.7	20	7.4
Tsetse risk areas										
Near home/office	3	7.5	4	6.67	17	13.6	1	2.7	25	9.5
In the bush/forest	17	42.5	26	43.3	52	41.6	17	49.9	112	42.7
In grazing areas when looking after livestock	12	30	16	26.7	18	14.4	5	15.5	51	19.5
Forest and bush roads	8	20	14	23.3	38	30.4	14	37.8	74	28.2
Sign for tsetse bite										
Pain/swelling on the site of tsetse bite	29	72.5	25	48.1	76	63.3	24	66.7	154	62.1
Fever and swelling on the site of tsetse bite	2	5	3	5.8	9	7.5	2	5.6	16	6.5
Fever	2	5	3	5.8	4	3.3	3	8.3	12	4.8
Nothing happened	7	17.5	21	40.4	31	25.8	7	19.4	66	26.6
Response after tsetse bite										
Visited health centre	6	15	4	7.8	11	9.2	4	7.8	25	9.5
Told family member	3	7.5	1	1.96	5	4.17	1	1.96	10	3.8
I did nothing	30	77.5	46	90.2	107	86.67	46	90.2	229	86.7
Season with more tsetse bites										
Dry season	26	63.4	28	45.9	71	55	28	75.7	153	57.0
Wet season	13	31.7	24	39.3	33	25.6	6	16.2	76	28.4
I do not know	2	4.9	9	14.8	25	19.9	3	8.1	39	14.6
Dangers associated with tsetse bite										
Disease/fever	17	41.5	24	39.3	51	39.5	14	37.8	106	39.6
Pain/injury	8	19.5	21	34.4	22	17.1	8	21.6	59	22.0
Disturbance	11	26.8	9	14.8	29	22.5	10	13.5	59	22.0
Anaemia	5	12.2	7	11.5	27	20.9	5	13.5	44	16.4

4.1.3 Livestock management and awareness of bovine diseases

Two livestock management system were distinct; crop-livestock and pastoral system the first being dominant over the other. Table 4.3 illustrates domestic animal possession, whereby, only (5.8%) of respondents reported not keeping animals. Cattle alone were kept by 10.1% of the respondents. The largest group (37.3%) of respondents kept cattle, goat, and sheep and also kept dogs. Whereas cattle, goat and dog (16.9%), cattle and dog (13.4%) and cattle and goat (10.5%). Domestic animal combinations with least number of respondents were cattle and sheep, and sheep, goat and dog by 1.31% each. Respondents with primary education were significantly the largest group compared to the ones with secondary education, college graduates and uneducated (P=0.018). The observed differences between age group, gender, occupation and village were not significant (P>0.05).

Table 4.3: Domestic animal ownership across villages

	Villages									
	Buganza		**Mwanyahina**		**Makao**		**Mwangudo**		**TOTAL**	
	N	%	N	%	N	%	N	%	N	%
No domestic animal	0	0.00	2	2.86	15	10.6	1	2.63	18	5.8
Cattle, goat, sheep and dog	24	42.9	26	37.1	48	33.8	16	42.1	114	37.3
Cattle and dog	6	10.7	4	5.71	27	19	4	10.5	41	13.4
Cattle, goat and dog	11	19.6	14	20.0	22	15.5	5	13.2	52	16.9
Cattle Goat and sheep	2	3.57	2	5.71	4	2.86	2	5.26	10	3.3
Cattle	4	7.14	9	12.9	12	8.45	6	15.8	31	10.1
Cattle and goat	7	12.5	11	15.7	10	8.45	4	10.5	32	10.5
Cattle and sheep	0	0.00	2	2.86	2	1.41	0	0.00	4	1.3
Sheep, goat and dog	2	3.57	0	0.00	2	1.41	0	0.00	4	1.3

The respondents mentioned economic livestock diseases in the study area as animal trypanosomosis (47.9%), East coast fever (ECF) (16.7%), Foot and Mouth Disease (FMD) (15.3%), Contagious Bovine Pleuropneumonia (CBPP) (1.8%) and Lumpy Skin Disease (LSD) (0.7%). Figure 4.2 illustrates the respondents varied consideration of the livestock

diseases by villages. Observed differences in livestock diseases awareness between village, gender, age group, education level and occupation were not significant (P>0.05).

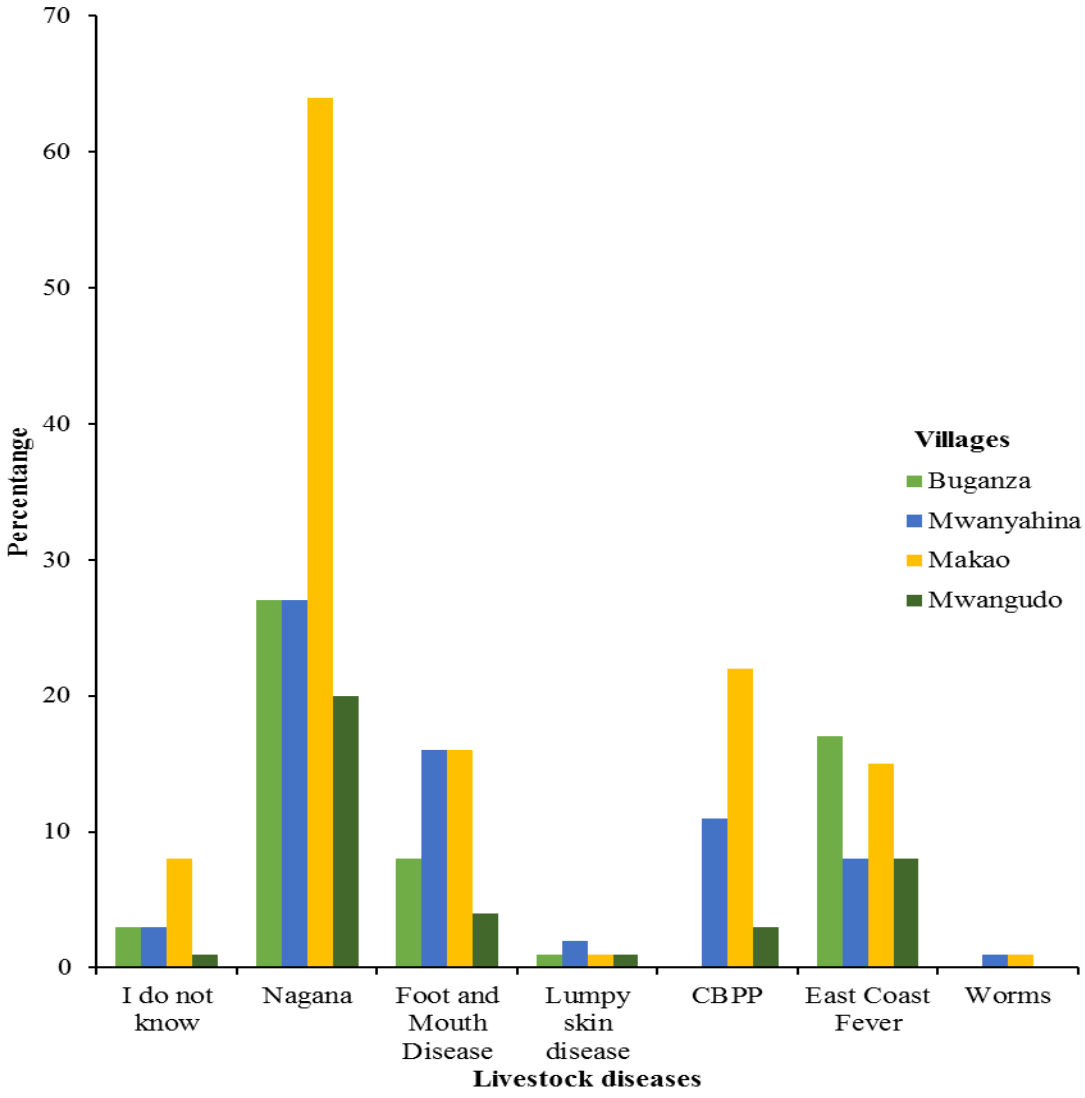

Figure 4.2: Percentages of respondent who mentioned different livestock diseases

4.1.4 Awareness, management and threat perception of animal trypanosomosis

Table 4.4 illustrates knowledge about nagana/bovine trypanosomosis. The disease was referred to as *"Bhusatu bho ngi"* by majority (66.0%) of the respondents.

The word is Kisukuma (the largest tribe found in the area) for fly disease. Eight percent referred it as "*ndorobo"* a Kiswahili word meaning tsetse fly, so they literally meant tsetse fly disease. About 9.0% referred it as "*ngaroti"* a Kimasai word which bears meaning equivalent to diarrhoea diseases and 18.5% did not know the local name. Tsetse flies were known as the source of nagana by 77.6% of the respondents. The disease was known more to the Makao village respondents than to other villages with fewest respondents from Buganza (P=0.0001). Differences in awareness on nagana among age groups, gender, education levels and occupation were not statistically significant (P>0.05).

Table 4.4: Bovine trypanosomosis awareness

	Villages									
	Buganza		**Mwanyahina**		**Makao**		**Mwangudo**		**TOTAL**	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Local name										
I don't Know	5	13.5	25	37.8	9	7.44	9	25.7	48	18.5
Bhusatu bho ngi	30	81.1	34	51.5	86	71.1	21	60.0	171	66.0
Ndorobo	2	5.41	3	4.55	12	9.92	1	2.86	18	7.0
Ngaroti	0	0.00	4	6.06	14	11.6	4	11.4	22	9.0
Source of nagana										
Tsetse bite	21	56.7	47	71.2	104	92.0	22	64.7	194	77.6
Other parasite	1	2.70	0	0.00	0	0.00	0	0.00	1	0.4
Witch craft	0	0.00	7	10.6	1	0.88	1	2.94	9	3.6
Other insects bite	0	0	0	0.00	1	0.88	0	0.00	1	0.4
I don't know	15	40.5	12	18.1	7	6.19	11	32.4	45	18

Table 4.5 shows respondents' knowledge on clinical signs, and their practices on treatment and their perceived effects of nagana. There was no statistically significant difference in awareness of clinical signs between gender, age group, education level, occupation and

Table 4.5: Respondents knowledge on clinical signs, their practices on treatment and perceived effects of nagana

	Villages									
	Buganza		Mwanyahina		Makao		Mwangudo		TOTAL	
Clinical signs of Nagana	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
I do not know	10	27.78	22	33.3	38	31.4	11	31.4	81	31.4
Anaemia	4	11.11	4	6.1	12	9.9	4	11.4	24	9.3
Abortion	0	0	8	12.1	10	8.3	7	20	25	9.7
Emaciation	7	19.4	12	18.2	23	19	1	2.9	43	16.7
Fever	3	8.33	7	10.6	14	11.6	2	5.7	26	10.1
Diarrhoea	5	13.89	1	1.5	7	5.79	0	0	13	5.0
Starry hair coat	4	11.11	5	7.6	9	7.4	6	17.1	24	9.3
Emaciation and starry hair coat	1	2.78	5	7.6	4	3.3	2	5.7	12	4.7
Emaciation and fever	1	2.78	2	3	1	0.8	0	0	4	1.6
Alopecia	1	2.78	0	0	3	2.5	2	5.71	6	2.3
Veterinary drug used in treatment									0	
Berenil	16	43.2	17	37.7	46	46.5	9	40.9	88	43.3
Novidium	2	5.4	6	13.3	8	8.1	1	4.6	17	8.4
Samorin	7	18.9	2	4.4	4	4	2	9.1	15	7.4
Berenil and Novidium	7	18.9	15	33.3	24	24.2	6	27.3	52	25.6
OTC	5	13.5	5	11.1	17	17.2	4	18.2	31	15.3
Frequency of the drug use										
Twice per week	3	8.1	16	24.2	39	34.2	10	28.6	68	27.0
Once per week	10	27	15	22.7	20	17.5	9	25.7	54	21.4
As recommended by vet shop	2	5.4	2	3	4	3.51	5	14.3	13	5.2
I cannot recall	22	59.5	33	50	51	44.7	11	31.4	117	46.4
Impact of Nagana in cattle herd										
Abortion and still births	11	29.7	22	33.3	36	29.5	13	37.1	82	31.5
Weight loss/ reduced meat production	13	35.1	27	40.9	49	40.2	4	11.4	93	35.8
High mortality	4	10.8	5	7.6	10	8.2	6	17.1	25	9.6
Increased treatment costs	9	24.3	12	18.2	27	22.1	12	34.3	60	23.1

villages (P>0.05). The reported clinical presentations perceived by the respondents to signs of nagana included emaciation (16.7%), fever 10.1%, abortion (9.7%), starry hair coat (9.3%), diarrhoea (5.0%), emaciation and starry hair coat (4.7%), alopecia, emaciation and fever (2.3%). Thirty-one percent of the respondents did not know exactly what were the clinical signs.

Drugs commonly reported by respondents to be used for treatment of nagana include Berenil (Diminazene aceturate) (43.3%), Berenil and Novidium (25.6%), Oxytetracycline (15.3%), Novidium (Homidium chloride) (8.4%) and Samorin (Isometamidium chloride) (7.4%). Difference in drug use preferences among age groups, villages, occupations, gender and education levels were not statistically significant (P>0.05). With regard to drug use frequency, 5.2% reported to follow veterinary shop instructions during treatment, while 27.0% and 21.4% reported administering drugs twice and once per week respectively. The remaining 46.4% could not recall. Statistical evidence associated village (P=0.024), gender (P=0.031) and occupation (P=0.025) with regard to the drug use. Respondents in Buganza and Mwanyahina did not follow veterinary shop drugs use guide in contrast to the other villages, whereas many of Makao respondents could not recall how they used drugs. Many males could not recall than females, while they also could not follow veterinary drugs use guides as well than their counterpart. While many agro pastoralists could not recall the drug use than other occupations, many of them followed veterinary shop drug use guides. None of the pastoralist observed veterinary drug use guides. The impact of nagana was considered similar across villages, gender, occupation, education level and age group (P>0.05), though emaciation and weight loss (35.8%) was the highly regarded impact of nagana viewed followed by abortion and still births (31.5%), increased treatment costs (23.1%) and mortality (9.6%).

4.1.5 Sleeping sickness and risk perception

Figure 4.3 illustrates HAT awareness and its clinical sings across villages. Sleeping sickness was known to only 40.3% of the respondents and significant difference was observed between age groups (P=0.045), gender (P=0.0004) and education level (P=0.008).

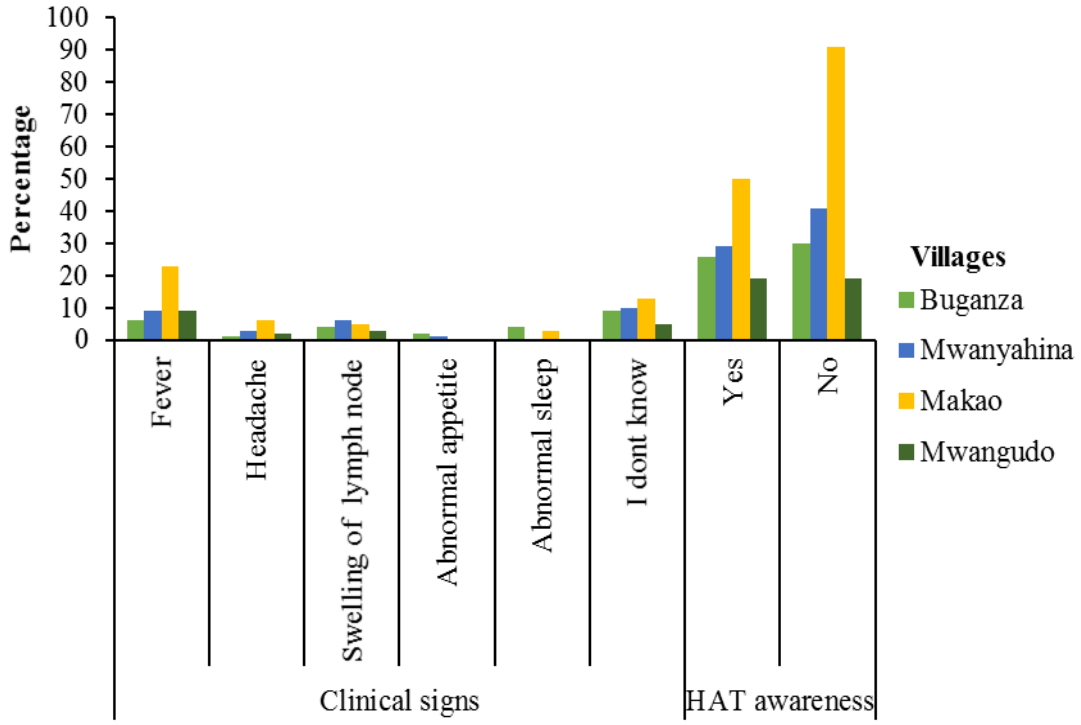

Figure 4.3: Sleeping sickness awareness and clinical signs

Respondents aged 18-29 knew less about sleeping sickness compared to other age groups, whereas fewer females were aware of HAT than males. Fewer primary school leavers knew about HAT compared to other education groups. With exception of age group (P=0.0002), knowledge on sleeping sickness clinical signs was the same across villages, gender, education level and occupation (P>0.05).

Mentioned sleeping sickness symptoms included fever (37.9%), swelling of the lymph nodes (14.5%), headache (9.7%), general body malaise and abnormal appetite (5.7%) and 29.8% did not know the symptoms of sleeping sickness. Respondent who aged above 50 years were many relative to other age groups.

The main sources of information were health centre and school (53%), friend/relative 24.5%, radio 16.5% and newspapers 5.8%. When tracked most of the information was delivered in recent years 2000's (48.6%), followed by early 1960-1989 (32.7%) and 1989-1999 (18.7%) (Figure 4.4).

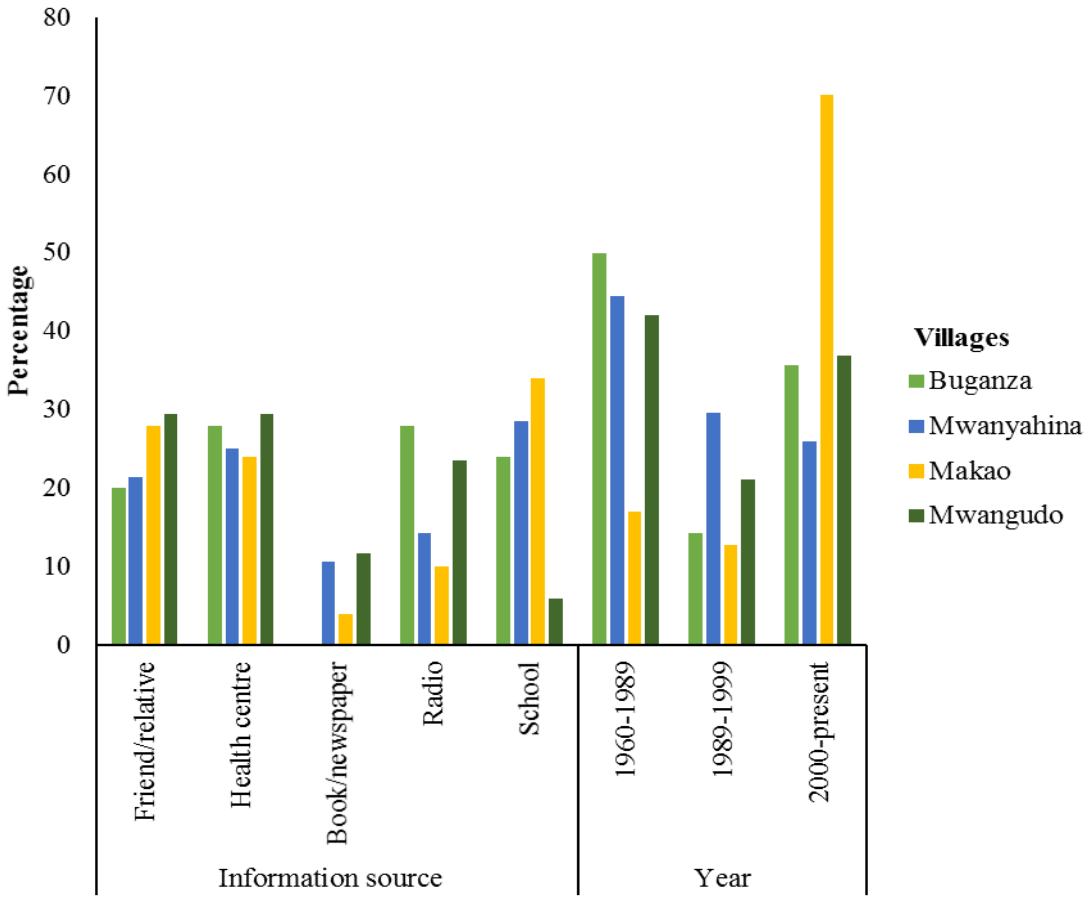

Figure 4.4: Source of sleeping sickness information and year

Knowledge about source of HAT was the same across villages, age groups, education level, gender and occupation (P>0.05). Seventy percent of the respondents knew tsetse fly as the vector of sleeping sickness, 20.2% reported mosquito while 2.4% associated it with witchcraft, 5.6% failed to mention any cause. Knowledge about the HAT risk areas was identical across gender, age group, education level occupation and villages (P>0.05). Identified sleeping sickness risky areas include bush/forest (61.8%), grazing areas (17.1%) and at home (6.5%) and in the bush roads (4.9%). About 11.4% respondents reported sleeping sickness to be a problem and 2.4% of the respondents did not know, whereas 86.2% reported that sleeping sickness was not a problem (Table 4.6)

Table 4.6: Sleeping sickness and risk perception attributes in the villages

	Villages									
	Buganza		**Mwanyahina**		**Makao**		**Mwangudo**		**TOTAL**	
Cause of HAT	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Tsetse fly	16	61.5	23	79.3	33	66	16	84.2	88	71.0
Other parasites	1	3.9	0	0	0	0	0	0	1	0.8
Witchcraft	0	0	1	3.5	2	4	0	0	3	2.4
Other insect	6	23.1	5	17.2	11	22	3	15.8	25	20.2
I do not know	3	11.5	0	0	4	8	0	0	7	5.6
HAT risk areas										
Home/office	3	11.5	2	7.1	2	4.1	1	5.3	8	6.6
Bush/forest	14	53.9	16	57.1	32	65.3	14	73.7	76	62.3
Grazing areas	4	15.4	7	25	7	14.3	3	15.8	21	17.2
Bush roads	3	11.5	1	3.6	1	2	0	0	5	4.1
I do not know	2	7.7	2	7.1	7	14.3	1	5.3	12	9.8
Is HAT a problem in your area										
Yes	8	32	1	3.5	2	4	3	15.8	14	11.4
No	16	64	26	89.7	48	96	16	84.2	106	86.2
I do not know	1	4	2	6.9	0	0	0	0	3	2.4
Accessibility of HAT treatment										
Yes	10	35.7	3	10.3	0	0	0	0	13	10.3
No	18	64.3	26	89.7	50	100	19	100	113	89.7
Measures taken for HAT sick person										
Taken to health centre	14	70	22	78.6	42	91.3	13	68.4	91	80.5
Taken to traditional healer	2	10	1	3.6	1	2.2	0	0	4	3.5
Nothing is done	1	5	0	0	1	2.2	1	5.3	3	2.7
I do not know	3	15	5	17.9	2	4.4	5	26.3	15	13.3
HAT control measures										
Yes	10	38.5	3	10.3	16	32	5	26.3	34	27.4
No	16	61.5	26	89.7	34	68	14	73.7	90	72.6

Measure taken to HAT patients was the same across the factors being considered in this study (P>0.05) with 80.5% sleeping sickness people being sent to hospital, 3.5% of respondents sent the sick people to traditional healers while 2.7% did nothing. Willingness to send a sleeping sickness patient to hospital is high (79.7%). Only 8.9% were willing to send the patient to traditional healer while 11.4% did not know what to do. Sleeping sickness treatments were not easily accessible in the area (89.6%). No current tsetse fly control activities were in place (72.6%) where the ongoing control (27.4%) which consist mainly insecticides spraying of livestock; 55.9% and 44.1% practiced bush clearing and bush fire during dry season. Figure 4.5 illustrates current trypanosomosis and perceived control measures.

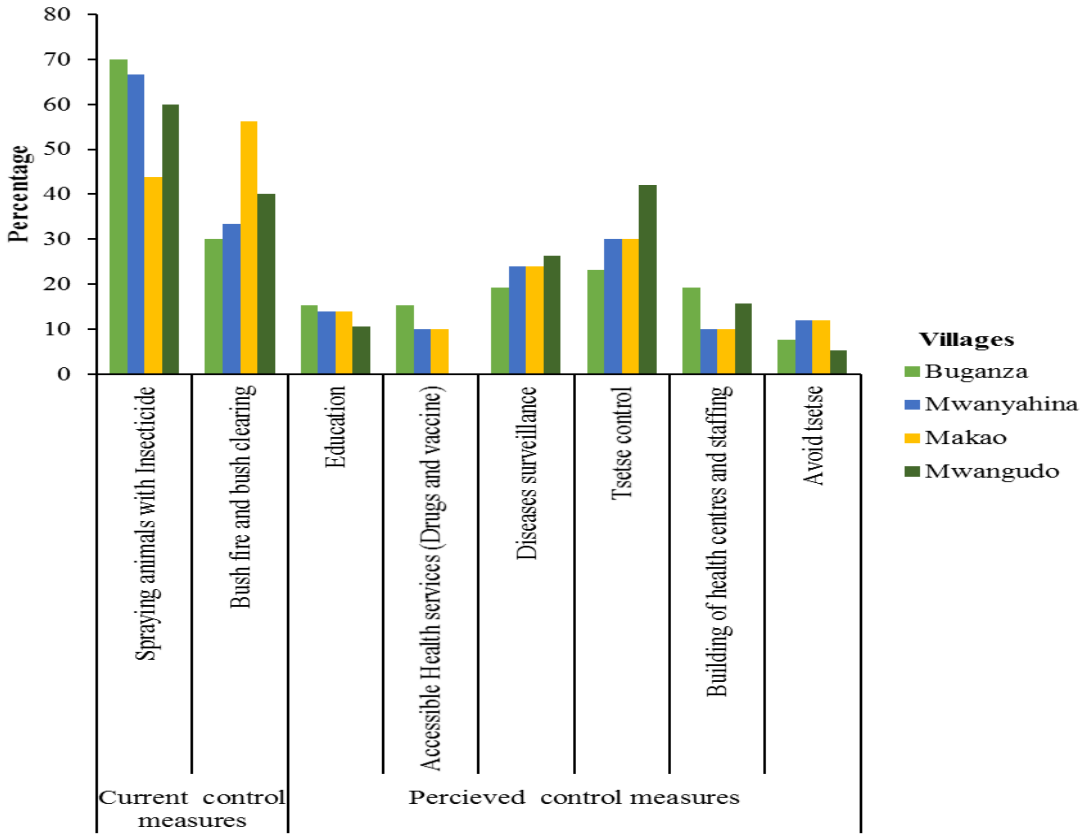

Figure 4.5: Current and perceived sleeping sickness control measures

The suggested measures by respondents included tsetse fly control (29.3%), disease surveillance (25.8%), building of health centres and staffing (12.9%), avoid tsetse fly (12.9%) and accessible health services (drugs and vaccine) 8.1%.

4.2 Tsetse fly collection

4.2.1 Tsetse abundances and mean catches

Table 4.7 shows the mean tsetse catches for the four villages surveyed and tsetse species trapped in the respective villages. There was highly significant difference in catches between villages (P=0.000).

Table 4.7: Mean tsetse catches by villages

Village	Overall mean	Tsetse species		
		G. pallidipes	*G. swynnertoni*	*G. morsitans*
Buganza	22.2±20.4	40.6±25.5	12.5±8.9	13.9±6.80
Mwanyahina	15.5±11.8	23.5±13.1	13.3±10.8	9.63±6.89
Makao	56.1±56.8	43.9±24.3	68.3±73.5	0
Mwangudo	51.0±21.2	59.3±25.7	42.7±12.1	0

Highest scores were observed in Makao and Mwangudo villages while the least scores were in Mwanyahina and Buganza villages. High apparent density/flies per trap per day (FTD) was observed in Makao and low apparent density was observed in Mwanyahina (Figure 4.6).

Three species namely *G. pallidipes*, *G. swynnertoni* and *G. morsitans* were identified Table 4.7). There was significant difference between species (P=0.012). *Glossina pallidipes* was the most prevalent species followed by *G. swynnertoni* and *G. morsitans* in that order. *Glossina morsitans* was found in Mwanyahina ward only.

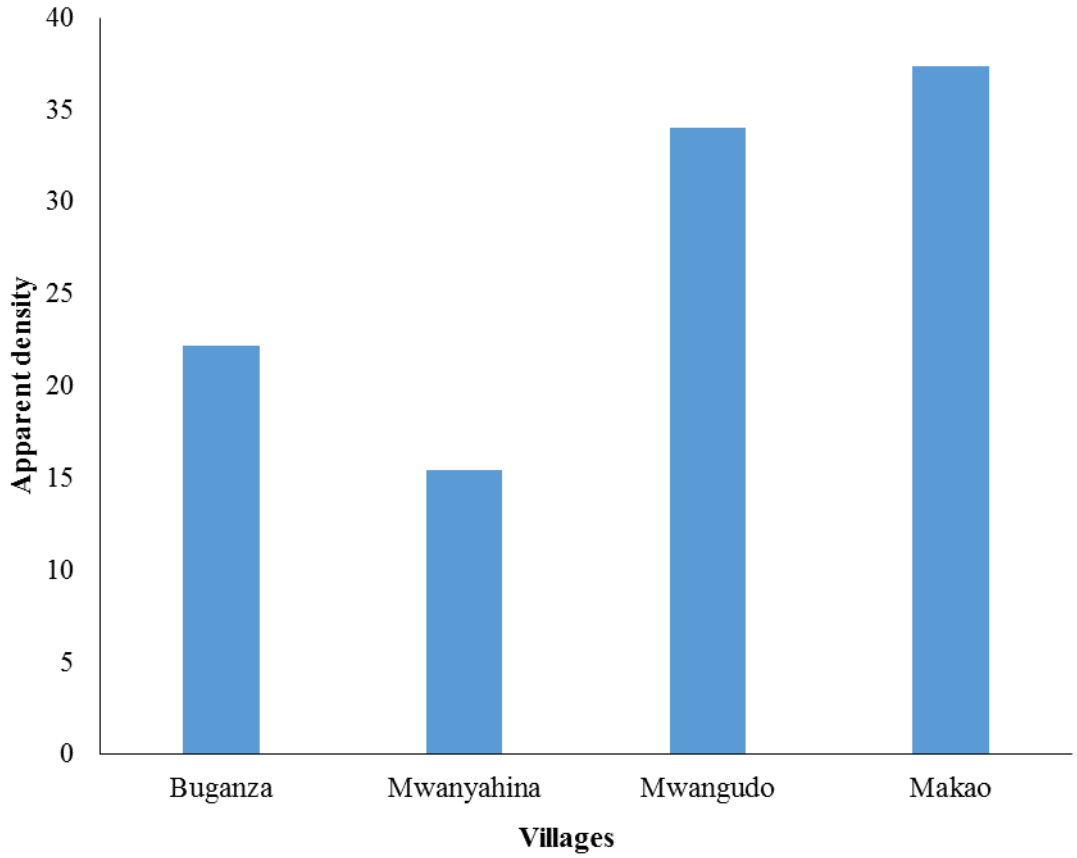

Figure 4.6: Tsetse apparent density by village

4.2.2 Performances of traps in catching different tsetse species

Figure 4.7 illustrates the performance of the four traps used in trapping tsetse species. Significant difference in the efficiency of trapping tsetse was observed in Biconical (P= 0.032) and NZI (P= 0.005) traps. Though the performance of traps in collecting tsetse species was the same (P= 0.377), NGU trap trapped *G. morsitans* and *G. swynnertoni* whereas *G. pallidipes* was trapped most by NZI trap. The S3 and Biconical traps collected the lowest number of flies of each of the three species.

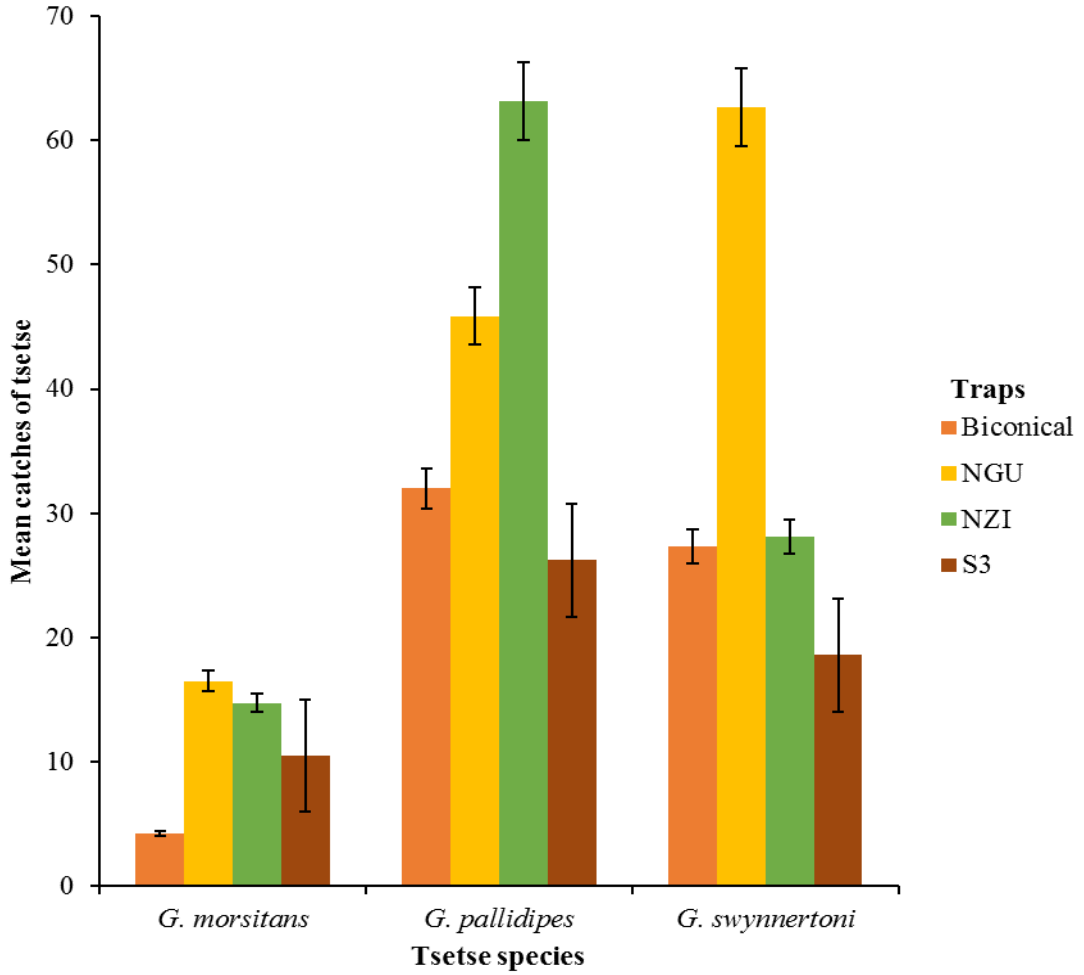

Figure 4.7: Performance of traps in catching tsetse species

4.2.3 Vegetation and tsetse species trapped

Three vegetation types were found in the four villages surveyed; open and scattered shrubs (Mwanyahina and Buganza), open woodland and woodland (Makao and Mwangudo). Except for *G. morsitans* which were caught in open shrubs the remaining species were trapped in all vegetation type. There was observed a highly significant difference (P=0.000) in mean catches between species caught in each vegetation type (Table 4.8). The mean catches were 57, 51, 19 flies for open woodland, woodland and open shrubs vegetation respectively.

Table 4.8: Mean tsetse catches in relation to vegetation where traps were deployed

Tsetse species	Vegetation type												P-value
	Open shrubs				Open woodland				Woodland				
	Mean	SD	95%CI		Mean	SD	95%CI		Mean	SD	95%CI		
G. morsitans	11.5	6.9	8.1	14.9	-	-	-	-	-	-	-	-	NIL
G. pallidipes	32.1	21.5	21.5	42.6	43.8	34.3	20.1	67.6	59.3	25.7	41.5	77.1	0.06
G. swynnertoni	14.6	11.6	9.1	20.1	69.1	73.2	18.4	119.9	41.8	12.8	32.4	51.3	0.006

Catches of *G. pallidipes* were not significantly different (P=0.06) between the three vegetation types whereas catches of *G. swynnertoni* were very significantly different (P=0.006).

4.3 Parasitological Results

4.3.1 Occurrence of trypanosomes and anaemia in cattle

Overall occurrence of trypanosomes of AAT was 2.4% (n=424). Despite the trypanosome occurrence being slightly higher in Makao and lowest in Mwangudo, (Table 4.9) the infections were the same between villages (P=0.212), and between cattle groups (P=0.940). Morphological identification (FAO, 1998) revealed *T. congolense* and *T. vivax* species. Average PCV of the animals sampled was 29.9% while the occurrence of anaemia was 8.3% (n=424). No statistical difference in anaemia was observed between cattle groups (P=0.635) and between villages (P=0.351).

Table 4.9: Mean PCV and occurrence of anaemia and AAT by village

Village	Mean PCV	Anaemia	AAT
Overall	29.9	8.3%	2.4%
Buganza	29.8	6.6%	2.3%
Mwanyahina	31.7	5.9%	1.4%
Mwangudo	29.6	13.7%	1.2%
Makao	29.3	9.4%	3.5%

4.3.2 Packed Cell Volume (PCV) of cattle in the villages

Average PCVs of cattle in the cattle sampled in the four villages are displayed on Table 4.9. Figure 4.8 illustrates the PCV values among various categories of cattle sampled. There was no statistically significant difference in PCV between cattle sampled in each village (P=0.062) and between cattle groups (P=0.310).

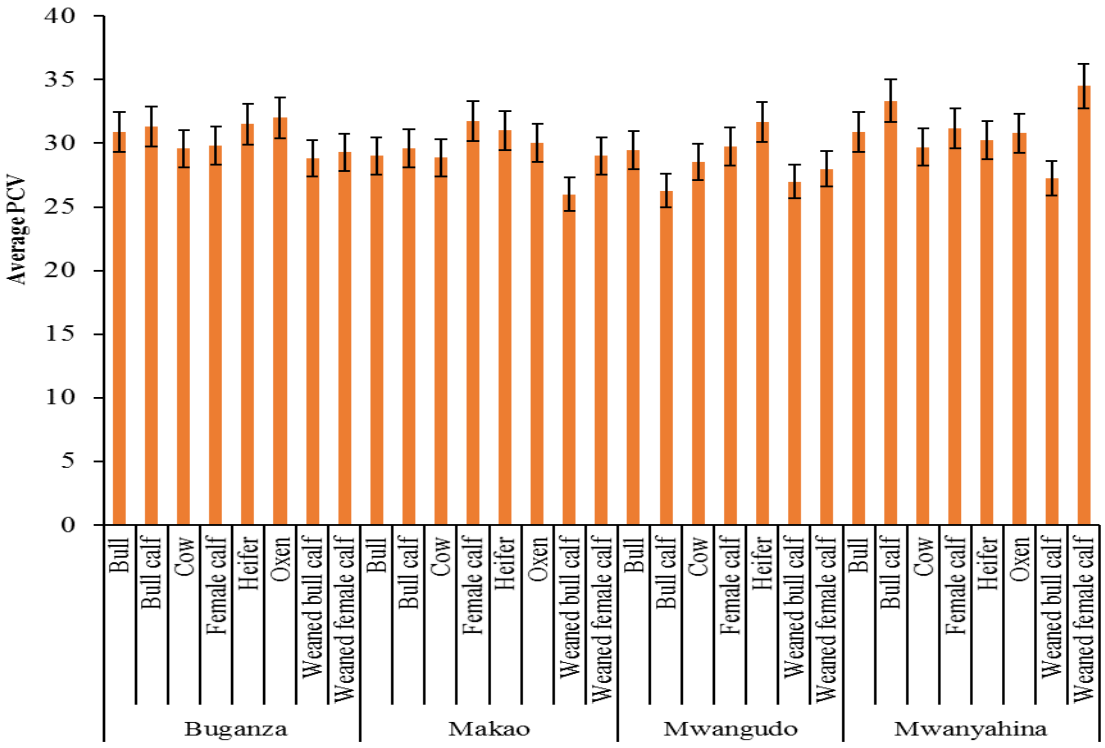

Figure 4.8: Mean percent PCV of different categories of cattle in the four villages

Eight percent of cattle sampled were anaemic, and there was observed highly significant difference between anaemic and non-anaemic cattle (P=0.000). Average PCV of anaemic cattle (<25% PCV) was slightly lower than normal cattle (≥ 25% PCV). A comparison of average PCVs between two factors; anaemic status and disease status (nagana) showed no statistical difference (Table 4.10).

Table 4.10: Comparison of mean cattle PCV between trypanosome negative and trypanosome negative cattle

Anaemia status	Disease status								P-value
	Trypanosome negative				Trypanosome positive				
	Mean	SD	95%CI		Mean	SD	95%CI		
Normal	30.6	4.0	30.2	31.0	30.0	3.4	27.7	32.3	0.677
Anaemic	21.9	2.9	20.9	22.8	21.5	2.1	18.6	24.4	0.869

4.4 Molecular Analysis Results

4.4.1 PCR detection of *Trypanosoma* species

Average trypanosomes infection occurrence was 15% (15/100) in cattle. Even though there was no significant difference in trypanosome infections between the villages (P=0.459) and between the cattle groups, (P= 0.225), the infections in bulls (17.1%) were higher compared to other cattle groups, whereas infections of cattle of Buganza village was higher than of other villages. The average occurrence of trypanosomes in tsetse was 1.2% (all infections were observed in Buganza village). All tsetse flies infected with trypanosomes were identified to be *G. pallidipes* (Table 4.11).

Table 4.11: Occurrence of trypanosomes in cattle and tsetse based on ITS1 PCR

Risk factor	No. samples	Infections	Occurrence	χ^2	P value
Village					
Buganza	25	6	24	2.59	0.46
Mwanyahina	14	2	14.3		
Makao	42	4	9.5		
Mwangudo	19	3	15.8		
Overall	**100**	**15**	**15**		
Cattle group					
Bull	31	6	17.1	9.41	0.23
Cow	21	2	9.5		
Bull calf	17	2	11.8		
Female calf	17	3	17.6		
Heifer	4	0	0		
Weaned bull calf	6	0	0		
Weaned female calf	3	1	33		
Oxen	1	1	1		
Overall	**100**	**15**	**15**		
Tsetse species					
G. morsitans	21	0	0	1.40	0.50
G. swynnertoni	58	0	0		
G. pallidipes	171	3	1.75		
Overall	**250**	**3**	**1.20**		
Village					
Buganza	65	3	4.6	8.64	0.03
Mwanyahina	27	0	0		
Mwangudo	43	0	0		
Makao	115	0	0		
Overall	**250**	**3**	**1.20**		

Figure 4.9 indicates the image of agarose gel on which trypanosomes identification was carried out withregard to band size. Lane 1-26 contains DNA from cattle buffy coat while lane 27-34 contains DNA from tsetse. Fifteen *T. congolense* were the only identified species and produced fragments with molecular weights of between 600-700 bp (lane 1, 2, 6, 8, 17 and 25). The species distribution proportions by village were 40% (n=25), 13% (n=14), 20% (n=19), 26.7% (n=2) in Buganza, Mwanyahina, Mwangudo and Makao villages respectively. In tsetse fly, *T. godfreyi* (lane 30) and two *Trypanosoma simiae* (lane 31 and 32) produced fragments with molecular weight of 300 and 400bp, respectively all being from *Glossina pallidipes* of Buganza village. Sleeping sickness pathogens were neither identified in tsetse nor in cattle. Co- infection in cattle and tsetse fly was not observed other than single infections.

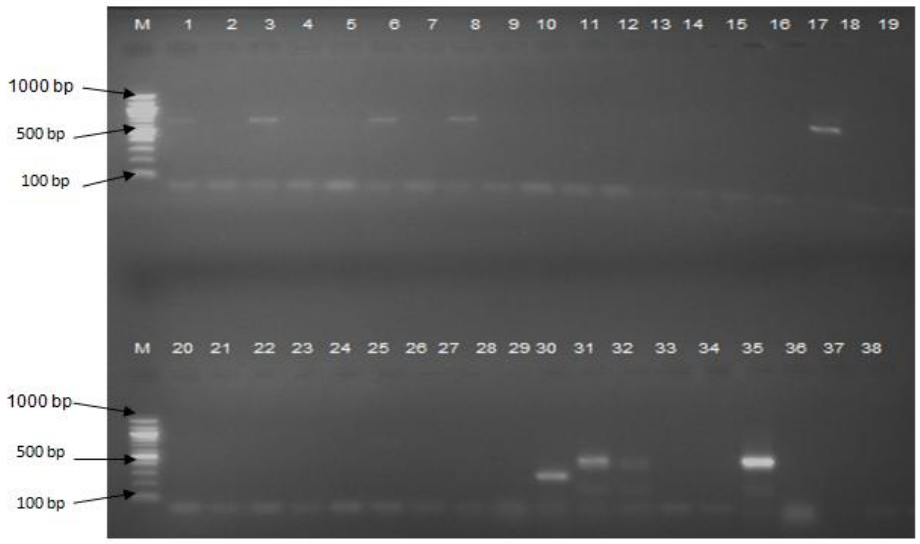

Figure 4.9: Agarose gel image showing bands from amplification of ITS1 region by PCR using DNA from tsetse fly and cattle. Lane M 100bp DNA ladder, lane 1-34 *Trypanosoma* DNA, lane 35 positive control and lane 36 negative control

4.4.2 Phylogenetic results

The evolutionary history was inferred using the Maximum Parsimony (MP) method. Tree which expressed few changes (most parsimonious tree) is shown in Figure 4.10. The analysis involved 21 nucleotide sequences. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) is shown next to the branches.

The light green highlighted in the topology indicates cattle isolates where the grey highlighted indicates the tsetse fly isolate. This analysis produced two closely related clusters; Cluster A composed of all sample sequences of this study which were closely related to *T. congolense* savannah from Serengeti, Tanzania. This pattern received a highly significant internal support (100 bootstrap); Cluster B in turn composed of *T. congolense* species from other locations, together with other salivaria species which were included in the phylogenetic analysis.

Different monophyl groups were distinct in the cluster A with variant significant support; TZSM013, TZSM016 and TZSM025 the monophyl group and the early branch that separated from the savannah type received significantly high support (100 bootstrap) compared to sequences of the isolates TZSM01, TZSM05 and TZSM08 which received a fair support (65 bootstrap). TZSM30 was distinct from the previous branch and from *T. congolense* from Ghana but this branch was not significant (40 bootstrap). Though *T. congolense* isolates from Burkina Faso and Kenya were similar to each other than to the TZSM01, TZSM05 and TZSM08 significant high support indicated that they were similar to *T. congolense* from Ghana (100 bootstrap).

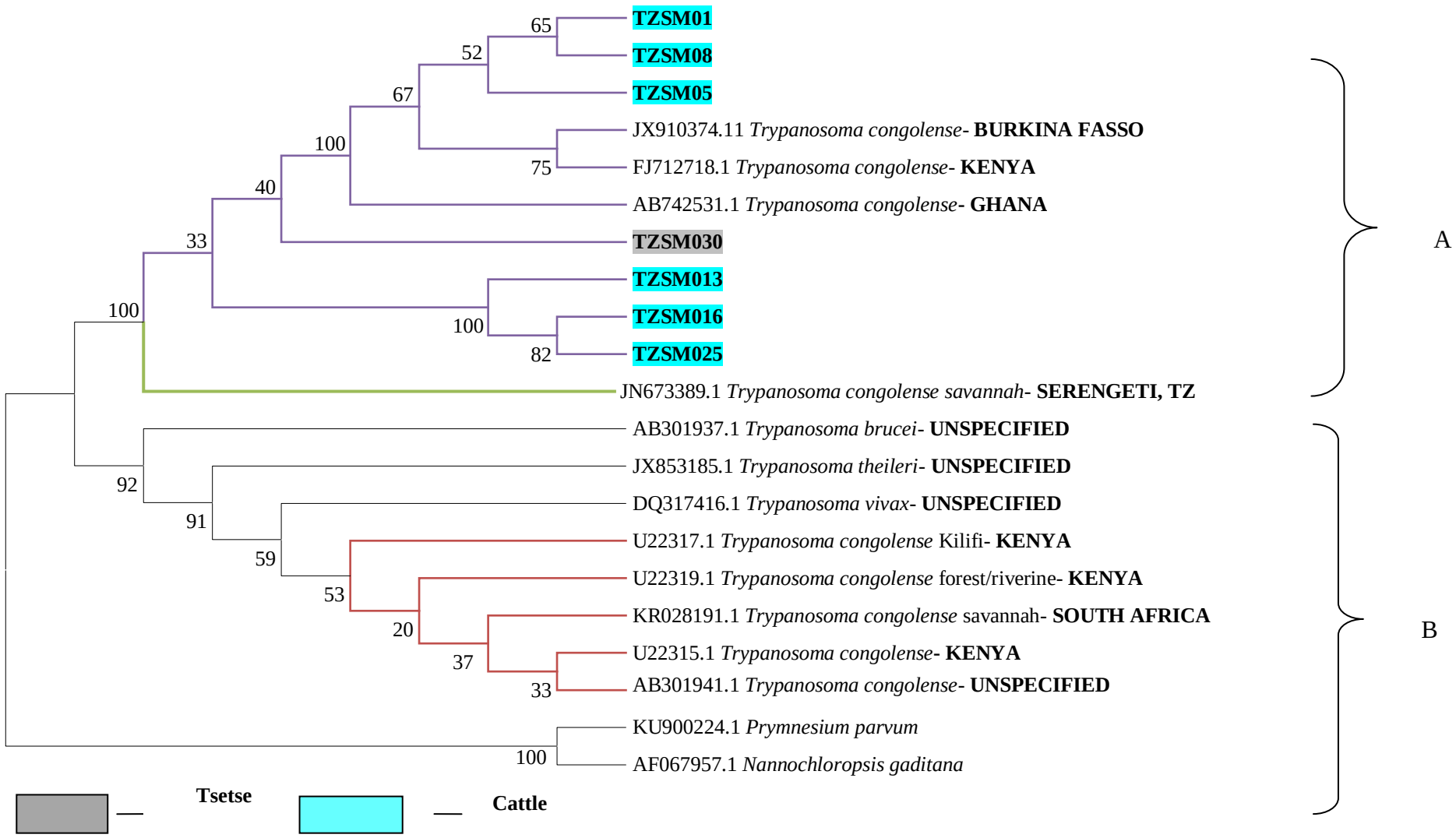

Figure 4.10: Maximum Parsimony based phylogenetic tree showing the relationship between *Trypanosoma* species of Meatu district with other *Trypanosoma* species

CHAPTER FIVE

5.0 DISCUSSION

The community was aware that tsetse fly is the source of trypanosomosis where bush /forest and grazing land are riskiest areas. With exception to Makao village in which awareness on AAT was high compared to Buganza, Mwanyahina and Mwangudo the awareness was low. This may be due to the pathogenicity observed in cattle and the understanding that bush and forests in the vicinity were the sources of infection. The game reserves and wildlife management areas provided alternative sources of pastures to their animals in all villages during drought despite strict bylaws and penalties that prohibit domestic animals from grazing in conservation areas.

Moreover, awareness on HAT was poor particularly among respondents aged 18-29 years, females and primary school graduates. This may be attributed to reduced cases of HAT in the area which may be due to high use of Diminazene aceturate which may have reduced *Trypanosoma brucei rhodesiense* circulation in livestock and vector as reported in Uganda (Fèvre *et al.*, 2001; Matovu *et al.*, 1997). However, reduced HAT awareness may be contributed by under reporting, which results from shared clinical signs with other fever causing diseases like malaria (Kennedy, 2013). Furthermore, contents of text books describe sleeping sickness poorly and their limited availability in schools especially in remote areas (where HAT is also endemic) might significantly affect teaching resulting in poor awareness of HAT amongst students. Therefore, there is a need to improve and create new HAT awareness channels essentially focusing on clinical signs awareness such as the use of social media whose use among youth is increasing. The use of social media in awareness creation has proved useful in building awareness on diseases for which there is

poor awareness in the community for example cancer (Lapointe *et al.*, 2014). Leaflets and pamphlets could convey information directly to the users if they will be made available. Moreover, schools can serve as a reliable information source if sleeping sickness clubs will be formed in schools within endemic areas and number of science text books will be made available to schools. These would increase awareness and subsequent reliable information disseminated to others.

The community in which this study was conducted takes AAT and HAT as important diseases. This is proved from the disease control efforts and medical seeking behavior indicated in the study. Nevertheless, the community views trypanosomosis control in a multidimensional approach rather than unidirectional.

Livestock keeping is a source of livelihood in Meatu district for many of its residents where the practice of keeping more than one domestic animal seems to be a way of diverging income sources. Whereas the challenge of bovine trypanosomosis was high, the chances of contracting sleeping sickness are high as well due to ownership of different domestic species (Ruiz *et al.*, 2015). Respondents with primary education are significantly at risk by owning more diverse domestic animals. The mixed management systems (pastoral and agro pastoral) present a threat of distribution of resistant strains (Selby *et al.*, 2013). Grazing livestock in the protected parks (game reserve) during drought increases chances of contracting trypanosomes as well as other parasites and vectors harboured in wildlife (Auty *et al.*, 2012a). Competition for available water sources with other uses during dry season limit effective insecticide application (both spraying and dipping) and thus caused dependency on chemotherapy only for control which is not only costly but also can lead to drug resistance strains emergency (Kibona *et al.*, 2006; Matovu *et al.*, 2001). The community was aware that nagana was a disease of economic importance in

cattle. This awareness may possibly have led to abusive use of trypanocides, that may unfortunately contribute to development of drug resistance amongst trypanosomes (Delespaux *et al.*, 2008). The application of insecticide pour on technique could help in minimizing the effects during drought (Rowlands *et al.*, 2001). Strategic training in clinical signs, and proper drug use particularly to respondents with primary education in Buganza and Mwanyahina villages, to pastoralists, agro-pastoralist and female members of the society is necessary. This is due to the respondent's treatment practices being based mostly on clinical signs that are shared by other diseases. Although there are possibilities of existence of the trypanotolerant livestock, local goats being identified by Mutayoba *et al.* (1989), limited research have been carried out to identify other domestic animals in Tanzania. The use of such animals could ease trypanosome control challenge to farmers (D'leteren *et al.*, 1988), while livestock intensification and use of prophylaxis instead of chemotherapy might reduce the costs which farmers do not see (FAO, 1998).

The ongoing vector control interventions are not environmental friendly because they are associated with clearing vegetation and although this can reduce tsetse infestations, in the long run it might have negative effects on climate change (Malele *et al.*, 2011b). The results from this study substantiate the existence of three species of *Glossina*; *G. pallidipes* being the most predominant species, followed by *G. swynnertoni and G. morsitans* at Mwanyahina and Mwangudo wards. Whilst the other two species being prevalent in both wards, *G. morsitans* was trapped at Mwanyahina ward only. A study carried by Salekwa *et al.* (2014) in Simanjiro district in areas adjacent to Tarangire National park reported that *G. swynnertoni* were the most abundant species compared to *G. morsitans* and *G. pallidipes.* The results of this study are also contrary to the study carried out in Serengeti ecosystem which involved sites of this study by Malele *et al.* (2007) who reported *G. swynnertoni* being the dominant species in the ecosystem. This difference may

be attributed by different tsetse traps used in each study. The traps used in the current study might have favoured capture of *G. pallidipes*.

Vegetation cover influenced catches as it has been indicated previously (Table 4.8). This information is useful in planning tsetse control by means of strategic traps and targets deployment. The fact that *G. pallidipes* was the prevalent species and was trapped most in all vegetation indicates the importance for control activities to focus on all vegetation types. It is clear that the fly apparent density was higher at Mwangudo than Mwanyahina ward possibly due to well established vegetation particularly in Makao village. Mwangudo ward was more characterised with dispersed, open shrubs posing higher tsetse bite risk.

Climate change and associated effects like shortage of feeds for livestock, shortage of food for the increased human population, create pressure to move into new virgin and fertile soils, which in most cases are protected parks and game reserves (McDermott *et al.*, 2001). In the surveyed villages, crop farms and residential houses were just few meters from the wildlife reserves. This practice increases the chances of contracting the vector borne diseases and intimidates economic development as well as environmental conservation initiatives. The findings are in line with the study carried out by Malele *et al.* (2011b) who reported trapping tsetse fly near wild animal parks. These findings justify the existence of trypanosomosis transmission in the wards, and the risks exposed to people and their livestock. Microscopy observation revealed the presence of animal trypanosomosis only. Similar findings have been reported in Manangwa *et al.* (2016) and Malele *et al.* (2011b). *Trypanosoma congolense* and *T. vivax* were the identified species. No statistically significant difference was observed in PCV between trypanosome infected and non-infected cattle; and there was no relationship between occurrence of nagana and PCV. However, a study conducted by Biyazen *et al.* (2014) reported differences in PCV

between infected and non-infected cattle. Observation of *T. congolense* in cattle is an evidence of cyclical transmission of trypanosomes by tsetse fly, but infection of *T. vivax* suggests transmission by other biting flies like *Tabanus* species, that were also trapped during the study and have been reported elsewhere by Desquesnes and Dia (2003). *Trypanosoma vivax* and *Trypanosoma congolense* are not only pathogenic to cattle but also other livestock species as well as in a number of wildlife (Anderson *et al.*, 2011).

The amplification of the ITS1 region identified 15 *T. congolense* in cattle, two *T. simiae* and one *T. godfreyi* in *G. pallidipes* only. The differential representation of *Glossina pallidipes* (n= 171) relative to other species in PCR analysis is due to its high relative abundance in the area (Table 4.7). *Trypanosoma congolense* strains are commonly reported AAT pathogen in Tanzania, *T. congolense* savannah sub type being highly pathogenic which causes high parasitemia accompanied by anaemia in cattle (Bengaly *et al.*, 2002). The current study reports occurrence of the pathogen in the study area and this signifies the importance of AAT where 8% of sampled cattle had anaemia. *Trypanosoma vivax* was not detected by PCR (but earlier detected by microscopy), that could be attributed by low trypanosomes in blood or genetic diversity of the species (Adams *et al.*, 2009). From the fact that other vector flies like *Tabanus* species are prevalent in the area (were also trapped during tsetse study), emphasizes the pathogen circulation and its effect in livestock cannot be ignored (Desquesnes and Dia, 2003). The PCR based occurrence of animal infective trypanosomes in cattle was higher than that reported by other studies (Manangwa *et al.*, 2016; Ruiz *et al.*, 2015). The higher occurrence explains the risk of the protected parks to livestock and people interaction, especially grazing animals in the areas during drought. The observed low trypanosome occurrence in tsetse fly (1.20%) also reported in Salekwa *et al.* (2014) is attributed to different factors, including tsetse refractoriness to trypanosome infections and abundant

presence of obligate enterobacteria symbionts; *Wigglesworthia* and *Wolbachia* species (Chen *et al.*, 1999; Hao *et al.*, 2001; Welburn and Maudlin, 1999). The fact infections impair reproductive fitness and flight activity the enterobacteria might be essential mechanisms for tsetse fly survival. Furthermore, the low trypanosome prevalence observed in tsetse fly may be partly due to block treatment effect brought by mass drug administration to cattle a phenomenon observed during sampling which may have resulted in reduced trypanosome circulation in vector (Matovu *et al.*, 1997).

Absence of human infective trypanosomes in cattle and tsetse fly has also been reported in similar studies Malele *et al.* (2011a), consequently Ruiz *et al.* (2015) speculated that that could be due to the active care given to cattle once detected symptomatic and therefore treated. However, this observation does not clear the doubts that there is sleeping sickness pathogen in the area. The presence of *T. godfreyi* and *T. simiae* in tsetse fly indirectly indicates that the vectors fed on wild pigs (warthog), which farmers were also mournful on the destruction they caused on their crops. These findings illuminate the HAT risks in the area as warthog are known reservoirs of human infective trypanosomes (Kaare *et al.*, 2007).

The detection of the seven samples (false negatives) which had packed cell volume (PCV) which was below 24% and earlier tested negative through microscopy suggests that the usefulness of including PCV parameter in trypanosomosis surveys (Marcotty *et al.*, 2008). However, the current PCR results highlights the trypanosomes circulating in the area but they cannot distinguish between mature and immature, current and passive infection tsetse, in animal or successfully treated cattle. The technique detects and amplifies trypanosome DNA (Dennis *et al.*, 2014). Phylogenetic analysis of 18S rDNA sequences of the salivaria trypanosomes indicated diversity within *T. congolense* group. The diversity

could be due to evolutionary forces, which might have caused different trypanosomes evolution rates within a location. This was vivid by occurrence of different isolates of the same species within the environment. The clustering pattern between samples and reference isolate JN673389.1, which was isolated from Lion (*Panthera leo*) of Serengeti, Tanzania (Northern Meatu) (Auty *et al.*, 2012a) indicate evidence of pathogen circulation between domestic animals and wildlife since both are found in the same ecosystem. Though there are ongoing debates regarding *Trypanosoma vivax* position in salivaria group and suggestions that the evolution rate in this species is higher than of the other species (Stevens and Rambaut, 2001) and thus its position in the phylogeny have been distant from other salivaria members (Auty *et al.*, 2012a; Stevens and Rambaut, 2001). Contrary to those findings *T. vivax* isolate downloaded from the Gene bank clustered within the salivaria group, this might be due to selection of species from families Chrysophyceae and Eustigmatophyceae are undisputed out group to kinetoplastids (Hughes and Piontkivska, 2003).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

From the current study, the following conclusions can be drawn;

i. Awareness on tsetse fly and tsetse-borne trypanosomosis among the public in the study area is higher for AAT than for HAT. The current practice of combating AAT elevates chances of emergence of resistant strains.

ii. Three tsetse species are prevalent in the area; *Glossina pallidipes* being the dominant, followed by *G. swynnertoni* and *G. morsitans.* The further identification of trypanosomes in this species describes their role in diseases transmission in the area.

iii. Human infective trypanosomes (*T. brucei rhodesiense*) were not identified in cattle and tsetse fly by the molecular techniques, but animal infective trypanosomes (*T. congolense*, *T. simiae* and *T. godfreyi*) were identified both in cattle and tsetse in Meatu.

6.2 Recommendations

There is need to create new awareness means on HAT like the use of social media whose use is increasing across age groups.

i. Training on proper drug use and clinical signs of AAT.
ii. Inclusion of all domestic animals in samples for establishment of *T. brucei rhodesiense* pathogen in sleeping sickness surveys.
iii. Tsetse fly control strategies should be instituted.

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APPENDICES

Appendix 1: Questionnaire

Location information

District	
Ward	
Village	
Sub village	
GPS location	

General information of respondent

Sex:	1=Male 2=Female	
Age	Years	
Education	0 = None 1 = Primary 2 = Secondary 3 = College 4 = University 5 = Primary school drop out	
Occupation	0 = Farmer 1 = Livestock keeper 2 = Civil servant 3 = Private employee 4 = Others (mention)	

Risk factors and risk perception

1	Do you know tsetse flies?	1 = Yes 2 = No	
2	Have you ever been bitten by tsetse flies?	1 = Yes 2 = No	
3	**If yes, in 2 above, in which area or location?**	1 = At home/office 2 = In the bush/forest 3 = In grazing areas when looking after livestock 4 = Other (specify) _____	
4	What happened after being bitten?	1 = Pain/swelling on the site of the bite 2 = Fever and Swelling on the site of the bite 3 = Fever 4 = Nothing happened	

		5 = Other (specify) _____	
5	What did you do after been bitten?	1 = Visited health centre 2 = Told family members 3 = Did nothing 4 = Other (specify) _____	
6	If you visited a dispensary/health centre, Did you get treatment?	1 = Yes 2 = No	
7	What season of the year do you experience more tsetse fly bites	1 = Dry season 2 = Wet season 3 = Dry and wet season 4 = Other (specify)	
8	If yes, what dangers are caused by tsetse bites?	1 = Disease (s)/fever 2 = Pain/injury 3 = Disturbance (s) 4 = Other (specify) ____	

Livestock ownership diseases and productivity

9	Species of animals kept	LIST	1 = Cattle 2 = Sheep 3 = Goat 4 = dog(s) 5=Others
10	What are the common diseases of your livestock? (List in order of importance)?	LIST IN ORDER OF IMPORTANCE	1. _____ 2. _____ 3. _____ 4. _____ 5.
11	Do you know a disease of livestock called trypanosomiasis? **If the answer to 11 is NO go to question 25**		1 = Yes 2 = No
12	List other local name or names that people in this area call the disease	LIST	1. _____ 2. _____ 3. _____ 4. _____
13	What do you think causes trypanosomiasis?		1 = Tsetse/insect bite 2 = Other parasites 3 = Witchcraft 4 = Other (specify) ____ 7 = I don't know
14	What are the clinical signs trypanosomosis in your herd?	LIST	1. _____ 2. _____ 3. _____ 4. _____ 5. _____ 6. _____
15	What trypanocides do	LIST	1. _____

	you use in treating animals and its price?		2. _____ 3. _____ 4. _____ 5. _____ 6. _____
16	How frequent do you administer the trypanocides?	MENTION	1. _____ 2. _____ 3. _____
17	What is the impact of trypanosomiasis in your livestock?	LIST	1. _____ 2. _____ 3. _____ 4. _____ 5. _____ 6. _____

History, awareness and attitudes on human trypanosomosis in the area

No	**Question**		**Answer**
18	Do you know a disease called sleeping sickness **If the answer to question 18 is No, Ends the questionnaire and thank the participant.**	1 = Yes 2 = No	
19	Where did you get the information about the disease?	1 = From a friend/relative 2 = From health centre 3 = Read a book/newspaper 4 = Other (specify) ____	
20	When did you get the information about the disease?	Year	
21	What do you think causes sleeping sickness	1 = Tsetse/insect bite 2 = Other parasites 3 = Witchcraft 4 = Other (specify) ____ 7 = I don't know	
22	Where do you think one can get sleeping sickness	1 = At home/office 2 = In the bush/forest 3 = In grazing areas when looking after livestock 4 = Other (specify) _____ 7 = Don't know	
23	Can you mention the signs of sleeping sickness? (**LIST**)	1. = Fever 2. =Headache 3. =Swelling of lymphnodes 4. =Abnormal appetite 5. =Abnormal sleep 6. =General malaise and body pain	

		7. = Don't know	
24	Is sleeping sickness a problem in your area	1 = Yes 2 = No 7 = Don't know	
25	What type of treatment will you use when you or a family member have sleeping sickness?	1 = Hospital medicines 2 = Traditional medicines 3 = Other (specify) _____ 4 = Do nothing	
26	Is it easy to get treatment in your area when one has sleeping sickness?	1 = Yes 2 = No	
27	What do you think should be done to people with sleeping sickness?	1 = Taken to health centre 2 = Taken to traditional healer (s) 3 = Left alone 4 = Other (specify) ____ 7 = Don't know	

Disease controls measures/practices

28	When a person has sleeping sickness, what actions are taken?	1 = Taken to health centre 2 = Taken to traditional healer (s) 4 = Nothing is done 5 = Other (specify) ____ 7 = Don't know	
29	Are there any measures that are taken to prevent tsetse bites or sleeping sickness in your area? **If the answer to 29 is NO go to question 32**	1 = Yes 2 = No	
30	If the answer is yes to 29 above, what are those measures?	LIST	1_____ 2_____ 3_____ 4_____ 5_____ 6_____ 7 = Don't know
32	Do you think those measures are effective in preventing tsetse bites or sleeping sickness?		1 = Yes 2 = No 3 = Don't know
32	What else do you think should be done to prevent people in your area from tsetse bites or sleeping sickness?	LIST	1_____ 2_____ 3_____ 4_____ 5_____ 6_____ 7= Don't know

Appendix 2: Cattle and tsetse nucleotide sequences

Source of sample	Sample ID	Sequence
Cattle	TZSM01	TACCTTTTGGAAGCAAAAGTCGTAACAAGGTAGCTGT AGGTGAACCTGCAGCTGGATCATTTTCCGATGATAAT ATATAT ATATATATACGTGTATATTATACACATGTGTGTGTGT GTTTTGAGAGGGTGTTGTTGTTGTGTGCGCGTGTGCG TGCGGG GCCCCTCTCTCTCGCGAATTATTCCCATCCCCATCCC CCCCCGTGTGGTGTGGGGTGTGTGTTGTGGGAACCGC ACGCGG GGGGGGGGGGGGGGTGTTGCCGGCGCCACACTCTAAA AAACACCCCTAAGAACACCCACGTGTAAAAACACGCC CCCCTG TCTCTCTCTTTTTCTTTTGTTTTGAGGGGGCGTACAG TTGTGTGCGCGCCCCCCCCGGGCAAAAAAAAAGAGGGG GGGGGG GAAAAAAAAAATTTTTTTTTTTCCCCCCCCCACTTTTT GTGTGTGCGGGCTGGCTCTTTTTTCTCTCTTTTTCTC CCCCCC CCCCACATTTTTTTAGAAACCCCCCCCATGTGTGTGG AGAGAAGAGAAGAAAATGTGTGTTTTTTTTAAAAAA
	TZSM05	TCCTTAGGGAGCAAAAGTCGTAACAGGTAGCTGTAGG TGAACCTGCAGCTGGATCATTTTCCGATGATAATATA TATATA AAAATGTACACATGTGTGTGTGTATATGCGGGTGTGG GTGTGCAGAGGGAGATGTTGTTGTTGGTGTGTGGTGT GCGTGT GCTTGCTCCTCCCCCTCGTTCCTGCTTCTCATCCCCA TCCCCCCCCCGCGTGGGGGGGGGGGGGGTGTGGGGGGGG GCCGAA CCGGGTGGGGGGGGGGTGGTGGTGTTGTGTCCCGGCCA CAATCTCAAAAACCCCCCTCGGAAAACCCCCACGTCC CAAAAC CCCCCTCTCCATGTCGCTCCCCTTCTTTTGTGATGAA AGGGTGCTTACTATTGTGCGCCCCCCGCGAAGGGGAA GAGAAG AGGGGGGTGGGGAGGACAACACGTTTTTTTTTCCCCC CCCACTTTTTTTGTGTGCGCCCTCTGTTTTTTCCCTT TTCCCC CTTTTCTTCTCCCTCTCCTCCTCATTTTTTTCTTGTT TTTCCATGTGTTGTGGGAGAGTGGAAGAAGAAGTGTG TGTTTT TGGAAGAAAAATATGCAGTGGGGAGAAATATGGAGAG TTCTTGTGTGTATACACCGGTGTGGTGCACGCCTCTC TCACAA CGTGTCGCGATGGATAACGTGTCTATCTATTTCAGAG AACAACACAAAAA
	TZSM08	GCCATTAGGAACAAGTCGTAACAGGTAGCTGTAGGTG AACCTGCAGCTGGATCATTTTCCGATGATAATATATA TATATA TATATATGTGTGTGTATATTACACGCGGGGGTGTGTG TTGAGAGATGTTTTTGTTGTTGTGTGCTCGTGTGCGC GCGGCC

		CCCCTCTCTCATAAATATTTCCCCCCCCCCCCCCCC CCCGGGGGGGGGGGGGGGGTTTGGGGAAAACCCCCG GGGGGG GGGGGTTTTTTTTTCCCCCCCCAAAATTTAAAAAACC CCCCCAAGAACACCCGTGTTTAAACCCCCCCCCCCCC CTTTTC TTTTTTTTTTTTTTGGGTGGGGGGGGTTTTATGGTGG GCCCCCCCCCCCCAAAGGAAAAAAAGGGGGGGGGGGG AAAAAA AAAATTTTTTTTTCCCCCCCCCCACCTTTTTGGGGGG GGCCCGGGTTTCTTTTTCCCCCCCTTTTTCCCCCCCC CCCCCT CTTTTTTCCCCCCCCTCCCCAGGTAGGAGGAAAAAAA AAAAAAAAATTTGTTTTTGTTTGAAAAAAAAGGGTGC AGGGAA AAAAATGTGGGGTGTGTTTGTGTGTGCGCGGGTGTGT TTTCCCCCCCCCTCACACAGCGGCACGAGGGAGATAA TTTTTC CTTTTTTTTAAAAAAAAAAAAAAAAAA
	TZSM013	AACCTGGGAACAAAAGTCGTAACAAGGTAGCTGTAGG TGAACCTGCAGCTGGATCATTTTCCGATGATAATATA TATATA CATATGCGTGTATATTATACACATGTGGGTGTGTGTT GTGAGAGGTTTTTTTTTTTGTGTGCTCGTGTGCGTGC GGGGCC CCCCTTTCATGCAAATTATTCCCCCCCCCCCCCCCC CGGTGGGGGGGGGGGGGTGGGTTGGGGAAACCACCCG GGGGGG GGGGGGGCTGGTTGTCGGCCCGCCTTCTTAAAAAACA CCCCCCAAGGACCCAGGTGTTCAAACACCCCCCCCCC TGTTGT TTTTTTTTTTTTTTTTTGGTAGGGGGGTTCCTGTTGT TGGCCGCCCCCCCCAGGGCGAAAAAAAAGGGGGGGGG GGGAAA AAACCACTTTTTTTTTCCCCCCCCCACTTTTTTTGTG GGCGCGGGGGCTTTTTTTTCCCCCTCTTTTTCCCCCC CCCCCC CCTTTTTTTCCCAACCCTCCCCATGTGTGGGGAGAAA AGGAAAAAAAAAATTTTTTTTTTTTGAAAAAAAAAAGG GCAGGG AAAAATAATGGGTGTGTGTTTGTGTGCGCCCGTGTGT TTTTGGCCCCTCTCCCTAAAACGCGGGGTGGATGGAA AGGCTT TCTCTTTTTTTGTAGAAAAAAAAAAAAAA
	TZSM016	TAAGCCTGCTTATCCATCGCGACACGTTGTGAGAGCC GTGACCAACACACCTGCGTACACACACAAGCACTCAC CATATT TCCCCACTGGACCTTCTTTCTCCAAACACACACACTT CCTCCTCCCCTCTCCCAACACACGTGGGAAGGGTTGG AAAAGA AGAAGAAGAAGAAGAAAAAAAGAAGGAAAAAAACCAA CCCGTGCCCCCCCCCATAAGCGTCCGGGGGGATAAAAA CACCTC GTTTCCTCCACCCCCCCTTCTTTTTGCCCTTGCGGGG

		CGCGCACCCCACCCAACCCCCCCCCCCCCACAAAAAAA AAAAAA AAAAAAGGGGGGGCGGGGGTTTGGGATTGGGCGTTTT CCTGGGCCGTTTTTAAAATTTGGGGCGGGGGACAACA CCACCC CCCCCCCCCGCGGGGGGCCCCCCCCACACACCCCACA CCACACCGGGGGGGGGAGGGGGGGGGTAATAATTCTT AAGAAG GAGGGGGGGCGCCCCCCCACCACCACCAAACAACAAC AACCTCTTCCCACACCCACCCCCGTGTTGTTAATAAC CCCCAT TTTTTTTTTTTATTTTATTTTCGAAAAAAAATCAACC TGCGGGTTGACCACCCCCACCTTTGTTGAGACTTTTT TTTCTT TCTTTATAGAAAAATCGGAAAATTTCGGGAGGG
	TZSM025	CCTGCTGCTTATCATCGCGACACGTTGTGAGAGCCGT GACCAACACACCTGCGTACACACACAAGCACTCACCA TATTTC CCCCACTGCACCTTCTTCCTCCAAACACACACACTTC CTCCCCCACTCTCCCCACACACGTGGGAAAGCGGGAA AAAGAT AAGGACGAGGAGAAAAAAAGAGAAAAAAGAAACCCGG TGCGCACACAATAAACGGCGGGGGGGGATAAAAAAACC TCTTCT CCTCCCCACCCCCCCCTTTTTTTCTTTTTGGGGGGGG CACAATAAAATAAACCTTCTCTCCACAAAAAAAAAAA AAAAAC ATGAGGGGGCGTGTTTTTTTACCACCTTTTTTTGTGC GGGGTTTTTTTTTTTAGGGGGGGGGCACCACAACACC CCCCCC CCCCGTGGTGCGCCCCCCACAACCCACACCACACCCC GGGGGGGGGGGGGGGGGGAAAATTTTATTTAAGAGAG GGGGGG GCCACACACACACAAACACAAAAAAAAAACAATCTCT CACAACACACACACGTGTGTATACACACACATATGTA TATATA TATATATTTTTTTTAAAAAGAGAGATCGGTGTGCGCG CACACACACATACCGCTACTTTTTTTTTTTTTTTCT CTATAA AAAGAATATATACGGGCTGCCGGGGGGGGG
Tsetse	TZSM030	GAAAGGGGGGCTGTTATCATGGGCGGACACGTTGTGT GAGCCGTGATCAACTACTAAACTTTCGTCAACCGGCA CGAGCA GAGAGAAGAAAAGCACGCACACACACACAGAGACACA CCAGGCATATAAAAACATATATATATATGCATAATAT ATATAT ATATTATATATGTGGTATATACACACAGGTGTGCGCG CCCCCCCCCCCCCACGTAAAATACTTTTACATATATAT ATATAT ATCACATCACACACGTCGTTCTGTGAAAATATATATA CACGTTTATATATCTCATGTTGCGGGGGTGCTCTGCT TGTCAT CAAAAAAAGATCCAGCTGCGGGTTCCCCTACCGCTCC CTTGTTACCACTTTTGCTTCCTCTATTGAAACAATAT CGGTGA ACTTCGGGG

Appendix 3: Published papers

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Journal of Parasitology and Vector Biology

Full Length Research Paper

An investigation on *Glossina* species and the prevalence of trypanosomosis in cattle in Meatu district, Tanzania

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A cross-sectional study which sought to identify *Glossina* species and the prevalence of trypanosomosis in cattle of Meatu district where sleeping sickness cases have been reported in villages that border protected wildlife parks, was carried out. Four phenols and acetone baited tsetse traps (NGU, NZI, S3 and Biconical) were used in the study and showed that three *Glossina* species occurs in the area namely *Glossina pallidipes*, *Glossina swynnertoni* and *Glossina morsitans*. *G. pallidipes* was the most prevalent species. Four hundred and twenty four (424) blood samples were collected from cattle and subjected to parasitological and hematological analysis. Analysis by microscopic and buffy coat showed an overall prevalence of animal trypanosomosis (AT) of 2.36% (n=10). Identified trypanosome species were *Trypanosoma congolense* (7/10) and *Trypanosoma vivax* (3/10). No mixed infection was identified. The packed cell volume (PCV) for hematological analysis revealed a prevalence of anemia of 8.25%. No statistical evidence implicated animal trypanosomosis as the cause of anemia. Identification of trypanosomes in screened animals implicate AT as a threat to cattle and other domestic and wild animals since the identified trypanosome species affect a wide range of animals. Tsetse control and proper treatment of livestock should be advocated to control the disease.

Key words: *Glossina*, animal trypanosomosis, sleeping sickness, Tanzania.

INTRODUCTION

Trypanosomosis is a disease caused by protozoan parasites of the genus *Trypanosoma* and is transmitted through a bite by tsetse flies of the genus *Glossina* (Wamwiri and Changasi, 2016). African Trypanosomosis refers to two forms of diseases: Human African Trypanosomosis (HAT) and Animal African

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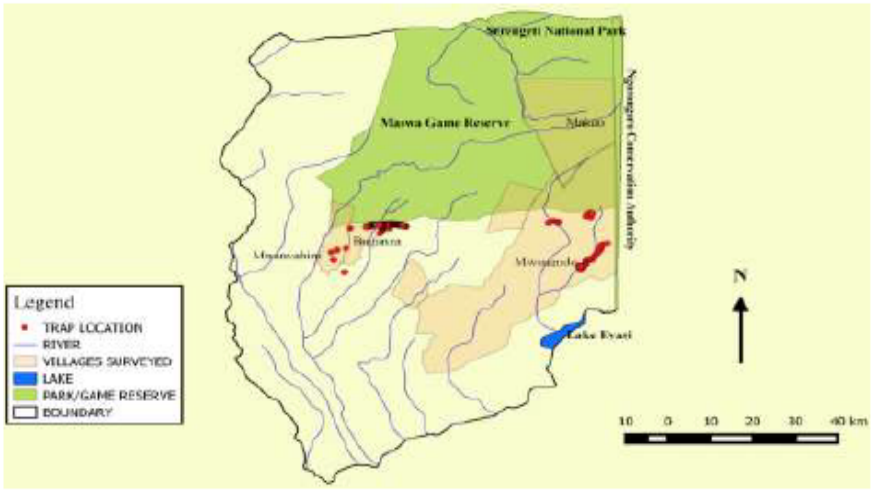

Figure 1. Map of Meatu District showing trap deployment in the villages surveyed.

Trypanosomosis (AAT). Human African Trypanosomosis is caused by *Trypanosoma brucei rhodesiense* (East and Southern Africa) and *T. brucei gambiense* (West and Central Africa) (WHO, 1998). AAT is caused by many trypanosome species including *T. brucei*, *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma simiae* (Kihamia et al., 1991; Leak, 1999). Trypanosomosis have resulted into serious economic impediment in 36 sub-Saharan African countries for years (WHO, 1998). While tsetse flies are well known as biological vectors of the diseases to both animals and human hosts, wildlife animals are also known to be reservoirs of all forms of trypanosomosis (FAO, 1982). Recent studies have confirmed domestic animals like cattle and pigs as being reservoirs of human infective trypanosomes (Haji et al., 2014; Hamill et al., 2013). In Tanzania, 302,465 km^2 of the land that accounts for thirty-two percent of the total land mass is tsetse infested where seven species of *Glossina* exist (Daffa et al., 2013).

From computations of Shaw et al. (2014), 4.5 Billion TSH (approximately 2 million USD) losses occur annually resulting from cattle mortality and reduced cattle productivity. The losses may be higher than 4.5 billion TSH if costs incurred by livestock owners in treating sick animals are included.

Nevertheless, one hundred cases of sleeping sickness cases are also being reported annually and the majorities are from Kigoma, Rukwa and Tabora regions (Simmaro et al., 2010).

Regular disease surveillance in the trypanosomosis endemic areas for identification of infective trypanosomes in susceptible hosts and key risk factors is one way to justify or estimate tsetse and trypanosomosis burden. The findings can be useful in influencing and guiding strategic vector and disease control for the protection of livestock and people. Therefore, determining population dynamics of the vector, that is, species, age-sex composition and identifying pathogens in the host, provides essential evidence on the transmissibility of pathogens between vector and hosts (Woo, 1970; Murray et al., 1977).

The aim of the current study was to identify *Glossina* species, establish their density and to determine the prevalence of trypanosomosis in cattle.

The domestic animal host was selected as it is abundant in the study area and it is known to be the reservoir of human infective trypanosomes (Simukoko et al., 2007).

MATERIALS AND METHODS

Study area and design

Meatu district is located at 34°41'8.34"E and 3°28'58.68"S. The district is among the five districts of Simiyu region; with an area of about 8,871 sq. km^2. In 2012, Meatu district had a population of about 296 616 people (NBS, 2012). The district has a bi-modal rainfall whereby short rains starts from October and ends in December and long heavy rains starts in March and ends in May. The average annual rainfall ranges from 600 to 900 mm. Its topography is characterized by flat, gently undulating plains and lowly sparsely vegetation and in some places, is covered with Miombo woodlands.

A cross-sectional study was conducted in Meatu district because of its proportion of land being occupied by the Maswa game reserve than other districts of Simiyu region and due to the fact that sleeping sickness was reported in previous years (district unpublished report). Entomological as well as parasitological data were collected in Buganza, Mwanyahina, Mwangudo and Makao villages in September 2015.

Figure 1 shows the surveyed villages selected based on their history of endemicity for trypanosomosis, vicinity to the protected areas and relative cattle population.

Table 1. Mean tsetse catches in Meatu district by villages.

Village	Overall mean	Tsetse species		
		G.pallidipes	*G. swynnertoni*	*G. morsitans*
Buganza	22.17±20.35	40.63±25.51	12.50±8.86	13.88±6.80
Mwanyahina	15.46±11.77	23.5±13.14	13.25±10.79	9.63±6.89
Makao	56.06±56.83	43.88±24.29	68.25±73.52	0
Mwangundo	51.02±21.18	59.30±25.65	42.74±12.09	0

Table 2. Tsetse apparent densities in Meatu district by village.

Village	Total catches	Traps	Trapping days	Apparent density (FTD)
Buganza	532	8	3	22.2
Mwanyahina	371	8	3	15.5
Mwangudo	816	8	3	34.0
Makao	897	8	3	37.4

Data collection

Collection of tsetse samples

Entomological data on tsetse species, sex and abundance were collected using Phenol® and Acetone® baited NZI (Mihok, 2002), NGU (Brightwell et al., 1987), S3 (Ndegwa and Mihok 1999) and Biconical (Challier et al., 1977) traps. Two sites were identified in each village in which the traps were randomly deployed at 100 m apart according to the vegetation type likely to influence visibility. Traps were rotated every day for three consecutive days and tsetse harvested after every 24 h. Tsetse species and sex were determined by taxonomic key as in FAO (1982) tsetse manual. After identification, a total of five non-teneral flies were pooled and preserved in 253 sterile vials containing absolute ethanol for further laboratory analysis.

Parasitological screening for trypanosome infection

Blood samples were collected from cattle after first obtaining oral consent from herd owners. A total of 424 cattle were sampled by collecting blood from the jugular vein using ethylene diamine tetra acetic acid (EDTA) vacutainer tubes, labeled and stored in ice packed cool box. Collected blood samples were later examined for packed cell volume (PCV) (Woo, 1970), buffy coat (Murray et al., 1977), and microscopically for the presence of trypanosomes in the field by the Giemsa-stained thick blood smears method. Cut off point of ≤24% PCV (Marcotty et al., 2008) was used for considering cattle anemic. A total of 100 buffy coat samples (including positives) were extruded on Whatman FTA® cards matrix, air dried before being stored for further analysis in the laboratory.

Data analysis

Both parasitological as well as entomological data collected from tsetse and animals from the four villages were entered in Microsoft Excel (2007) and Fly per Trap per Day (FTD), prevalence of nagana and anemia were computed. Furthermore, the records were transferred to Epi Info7 (CDC, 2014) analytical software for analysis. One-way analysis of variance (ANOVA) was used to analyze the variations of tsetse catches and cattle PCV in the villages.

Tsetse counts were used as the dependent variable while trap type, tsetse species, vegetation type and village were used as the independent variables. The overall comparison of tsetse species and the trap types regardless of the tsetse fly species was done using generalized linear model analysis. Packed cell volume was used as the dependent variable whereas nagana, species and village were independent variables. Separation of means was done at 95% confidence interval (CI) and the significance level of 5% ($P< 0.05$) in all statistical tests.

RESULTS

Collection of tsetse samples

The overall mean tsetse catches for the four villages was 654.08, three species namely *Glossina pallidipes, Glossina swynnertoni* and *Glossina morsitans* were identified. *G. pallidipes* was the most abundant tsetse species followed by *G. swynnertoni* and *G. morsitans* in descending order. *G. morsitans* was found at Mwanyahina and Buganza villages only. The highest scores were observed at Makao and Mwangudo villages, while the lowest scores were observed at Mwanyahina and Buganza villages (Table 1). The differences in catches among species and between villages were highly significant ($P<0.000$). High FTD was observed at Makao (37.4) while low apparent density was observed at Mwanyahina (15.5) (Table 2).

The performance of traps in tsetse catching is shown in Figure 2. NGU trap obtained the highest score for the two species: *G. morsitans* and *G. swynnertoni* while G. *pallidipes* were mostly trapped by NZI trap. S3 and

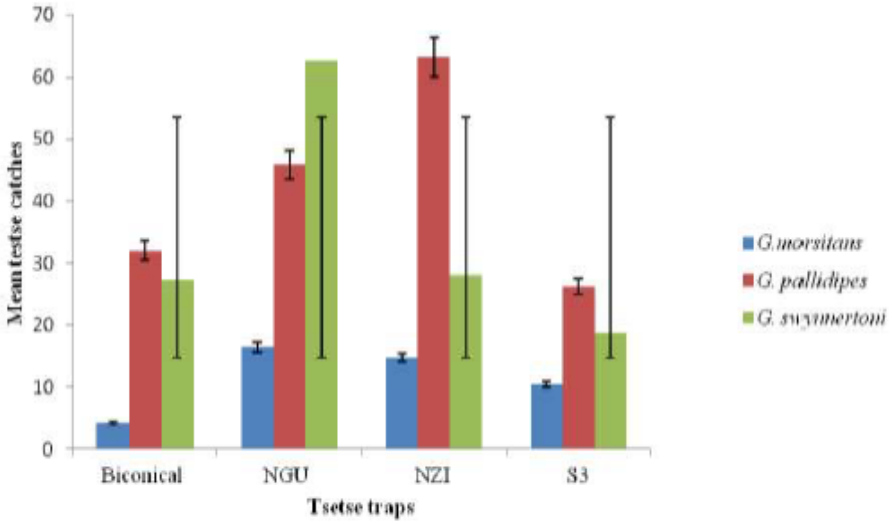

Figure 2. Performance of traps in catching *Glossina* species.

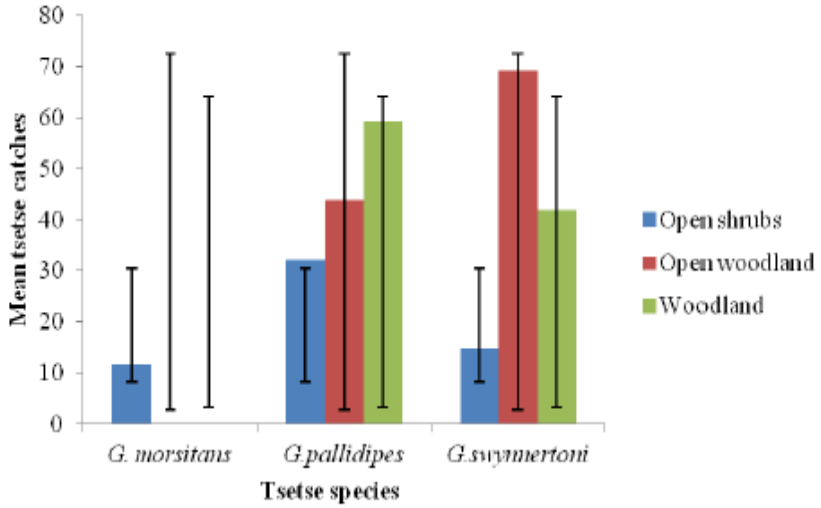

Figure 3. Mean tsetse catches in relation to vegetation where traps were deployed.

Biconical trapped the lowest numbers of all three species (Figure 2). Three vegetation types were found in the villages surveyed. Open and scattered shrubs (Mwanyahina and Buganza) open woodland and woodland (Makao and Mwangudo). *G. pallidipes* and *G. swynnertoni* were trapped in all vegetation types, except for *G. morsitans* which were caught in open shrubs only (Figure 3). The catches of tsetse in the three vegetation types were significantly different (P<0.001).

Parasitological screening for trypanosome infection

Table 3 summarizes PCV scores, the prevalence of trypanosomosis (nagana) and the prevalence of anemia in cattle in the sampled villages. The overall prevalence of trypanosomosis in cattle was 2.36% (n=10), being highest at Makao village (3.50%) and lowest at Mwangudo village (1.23%). Morphological identification (Uilenberg, 1998) revealed *T. congolense* (7/10) and *T.*

Table 3. Haematological and parasitological attributes of cattle in Meatu district by village.

Village	Anaemia	Nagana	Mean PCV
Buganza	6.56%	2.31%	29.78
Mwanyahina	5.88%	1.43%	31.69
Mwangudo	13.70%	1.23%	29.61
Makao	9.35%	3.50%	29.29
Overall	8.25%	2.36%	29.86

Table 4. Comparison of the effect of nagana on cattle PCV and anaemia.

Class	Level	Mean PCV (± SD)
Anaemia status	Normal	30.58±4.01[a]
	Anaemic	21.83±2.84[b]
Infection status	Nagana negative	30.60±4.03[a]
	Nagana positive	30.13±3.31[a]

vivax (3/10). *T. congolense* was the most dominant tsetse species. Mean percent PCV of the animals sampled was 29.86%, while the prevalence of anemia was 8.25%. Two anemic cattle were found to be infected with *T. vivax*. Since a PCV value of ≥25% was used as a cut of point for cattle to be considered normal, a comparison between normal and anemic cattle in Table 4 revealed a significant difference (P<0.001). There was no statistical evidence for significant differences in PCV values between cattle which were nagana positive and those which were nagana negative (P=0.3103).

DISCUSSION

The findings of this study clearly substantiates the existence of three species of *Glossina*; *Glossina pallidipes, Glossina and swynnertoni* at Mwanyahina, *Buganza*, *Makao* and Mwangudo villages which are adjacent to Maswa Game reserve, whereas *G. morsitans* was trapped at Mwanyahina and *Buganza* villages only. An investigation carried out by Salekwa et al. (2014) in Simanjiro and areas of Tarangire National park reported that *G. morsitans* were the most abundant species as compared to *G. swynnertoni* and *G. pallidipes*. This difference may be attributed to type of vegetation cover. These results are contrary to the study carried out by Malele et al. (2007) who reported existence of four tsetse species namely *G. swynnertoni*, *G. pallidipes*, *G. m. morsitans* and *G. brevipalpis*. Whereby, *G. swynnertoni* was the most dominant species, a study that included some of the sites surveyed during the present study. It is clear that the Fly per Trap per Day (FTD) is higher at Mwangudo and *Makao* than at Mwanyahina and *Buganza* villages, posing higher tsetse bite risk in the former village than in the later. These findings confirm the existence and transmission of trypanosomosis in the study area and hence the risks of exposure to people and their livestock. Climate change and associated effects such as shortage of feeds for livestock, shortage of food for increased human population create pressure to move into new virgin and fertile lands (McDermott et al., 2001) which in most cases are protected parks and game reserves. The villages surveyed and their crop farms are located within few meters from the Maswa game reserve which increases the chances of contracting the vector-borne diseases. The good performance of NGU traps relative to other traps which in line with findings of the study performed by Malele et al. (2016) validates further its suitability for survey and control purposes in the Serengeti ecosystem.

Parasitological screening for trypanosome infection

This study provides an explanation on the presence of animal trypanosomosis and not human trypanosomosis in cattle by microscopy, where *T. congolense* and *T. vivax* were identified. Similar findings have been reported by Haji et al. (2014). No significant statistical difference was observed in PCV between trypanosome infected and non-infected cattle, and there was no relationship between nagana and PCV. The observation of trypanosome infection in cattle is an evidence of cyclical

transmission of trypanosomes by tsetse but infection of *T. vivax* suggests transmission by other biting flies like *Tabanus* species which were also trapped during the study and have been reported elsewhere in Desquesnes and Dia (2003). *T. vivax* and *T. congolense* are not the only pathogens of importance to cattle but also other livestock species as well as in wildlife (Kaare, 2007; Anderson et al., 2011).

Conclusion

The aim of this study was to identify *Glossina* and trypanosomes species present in cattle in the study area, and the findings also gave the picture of the vector composition and trypanosome species in cattle. Further studies will be done at the VVBD to identify the pathogens in the vectors and cattle using more sensitive molecular tools.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Assessment of community knowledge, attitude and practices for sustainable control of tsetse and tsetse-borne trypanosomosis in Meatu district, Tanzania

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Abstract

This paper reports the knowledge, attitude and practices of respondents in selected villages of Meatu district on trypanosomosis collected through a cross-sectional study which involved 306 participants. Tsetse was well known vector of trypanosomosis by about 70 % of participants. Animal African Trypanosomosis (AAT) was known by 90.2 % (259) while Human African Trypanosomosis (HAT) was known by 40.3 % (123). Seventy percent of respondents knew the correct clinical signs of HAT while 68.6 % (177) knew the clinical signs of AAT. The use of trypanocidal drugs was up hazard with only 5.2 % observed recommended veterinary drug use and 46.4 % could not recall how they used drugs in treating AAT. The awareness on HAT trend has shown to be increasing in recent years compared to previous years with school and health centres playing a front role. Since HAT health services are inaccessible and current trypanosomosis control techniques are not sustainable an integrated approach involving community is essential.

Keywords: *community, nagana, sleeping sickness*

Introduction

Trypanosomosis is a neglected tropical disease that continues to be a constraint to livestock-development in about 38 African countries and causing a fatal human disease (Allsopp, 2001). Tsetse borne sleeping sickness and nagana seriously impede agricultural sector development which employs about 80 % of rural people in the country. It threatens hundreds of lives the productive force resulting into gravely economic losses.

The tsetse borne trypanosomosis is mainly managed through vector control and/or parasite control. Vector control is done by use of traps and Insecticide treated targets (ITTs) the main technique employed in national parks, game reserve and areas adjacent to reserved areas (Daffa et al 2003). Insecticides application utilizes synthetic pyrethroids which are available through government subsidy (URT 2011; URT, 2016). Hand spraying and dipping constitute major control methods. The application of Sterile Insect Technique (SIT) led to eradication of *Glossina austeni* in Zanzibar (Msangi et al 2000) and has not been extended in other parts of the country due to several constrains, including cost. Parasite control is done through the application of chemotherapy and chemoprophylaxis. The dependence on trypanocidal drugs alone is not sustainable because of prohibitive prices, unreliable supply and the risk of emergence of trypanosome resistant strains (Matovu et al 2001).

Attitude and knowledge plays a significant role in practices taken towards any disease trypanosomosis inclusive. At community the knowledge, attitudes and practice on trypanosomosis have been inherited, acquired and shared within the community for thousands of years and through all these years the community has evolved a way of surviving in the deadly environment. Though assessment on community Knowledge Attitude and Practice (KAP) on the vector and the diseases have been conducted in Tanzania (Kinungh'i et al 2006; Byamungu et al 2016) their application in tsetse control is limited leading to re-infestation of tsetse in controlled areas. In most cases communities are implementers of the projects activities ignoring the fact that the vector and diseases have evolved within the community for years. Double control costs emerge as a result of non integrative and non participatory approaches. Entomological as well as parasitological surveys when coupled by a study on the community KAP gives an immense understanding of the community, disease and vector therefore increases advantages of intervention success.

This paper presents knowledge, attitude and practices of four villages (Buganza, Mwanyahina, Makao and Mwangudo) of Meatu district on tsetse borne diseases with a view to adding to how the information could be best tapped, addressed and integrated in the control.

Methodology

Study area

The study was conducted in Meatu district located between longitude 34°8' and 34.49'E and between latitude 2°57' and 4.9"S in two wards (Mwanyahina and Mwangudo) in their four villages namely Mwanyahina, Buganza, Mwangudo and Makao (figure 1). The district is among five districts of Simiyu region. The district has the size of about 8 871 km ^{2} and a population size of about 296 616 people according to the 2012 Tanzania National Census (URT, 2012). Meatu experience a uni-modal rainfall which usually starts from October up to May. The average annual rainfall ranges from 400 mm to 900 mm. Its vegetation is mainly open bush savannah dominated by acacia species. The district borders Maswa Game reserve to the North and Ngorongoro Conservation Area (NCAA) on the North eastern part. These wildlife conservation areas are active source of tsetse infestations and hence making the district a viable candidate for the study.

Sampling techniques and data collection

Purposive sampling technique was used in the wards and villages selection. The relative position to Game reserve and National Parks and cattle population were the criteria. A cross sectional study was conducted in September 2015 to assess community's knowledge, attitude and practice on tsetse borne trypanosomosis using semi structured questionnaire. The questionnaire was addressed to subjects regardless of gender to respondents aged 18 years and above. A pre-test and introduction of the study objectives to the village leaders were done before the study was conducted. Oral consent was obtained before a questionnaire was administered to an individual. Sample size of 306 respondents was interviewed.

Data management and analysis

Data was entered, coded and summarized using Microsoft Excel (2007) spreadsheets and then analysed by using Epi info 7 software (CDC, 2014). The coding involved assigning codes to open ended responses after structuring them. Descriptive analysis concentrated on frequencies and percentages.

Results

Socio-demographic characteristics

Information on Knowledge, Attitude and Practice (KAP) was collected from 306 respondents from

the selected two wards covering four villages. Table 1 summarizes socio-demographic information. Makao village contributed 46.4 % of all respondents followed by Mwanyahina, Buganza and Mwangudo. Among respondents 52.3% were females and males constituted 47.7 %. The largest group of the respondents aged 19-29 years (32.7 %) while the least group had 40-39 years (21.6%). Seventy percent of the respondents were literate where by 60.1 % (184) had attained primary education, 8.17 % secondary education while 2.61% were college graduates. Three major means of livelihood in descending order were agro-pastoralism, pastoralism and other activities (of bee-keeping, retail shops) corresponding to 74.2 %, 11.8 %, and 6.54 % respectively. Women aged 18-29 years contributed a fairly large proportion of agro-pastoral. Pastoralism in contrast was a male's activity.

Table 1. Socio-demographic characteristics of the respondents by wards

	Wards					
	Mwanyahina		Mwangudo		TOTAL	
	N	%	N	%	N	%
Age (In years)						
18-29	46	36.0	54	30.3	100	32.7
30-39	24	18.8	46	25.8	70	22.8
40-49	28	21.9	38	21.4	66	21.6
>50	30	23.4	40	22.5	70	22.8
Gender						
Male	78	60.9	68	38.2	146	47.7
Female	50	39.1	110	61.8	160	52.3
Education level						
None	39	30.5	50	28.1	89	29.1
Primary	78	60.9	106	59.6	184	60.1
Secondary	9	7.0	16	8.99	25	8.17
College	2	1.56	6	3.57	8	2.61
Occupation						
Agro pastoral	95	74.2	132	74.2	227	74.2
Pastoralists	16	12.5	20	11.2	36	11.8
Civil Servant	9	7.03	14	7.87	23	7.52
Other activities	8	6.25	12	6.74	20	6.54

Livestock management and awareness of animal trypanosomosis

Two livestock management system were distinct; crop-livestock and pastoral system the previous being dominant than the latter. Table 2 illustrates livestock possession whereby owning more than one livestock species was common (98.4 %). Cattle were kept by 10.1 % of respondents whereas the largest group (37.3 %) of respondents kept cattle, goat, sheep and dog followed by cattle, goat and dog (16.9 %), cattle and dog (13.4 %), cattle and goat (10.5 %). Least groups were cattle and sheep and sheep, goat and dog 1.31 % each.

Table 2. Livestock ownership across villages

	Villages									
	Buganza		Mwanyahina		Makao		Mwangudo		Total	
	N	%	N	%	N	%	N	%	N	%
No Livestock	0	0.00	2	2.86	15	10.6	1	2.63	18	5.8
Cattle, goat, sheep and dog	24	42.9	26	37.1	48	33.8	16	42.1	114	37.3
Cattle and dog	6	10.7	4	5.71	27	19	4	10.5	41	13.4
Cattle, goat and dog	11	19.6	14	20.0	22	15.5	5	13.2	52	16.9
Cattle Goat and sheep	2	3.57	2	5.71	4	2.86	2	5.26	10	3.27
Cattle	4	7.14	9	12.9	12	8.45	6	15.8	31	10.1
Cattle and goat	7	12.5	11	15.7	10	8.45	4	10.5	32	10.5

Cattle and sheep	0	0.00	2	2.86	2	1.41	0	0.00	4	1.31
Sheep, goat and dog	2	3.57	0	0.00	2	1.41	0	0.00	4	1.31

Knowledge on bovine trypanosomosis

Table 3 below illustrates knowledge about nagana/bovine trypanosomosis. The disease was mainly referred to as *"Bhusatu bho ngi"* (66.0 %) word equivalent to fly disease by kisukuma language (a largest proportion tribe found in the area). Eight percent referred it as "*ndorobo*" a Kiswahili word meaning tsetse fly so they literally meant tsetse fly disease. About 8.95 % referred it as "*ngaroti*" a Kimasai word meaning diarrhea disease and 18.5 % did not know the local name. Tsetse was known as the source of nagana (77.6 %) though other still believed it is associated with witchcraft (3.60 %).

Table 3. Knowledge on bovine trypanosomosis by village

	Villages									
	Buganza		Mwanyahina		Makao		Mwangudo		Total	
	N	%	N	%	N	%	N	%	N	%
Local name										
I don't Know	5	13.5	25	37.8	9	7.44	9	25.7	48	18.5
Bhusatu bho ngi	30	81.1	34	51.5	86	71.1	21	60.0	171	66.0
Ndorobo	2	5.41	3	4.55	12	9.92	1	2.86	18	6.95
Ngaroti	0	0.00	4	6.06	14	11.6	4	11.4	22	8.95
Source of nagana										
Tsetse bite	21	56.7	47	71.2	104	92.0	22	64.7	194	77.6
Other parasite	1	2.70	0	0.00	0	0.00	0	0.00	1	0.4
Witch craft	0	0.00	7	10.6	1	0.88	1	2.94	9	3.6
Other insects bite	0	0	0	0.00	1	0.88	0	0.00	1	0.4
I don't know	15	40.5	12	18.1	7	6.19	11	32.4	45	18

Figure 1 illustrates bovine diseases of economic importance in Meatu where bovine trypanosomosis was ranked the first in respect to other diseases followed by East coast fever (ECF).

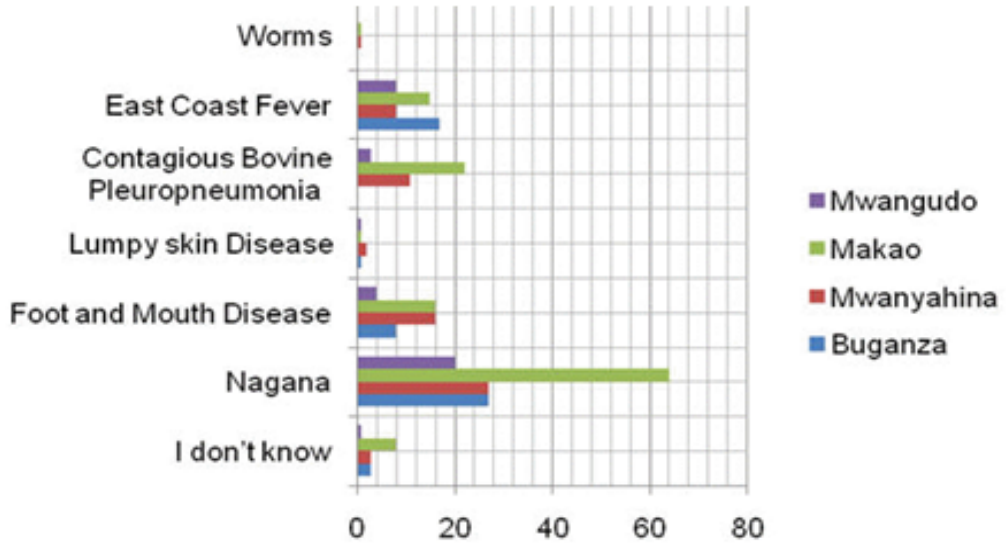

Figure 1. Cattle disease of economic importance in Meatu by village

Clinical signs and perceived impact of the disease are shown in figure 2, the first recognized signs by respondents were emaciation and starry hair coat, abortion and anemia while a proportion of

respondents did not knew the signs. Respondents associated nagana with abortions and still births, weight loss and increased treatment costs while high mortality was ranked the last.

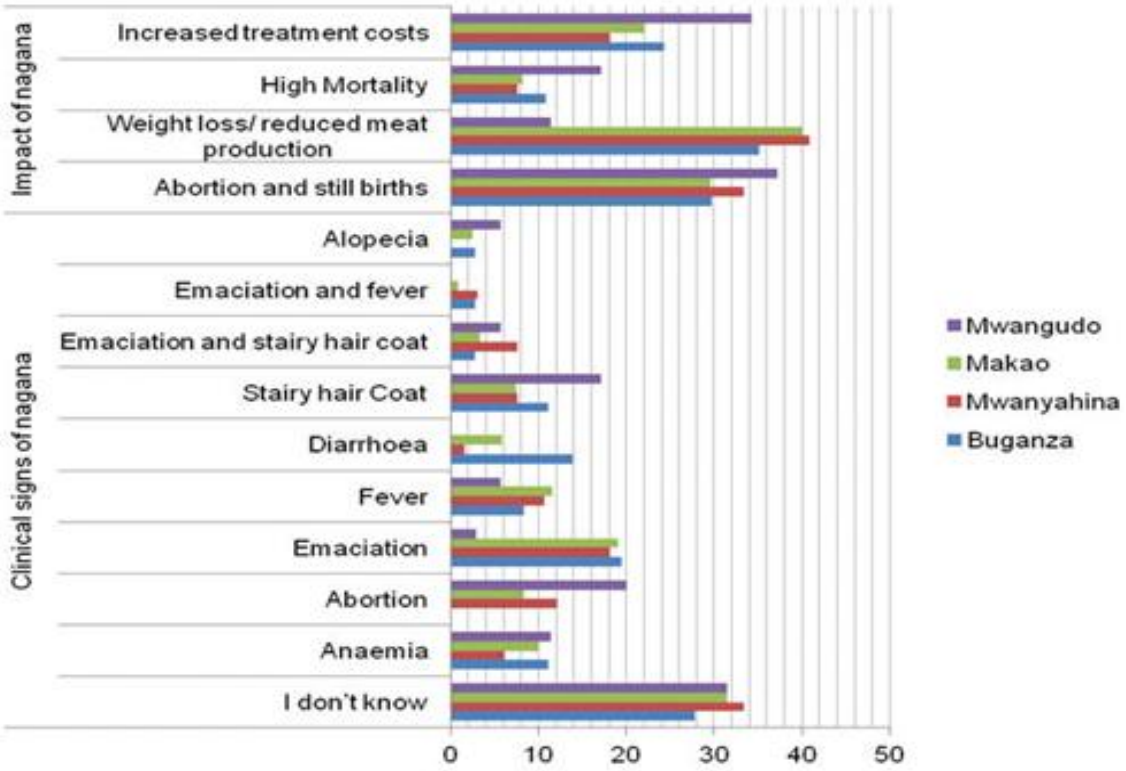

Figure 2. Clinical signs and perceived impact of Nagana

The drugs for treatment of nagana are shown in figure 3. It was observed that Berenil (Diminazene aceturate) was widely used for treatment against AAT, followed by Oxy tetracycline (OTC), Samorin (Isometamidium chloride) and Novidium (Homidium chloride). A proportion followed the instruction recommended for veterinary drug use with the majority could not recall which drugs were used in livestock and some of the respondents reported once to twice per week drug administration.

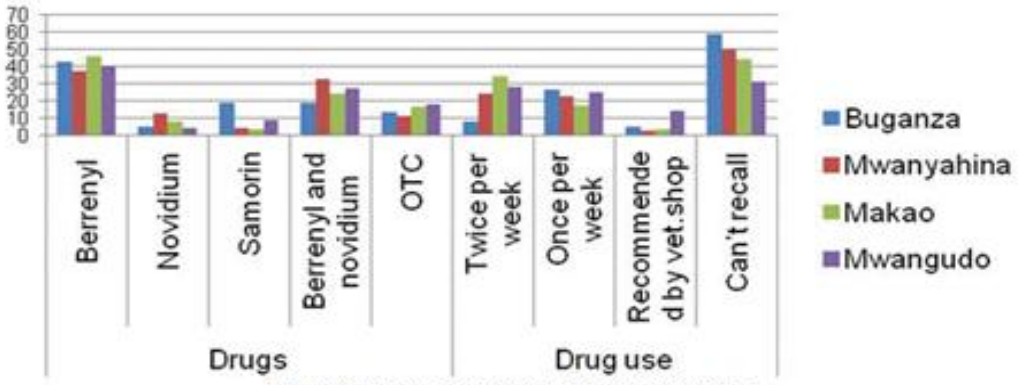

Figure 3. Drug use and drug use frequency by village

Awareness of sleeping sickness and risk perception

Sleeping sickness was known to 40.3 % (123) of the respondents while 70.2 % (87) mentioned sleeping sickness symptoms; fever (37.9 %), swelling of the lymph nodes (14.5 %), headache (9.68

%), abnormal appetite (5.65 %) and general body malaise (2.42 %) while 29.8 % did not know the symptoms of sleeping sickness (Figure 4).

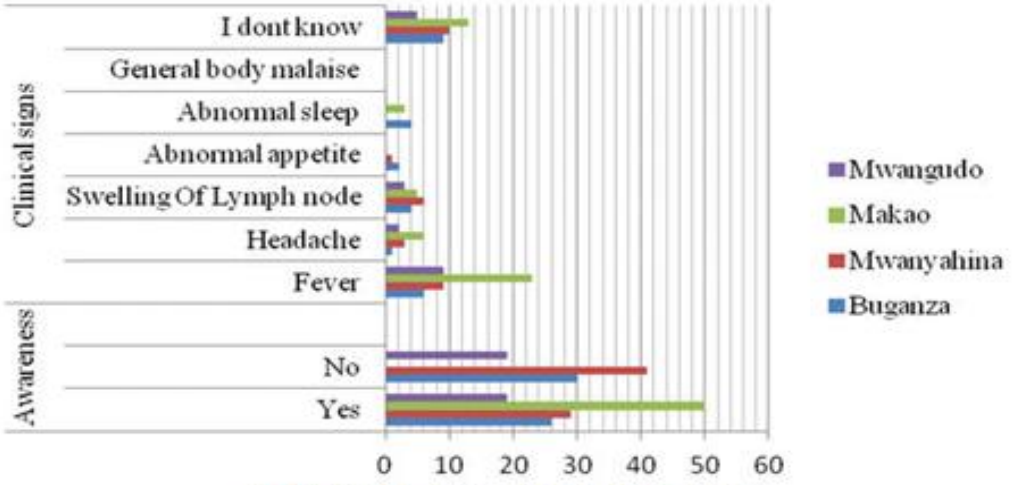

Figure 4. Sleeping sickness awareness and clinical signs

Figure 5 indicated the trend by which the information on sleeping sickness was delivered. The information on the disease revealed to be increased in 2000s (48.6 %), followed by early 1960-1989 (32.7 %) and 1989-1999 (18.7 %). Health centres and schools (53 %) play a half role in dissemination information, followed by friend relative or friend (24.8 %), radio (16.5 %) and newspaper (5.79%).

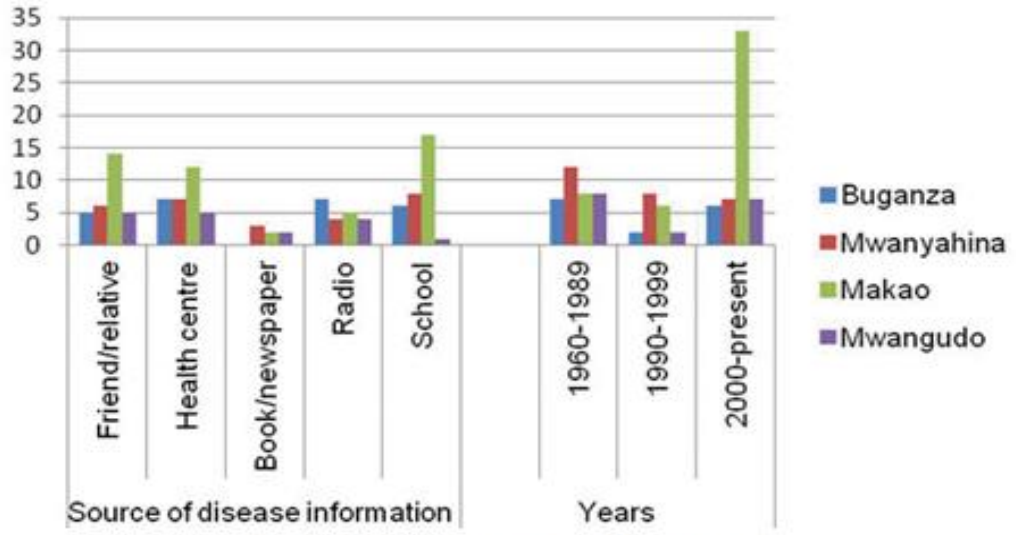

Figure 5. Source of sleeping sickness information and year

Table 4 summarizes further HAT information collected in the area. About 11.4 % respondents reported sleeping sickness to be the problem whereas 2.44 % did not know whether sleeping sickness was a problem and 84.8 % reported that sleeping sickness was not a problem. About 70.9 % of the respondents identified tsetse as source of sleeping sickness, 20.2 % respondents reported mosquito, 2.42 % associated it with witchcraft, 0.81 % other parasite and 5.65 % did not know the source.

Table 4. Knowledge about Human African trypanosomosis across villages

	Villages

	Buganza		Mwanyahina		Makao		Mwangudo		TOTAL	
	N	%	N	%	N	%	N	%	N	%
Source of HAT										
Tsetse bite	16	61.5	23	79.3	33	66.0	16	84.2	88	70.9
Other parasites	1	3.85	0	0.00	0	0.00	0	0.00	1	0.81
Witchcraft	0	0.0	1	3.45	2	4.00	0	0.00	3	2.42
Mosquito	6	23.1	5	17.2	11	22.0	3	15.8	25	20.2
I don't know	3	11.5	0	0.00	4	8.00	0	0.00	7	5.65
Season with more tsetse bites										
Dry	26	63.4	28	45.9	71	55.0	28	75.7	153	57.1
Wet	13	31.7	24	39.3	33	25.6	6	16.2	76	28.4
I don't know	2	4.88	9	14.8	25	19.4	3	8.11	39	14.6
HAT risk areas										
Home/office	3	11.5	2	7.14	2	4.08	1	5.26	8	6.56
Bush/forest	14	53.9	16	57.1	32	65.3	14	73.7	76	62.3
Grazing areas	4	15.4	7	25.0	7	14.3	3	15.8	21	17.2
Bush roads	3	11.5	1	3.57	1	2.04	0	0.00	5	4.10
Don't know	2	7.67	2	7.14	7	14.3	1	5.26	5	9.84
Is HAT a problem in your area										
Yes	8	32.0	1	3.45	2	4.00	3	15.8	14	11.4
No	16	64.0	26	89.7	48	96.0	16	84.2	106	84.8
I don't Know	1	4.00	2	6.90	0	0.00	0	0.00	3	2.4
Accessibility of HAT treatment										
Yes	10	35.7	3	10.3	0	0	0	0.0	13	10.3
No	18	64.3	26	89.7	50	100	19	100	113	89.7
Measures taken to HAT sick person										
Taken to health centre	14	70.0	22	78.6	42	91.3	13	68.4	91	80.5
Taken to traditional healer	2	10.0	1	3.57	1	2.17	0	0.00	4	3.54
Nothing is done	1	5.0	0	0.0	1	2.17	1	5.26	3	2.65
Don't know	3	15.0	5	17.9	2	4.35	5	26.3	15	13.3
HAT control measures										
Yes	10	38.5	3	10.3	16	32.0	5	26.3	34	27.4
No	16	61.5	26	89.7	34	68.0	14	73.7	90	72.6

Identified sleeping sickness risk areas included bush/forest (62.3 %), grazing areas (17.2 %), at home (6.56 %) and in the bush roads (4.10 %) and 9.84 % did not knew. Eighty percent of diseased people are sent to hospital, 3.54 % are sent to traditional healers while 2.65 % did nothing, while 13.3 % did not knew what to do. Sleeping sickness treatments were not easily accessible in the area (89.7 %) and there are no sustainable tsetse control activities in place (72.6 %). The ongoing tsetse control methods reported by 27.4 % of the respondents were insecticides spraying of livestock, 55.9 % bush clearing and 44.1 % bush fire. (Measures suggested by respondents included tsetse control (29.0 %), disease surveillance (25.8 %), building of health centres and staffing (12.9 %), avoid tsetse (12.9 %), accessible health services (drugs and vaccine) 8.06 % (Figure 6).

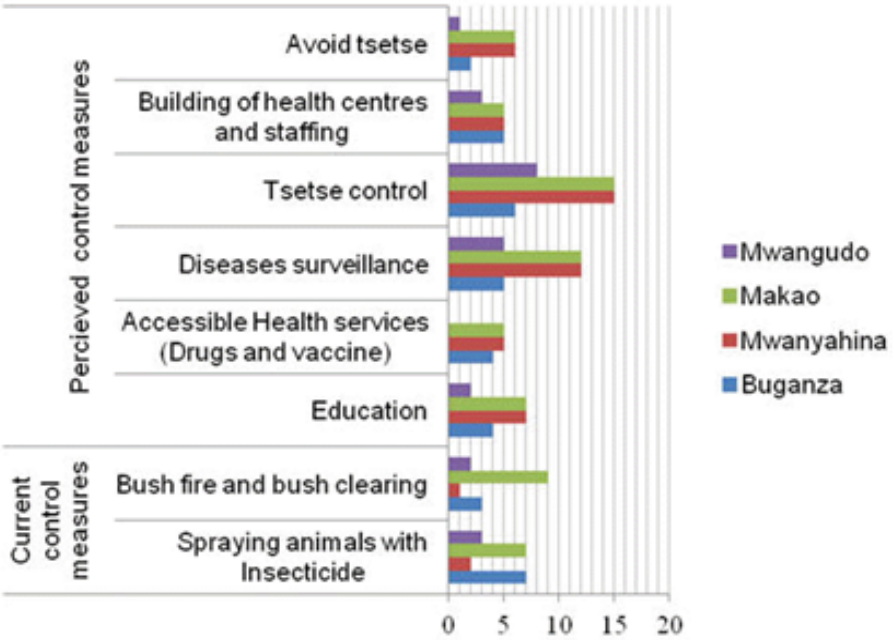

Figure 6. Current and perceived sleeping sickness control measures

Discussion

Knowledge

Community is aware that tsetse is the source of trypanosomosis where bush /forest and grazing land are most risky areas. Awareness on AAT is high compared to HAT. This may be due to the pathogenicity observed in cattle and the understanding that bush and forests which they are near to are the sources of infection. Game reserves and wildlife management areas offer alternative sources of pastures to their animals during drought despite strict bylaws and penalties that prohibit animals from grazing. Poor awareness of HAT on the other hand is attributed to reduced cases of HAT in the area which may be due to high use of Diminazene aceturate (Figure 3) which reduced *Trypanosoma brucei rhodesiense* circulation in livestock and vector in Uganda (Matovu et al 1997; Fevre et al 2001). However, reduced HAT awareness may be contributed by under reporting which results from shared clinical signs with other fever causing diseases (Kennedy 2012). Furthermore, contents of text books describe poor sleeping sickness disease while their availability in schools especially in remote areas (where HAT is also endemic) significantly affect teaching resulting to poor awareness of HAT to students. Therefore, there is a need to improve and create new HAT awareness channels such as the use of social media (Olet and Othieno 2015) which their use among youth is increasing. The use of social media in awareness creation has proved useful in cancer awareness (Laponte et al 2014). Leaflets and pamphlets could convey information directly to the users if they will be made available. Moreover, Schools can serve as a reliable information source if sleeping sickness clubs will be formed to schools found in endemic areas and number of science text books will be made available to schools. These would increase awareness and subsequent reliable information disseminated to others.

Attitude

Community views AAT and HAT as important diseases. This is vivid from positive disease control efforts and medical seeking behavior the community has. Nevertheless the community view trypanosomosis control in a multidimensional approach rather than unidirectional (Figure 6). In this way the community is prepared to participate in tsetse and trypanosomosis control which will be advocated their area.

Practice

Livestock keeping is a source of livelihood in Meatu district for many of its residents where the practice of keeping more than one livestock species seems to be a way of diverging income sources. While the challenge of bovine trypanosomosis was high, the chances of contracting sleeping sickness are high as well due to ownership of different domestic species (Ruiz et al 2015). The mixed management systems (pastoral and agro pastoral) present a threat of distribution of resistant strains (Selby et al 2013). Grazing livestock in the protected parks (game reserve) during drought increases chances of contracting trypanosomes as well as other parasites and vectors harboured in wildlife (Auty et al 2012). Competition for available water sources with other uses during dry season limit insecticide application (both spraying and dipping) and cause dependency on chemotherapy only for control which can lead to drug resistance strains emergency (Matovu et al 2001; Kibona et al 2006). The application of insecticide pour on technique could help in minimizing the effects during drought (Rowlands et al 2001). Since farmers treatment regimes is based mostly on clinical signs which are shared by other diseases there is need for strategic education in specific nagana clinical signs and proper drug use. While the utilisation of trypanotolerant livestock in Tanzania is limited (Mutayoba et al 1989) their use could ease trypanosome control challenge to farmers (D'leteren et al 1998). Livestock intensification and use of prophylactic than chemotherapy would reduce the costs which farmers do not see (FAO, 1998).

The ongoing vector control interventions are not environmental friendly because they are associated with vegetation destruction which can reduce tsetse belt in a while but contributing to climatic change in the long run (Malele et al 2011).

Conclusion

- The awareness of AAT is higher than HAT among village residents; new awareness dissemination means need to be instituted.
- Since chemo treatment plays as main trypanosomosis control which is a risk factor for emergency of resistant strains, prophylactic use should be promoted.
- Training and advocacy of integrative and participatory tsetse and trypanosomosis control approaches following what is perceived important by community are is essential for realization of control success.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgement

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Appendix 4: Manuscript submitted to scientific journal

Phylogenetic analysis of trypanosomes isolated from cattle and *Glossina* spp of Meatu district, Tanzania

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Abstract

Trypanosomes are etiological agents of trypanosomosis. Thus their identification and characterization into species and subspecies and the population circulating in vectors and hosts is crucial for effective control of the diseases they cause. This study analysed samples collected from cattle and tsetse of Meatu district in order to infer their evolutionary relationship. Internal transcribed spacer 1 (ITS1) PCR and Maximum Parsimony (MP) techniques were used to characterize trypanosomes and infer their evolutionary relationship respectively. Prevalence of trypanosomes in cattle was 15 % (n=100) while in tsetse was 1.20 % (n=250). *Trypanosoma congolense* were identified in cattle whereas, *T. simiae* and *T. godfreyi* were identified in *Glossina pallidipes*. The MP phylogenetic analysis supported monophyletic nature of the salivaria trypanosomes. It has further revealed trypanosomes of Meatu were genetically similar not only to others found in Serengeti ecosystem but also to others in eastern and southern Africa.

Key words: Phylogeny, Tanzania, trypanosomes, trypanosomosis

Introduction

Characterization of tsetse transmitted trypanosomes since late 18th century has been a subject of research upon their discovery being etiological agents of nagana in cattle and later sleeping sickness in humans (Steverding 2008; Adams et al. 2008). The trypanosomes hamper livestock sector development sub-Saharan Africa whereby thousands of human lives are threatened and the potential agricultural areas are underutilized (Ilemobade 2009). The research on characterization of pathogens in vectors and vertebrates have enabled its description to genus and sub-genus levels, distinction of African trypanosomes from American trypanosomes, discovery of new species and subspecies and presence of wide range of reservoirs hosts (Lloyd &Johnson 1924; Hoare 1972; Hamilton et al. 2008). Through the mentioned efforts, proofs have been generated to infer that animal and human trypanosomes are different in respect to their pathogenicity and host range. The advances in molecular techniques have offered prospects of vaccine development against trypanosomosis.

In Tanzania the seven known species of *Glossina* are infested by *Trypanosoma congolense, T. vivax, T. brucei brucei, T. godfreyi, T. simiae* which cause nagana in livestock whereas *T. brucei rhodesiense* is reported as the sole cause of sleeping sickness in humans (Daffa et al. 2013).

Characterizing human pathogens in relation to animal pathogens is of public health importance and suits better allocation of the limited resources for diseases surveillance and control (Shaw et al. 2014). Specification of the pathogens also facilitates combating sleeping sickness, which is usually expensive to control and highly life threatening (Sindato et al. 2008).

Traditionally, trypanosomes detection in tsetse entails dissection and microscopic observation of the parasite in their respective maturation sites (salivary gland, midgut and proboscis) (Lloyd &Johnson 1924). In vertebrate hosts, wet, thick and thin Giemsa stained blood smear are examined under light microscope for detection of trypanosomes. Due to low parasitemia buffy coat technique is used to ascertain trypanosome infections by concentrating trypanosomes at the blood plasma-junction, whereas animal inoculation is widely used increase number of parasites in *in vivo* before confirmation through microscopy/PCR. Packed Cell Volume (PCV) readings are used together with buffy coat technique to establish presence of trypanosomes by determining the proportion of Red Blood Cells (RBCs) since the pathogens reduce their concentration in infected vertebrate

hosts. Parasitological techniques are not only reference techniques they also are useful in where laboratory settings. Conversely, molecular based techniques are reliable, sensitive and specific compared to parasitological and serological methods. Satellite based molecular techniques; DNA probes and PCRs (Masake et al. 1997; Gibson et al. 1988; Moser et al. 1989; Masiga et al. 1992; Welburn et al. 2001) are highly specific but are laborious to perform and cannot identify new species. Pan trypanosome PCR assays in addition to identification of new species it identifies multiple infections in the sample in one PCR test and therefore save time and cost and are ideal for large sample size examination (Njiru et al. 2005). Phylogenetic analysis has less been based on protein coding rather than non-coding gene sequences (satellite and spacer DNA) genes due highly conserved nature and few available reference sequences in Gene Bank and other sources. Nevertheless, 18S gene has extensively been used in favour of satellite DNA to infer phylogenetic relationship due to highly conserved sequences and availability in high copy number (Auty et al. 2012). In this study ITS1 PCR was used to detect trypanosome while 18S gene was utilised to analyse their evolutionary relationship as identified in cattle and tsetse. Lesser information on sleeping sickness in Meatu district is available although some cases were reported in the district in recent years (District unpublished report, 2015). Sleeping sickness pathogens in tsetse flies were identified by Malele et al. (2007) in a study that was carried in Serengeti ecosystem which involved some villages in the district. There was a need to generate information on sleeping sickness and nagana status in the district which would assist in planning strategies for tsetse control to improve human health and food security in the area.

Materials and Methods

Study area

The study was conducted in Meatu district located between longitude 34°8' and 34.49"E and between latitude 2°57' and 4.9"S in two wards (Mwanyahina and Mwangudo) in their four villages namely Mwanyahina, Buganza, Mwangudo and Makao (Figure 1). Meatu is one of five districts of Simiyu region. The district has the size of about 8 871 km^2 and a population size of about 296 616 people according to the 2012 Tanzania National Census (URT 2012) . Meatu experience a uni-modal rainfall which usually starts from October up to May. The average annual rainfall ranges from 400 mm to 900 mm. Its vegetation is mainly open bush savannah dominated by acacia species. The district borders Maswa Game reserve to the North (two-third of its size is located in the district) and Ngorongoro

Conservation Area (NCA) on the North eastern part. Furthermore active wildlife conservation (Wildlife Management Areas) initiatives are going on in some of the wards adjacent to the protected parks.

Sample collection

Tsetse fly cluster samples (n=250) composed of three species: *Glossina pallidipes* (n=171), *G. swynnertoni* (n=58) and *G. morsitans* (n=21) and buffy coats (n=100) from cattle collected from four villages (Buganza, Mwanyahina, Mwangudo and Makao) of Meatu district were used. Detailed method on collection and preservation of the fly and blood samples is described in (Malulu et al. 2017). In brief, tsetse fly samples were collected by Phenol® and Acetone® baited NZI, NGU, S3 and Biconical tsetse traps. The species of the trapped tsetse flies were determined by taxonomic key as described in (FAO 1982) tsetse manual and preserved in absolute ethanol. Buffy coats were obtained in accordance to (Murray et al. 1977) protocol and preserved on Whatman FTA® cards.

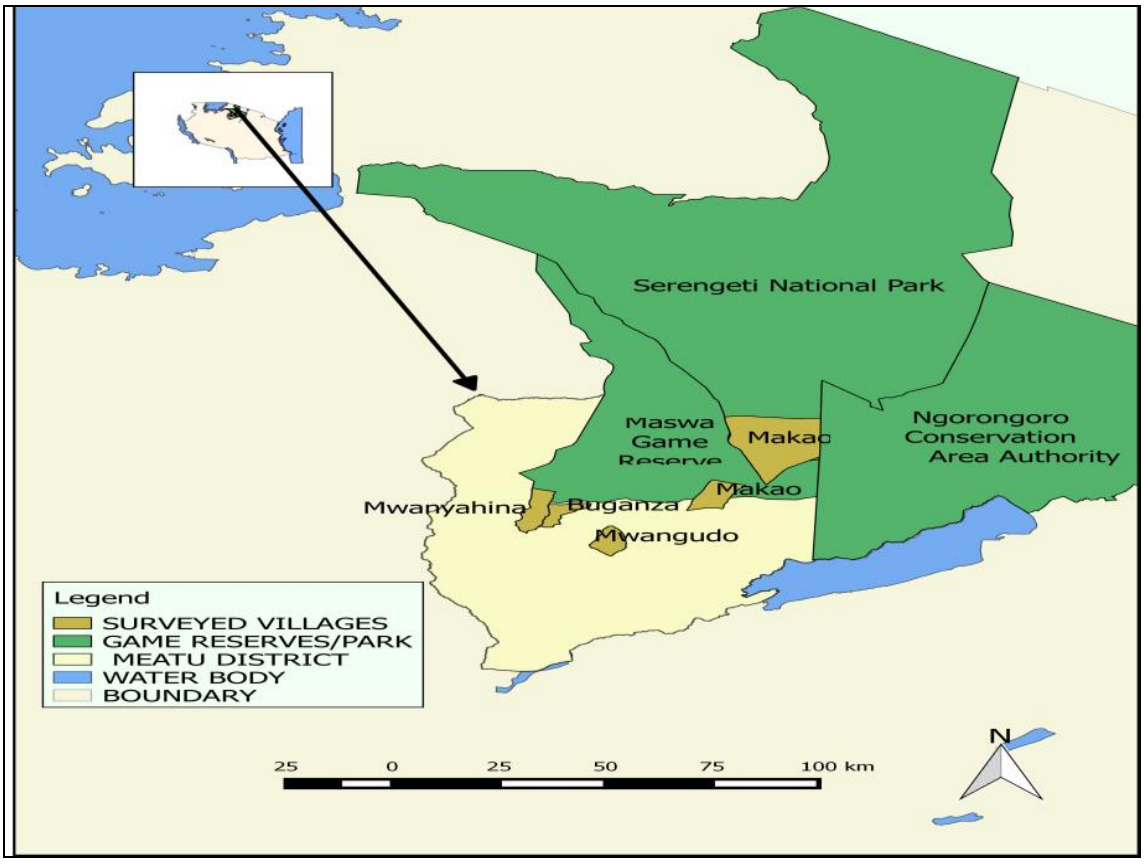

Figure 1. A map showing the surveyed villages from which samples were drawn

DNA extraction

Deoxy-ribose nucleic acid (DNA) extraction and PCR were conducted at Vector and Vector Borne Diseases Institute (VVBDI), Tanga. DNA was extracted from whole tsetse flies by Ammonium acetate precipitation (Bruford et al. 1988). Absolute ethanol was poured off from the sample and the flies ground using sterile hand pestles after being air dried overnight under room temperature. Phosphate Buffered Saline (PBS) 500µl was added for homogenisation and from which 100 µl of supernatant was used for DNA extraction. Thereafter 250 µl of Digisol buffer and 10µl of proteinase K (stored at -20^0C) were added to the sample and vortexed for 30 seconds and incubated at 55.0^{o}C for 1 hour. Some 300µl of Ammonium acetate was added to each Eppendorf tubes and vortexed for 30 seconds repeatedly for 5 minutes then supernatant transferred to new clean and sterile Eppendorf tubes. Then 1ml 100% ethanol was added to the supernatant, vortexed and inverted gently 10-20 times for DNA precipitation followed by centrifugation at 21952g for 15 minutes. Supernatant was poured and pipetted off and 900µl of cold ethanol was then added and the Eppendorf tubes inverted gently 10 times to rinse the DNA pellet and the DNA was air dried overnight at room temperature. Thereafter 100µl of Tris EDTA buffer were added to each Eppendorf tubes and stored at -20^0C until used for PCR.

Chelex® resign (20%) was used in the DNA extraction of buffy coat samples preserved on FTA cards as described in (Ahmed et al. 2011) and (Ruiz et al. 2015). In brief, 10 hole punches (1.2mm) were taken from the FTA® card matrix using Harris® Micro-punch and added into 1500µl sterile Eppendorf tubes, 200µl of 20% Chelex® resign and vortexed for 20 second to ensure the beads are distributed evenly and incubated at 55^{o}C for 1 hour. Thereafter, the samples were agitated and centrifuged at 21952g for 10 seconds followed by boiling for 10 minutes. Supernatant of 100µl were transferred to a new sterile Eppendorf tubes. All DNA samples were stored at -20^0c until used for PCR.

DNA amplification

ITS1 amplification was carried out in 25 µl reaction mixture containing 2 µl DNA template, 9.5µl distilled water, 12.5µl *Taq*1× Master mix (New England Biolabs MO270L) and 0.5µl of each ITS1 primers (CF: 5'-CCGGAAGTTCACCGATATTG-3' and BR:5'-TTGCTGCGTTCTTCAACGAA-3'). Primers and PCR conditions used were as described by (Njiru et al. 2005). Briefly an initial denaturation was done at 94°C for 30 seconds,

followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing step at 58°C for 45 seconds, elongation and extension steps each at 68°C for 5 minutes. PCR products were visualized in 1.5% agarose gel stained with Ethidium bromide through the ultra Violet-Trans-illuminator. DNA extracted from *T. evansi* isolated and characterised from Sudan (kindly donated by Dr. Hamid Ibrahim Noor from Khartoum University, North Sudan) was used as positive control and PCR water as negative control. A sample was classified in a respective species if found within base pair as indicated in brackets; *Trypanozoon* (480 bp), *T. congolense; T. congolense* savannah (700 bp), *T. congolense* forest (710 bp), *T. congolense* Kilifi (620 bp), *T. Simiae* Tsavo (370 bp), *T. simiae* (400 bp), *T. godfreyi* (300 bp) and *T. vivax* (250 bp). Seven trypanosome PCR products were sent to Bioneer, Korea for purification and sequencing of 18S gene. The identified 18S nucleotide sequences were used in phylogeny analysis.

Data analysis

Occurrence of trypanosomosis

Data were entered in Microsoft Excel sheets, occurrence of trypanosomes infections in tsetse and cattle was calculated by determining total positives out of the total number of samples. Chi-square (χ^2) test was used in comparing infections between cattle groups and between villages, and between tsetse species and village for cattle and tsetse respectively using Medcalc® statistical software version 11.3.1.0.

Phylogeny analysis

Sequence Alignment

MUSCLE (Multiple Sequence Comparison by Log Expectation) method (Edgar 2004) was used in the alignment of the 21 18S gene containing sequences; 7 new 18S sequences, 12 18S sequences of other *Trypanosoma* species were downloaded from Gene Bank using mega BLAST algorithm (Table 1) (being selected based on E-value and sequence similarity with the seven new sequences), and 2 out group 18S sequences of *Prymnesium parvum* and *Nannochloropsis gaditana* (free living flagellates which belong to families Chrysophyceae and Eustigmatophyceae, the definite out group to kinetoplastids were used (Stevens &Rambaut 2001). The aligned sequences were used to construct a phylogenetic tree by the use of Maximum Parsimony (MP) method by use of MEGA (Molecular Evolutionary Genetic Analysis) 6 software default settings (Tamura et al. 2013).

Table 1. Gene Bank reference of sequences used in the phylogenetic analysis

Accession number	Source	Species	Percent identity
U22315.1	Brown rat	*T. congolense*	86%
DQ317416.1	Unknown	*T. vivax*	70%
JN673389.1	Lion	*T. congolense* Savannah	85%
AB301937	Mouse	*T. congolense*	76%
JX910374.1	unknown	*T. brucei*	80%
KR028191	cattle	*T. congolense* Savannah	70%
FJ712718.1	Mouse	*T. congolense*	85%
JX853185.1	Cattle	*Trypanosoma theileri*	82%
U22317.1	Brown rat	*T. congolense* Kilifi	98%
AB301941	Deer	*T. congolense*	84%
U22319	Brown rat	*T. congolense* forest	93%
AB742531	Tsetse fly	*T. congolense*	76%

Results

PCR detection of *Trypanosoma* species

Occurrence of trypanosomes infection in cattle was 15 % (15/100) and in 1.2% (3/250) in tsetse. Though there was no significant difference in trypanosome infections in cattle between villages (P=0.459). Though infections between cattle groups was the same (P= 0.225) the infections in bulls (17.1%) were higher compared to other cattle groups. The number of trypanosomes infections was different between villages (P=0.034) though all infections were in from Buganza village. No significant difference in infections between *Glossina* species was observed (P= 0.495) with all infections were from *G. pallidipes* (Table 2).

Table 2. Occurrence of trypanosomes in cattle and tsetse based on ITS1 PCR

Risk factor	Number of samples	Total infections	Prevalence	χ^2	P value
Village					
Buganza	25	6	24	2.59	0.4591
Mwanyahina	14	2	14.3		
Makao	42	4	9.5		
Mwangudo	19	3	15.8		
Overall	**100**	**15**	**15**		
Cattle group					
Bull	31	6	17.1	9.41	0.225
Cow	21	2	9.5		
Bull calf	17	2	11.8		
Female calf	17	3	17.6		
Heifer	4	0	0		

Weaned bull calf	6	0	0		
Weaned female calf	3	1	33		
Oxen	1	1	1		
Overall	**100**	**15**	**15**		
Tsetse species					
Glossina morsitans	21	0	0	1.40	0.495
Glossina swynnertoni	58	0	0		
Glossina pallidipes	171	3	1.75		
Overall	**250**	**3**	**1.20**		
Village					
Buganza	65	3	4.6	8.64	0.0344
Mwanyahina	27	0	0		
Mwangudo	43	0	0		
Makao	115	0	0		
Overall	**250**	**3**	**1.20**		

Fifteen *T. congolense* produced fragments with molecular weights of between 600-700 bp (figure 2, lanes1, 3, 6, 8, 17 and 25) and was the only species identified in cattle in all villages. In tsetse flies, one *T. godfreyi* (Lane 30) and two *T. simiae* (Lanes 31 and 32) produced molecular weight of 300 and 400 bp, respectively all being from *Glossina pallidipes*. Sleeping sickness pathogens were neither identified in tsetse flies nor in cattle. Co infection was not observed other than single infection of *T. congolense*.

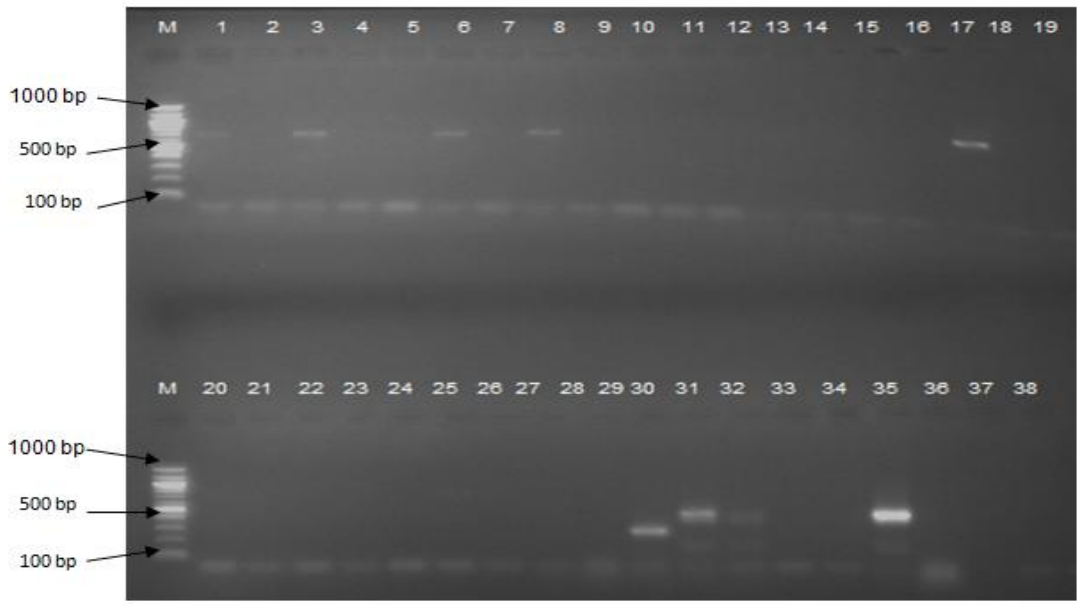

Figure 2. Identification of Trypanosomes by ITS1 PCR. Lane M 1000 bp DNA ladder, lane 1-34 *Trypanosoma* DNA, lane 35 positive control, lane 36 negative control.

Phylogenetic results

The evolutionary history was inferred using the Maximum Parsimony (MP) method. Tree which expressed few changes (most parsimonious tree) (length = 3324) is shown (Figure 3). The consistency index is 0.682639, the retention index is 0.743736 and the composite index was 0.562948 (0.507704) for all sites and parsimony-informative sites. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) is shown next to the branches. The MP tree was obtained using the sub tree-Pruning-Regrafting (SPR) algorithm.

The light green highlighted in the topology indicates cattle-originated isolates where the grey highlighted indicates the tsetse-originated isolates. This analysis produced two closely related clusters. The cluster A composed of all sample sequences of this study which were closely related to *T. congolense* savannah from Serengeti, Tanzania. This pattern received a highly significant internal support (100 bootstrap). Cluster B in turn composed of *Trypanosoma* species from others location were included in phylogeny. Different monophyl groups were distinct in the cluster A with variant significant support; TSM013, TSM16 and TSM25 the monophyl group and the early branch that separated from the savannah type received high significant support (100 bootstrap) compared to sequences of the isolates TSM 01, 05 and 08 which received a fairly support (65 bootstrap). TSM30 was distinct from the previous branch and from *T. congolense* from Ghana but this branch was not significant (40 bootstrap). Though *T. congolense* isolates from Burkina Faso and Kenya were similar to each other than to the TSM01, TSM05 and TSM08, high significant support indicated that they were similar to *T. congolense* from Ghana (100 bootstrap).

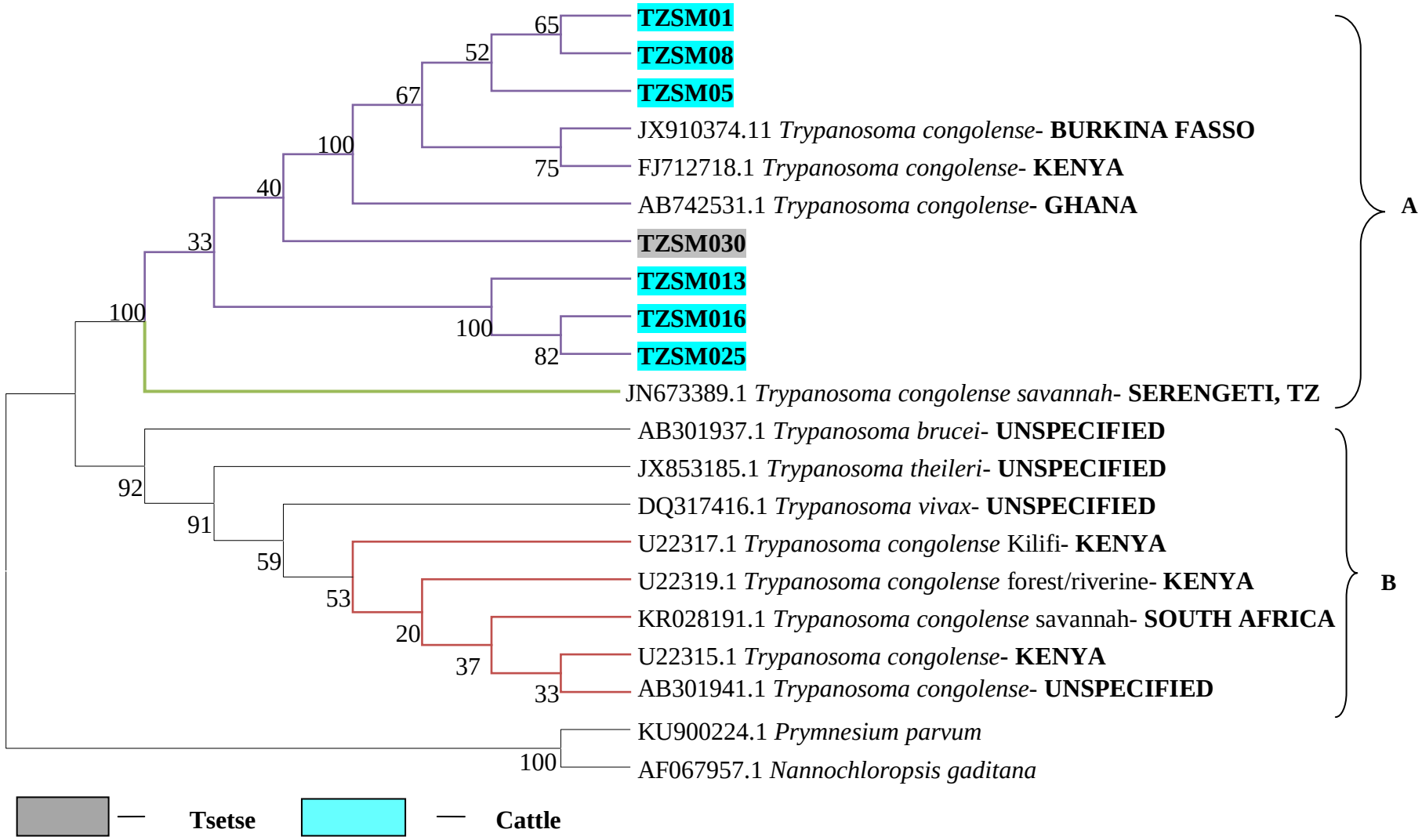

Figure 3: The Maximum Parsimony Phylogenetic tree showing the relationship between *Trypanosoma congolense* of Meatu district and other species

Discussion

ITS1 PCR analysis identified animal trypanosomes; *T. congolense* in cattle and *T. simiae* and *T. godfreyi* in tsetse flies. The occurrence of animal trypanosomes in cattle was higher than of other studies conducted in nearby areas (Manangwa et al. 2016; Ruiz et al. 2015). Nevertheless, *Trypanosoma vivax* that was detected in cattle through a parasitological study reported by Malulu et al. (2017) was not detected. This could be due to genetic diversity of the species in Tanzania (Adams et al. 2009) that the current primers could not amplify or low DNA quantity/quality in sample. However, the trapping of the *Stomoxys and Tabanus* species during the entomological study explain the circulation of the species since are known mechanical transmitters of the pathogen. The observed low trypanosome occurrence in tsetse (1.20%) has also been reported by Salekwa et al. (2014) and this occurrence may be attributed to combination of factors including tsetse refractoriness to trypanosome infections and obligate enterobacteria symbionts; *Wigglesworthia* and *Wolbachia* species (Chen et al. 1999; Welburn &Maudlin 1999; Hao et al. 2001). The mentioned factors are essential mechanisms for tsetse survival since trypanosomes infections impairs reproductive fitness and flight activity. Furthermore, low sensitivity of the ITS1 PCR assay compared to the species specific assay may explain the observed low trypanosomes prevalence, similar observation were reported by Malele et al. (2003) and led into suggestion that these primers are not compared to species specific as they cannot pick sequences that they were not designed for.

Absence of human infective trypanosomes in cattle and tsetse have also been reported by Malele et al. (2011). However, Ruiz et al. (2015) speculated this to be due to active treatment of cattle with trypanocidal drugs. However, this observation does not clear the doubts that there is sleeping sickness pathogen in the area. The observation of *T. godfreyi* and *T. simiae* in tsetse suggests that the vectors fed on wild pigs (warthog) which farmers were also mournful on the destruction they caused on their crops. These findings illuminate the HAT risks in the area as warthogs are known reservoirs of human infective trypanosome (Kaare et al. 2007).

Phylogenetic analysis of 18S rDNA sequences of the salivaria group indicates the diversity within *T. congolense* group where the clustering of the trypanosomes in this group was not uniform. Different evolutionary forces might be behind these changes which have resulted into occurrence of different isolates of the same species within the same environment. The clustering patterns between samples and reference isolate

JN673389.1 which was isolated from Lion (*Panthera leo*) of Serengeti, Tanzania (Northern Meatu) which was also highly significant (100 bootstrap) indicate circulation of trypanosome species between wildlife and domestic animals since both are found in the same ecosystem.

Conclusion

The current study did not find *T. brucei rhodesiense* in tsetse and cattle. However African Animal Trypanosomosis (AAT) pathogens were observed in tsetse and cattle and poses constrain for the livestock production. The phylogenetic analysis supports monophyletic of salivaria trypanosomes which were closely related to other trypanosomes found in others in sub-Saharan Africa countries but they formed monophyl groups to themselves.

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