PREVALENCE AND RISK FACTORS ASSOCIATED WITH TRYPANOSOMOSIS IN CATTLE HERDS IN KILWA DISTRICT LINDI REGION OF TANZANIA

By

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A dissertation submitted to the University of Zambia, School of Veterinary Medicine, Department of Paraclinical Studies, in partial fulfillment of the requirements for the Degree of Master of Science in One Health Analytical Epidemiology

UNIVERSITY OF ZAMBIA

DECLARATION

I, **EMMANUEL NICHOLAUS KASSIAN** do hereby declare that this dissertation is full representation of my own work and that its contents is only for this original work submitted by me to the University of Zambia for the degree of Master of Science in One Health Analytical Epidemiology has not been submitted at any other institution before.

Date..... Signature.....

CERTIFICATE OF APPROVAL

This dissertation of Emmanuel Nicholaus Kassian is approved as fulfilling part of the requirements of the award of the Degree of Master of Science in One Health Analytical Epidemiology of the University of Zambia.

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ABSTRACT

African Animal trypanosomosis (AAT) or *Nagana* and Human African Trypanosomosis (HAT) or Sleeping Sickness are complex chronic, debilitating, emaciating and often fatal diseases of animals and humans, respectively. This cross-sectional study was conducted to determine the prevalence and risk factors associated with bovine trypanosomosis in tsetse-infested Kilwa district, Lindi region, Southern Tanzania. Blood samples were collected from 420 cattle randomly selected from 86 herds from ten villages. A maximum of ten herds per village and at maximum six animals from each herd were selected for sampling. At the same time a questionnaire was administered. Individual animal samples were analysed using microscopy and pooled sample at herd level were analysed by loop mediated isothermal amplification (LAMP). A herd was considered positive if at least one animal in the herd was positive for AAT.

A herd prevalence of 9.3 % (95% CI: 2.9-14.9) was recorded for AAT by microscopy, mainly caused by *Trypanosoma congolense* 5.8% (95% CI = 0.9-10.7), *Trypanosoma brucei* species 5.8% (95%, CI = 0.9-10.7) and *Trypanosoma vivax* 3.5% (95% CI = 0-7.4). Loop mediated isothermal amplification (LAMP) recorded a heard prevalence of 41.9% (95% CI: 30.0-51.4%), mainly caused by *T. congolense* 30.2% (95% CI: 20.5-39.9), *T. brucei* species 25.6% (95% CI: 16.4-34.8) and *T. vivax* 20.9% (95% CI: 12.3-29.7). Most of the cattle herds had mixed infections of these parasites. According to LAMP, Miteja and Matandu villages both had the highest AAT prevalence of 57% (95% CI: 20.3-93.7) while Mavuji had the lowest prevalence of 14% (95% CI: 0-39.7). Data from the present study suggest that district of origin, grazing in Game Reserve, season of increased vector, form of watering point, and affordability of the anti-trypanosomal drugs are risk factors associated with AAT in Kilwa district, southern Tanzania. Use of tsetse trapes and targets, continuous surveillance and monitoring of AAT using more sensitive and specific molecular tests, discouraging settlement and graing in the game reserve are recommended.

DEDICATION

To my mother Petronia Petrol Kassian who gave me life, nurtured me, taught me, dressed me, fought for me, and kissed me unconditionally.There is no woman like her.

My brother Odass Kassian for supporting my early childhood education that made me reache the level that I am able to conduct this study.

My wife Hellene, my son Jovin and my daughter Careen for the patience, encouragement and support they rendered for the whole time when I was away for my studies.

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LIST OF ABBREVIATIONS

µl	Micro liter
^{0}C	Degrees Celsius
AAT	Animal African Trypanosomosis
CATT	Card Agglutination Test for Trypanosomosis
CDC	Center for Disease Control and Prevention
CI	Confidence Interval
CSF	Cerebrospinal Fluid
DDW	Double Distilled Water
DED	District Executive Director
DNA	Deoxyribonucleic Acid
EDTA	Ethly Diamine Tetra acetic Acid
ELISA	Enzyme-linked immunosorbent assay
FTA	Flinders Technology Associates
HAT	Human African Trypanososmosis
IM	Intra muscular
IV	Intra venous
LAMP	Loop-mediated isothermal amplification
PCR	Polymerase chain reaction
PCV	Packed Cell Volume
RIME	Repetitive Insertion Mobile Element
rRNA	Ribosomal Ribonucleic Acid
SAT	Sequential Aerial Spray

SC	Sub cutenous
SIT	Sterile Insect Technique
SPSS	Super perfoming Statistical Software
SRA	Serum Resistant Associated genes
UNZA	University of Zambia
Uv-light	Ultra violet light
VSG	Variant Surface Glycoprotein
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

African Animal trypanosomosis (AAT) or *Nagana* and Human African Trypanosomosis (HAT) or Sleeping Sickness are complex chronic, debilitating, emaciating and often fatal diseases of animals and humans, respectively. The outcome of the infection differs substantially among trypanosome species, among humans, livestock species and within livestock species and breeds (Connor and Van den Bossche, 2004). The disease is endemic in sub-Saharan Africa between latitudes 14^{o}N and 29^{o}S (Fig 2) (WHO, 2000). Human African Trypanosomosis is caused by trypanosomes belonging to the subgenus Trypanozoon namely *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. *T. b. rhodesiense* causes disease in eastern and southern parts of Africa, while *T. b. gambiense* occurs in west and central Africa (Brun *et al*., 2010). HAT is invariably fatal when untreated. Because of the difficulty and cost of surveillance and treatment, HAT has been considered to have profound impact on the socio-economic development of Africa (Mersha *et al.*, 2013). It affects mainly people in poor communities and therefore is not accorded high priority by pharmaceutical companies (Kibona, 2002). According to Kibona (2002), the disease was first recorded in Tanzania in 1922 in Maswa district, south of Lake Victoria. It then spread throughout mainland Tanzania such that it is currently endemic in 9 regions namely Arusha, Manyara, Mara, Lindi, Ruvuma, Kagera, Tabora, Mbeya and Rukwa.

In livestock, AAT is caused by, *T. congolense, T. vivax, T. brucei brucei, T. evansi, T. simiae, T. suis* and *T. equiperdum*. Trypanosomosis is one of the most intractable diseases affecting most species of livestock in Africa (Zewdu *et al.,* 2013). Infection results in high mortality rate in acute cases and in a severe loss of production in chronic cases, thus, effectively retarding agricultural development in large areas of the continent (Zewdu *et al.,* 2013). The main economic losses attributed to AAT are related to cattle mortality and morbidity, diagnosis and treatment costs, the reduction in meat and milk production and the reduction of livestock production areas (Oluwafemi *et al.*, 2007). Because of the complexity of the disease-vector inter-relationship, little progress has been made in controlling the disease since its discovery by Bruce, late in the nineteenth century (Murray and Gray, 1984).

Within the Lindi region, the Kilwa people are traditionally non-livestock keepers, save for small stock of goats and poultry kept by the local communities. In 1996, Kilwa district had 1,436 cattle, 4,879 goats and 5000 sheep (DED Kilwa, 2009). Since 2007, following the evacuation of livestock from Usangu and Ihefu areas in Mbarali district (Mbeya region), which were declared conservation areas and the key water sources for hydroelectric power generation, pastoralist and agro pastoralist have been migrating to Kilwa from the southern highland and western circuits of the country, bringing with them large herds of cattle (Ngailo, 2011). Following these movements, Kilwa district received 12,554 cattle, 18,110 goats and 2,173 sheep from Ihefu (Mbeya region) and other parts of Tanzania. This has brought the number of livestock kept in Kilwa to increase to 14,756 cattle, 28,742 goats and 5,143 sheep (DED Kilwa, 2009). Many pastoralists opted to settle in the

district which has a low human population density, hence ensuring ample grazing land for their animals (Malele *et al.,* 2011). However, animal populations in Kilwa district increased rapidly as a result of this eviction from the wetland sources, settlement of these animals came without much preparation to avert the problem caused by tsetse and AAT in the pasture areas (Pingo *et al.,* 2007). Despite the availability of plenty of grazing areas for animals, the pastoralists were confronted with a serious challenge of tsetse and AAT, which has become a major stumbling block to livestock sector development in Kilwa. Before this study there was little documentation on the prevalence of AAT in Kilwa District (Connor and Halliwell, 1987).

1:2 STUDY JUSTIFICATION

Trypanosomosis has frequently been reported in Tanzania (Connor and Halliwell, 1987; Nonga and Kambarage, 2009; Karimuribo *et al.,* 2011; Haji *et al.,* 2015). Connor and Halliwell, (1987) reported a 16% prevalence of Trypanosomosis in the southern zone of Tanzania. Kilwa Distitrict was formerly inhabited mainly by crop farmers, but in recent years, it has been receiving pastoralists from different parts of the country (Ngailo, 2011). According to Eloy and Lucheis, (2009), many villages in Kilwa District which have good areas for livestock grazing, are bordering Selous Game Reserve. Livestock and humans living in wildlife-domestic animal-human interface areas are at high risk of getting trypanosomosis (Kinung'hi *et al.,* 2006). The role played by wild animals in transmission of AAT in Tanzania is not well documented; the increase in livestock keeping in the area and high movement of pastoralist with their livestock from outside and within the district has increased the contact between human, livestock and wild animals. This

increases the chance of human and livestock getting infected with HAT and AAT, respectiverly. Despite the conducive environment for transmission of trypanosomes between people and animals in Kilwa, little research has been done to determine the prevalence of the disease in the district. Thus there was a need to determine the prevalence of trypanosome species among cattle and determine if those animals carry the human-infective trypanosomes.

1:3 HYPOTHESIS

H_o: Cattle in Kilwa district do not habour trypanosomes so there is no variation in the prevalence of trypanosomosis among different host, social and environment factors.

1:4 OBJECTIVES

1:4.1 GENERAL OBJECTIVES

To determine the prevalence and risk factors associated with AAT in cattle in Kilwa District, Lindi Region, Southern Tanzania.

1:4.2 SPECIFIC OBJECTIVES

1.4.2.1 To determine the prevalence of AAT in cattle in Kilwa district, Southern Tanzania

1.4.2.2 To identify the trypanosome species infecting cattle in Kilwa district

1.4.2.3 To determine the prevalence of human-infective trypanosomes in cattle of Kilwa District.

1.4.2.4 To determine the risk factors associated with AAT in cattle in Kilwa district.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE TRYPANOSOME PARASITES

Trypanosomes are extracellular flagellated parasites belonging to the Kingdom Protista, Subkingdom Protozoa, Phylum Sarcomastigophora, and Subphylum Mastigophora, class Zoomastogophora, order Kinetoplastida, Suborder trypanosomatina, Family Trypanosomatidae, genus *Trypanosoma*. The group presents flagella and an organelle recognized by its kinetoplast and DNA condensation (Eloy and Lucheis, 2009) (Fig. 1). Trypanosomes were originally enzootic and affected only wild animals, including mammals and birds, which served as reservoirs (Auty *et al*., 2012). Later it spread to domestic animals such as cattle, horses and dogs. The disease became a zoonosis when contact between rural inhabitants and natural *Trypanosoma* foci occurred, due to ecological imbalances and increasing migration (Eloy and Lucheis, 2009). Cattle are significantly involved in this context, because they are the main domestic animals kept by pastoralist and contribute to the transmission and maintenance cycles of these parasites. The presence of a wide range of different types of host animals is essential component of tsetse fly distribution (Eloy and Lucheis, 2009).

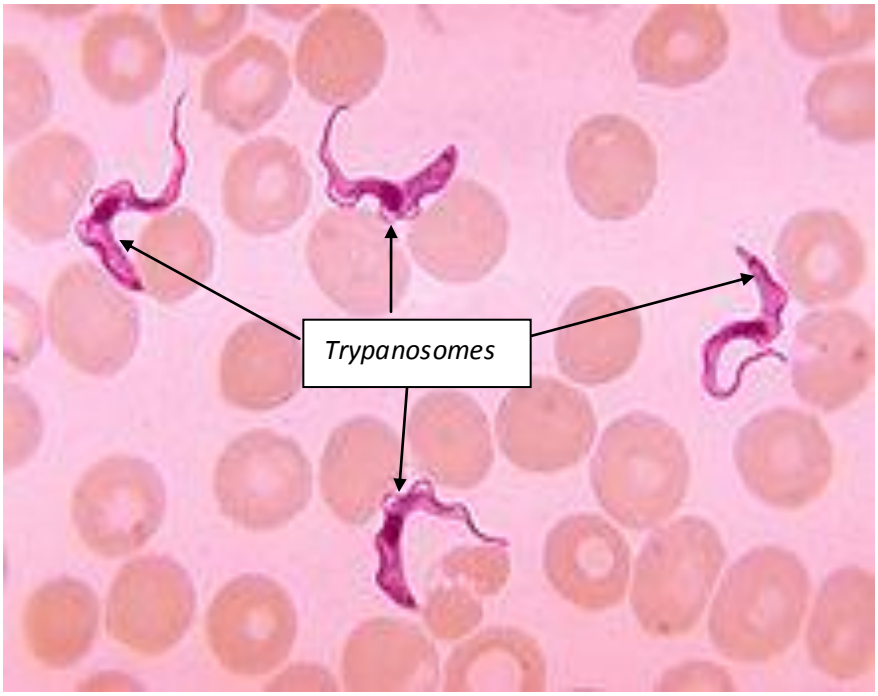

Figure 1. Trypanosoma brucei (CDC, 2012)

Tryypanosoma brucei. rhodesiense and *Trypamosoma bruei. gambiense* cause disease in humans (HAT). On the other hand, *T. congolense*, *T. vivax*, *T. brucei brucei*, *T. evansi*, *T. simiae*, *T. suis* and *T. equiperdum* cause AAT (Abenga *et al.*, 2002). While most of these trypanosome species are transmitted by the tsetse fly, *T. evansi* is mechanically transmitted by biting flies while *T. equiperdum* is sexually transmitted (Hamilton *et al.*, 2004; Namangala, 2012) (Table 1).

African trypanosomes are directly exposed to the host immune system; as such the plasma membrane is completely surrounded by a thick coat, which is impermeable to macromolecules and protective against lytic elements in the host's serum. This surface coat is composed of a repeat of 10 million single species of variant surface glycoprotein (VSGs) copies fixed to the outer membrane by a glycosylphosphatidyl inositol (GPI) anchor (Borst, 2002; Toya, 2009). These molecules are so tightly packed that hidden epitopes and invariant proteins are protected from immune recognition. The highly immunogenic VSG undergoes

antigenic variation, enabling the parasite to escape immune reactions (Borst, 2002; Namangala, 2011). Antigenic variation in trypanosomes is extremely sophisticated and potentially unlimited, in contrast to the limited range for *Plasmodium* (Donelson *et al.*, 1998). The VSGs have two functions. Firstly, they elicit an efficient antibody response that reduces the number of parasites, allowing prolonged extracellular parasite survival in the blood compartment (Morgan *et al*., 2002). Secondly, they allow some parasites to escape destruction through periodic variation of surface epitopes where VSG gene expressions switch from one VSG to another. Moreover, endocytosis activity is very high in trypanosomes, enabling the whole VSG coat to be turned over within only 12 min (Morgan *et al*., 2002). Consequently, antibody/VSG complexes are internalized quickly into the parasite and digested in lysosomes, whereas VSGs are recycled on the cell surface. Such an exceptional rate of intracellular membrane trafficking has been identified as a new role of the VSG in immune system evasion (Morgan *et al*., 2002).

Table 1: Distribution of vertebrate hosts and mode of transmission of salvarian trypanosomes.

Subgenus	Species	Geographical Distribution	Vertebrate host	Cyclic transmission	Non- cyclic Transmission	Importance
Duttonella	*T. vavix*	West, Central and East Africa	Wild and domestic Ungulates	*Glossina*	Biting Diptera	Major disease in cattle and ungulates.
	T. uniforme	West indies, South America		None		Localised mild disease.
Nannomonas	*T. congolense*	West, Central and East Africa	Ungulates+ carnivores	Glossina	Biting Diptera	Major disease in cattle and ungulates.
	T. simiae	West, Central and East Africa	mainly siuds	Glossina		Acute disease in domestic pigs.
	T. godfreyi	West, Central and East Africa	mainly siuds	Glossina		Chronic disease in domestic pigs
Pycnomonas	*T. sius*	Central Africa	Suids	Glossina	Not known	Pathogenic only to pigs
Trypanozoon	*T. b brucei*	West, Central and East Africa	Wild & domestic & humans	Glossina	Biting Diptera	Acute disease in dogs
	T. b. rhodesiense	East and southern Africa				Acute disease in humans.
	T.b.gambiense	West, Central and East Africa	Wild & domestic & humans	none		Chronic disease in humans.
	T. evansi	North Africa, Asia,Central and South America	Wild & domestic & humans	none	Coitus and Biting Diptera	Major disease of camels
	T. equiperdum	Africa, Asia, SouthAmerica	Wild & domestic Animals		Coitus	Major disease of horses
			Wild & domestic equines			

Source: Hamilton *et al.*, 2004

2.2 TRYPANOSOMES AFFECTING LIVESTOCK

Tsetse-transmitted bovine trypanosomosis is considered to be one of the most important diseases constraining livestock production on the African continent, preventing full use of the land to feed the rapidly increasing human population (Murray and Gray, 1984). The main tsetse-transmitted trypanosomes include *T. congolense*, *T. vivax*, *T. brucei brucei* and *T. simiae* (Abenga *et al.*, 2002; Barret *et al*., 2003; Namangala, 2011)**.** However, *T. vivax* may also be transmitted mechanically on the mouthparts of biting dipteral, other arthropods and instruments like syringes (Desquesnes and Dia, 2004). *Trypanosoma evansi and T. equiperdum* are transmitted mechanically and sexually, respectively (Hamilton *et al.*, 2004; Namangala, 2012).

An infection with one of the above trypanosome species may result in a chronic, debilitating, emaciating and often fatal disease but the outcome of the infection differs substantially between trypanosome, between livestock species and within livestock species among breeds (Connor and Van den Bossche, 2004). In cattle, *T. congolense* (subgenus *Nannomonas*) is considered the most pathogenic trypanosome species causing disease in ruminants in Southern and East Africa (Marcotty *et al.*, 2007). Based on the molecular markers, *T. congolense* has been divided into four sub-groups, i.e. Savannah, Forest, Kilifi and Tsavo (Bengaly *et al*, 2002a, b). Limited experiments comparing the virulence of one strain of each subgroup in mice and cattle have shown differences between the subgroups, with the *T. congolense* strain of the Savannah subgroup being the most virulent (Hide and Tait, 2004). In wild animals, such as warthogs, bushbucks, kudus or buffalos, trypanosomes become established but do not produce disease. This is because these animals and the parasites have evolved for

many years resulting in a balanced host/parasite relationship (Namangala, 2011). In domestic animals relationship with the parasite has not fully developed; leading to development of the disease (Namangala, 2011). Among the domesticated animals, humpless cattle such as the N'dama of West Africa (*Bos indicus*) were the first to be introduced into northern and western Africa from about 4,500 BC,-and hence they are adapted to tsetse-transmitted trypanosomes and are thus trypanotolerant (d'Ieteren *et al*., 1998).

2.3 TRYPANOSOMES AFFECTING HUMANS

The tsetse-transmitted *T. b. rhodesiense* and *T. b. gambiense* are the known species which cause disease in humans (WHO, 2000). While *T. b. gambiense* is anthropodic, *T. b. rhodesiense* is zoonotic (Cecchi *et al*., 2009). After tsetse fly inoculation of the parasites at the bite site during a blood meal, parasite rapidly transform by binary fission into blood trypomastigotes resulting, in some cases, into chancre development (Richter *et al*., 2012; Kennedy, 2013). In 1998, about 60 million people were believed to be at risk of HAT while about 40,000 new cases were reported, 300,000 cases remain undiagnosed and therefore remain untreated each year (WHO, 2014). HAT is re-emerging in several sub-Saharan countries (WHO, 2015). Humans are resistant to the other animal-infective trypanosome species which are normally destroyed by the trypanolytic factor (APOL-1) occurring in normal human serum. In contrast, *T. b. rhodesiense* and *T. b. gambiense* cause HAT because they are naturally resistant to APOL-1 (Truc *et al*, 2006; Barrett *et al*., 2007). In very rare cases,

immunocompromised humans are infected with animal-infective trypanosomes species, such as *T. b. brucei, T. congolense* and *T. evansi* (Brun *et al*., 2010).

2.4 THE TSETSE VECTOR.

Tsetse flies are the main vectors responsible for the transmission of AAT and HAT in the 37 sub-Saharan African countries between latitudes 14^0N and 29^0S (Fig 2). Over 11 million km^2 of Africa (about 37% of the continent) is tsetse infected, putting millions of humans and livestock at risk of contracting trypanosomosis (Namangala, 2011). Tsetse flies comprise about 30 species and subspecies (Schofield, 2008; WHO, 2000, Despommier *et al*., 2005); classified into (i) savannah, (ii) riverine and (iii) forest groups (Geiger *et al*., 2005).

On the basis of morpholology and general structure, groups are also known as (i) morsitans, (ii) palpalis and (iii) fusca group (Eloy and Lucheis, 2009; Ugochukwu, 2008). The savannah group includes *G. morsitans, G. swynnertoni, G. longpalpis, G. pallidipes* and *G. austeni* (Swynnerton, 1936). This group, which is found at 900–1800 m above sea level, is a vector of both AAT and HAT in East and Southern Africa (Hargrove *et al*., 2012). The presence of wildlife is the key factor controlling its distribution. (Kaare *et al*., 2007). The riverine group, which prefers the shaded habitat, riverine and lakes, includes *G. palpalis, G. fuscipes, G. martinii, G. quanzensis, G. caliginea, G. pallicera and G. tachinoides* are mainly found in west and central Africa, transmitting *T. b. gambiense*, responsible for about 90% of all HAT cases in sub-Saharan Africa (Katunguka *et al*., 1996). The forest group includes *G. fusca, G.*

tabaniformis, G. nigrofusca, G. fuscipleuris, G. schwetzi, G. severini and *G. vanhoofi*. This group occurs in forest and savannah land, are not important in trypanosomosis transmission since cattle are not kept in thick forests (Kaare *et al*., 2007). Their distribution depend on the forest vegetation and environmental climate, most species in this group inhabit the moist evergreen forests (Jordan, 1986).

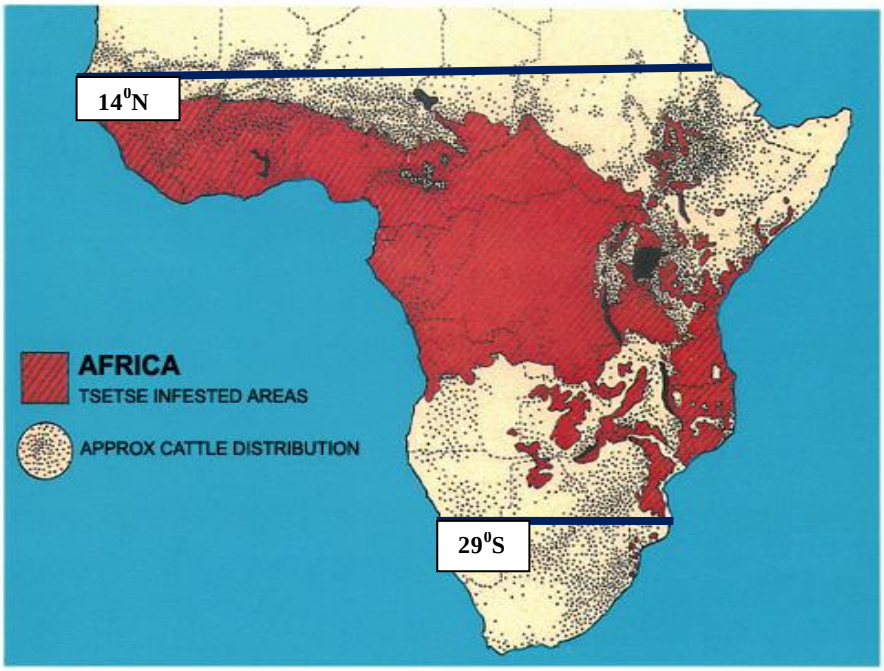

Figure 2: Africa tsetse fly infested areas (WHO, 2000)

In Tanzania, different species of tsetse flies occupy up to two thirds of the land. The species include *G. morstans, G. pallidipes, G. swynnertoni* and *G. austeni*, occupying savannah areas, *G. fuscipes* occupying riverine and lake areas especially around Lake Victoria and Tanganyika and *G. longipennis* and *G. brevipalpis,* occupying fringe forest areas (Ford and Katondo, 1977; Moloo, 1982).

2.5 THE MAMMALIAN HOST

2.5.1. PATHOGENESIS OF TRYPANOSOMOSIS.

Animal African Trypanosonomosis in all species is a progressive and often fatal disease. The pathogenesis of AAT depends on the strain of trypanosome involved, breed of the host animals, age and sex (Leak *et al.*, 1987). Animals African Trypanosomosis is caused by *T. congolense, T. vivax, T. evensi, and T.equiperdum and T. simiae* and to the lesser extent *T.b. brucei* (Blood and Rodostitis, 2007). Although AAT is mainly transmitted by tsetse flies, mechanical transmissions by haematophagous flies also occur. Although AAT affects several domestic animals (Masiga *et al.*, 2002), it is of major importance in cattle (Delespaux *et al.*, 2008; Hotez *et al*., 2009). *Trypanosoma congolense* causes chronic disease, *T. vivax* a more acute disease and *T. b. brucei* a mild to chronic disease in cattle (Blood and Rodostitis 2007)

Trypanosoma vivax usually multiplies rapidly in blood and is evenly dispersed throughout the cardiovascular system, whereas *T. congolense* tends to aggregate in small blood vessels and capillaries of the heart, brain and skeletal muscle from where a small proportion of parasites enter the blood circulation (Stuart *et al.*, 2008). *Trypanosoma brucei*, and rarely *T. vivax*, has the added capability of passing out of the capillaries into the interstitial tissues and serous fluids of body cavities where they continue to multiply (*Luckins et al*., 1994). *Trypanosoma vivax* and *T. congolense* exert their effect mainly by causing severe anaemia and mild to moderate organ damage. Trypanosomes can also pass through the placenta and into the fetus in pregnant animals, as a result some cows abort and cause premature and still birth and

sometimes via mother to offspring transmission (WHO, 2010). A cerebral form of the disease occurs with *T. brucei* alone or in mixed infections with the other species. Onset and severity of the anemia is directly related to the appearance of the parasite in the blood and to the level of the parasitaemia. The affected animals develops clinical signs like undulating fever, progressive anemia, lymphadenopathy, emaciation and loss of body condition, decreased milk production in dairy animals, neurological signs, oedema, diarrhoea, keratitis, lacrimation, loss of appetite, stress, abortions, premature births and finally if not treated death (Urquart *et al*., 1995; Uilenberg, 1998; Blood and Rodostitis 2007; Adeiza *et al*., 2008; Namangala, 2012).

The progression of HAT is grouped into two stages, the first and the second stage (Kuepfer *et al*., 2011; Kagira *et al*., 2011). HAT takes two forms, depending on the parasite involved, chronic and acute form. **C**hronic form if caused by *Trypanosoma brucei gambiense,* which is found in 24 countries in west and central Africa (WHO, 2015). Currently accounts for over 98% of reported cases of sleeping sickness. A person can be infected for months or even years without major signs or symptoms of the disease, it takes an average of 18 months for the disease to reach second stage. When symptoms emerge, the patient is often already in an advanced disease stage where the central nervous system is affected. (Kristensson *et al*., 1995, Atouguia and Kennedy, 2000). On the other hand acute form of the disease is caused by *Trypanosoma brucei rhodesiense* which is found in 13 countries in eastern and southern Africa (WHO, 2015). This form represents fewer than 2% of reported cases (WHO, 2015). Signs and symptoms are observed a few months or weeks after

infection. And the disease develops rapidly and invades the central nervous system (WHO, 2015).

The first stage starts once the metacyclic trypanosomes are injected into the host by the fly during feeding, they multiply at the subcutaneous site and in human, provoking a local skin reaction called a chancre which is most pronounced in a fully susceptible host and may be slight or absent with some strains or species of trypanosomes (Brun *et al*., 2010). Within a chancre, metacyclic parasites change to trypomastigote form and enter the blood stream directly or through the lymphatics. The appearance of a chancre is accompanied by the development of early clinical signs including undulating fever, headache, pruritus, itching, and lymphadenopathy (Kennedy, 2004, Brun *et al*., 2010). As the lesion decreases in size, an increased numbers of mature plasma cells, macrophages, eosinophils, and mast cells are found within it. This composition of cells within the chancre suggests an initial immune response. However this largely depends on the species of infecting trypanosomes. The second stage occurs when the parasite crosses blood brain barrier and invades the central nerves system causing damage which leads to development of several sign including psychiatric disorders, depression and altered sleep–wake patterns (Atouguia, and Kennedy, 2000). This sleeping disorder is characteristic and leads to the name sleeping sickness, (meningo-encephalitis) (Brun *et al*., 2010; Namangala, 2012),. Trypanosomes and/or increased numbers of leukocytes are found in the cerebrospinal fluid (CSF) **(**WHO, 2002a). If not treated it leads to coma and death (Brun *et al*., 2010; WHO, 2010; Kennedy 2013).

2.5.2 WILDLIFE RESERVOIRS

Trypanosomes are true multi-host parasites capable of infecting a wide range of wildlife species that constitute a reservoir of infection for both man and domestic animals. Wildlife animal species like bushbuck, duiker, giraffe, impala, lion, warthog, waterbuck, zebra, leopard and buffalo, are believed to habour human infective trypanosomes (Anderson *et al*., 2011). Animal African Trypanosomosi can be maintained in a closed ecosystem for more than a century as long as ecological factors that favor adequate interplay between tsetse and wildlife reservoir hosts and livestock are kept in balance in a suitable habitat. The persistence of AAT in wildlife in an ecosystem is dependent on interplay of several biological and ecological factors that include vector tsetse flies, wildlife, habitat, and conservation strategy. The relative abundance of wild game has a significant influence on the survival of tsetse flies (Harriet *et al*., 2012). Seasonal variations in the movement of wild hosts have been reported to significantly influence the ecological behavior of *Glossina* species (Newberry *et al*., 1982). During the rainy season, wild bovid are widely dispersed covering large areas within the national parks, with water supplies being widely distributed. However, during the dry season, as the water sources dry up, animals move to areas close to the river with increasing tsetse densities. This trend of seasonal movement has a significant influence on the distribution of tsetse flies, rendering the riverine area to be the most densely populated within tsetse populations. This reflects the ability of animal hosts to maintain infectious trypanosomes transmissible to other naive wild and domestic animals (Robison, 1998). In the wild ecosystem, both host and parasite have evolved over time to establish a balanced relationship that does not

make them susceptible to the extent of producing disease, unless stressed, hence making them play an important role as reservoir hosts from which a vector gets the infection and transmits it to the susceptible host either human or domestic animals (Aksoy *et al*., 2003; Brun *et al*., 2010).

2.5.3 ANIMAL TRYPANOSOMOSIS SITUATION IN TANZANIA

In Tanzania, tsetse-borne bovine trypanosomosis continue to be the menace in the livestock industry despite the age long attempts to control the disease (Connor, Halliwell, 1987; Nonga and Kambarage 2009; Karimuribo *et al*., 2011; Haji *et al*., 2015). Bovine trypanosomosis is among the most important diseases that are responsible for reduced livestock productivity and is responsible for up to 75% of the morbidities and mortalities in cattle (Jahnke *et al.*, 1988). Southern Tanzania is one of the areas in the country that is tsetse infested and keeping of livestock has been severely constrained by tsetse transmitted AAT. Connor and Holliwell (1987) reported that 16% of cattle sampled from southern zone of Tanzania were infected with AAT. Out of these, 56% were infected with *T. Congolese*, 17% were infected with *T. vivax,* and 2.2% were infected with *T. brucei,* while the rest had mixed infections.

2.5.4 HUMAN TRYPANOSOMOSIS SITUATION IN TANZANIA

In Tanzania, like in most other Southern and East African countries, HAT due to *T. b. rhodesiense* is the only form of the disease known to occur (Stich *et al.*, 2002). Tsetse distribution in Tanzania has been altered due to several reasons including change in land use like human settlement and their activities, infrastructure development and

land reform policies (Malele *et al*., 2011) According to the Tanzania tsetse distribution map, the area which is tsetse infected with high to low risk is 27% and non risk area is 57%. The overall tsetse infested risk area is about 33% (1/3) of the country and over 4 million people living in rural areas are at risk of contracting HAT (Ford and katondo, 1977, MLFD, 2011, Daffa *et al* 2012). The rural populations whose livelihoods depend on agriculture, animal husbandry or hunting are the most exposed to the tsetse bites. However, only less than 1% of the people at risk of infection are under regular medical surveillance (Kibona *et al.*, 2006). Between 1996 and 2005, a total of 2571 cases of HAT were reported in Tanzania (Mcharo and Kitua, 2001).

In Tanzania HAT presents a serious threat to human health with an annual incidence of 400 cases (Mcharo and Kitua, 2001). The animal reservoir for *T. b. rhodesiense* in Tanzania comprises both wild and domestic animals, all of which are documented to play a role in maintaining the disease (Mwambu and Mayende, 1971). However, the number of cases of sleeping sickness reported among tourists visiting the Tanzanian National Parks increased from 6 cases between 1998 and 2000 to 13 in 2001 (Kaare *et al.*, 2006). One of the major constraints in HAT control in Tanzania is insufficient information regarding the actual extent of the disease in the country. This among other factors has been attributed to a weak disease surveillance system, poor diagnostic capability and insufficient trained staff. While vector control is necessary for the reduction of human vector contact and thus reduction in transmission of the disease, surveillance is imperative for early and effective diagnosis (Atouguia and Kennedy, 2000).

2.6 DIAGNOSIS OF TRYPANOSOME INFECTIONS

Besides clinical diagnosis, direct (parasitological) and indirect (serological) diagnostic methods with varying degrees of sensitivity and specificity are available for diagnosis of AAT and HAT (Jamonneau *et al.,* 2003). Due to the multisystemic nature of trypanosomosis and the variety of clinical signs, diagnosis faces a lot of challenges. Novel sensitive molecular diagnostics including polymerase chain reaction (PCR) (Jamonneau, *et al.,* 2003) and loop-mediated isothermal amplification (LAMP) (Kuboki *et al.,* 2003) are more useful diagnostic methods. Diagnosis of trypanosome infections in humans or domestic livestock is a basic requirement for prevalence determination as well as for planning and implementing chemotherapy and for monitoring vector control operations. Early and accurate detection of these parasites is essential for successful interventions of HAT (Brun *et al*., 2010).

2.6.1 CLINICAL AND POST-MORTEM SIGNS

Intermittent fever, anemia and loss of body condition are important parameters used routinely for tentative diagnosis of trypanosomosis in areas where this disease is endemic and laboratory services are not available (Atouguia and Kennedy, 2000). However, diagnosis of trypanosome infection based on clinical signs alone is unreliable (Wastling and Welburn, 2011). Necropsy of an animal that died of AAT reveal generalized carcass emaciation, enlarged lymph nodes, enlarged liver and petechial haemorrhages of the serosal membranes, especially in the peritoneal cavity (Taylor and Authie, 2004). However, like clinical signs, these pathological finding are

not pathognomonic but they can help in giving a tentative diagnosis of AAT in a herd (Taylor and Authie, 2004).

Both Gambian and Rhodesian HAT have smillar clinical features, but differ significantly in the course of disease. The early stage of the forms of HAT occurs when the parasite presence is mainly circulating in blood and lymphatic vessels and is characterized by fever, malaise, generalized rash, headache, myalagia, prurites, transient oedema, lymphadenopathy, spleenomegaly and hepatomegaly. The late stage HAT (meningoencephalitis or neurological stage) begins after the parasite crosses the brain blood barrier (BBB) into the CNS, and is characterized by headache, sensory disturbance, poor coordination, mental and physical lethargy. Psychrogical and behavioral change may preced clinical neurological like tremor, fasculation, cerebellar ataxia and signs of meningitis or encephalitis, loss of consciousness, coma and death.

2.6.2 MICROSCOPY

The diagnosis of AAT is made through laboratory tests, because the clinical features of infection are not sufficiently specific. The diagnosis rests on finding the parasite in body fluid or tissue and lymph node biopsy by microscopy (Chappuis *et al*., 2005; WHO, 2006). Blood and lymph fluid can be directly examined as wet smears and trypanosomes are detected to be present by light microscopy using unstained wet smears or Giemsa-stained thick and thin smears (Chappuis *et al*., 2005). Although highly specific, microscopy often lacks sensitivity (Connor and Halliwell, 1987). This may be improved by centrifugation of blood and microscopic examination of the resultant buffy coat (De Rissio *et al.*, 2010).

In human HAT diagnosis rest up on demonstration of trypanosomes by microscopic examination of chancre fluid, lymphnode aspirates, blood, bone marrow or in the late stages of infection cebral spinal fluid (CSF). A wet preparation smear is eamined for the motile trypanosome, and in addition, a smear should be fixed, stained with giemsa and examined. Concentration techniques can be used prior to microscopic examination. For blood sample this includes centrifugation followed by examination of the bufy coat. For other samples such as spinal fluid, concentration techniques include centrifugation followed by examination of the sediments.

2.6.3 SEROLOGY

2.6.3.1 CARD AGGLUTINATION TEST FOR TRYPANOSOMIASIS (CATT).

Diagnosis of HAT in active screening campaigns involves a primary serological screening using the Card Agglutination Test for Trypanosomiasis (CATT), which targets antibodies produced against the variant surface antigen which is expressed early in most *T. b. gambiense* infections. (Chappuis *et al*. 2005). However, variable sensitivity (and failure to detect *T. b. rhodesiense* parasites which lack the CATT antigen) means microscopic identification of parasites in the lymph or blood is required for confirmation, while diagnosis at Stage 2 requires the identification of either parasites or white blood cells in Cerebral Spinal Fluid (CSF) (Chappuis *et al*. 2005).

However, the use of this technology in diagnosis of AAT is limited by the by the fact that ant-body ELISA are not species-specific because of strong cross reactions between the pathogenic *trypanosoma* species and lack of distinction between the past

and present infection hence easy to get false positive and negative. (Desquesnes *et al.*, 2001)

2.6.3.2 ELISA

The antibody tests in current use suffer from a lack of well-defined antigens necessary for designing simple and accurate tests that are easily adaptive for field use. Secondly, the detection of ant-trypanosoma antibodies in serum cannot distinguish between an active infection and a past infection (Voller *et al.,* 1978 Desquesnes *et al.,* 2001). Luckins and Mehlitz (1976) used micro plate-ELISA system in their study of bovine trypanosomosis and found that cattle developed positive ELISA values after infection but it was not possible to differentiate between *T. vivax, T. congolense, T. b. brucei*, *T. b. rhodesiense* or *T. b. gambiense*. The length of time taken for antibodies to disappear from circulation after a successful therapy of cattle is not yet clear. However the detection of circulating trypanosome antigens may be a more sensitive means of practical diagnosis and could increase the reliability of detection of current infection in animals undergoing trypanocidal drug therapy during a period at which it is not possible to isolate parasite from the peripheral circulation (Voller *et al.,* 1978).

2.6.4 MOLECULAR TECHNIQUES

Molecular techniques such as PCR and LAMP have significantly improved the sensitivity and specificity of trypanosome diagnosis of AAT/HAT compared to the traditional parasitological methods (Mugittu *et al.,* 2001).

2.6.4.1 POLYMERASE CHAIN REACTION (PCR)

Polymerse Chain Reaction (PCR) which is used to amplify selected sections of DNA or RNA, is highly efficient in that it is based on the use of an enzyme, DNA polymerase, which amplifies sequences of DNA bases until sufficient material is produced to be detected (Geysen *et al*.,2003). The PCR permits identification of parasites at levels far below the detection limit of the commonly used parasitological techniques using species-specific DNA hybridization probes (Majiwa *et al*., 1985; Majiwa and Webster, 1987). However, this technique was shown to be relatively insensitive and in some cases was problematic for detection of *T. vivax*. The two internal transcribed spacer PCR (ITS –PCR) based assays utilizing the same diagnostic target which has a higher diagnostic capacity is used instead of the species specific tests. This is used as universal primers as it can detect all the pathogenic trypanosome species (Thumbi *et al*, 2008; Nakayima *et al*. 2012). The test detects many Animal /Human pathogenic *Trypanosoma* species in a single PCR thereby reducing the number of reactions per sample (Nakayima *et al* 2012). This saves time and costs as compared to species-specific PCR. The expected products of ITS-PCR are species-specific with size differences as the technique is able to detect the following African trypanosome species: Trypanozoon, *T. congolense* (River/ Forest), *T. congolense* (Kilif), *T. congolense* (Savannah), *T. vivax, T. simiae, T. evansi, T. congolense* (Kenya Coast), *T. godfreyi* and *T. theileri*. It is able to detect a single trypanosome genome/40µl of blood and has been optimised for PCR amplification of blood applied to paper (Whatman FTA) permitting direct PCR analysis of wide material. Polymerase chain reaction (PCR) is a method used to analyze a short

sequence of DNA (or RNA) even in samples containing only minute quantities of DNA or RNA (Desquesnes *et al*., 2001; Adams *et al*., 2006). Polymerase Chain Reaction (PCR) has a wide range of applicability as the fundamental tool for promised better sensitivity and it has remained the most appropriate method for laboratory based diagnosis compared to parasitological methods (Masiga *et al*., 1992; Masake *et al*., 1997; Morlais *et al*., 2001). Its use in the routine diagnosis in clinical settings in Africa has been limited in that it is very expensive in terms of cost, requirement for skilled manpower, need for precision instruments and elaborate visualisation methods, (Njiru *et al*., 2008; Namangala *et al*., 2012).

2.6.4.2 LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

Loop mediated isothermal amplification (LAMP) is a novel strategy which amplifies DNA with high sensitivity and rapidity under isothermal conditions (60-65°C), producing large quantities of DNA within 30-60 minutes (Notomi *et al*., 2000; Thekisoe *et al*., 2007). This allows visual detection of amplicons by naked eyes or through measurement of turbidity or fluorescence (Wastling *et al*., 2010). This method employs a DNA polymerase and a minimum set of four specially designed primers that recognize a total of six distinct sequences on the target DNA (Notomi *et al*., 2000). An inner primer containing sequences of the sense and antisense strands of the target DNA initiates LAMP. The strand displacement DNA synthesis primed by an outer primer releases a single-stranded DNA. This serves as template for DNA synthesis primed by the second inner and outer primers that hybridize to the other end of the target, which produces a stem–loop DNA structure (Notomi *et al*., 2000). This

technique has an advantage that it can be carried out with minimal equipment especially in HAT/AAT endemic countries (Picozzi *et al*., 2002; 2008). The LAMP method has several advantages over PCR in that: (i) LAMP amplification can be achieved using simple heating device that maintains temperature at isothermal (60–65^{0}C) conditions, (ii) amplification can be achieved using partially or non-processed template therefore DNA extraction may not be necessary, (iii) reactions are rapid and require shorter time (iv) sensitivity is equal or higher than that of PCR and (v) the technology allows the use of varied product detection formats. These characteristics make LAMP strategy ideal for AAT/HAT diagnosis in resource poor endemic regions. (Nagamine *et al*., 2002, Kaneko *et al*., 2007; Njiru *et al*., 2008)

2.7 TREATMENT AND CONTROL

2.7.1 TREATMENT OF TRYPANOSOME INFECTIONS IN LIVESTOCK

Trypanocidal drugs remain the principal method of AAT control in most African countries. The chemotherapy and chemoprophylaxis of AAT relies essentially on several drugs (Table 2) as follows: Diminazine aceturate is used as curative drug in cattle against *T. vivax, T. congolense* and *T. brucei* (Uilenberg, 1998). This drug has an advantage of having low toxic effects, easy utilization and being effective against trypanosome strains which are resistant to other drugs (Taylor, 1998). Homidium bromide and Homidium chloride are used as a curative and prophylactic drug in cattle and equids against *T. vivax* and *T. congolense* (Brander *et al*., 1991). Isometamedium is used as a curative and prophylactic drug in cattle against *T. vivax* and *T. congolense* (Delespaux *et al*., 2010). Quinapyramine are effective drug in horses, camels, pigs and

dogs against *T. vivax, T. congolense, T. brucei, T. evansi, T. equiperdum* and *T. simiae* (Tuntasuvan *et al*., 2003). Suramin is used as a curative and some times prophylactic drug in horses, camels, and dogs against *T. brucei* and *T. evansi* (Tuntasuvan *et al*., 2003). Melarsomine dichlorhydrate is used as a curative and prophylactic drug in camels against *T. evansi* (Merck Veterinary Manual). Development of resistance against many drugs which are in the market is the main problem in treatment of AAT (Geerts *et al*., 2001; Sinyangwe *et al*., 2004).

Table 2: Drugs used for treatment of trypanosomosis in livestocks

Generic name	Trade name	Solution used	Dosage rate	Route	Remarks
Suramin	Naganol	10%	10mg/kg(1ml/10kg)	IV	Mainly used in camels (*T.evansi*)
Diminazine aceturate	Berenil,Trypazen	7%	3.5-7mg/kg(1-2ml/20kg)	IM	Treats several animal species
Homidium bromide	Ethidiumbromide	2.5%	1mg/kg(1ml/25kg)	IM	Used in cattle, small ruminants. Soluble in hot water (Potential carcinogenic)
Homidium chloride	Ethidium C, Novidium	2.5%	1mg/kg (1ml/25kg)	IM	As above but soluble in cold water.
Quinapyramine methyl ulphate	Antrycide,Trypacide	10%	5mg/kg(1ml/20kg)	SC	Treats *T. evansi & T. brucei* in camels & horses.
Melcy	Cymelarsan	0.5%	0.25-0.5mg/kg (1-2ml/20kg)	IM/SC	Used only in camels against *T. evansi*.
Isometamidium chloride	Samorin, Trypamidium	1%	0.25-.5mg/kg(1.25ml/50kg)	IM	Used mainly in cattle and contains Homidium (Potentially carcinogen)

2.7.2 TREATMENT OF TRYPANOSOME INFECTIONS IN HUMANS

Treatment of HAT relies on a limited number of highly toxic drugs, (Brun *et al*., 2010). Four drugs are registered for the treatment of sleeping sickness depending on the stage of the diseases (Richter *et al*., 2012). Pentamidine is used for the treatment of the first stage of HAT due to *T. b. gambiense* (Richter *et al*., 2012). Despite non-negligible undesirable effects, it is in general well tolerated by patients. It is given at 4 mg / kg once per day, usually over a 7-day period, intramuscularly. Intravenous injection induces a potentially dangerous hypoglycaemic response (Waskin *et al* 1988). Injection-site colouration, nephrotoxicity, leucopenia and liver enzyme abnormalities are common adverse events (WHO, 2014). Suramin is used for the treatment of the first stage of *T. b. rhodesiense* (WHO, 2015). It provokes certain undesirable effects, in the urinary tract and allergic reactions. In a typical course it is given by slow intravenous injection once every 3–7 days, over a 4 week period (Brun *et al*., 2010). Suramin is more than 99% protein bound in serum with a terminal half-life of 41–78 days. Blood–brain barrier permeation is minimal, hence restriction for the first stage treatment of *T. b. rhodesiense* HAT. The uptake into trypanosomes is via ISG75 receptor-mediated endocytosis and interference with multiple points in the endocytic pathway leads to decreased suramin sensitivity, although how it actually kills cells once inside remains unknown. Adverse reactions include pyrexia, nephrotoxicity, nausea, urticaria, neuropathy and anemia, (WHO, 2014).

Melarsoprol is used to treat the second stage of both forms of HAT. It is derived from arsenic and has many undesirable side effects. The most dramatic is reactive

encephalopathy (encephalopathic syndrome) which can be fatal (3% to 10%) (Atouguia and Kennedy, 2000; Legros *et al.,* 2002). Melarsoprol is given by intravenous injection as a 3.6% solution in propylene glycol, usually over a 10-day course. An increase in resistance to the drug has been observed in several foci particularly in central Africa (Kennedy, 2004). However, an often fatal reactive encephalopathy afflicts 5–10% patients taking the drug, other adverse effects include pyrexia, headache, pruritus, thrombocytopaenia and heart failure (Atouguia and Kennedy, 2000; Legros *et al.,* 2002). Eflornithine is a drug that is less toxic than melarsoprol, and is effective against *T. b. gambiense* (WHO, 2015). The regimen is strict and difficult to apply (WHO, 2014). A combination treatment of nifurtimox and eflornithine is used to simplify the use of eflornithine in monotherapy, but unfortunately it is not effective for *T. b. rhodesiense* (Brun *et al*., 2010). Eflornithine inhibits the polyamine biosynthetic enzyme ornithine decarboxylase, which turns over more rapidly in man than trypanosomes, partly explaining the selective effect of the drug. In monotherapy it is given at 100 mg kg body weight at 6 h intervals (i.e. 400 mg/kg per day) by intravenous infusion for 14 days (Legros *et al.,* 2002). Adverse effects include fever, headache, hypertension, macular rash, peripheral neuropathy and tremor, gastrointestinal problems including diarrhoea. Infusion-associated septicaemia is a major issue (WHO, 2014).

2.7.3 VECTOR CONTROL

Trypanosomosis is controlled either directly through the use of trypanocidal drugs or indirectly by controlling tsetse fly and there by breaking the disease transmission cycle

(Jordan, 1986). A wide variety of tsetse control techniques have been developed and have undergone trial, and gave satisfactory control of tsetse within any given target region. These control techniques include: clearing of vegetation, game elimination/exclusion, Insecticides Ground spraying, Sequential aerial technique (SAT), Traps and targets and use of sterile insect technique (Allsopp, 2001, Vreysen *et al*., 2004).

2.7.3.1 BUSH AND GAME CLEARANCE

Early attempts to control tsetse included extensive bush clearance (designed to eliminate the shaded places where tsetse rest and lays their larvae) and extensive shooting of wild game animals (designed to eliminate the wild blood sources used by the tsetse flies). Although widely effective, such methods can no longer be recommended on environmental conservation grounds (Allsopp, 2001; Aksoy *et al*., 2003).

2.7.3.2 INSECTICIDE GROUND SPRAYING

The application of residual insecticides to tsetse resting sites was very widely used, but is now discouraged due to concerns about effects on non-target organisms (Barret *et al*., 2003).

2.7.3.3 SEQUENTIAL AERIAL TECHNIQUE (SAT)

Because of the tsetse flies exquisite susceptibility to modern insecticides including pyrethroid such as deltermethrin, high levels of tsetse control can be achieved by sequential aerial spraying of ultra low dosages of biodegradable products. Using

modern global positioning systems (GPS), SAT can now be applied highly accurately along pre-planned flight lines. This method can be used to eradicate tsetse flies but it has the set back of being expensive (http://www.africa-union.org/PATTEC/home.htm; Aksoy *et al*., 2003). The SAT was used in Botswana to control Tsetse fly (*G. m. centralis*) in Okavango Delta and the adjacent Kwando and Linyati area. Also in Ghana, SAT was used to reduce tsetse fly species *G. tachinoides* and *G. palpalis gambiense* (Kgori *et al*., 2006; Allsopp & Phillemon Motsu, 2002). In Tanzania this method was used extensively in Northern part especially Arusha region, Babati area using either residual or non residual insect cide (Tarimo, 1974). In addition, it has a disadvantage of affecting non-target speies.

2.7.3.4 TRAPS AND TARGETS.

Trapping techniques have been greatly enhanced by development of designs that mimic the fly's perception of vertebrate hosts. These generally use blue and black cloths which are impregnated with insecticide and there in a shape that attracts the flies and then funnel them upwards into a netting trap – usually in the form of a monoconical (pyramidal) or biconical shape (Lancien *et al*., 1981; Barret *et al*., 2003; Hargrove, 2003a). For tsetse control, a simpler and cheaper device involves a suspended screen of blue and black cloth (often known as a tsetse target) impregnated with a biodegradable pyrethroid insecticide such as deltamethrin. Flies are attracted by the blue segments and land on the black segment, quickly succumbing to the insecticide. The effectiveness of traps and targets can greatly be enhanced by addition of appropriate odour bait like acetone or octenol (Jordan 1993). In Tanzania, traps and

targets have been used in some areas of the country which are tsetse infested including Mkwaja, North and Central Tanzania and Kasulu (Gao *et al*., 1990; Muagirwa *et al*., 1994; Daff *et al*., 2003). In Kilwa district area targets are in use although in small quantity.

2.7.3.5 LIVE BAIT TECHNIQUES

This is done using live animals or moving objects like sprayed vehicles. It has become the more preferred method of tsetse control in most African countries (Green, 1994). Similar to the concept of traps and targets, the live bait techniques involves treating cattle with insecticide formulations pyrethroids such as deltermethrin, usually by means of cattle dips, or as pour-on, spot-on, or spray-on (Thompson *et al.*, 1991). These are highly effective against tsetse flies, and have the additional advantage of controlling other flies and cattle ticks (Chizyuka and Liguru, 1986). In Tanzania, this method has reduced tsetse flies and ticks in ranches like Mkwaja and Kagera and currently is the most common method which has been easily adopted by farmers to control tsetse flies (Fox *et al*., 1991, Hargrove *et al*., 2003).

2.7.3.6. STERILE INSECT TECHNIQUE (SIT)

This is the biological tsetse control method which involves sustained and systematic release of sterile male insects among the indigenous target population. It exploits the particular mating behavior of tsetse, whereby female flies rarely mate more than once (Mkanyi and Feldmann, 2000; Barret *et al*., 2003; Vreysen *et al.,* 2004). In this method of control, male flies are mass reared in the laboratory, sterilized by

irradiation, and released to mate with wild females. Females mated with sterile males are unable to produce offspring. Unlike all other tsetse control techniques, SIT has no effect on non-target organism, is more efficient at lower fly densities, and is ideally suited to the final phase of local tsetse eradication (Moloo, 1982). For this technique to be effective, it requires pre-implementation of other methods to reduce population of tsetse and is more successful in small areas (Mkanyi and Feldmann, 2000; Vreysen *et al*., 2004). In Tanzania it was first conducted against *G. m. morsitans* in Mkwaja ranch and then used to control *G. austeni* in Zanzibar Island in Unguja where currently there are no cyclically transmitted trypanosomoses (Msangi *et al*., 2000).

2.8 EPIDEMIOLOGY

2.8.1 EPIDEMIOLOGY OF ANIMAL AFRICAN TRYPANOSOMOSIS

The tsetse-fly-infested area of Africa extends from the southern edge of the Sahara desert (lat. 14° N) to Angola, Zimbabwe, and Mozambique (lat. 29° S). This puts over 29 million people 45 million cattle and unknown million of sheep and goats in Africa being at risk of contracting trypanosomosis from tsetse fly (Ford and Katondo, 1977, FAO, 1979, Deichmann, 1996). Of the three main African animal trypanosomes, only *T. vivax* is known to be transmitted both cyclically in Africa and mechanically in Africa and America (Gardiner and Mahmoud, 1990). *Trypanosoma congolense* is considered to be the most important cause of AAT in East and West Africa and is a major cause of the disease in cattle. Sheep, goats, horses, and pigs may also be seriously affected. Indigenous breeds of dogs have resistance towards *T. congolense* compared to exotic breeds and often develop chronic infection (Boyt, 1988).

Trypanosoma vivax primarily affects cattle, sheep and goats is the most important cause of AAT in West Africa. This trypanosome readily persists in areas free of tsetse flies (for example, in Central and South America and in the Caribbean), where it is transmitted mechanically by biting flies or contaminated needles, syringes, and surgical instruments (Curasson, 1943; Desquesnes and Gardiner, 1993; Desquesnes and Dia, 2003, 2004). *Trypanosoma brucei brucei* affects cattle, horses, dogs, cats, camels and pigs throughout sub-Saharan Africa. Infection of cattle, sheep, goats and sometimes pigs mainly results in mild or asymptomatic infection (Boyt, 1988; Matete, 2003).

Cattle, sheep, goats, pigs, horses, camels, dogs, cats, and monkeys are susceptible to AAT and may suffer syndromes ranging from subclinical mild or chronic infection to acute fatal disease. More than 30 species of wild animals can be infected with pathogenic trypanosomes. However, the presence of trypanosomes in wildlife does not necessarily make them susceptible to disease unless stressed; hence they act as carriers or reservoir hosts from which tsetse flies acquire infection (Brun *et al*., 2010). Livestock grazing in the interface areas with wild animals are easily infected as the latter act as reservoir for trypanosomes. Genetic resistance to AAT has been attributed to certain breeds of livestock, e.g. West African N'dama. This resistance is manifested by ability to withstand the adverse effects of trypanosomes by regulating parasite growth and their ability to prevent or reduce the rate and degree of development of anemia (Seifert, 1996).

2.8.2 EPIDEMIOLOGY OF HUMAN AFRICAN TRYPANOSOMOSIS

People living in an area which is tsetse infested are at high risk of being infeted by HAT. Equally livestock headsmen are also at risk of getting HAT either from reservoir wild animals or from the infected livestock. Travelers undertaking outdoor activities such as hiking, camping and ecotourism in HAT endemic countries are at higher risk (Eloy and Lucheis, 2009). *Trypanosoma brucei rhodesiense* which causes Rhodesian HAT is found in focal areas of Eastern and South East Africa. Over 95% of the Rhodesian HAT cases occur in Tanzania, Uganda, Malawi, and Zambia. Animals are the primary reservoir of infection (Eloy and Lucheis, 2009). On the other hand, *T. b. gambiense* which causes Gambian HAT is found predominantly in Central Africa and in limited areas of West Africa. Most of the HAT in sub-Saharan Africa is caused by this form of the parasite. Over 95% of the cases of Gambian HAT are found in Democratic Republic of Congo, Angola, Sudan, Central African Republic, Chad, and northern Uganda. Because of the prolonged course, humans serve as important source of infection of *T. b. gambiense* although domestic animals such as pigs, dogs, goats and sheep may also serve as reservoirs (Eloy and Lucheis, 2009).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1 STUDY AREA

The study was conducted in Kilwa district, Lindi region, Southern Tanzania (Fig. 3). Kilwa district lies on Latitude 8°20's to 9°56's and Longitude 38°36'E to 39°50' E. To the north it borders Rufiji District, Coast Region, Lindi and Ruangwa Districts in the South, Liwale District in the West and to the East, the Indian Ocean (MacLennan, 1980). The total area of Kilwa district is 13,347.50 square Kilometers (1,334,750 ha) of which 12,125.9 square kilometers is surface land and 1,221.52 square kilometers is the ocean. The total human population in 2002 was 171, 057 living in 36,549 households (National Statistics Beural, 2009). Kilwa District is administratively divided into 6 divisions, 20 wards, and 96 villages, 14 villages are occupied by agro pastoralists and their livestock (DED Kilwa, 2009). The district has a coastal climate which is hot and humid with the average temperature ranging between 22°C to 30°C. Humidity is high during the long rains. The district receives a total rainfall of 800-1400 mm/year and its distribution varies according to locality. The Northern part of district receives more rainfall (1000-1400 mm/year), than the Southern part (800-1400 mm/year).

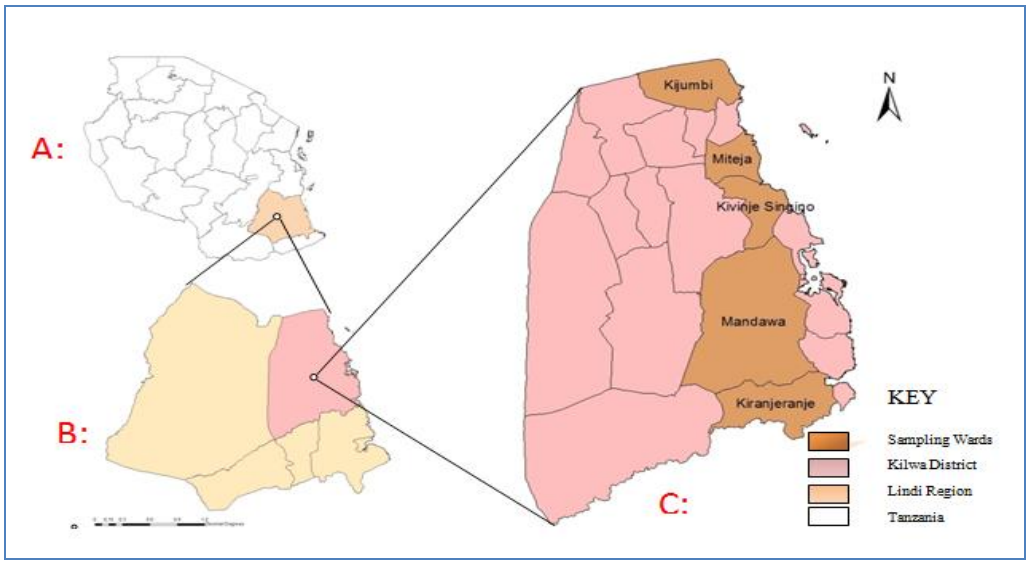

Figure 3: Map of the study area showing (A) Tanzania (B) Lindi Region and (C) Kilwa District showing sampling Wards.

The period of rainfall coincides with the onset of each monsoon wind (the long period of rainfall, from about Mid-March to May, and the short period of rainfall from about late October to December). Kilwa District falls under two major vegetation zones. Along the low lying coastal strip, the vegetation is characterized by the East Africa Coastal forest and thickets. The vegetation is thus predominantly deciduous shrubs with scattered trees. The upland parts of Kilwa district are on the eastern edge of the central African Miombo belt where the vegetation is heterogeneous, with a variety of vegetation types associated with the miombo region and other types of vegetation associated with the coastal strip of East Africa. Most of the Miombo lies to the west of the coastal forest and is interspersed with bush land and seasonally flooded open areas of grassland. The original tribes of Kilwa District were traditionally do not keep livestock save for small stock of goats and poultry kept by the local communities. In 1996, Kilwa district had 1,436 cattle, 4,879 goats and 5000 sheep (National statistics

beural, 2009). In the past few years however pastoralist and agro-pastoralist migrated into Kilwa from the northern and western circuits in the country, bringing with them large herds of cattle. This brought the number of livestock kept in Kliwa today to 14,756 cattle, 28,742 goats and 5,143 sheep (National statistics beural, 2009).

3.2 STUDY AND SAMPLING DESIGN

A cross-sectional survey was conducted in the tsetse-infested Kilwa district, Lindi region, Southern Tanzania. Multistage sampling was used. The Wards were the primary, villages secondary and herd tertiary sampling units. The five wards which keep cattle in the district were included in the study (conviniently sampled). A total of 14 villages kept cattle in the five wards. From, these ten were randomly sampled. The sampling frame of all household keeping cattle in each village was obtained from the district veterinary office. All names of households known to keep cattle were entered in Microsoft excel spreadsheets and simple random sampling was used to select sampling units. A maximum of ten herds was selected from each village and up to six cattle were selected for sampling from each herd.

3.3 SAMPLE SIZE DETERMINATION

Currently there is no established (known) prevalence of AAT in cattle in Kilwa district. As such, the 16% prevalence of AAT reported by Connor and Holliweell (1987) in cattle in southern zone of Tanzania was used to estimate the cattle herd sample size using the formula described by Chulaluk (2009).

$$N = \frac{Z^2 \text{ x } P \text{ x } Q}{L^2}$$

Where

N = is required sample size

Z = critical value for a given 95% confidence level = 1.96

L = desired absolute precision = 0.1

P =Estimated prevalence of trypanosomosis in the Study = 16%.

Q =1-P

Based on the above formula, the calculated sample size (N) was 92 cattle herds.

The Finite sample of herds was calculated using the formula described by Godden William (2004):

$$FSS = \frac{ss}{(1 + (\frac{ss - 1)}{Pop}}$$

Where

FSS = Finite sample size

Pop = Number of herds in the District =350

SS = Sample size = 92 cattle herds

Thus, after adjusting the number of herds of cattle in the district, the finite sample size was reduced to 73 herds.

According to the above sample size calculation, a minimum of 73 cattle herds were required for blood sampling. However, a total of 86 cattle herds were sampled in this

study from 10 villages, with a maximum of 10 herds per village. On average each herd had 60 cattle, from which up to 6 cattle (10%) were randomly selected for sampling, resulting in a total of 420 cattle sampled during the study.

3.4 BLOOD SAMPLE COLLECTION AND QUESTIONAIRE ADIMISTRATION.

Blood samples from 420 cattle were drawn into EDTA vacutainer tubes from the jugular vein using vacutainer needle for microscopic analysis. For each sample, approximately 200µl of whole blood was spotted onto Whatman® FTA card air-dried, kept in zip locked plastic bags containing silica and stored at room temperature before they were transported to UNZA, School of Veterinary Medicine, for LAMP analysis. The samples in EDTA tubes was transported to Sokoine University of Agriculture, Faculty of Veterinary Medicine, Morogoro, for laboratory analysis on ice in cooler boxes. In addition, information on the age, sex and breed of each animal sampled was collected. At the same time of blood collection, a structured questionnaire (Appendix A) was administered to the head of each household whose animals were sampled. The information that was collected included demographic data, livestock species, numbers and their management and when they arrived in Kilwa, just to mention a few.

3.5 LABORATORY ANALYSIS OF SAMPLES

At the laboratory fresh blood samples from an individual animals in capillary tubes was centrifuged at 12,000 RPM for five minutes. The resultant buffy coats were processed into thin and thick smears, dried, fixed with absolute methanol and stained with 10% Giemsa

solution. The slides were packed in the slide boxes before transportation to the the University of Zambia (UNZA), School of Veterinary Medicine laboratory where they were examined under oil emersion field microscopy to identify trypanosomes species as previously described (Murray *et al*., 1977). The trypanosomes on stained Giemsa preparation were differentiated to species level based on their morphological characteristic features including the size, shape, flagellum, undulating membrane, nucleus, kinetoplast and basal body.

3.6 DNA EXTRACTION.

Blood samples collected on FTA cards from 420 cattle were dried throroughly at room temperature. Four to five discs (about 3 mm) of blood spot were punctured from the highly concentrated areas of each whatmans FTA® Elute card using Harris micro punch and placed into one labeled 0.5 ml appendorf tubes. The discs were rinsed twice each in 750 µL of double distilled water (DDW) by vortexing for 5 seconds and then discarding the DDW. Fresh DDW (30 µl) was added, vortexed for 5 seconds and centrifuged for one minute. The resulting DNA was eluted by rapid boiling at 95°C for 30 minutes using a heat block as described by Lehane *et al*, (2000) and Duscher *et al*., (2009). Tubes were removed from the heating block, pulse votexed and centrifuged for 1 minute. Using sterile pipette tip, the FTA Elute matrix disk were removed and discarded. In order to circumvent the limitated LAMP reagents, 1.0 µL of the obtained DNA from each of the 10 cattle was then pooled to make one tube and stored at minus 20°C until use.

3.7 IDENTIFICATION OF TRYPANOSOME SPECIES BY LOOP

-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

Extracted DNA was subjected to a trypanosome-species-specific LAMP in a total reaction volume of 25 µl using a Loop amp DNA Amplification Kit (Eiken Chemical, Tochigi, Japan) as described by Thekisoe *et al.*, (2007). Primers used are summarized in table 3 and include the primers specifically targeting the 18S rRNA gene of *T. congolense* (CON2-LAMP) (Thekisoe *et al*, 2007a), the repititve insertion mobile element (RIME) gene of the *Trypanozoon* subgroup (RIME- LAMP), and Satellite DNA of *T. vivax* (VIVAX-LAMP) (Njiru *et al*, 2011), The reaction mixture was incubated at 64°C for 30 minutes in a heat block (Dry Thermounit DTU 1B, TAIEC Co., Saitama, Japan) and then at 95°C for 2 minutes to terminate the reaction. The LAMP products were visualized using a transilluminator (WD, H19, Good design award Co., Japan).

Table 3: List of trypanosome species specific LAMP primers sets

Target gene	Primer	Sequence	Specificity	Reference
RIME	FIP	5'-GGAATACAGCAGATGGGGCGA GGCCAATTGGCATCTTTGGGA-3'	*Trypanozoon*	*Njiru et al. 2008a*
	BIP	5'-AAGGGAGACTCTGCCACAGTC GTCAGCCATCACCGTAGAGC-3'		
	F3	5'-CTGTCCGGTGATGTGGAAC-3'		
	B3	5'-CGTGCCTTCGTGAGAGTTTC-3'		
	LF	5'-GCCTCCCACCCTGGACTC-3'		
	LB	5'-AGACCGATAGCATCTCAG-3'		
SRA	FIP	5'-GGACTGCGTTGAGTACGCATC CGCAAGCACAGACCACAG-3'	*T. b. rhodesiense*	Njiru *et al*. 2008b
	BIP	5'-CGCTCTTACAAGTCTTGCGC CCTTCTGAGATGTGCCCACT-3'		
	F3	5'-GCGGAAGCAAGAATGACC-3'		
	B3	5'-TCTTACCTTGTGACGCCTG-3'		
	LF	5'-CGCGGCATAAAGCGCTGAG-3'		
	LB	5'-GCAGCGACCAACGGAGCC-3'		
CON2 18s rRNA	FIP	5'-GCGCATGCGTCGGTGTTATT TTCGCGTGTGTGTTCATGTCA-3'	*T. congolense*	Thekisoe *et al*. 2007
	BIP	5'-ACTCTCCCCCCAAAATGGTT GTCCAAGCACGCAAATTCACAT-3'		
	F3	5'-TGTGTGTTTGTCGTGGAAGC-3'		
	B3	5'-ATTCGTGACCGCGTCAAA-3'		
Satellite DNA	FIP	5'-GTGGAGCGTG- CCAACGTGG CACCCGCTCCCAGACCATA-3'	*T. vivax*	Njiru *et al*. 2011
	BIP	5'-TGTCT- AGCGTGACGCGATG GAAGAGGGAGTGGGGAAGG-3'		
	F3	5'-TGTTCTGGTGG- CCTGTTGC-3'		
	B3	5'-GGCCGGAGCGAGAGGTGC-3'		
	LF	5'-CACATGGAGCATCAGGAC-3'		
	LB	5'-CCGTGCACTGTCCCGCAC-3'		

3.8 POLYMERASE CHAIN REACTION (PCR) FOR DETECTION OF *T. B. RHODESIENSE*

Detection of the *T b rhodesiense* was carried out by PCR, using species specific primer targeting SRA gene where all pooled samples from 86 herds were analysed for *T. b. rhodesiense*.

3.9. DATA ANALYSIS

Data were entered into MS-excel and analyzed using SPSS (IBM, USA, and version 20.0). Chi-square (χ^2) or the Fisher's exact test was used to determine association between categorical variables. Multivaliable logistic regression was employed to determine predictors of herds being positive to trypanosomes. All variables with P-values less than or equal to 0.250 in the univariate analysis were included in the model. A significant Omnibus Test for Model Coefficents ($P \leq 0.05$) and a non-significant Hosmer and Lemeshow test ($P > 0.05$) were used to determine whether the model fitted the data. All statistics were considered significant at $P < 0.05$).

3.10 ETHICAL CONSIDERATIONS

Approval and clearance for the study was obtained from the District Authorities (reference No 10/20/I/Vol.III/181). Consent forms were filled signed by all the interviewees before proceeding with the study (Appendix B). All ethical guidelines were adhered to and any information deemed sensitive was kept confidential.

CHAPTER FOUR

4.0 RESULTS

4.1 MICROSCOPY RESULTS

Blood samples from 420 cattle sampled from 86 herds from ten villages were microscopically examined individualy for presence of trypanosomes but keeping samples from one herd as one group. A herd was regarded positive if at least one animal was found to be trypanosome positive. Figure 4 A-C is a representative illustration of the trypanosome species observed under the microscope. Apart from trypanosomes, *Anaplasma marginale* was the only other hemoparasite which was found to infect cattle in Kilwa district (Fig. 4 D).

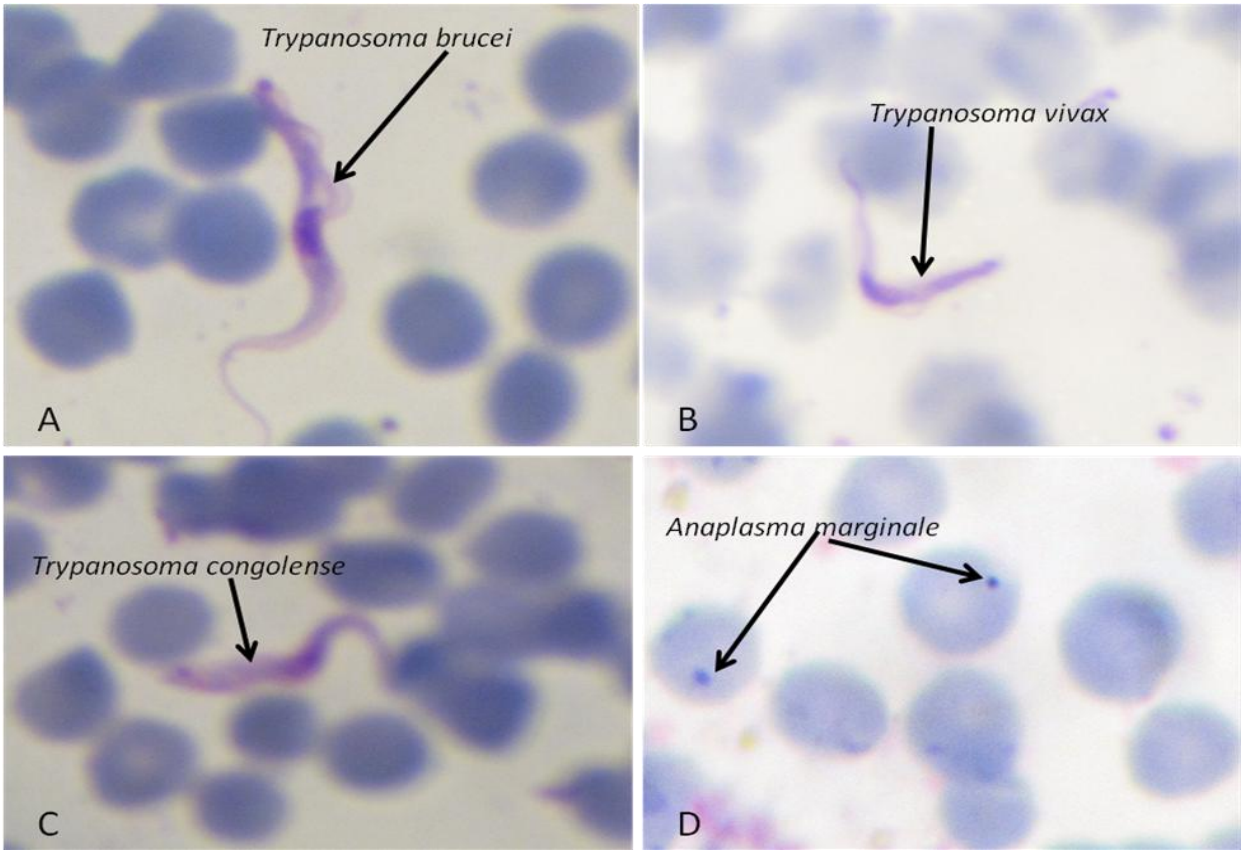

Figure 4: Giemsa stained buffy coat smear showing (A) T.brucei, (B) T.vivax, (C) T.congolense (D) Anaplama Marginale.

The results obtained by microscopic examination of the Giemsa-stained cattle blood smears indicated that 9.3%, (95% CI = 3.2-15.4) of the cattle herds were infected with trypanosomes (Table 4). About 5.8% (95% CI = 0.9-10.7) of the herds were infected with *T.congolense*, *T. brucei* accounted for 5.8% (95% CI = 0.9 -10.7); while *T. vivax* accounted for 3.5% (95% CI = 0-7.4) (Table 4.1). Out of those infected herds 2.3% (95% CI = 0-5.5) and 1.2% (95% CI = 0-3.5) were monolythic infections with *T. brucei* and *T. vivax,* respectively. Furthermore, 3.5% (95% CI = 0-7.4) of the herds had mixed infections of *T. brucei* and *T.congolense,* and 2.3% (95% CI = 0-5.5) had mixed infection of *T. vivax* and *T.congolense*. (Table 4)

Anaplasma marginale, the only other hemoparasite detected by microscopy in the Kilwa cattle blood samples, recorded a herd prevalence of 14% (95% CI = 6.7-21.3) (Table 5). Of those, about 12.8% (95% CI = 5.7-19.9) of the herds were mixed infections with trypanosomes. Only 1.2% (95% CI = 0-3.5) of the herd had monolythic *Anaplasma maginale* infections (Table 5).

Table 4: Prevalence of trypanosome species at village level by Microscopy Technique.

Name of the Village	Number of cattle sampled /Village (n)	Number of herds sampled/Village (n)	Numberof positive herds/Village (n)	Prevalene of trypanosomes 95% CI				Overall prevalence: % (95% CI)
				T.v	***T.b***	***T.c +T.v***	***T.c + T.b***	
Marendego	42	10	2	**10%(0-28.6)**	**0**	**10.0%(0-28.6)**	**0**	**20%(0-44.8)**
Somanga	45	8	0	**0**	**0**	**0**	**0**	**0**
Miteja	40	7	1	**0**	**0**	**0**	**14.3%(0-40.2)**	**14.3%(0-40.2)**
Kiranjeranje	42	9	1	**0**	**0**	**11.1%(0-31.6)**	**0**	**11.1%(0-31.6)**
Mbwemkuru	40	8	1	**0**	**0**	**0**	**12.5%(0-35.4)**	**12.5%(0-35.4)**
Hotel tatu	42	10	0	**0**	**0**	**0**	**0**	**0**
Kiwawa	44	10	0	**0**	**0**	**0**	**0**	**0**
Matandu	42	7	1	**0**	**0**	**0**	**14.3%(0-40.2)**	**14.3%(0-40.2)**
Mavuji	41	7	2	**0**	**28.6%(0-62.1)**	**0**	**0**	**28.6%(0-62.1)**
Makangaga	42	10	0	**0**	**0**	**0**	**0**	**0**
Total	**420**	**86**	**8**	**1.2, 0-3.5**	**2.3, 0-5.5**	**2.3,0-5.5**	**3.5,0-7.4**	**9.3, 3.2-15.4**

Key: Tryp=Trypanosome; T.b=*Trypanosoma brucei;* T.v=*Trypanosoma vivax;* T.c= *Trypanosoma congolense*

Table 5: Prevance of Anaplasma species at village level by Microscpy technique.

Name of the Village	Number of cattle sampled /Village (n)	Number of herds sampled/Village (n)	Numberof positive herds/Village (n)	Prevalene of Anaplasma 95% CI		Anaplasma overall
				A.M	A.M + Tryps	
Marendego	42	10	2	**0**	**20%(0-44.8)**	**20%(0-44.8**
Somanga	45	8	1	**12.5%(0-35.4)**	**0**	**12.5%(0-35.4)**
Miteja	40	7	1	**0**	**14.3%(0-40.2)**	**14.3%(0-40.2)**
Kiranjeranje	42	9	2	**0**	**22.2%(0-49.4)**	**22.2%(0-49.4)**
Mbwemkuru	40	8	1	**0**	**12.5%(0-35.4)**	**12.5%(0-35.4)**
Hotel tatu	42	10	1	**0**	**10%(0-28.6)**	**10%(0-28.6)**
Kiwawa	44	10	2	**0**	**20%(0-44.8)**	**20%(0-44.8)**
Matandu	42	7	0	**0**	**0**	**0**
Mavuji	41	7	1	**0**	**14.3%(40.2)**	**14.3%(40.2)**
Makangaga	42	10	1	**0**	**10%(0-28.6)**	**10%(28.6)**
Total	**420**	**86**	**12**	**1.2%, 0-3.5**	**12.8%, 5.7-19.9**	**14%, 6.7-21.3**

KEY: A.M=*Anaplasma marginale;* Tryps = Trypanosomes

4.2 LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) RESULTS

Pooled DNA samples extracted from the 86 cattle herds was then subjected to LAMP analysis using trypanosome-species-specific primers (CON2-LAMP, *VIVAX*-LAMP, RIME-LAMP). The LAMP positive samples (yellowish) could clearly be distinguished from the negative ones (brown) through colour change (Fig. 5). All samples which were positive by microscopy were also positive on LAMP.

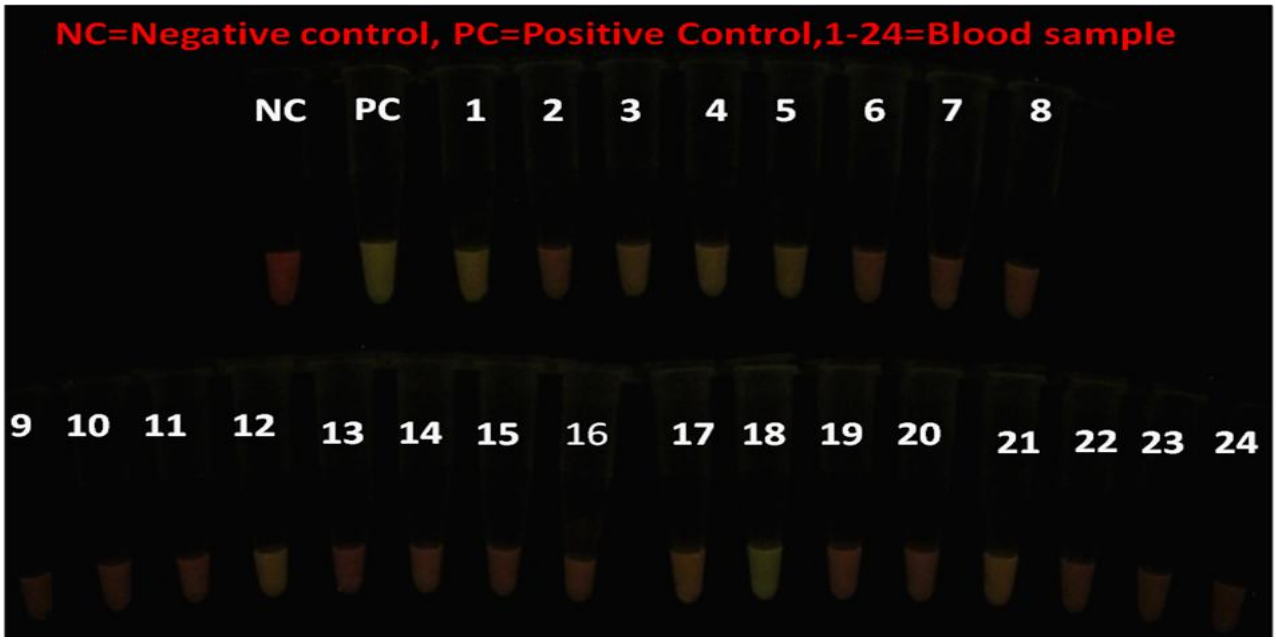

Figure 5: Visual appearance of representative results for LAMP (VIVAX-LAMP).

In contrast to the brown background fluorescence in negative samples, positive samples exhibit a bright fluorescent yellowish colour when visualized under the transilluminator. NC: Negative Control (distilled water); PC: Positive Control (*T. vivax*); 1- 24: cattle blood samples from which samples 1,3,4,5,12,17,18 and 21 were positive while the rest were negative.

The results of the LAMP analysis are further summarized in table 6. The overall prevalence of trypanosomosis according to LAMP was 41.9% **(**95% CI = 31.5-52.3). Of these, 30.2% (95% CI = 20.5-39.9) were infected with *T. congolense*, 25.6% (95% CI = 16.4-34.8) *T. brucei* and 20.9% (95% CI = 12.3-29.5) *T. vivax* (Table 6). No *T. b. rhodesiense* was recorded after screening of RIME-LAMP (*T. brucei*) positive samples with SRA-PCR (not shown). In this study 15.1% (95% CI = 7.5-22.7) of the herds were infected with single infections: 8.0% (95% CI = 2.3-13.7) being infected with *T. congolense*, 6.0% (95% CI = 1.0-11.0) infected with *T. vivax* and 1.1% (95% CI = 0-3.1) infected with *T. brucei*. Mixed infections accounted

for 23.3% (95% CI = 14.4-32.2) of the total herds trypanosome infections: 8.1% (95% CI = 2.3-13.9) co-infected with *T. congolense* and *T.brucei,* 7.0% (95% CI = 1.6-12.4) co-infected with *T. congolense* and *T. vivax* and 1.2 % (95% CI = 0-3.5) were co-infected with *T. vivax* and *T. brucei* (Table 6). About 7.0% (95% CI = 1.6-12.4) of the herds were co-infected with all the three trypanosome species (*T.* congolense, *T. brucei*, *T. vivax*). There was variation in prevalence of trypanosome infections among the villages ($p > 0.05$). As shown in table 6, Miteja and Matandu villages had the highest prevalence of 57.1% (95% CI = 20.4-93.8) while Mavuji had the lowest prevalence of 14.0% (95% CI = 0-39.7). The prevalence of *T. vivax* was highest in Hotel tatu village (40.0%, 95% CI = 9.6-70.4) and no herd tested positive for that *trypanosome spp* in Somanga village. On the other hand, Miteja village had the highest prevalence 57.1% (95% CI = 20.4-93.8) of *T. congolense* while no herd tested positive in Makangaga village. Similarly, Miteja village had the highest prevalence of *T. brucei* (57.1%, 95% CI = 20.4-93.8), followed by Mbwemkuru which had a prevalence of 50.0%, (95% CI: 28.4-95.6) while no herd was positive for *T. brucei* in Kiwawa and Matandu villages (Table 6).

Table 6:Prevalence of trypanosome at village level by LAMP test.

Name of the Village	Number of cattle sampled /Village (n)	Number of herds sampled/ Village (n)	Number of positive herds/Village (n)	Infected with : % (95% CI)							Overall prevalence: % (95% CI)
				T.c	***T.v***	***T.b***	***T.c +T.v***	***T.c + T.b***	**T. *v* + *T.b***	***T.c+ T.v + T.b***	
Marengego	42	10	5	30.0% (1.6-58.4)	30.0% (1.6-8.4)	40.0% (9.6-70.4)	0.0	10.0% (0-28.6)	10.0% (0-8.6)	10.0% (0-28.6)	50.0% (28.4-95.6)
Somanga	45	8	3	37.5% (4.0-71.1)	0.0	37.5% (3.9-71.1)	0.0	25.0% (0-55.0)	0.0	0.0	37.5% (3.5-70.5)
Miteja	40	7	4	57.1% (20.4-93.8)	14.3% (0-40.2)	57.1% (20.4-93.8)	0.0	14.3% (0-40.23)	0.0	14.3% (0-40.2)	57.1% (20.3-93.7)
Kiranjeranje	42	9	4	33.3% (2.5-64.1)	22.2% (0-49.4)	33.3% (2.5-64.1)	11.1% (0-31.6)	0.0	0.0	11.1% (0-31.6)	44.4% (11.9-76.9)
Mbwemkuru	40	8	4	50.0% (15.4-84.7)	25.0% (0-55.0)	50.0% (15.4-84.7)	25.0% (0-55.0)	0.0	0.0	0.0	50.0% (9.4-70.4)
Hotel tatu	42	10	4	30.0% (1.6-58.4)	40.0% (9.6-70.4)	20.0% (0-44.8)	20.0% (0-44.8)	0.0	0.0	10.0% (0-28.6)	40.0% (9.4-70.4)
Kiwawa	44	10	5	30.0% (1.6-58.4)	30.0% (1.6-8.4)	0.0	10.0% (0-28.6)	10.0% (0-28.6)	0.0	10.0% (0-28.6)	50.0% (28.4-95.6)
Matandu	42	7	4	42.9% (6.2-79.6)	14.3% (0-40.2)	0.0	0.0	28.6% (0-62.1)	0.0	14.3% (0-40.2)	57.1% (20.3-93.7)
Mavuji	41	7	1	0.0	14.3% (0-39)	14.3% (0-39)	0.0	0.0	0.0	0.0	14.3% (0-39)
Makangaga	42	10	2	0.0	20.0% (0-44.8)	20.0% (0-44.8)	0.0	0.0	0.0	0.0	20.0% (0-44.8)
Total	**420**	**86**	**36**	**30.2% (20.5-39.9)**	**20.9% (12.3-29.5)**	**25.6% (16.4-34.8)**	**7.0% (1.6-12.4)**	**8.1% (2.3-13.9)**	**1.2% (0-3.5)**	**7.0% (1.6-12.4)**	**41.9% (31.5-52.3)**

Key: *T.b = Trypanosoma brucei; T.v = Trypanosoma vivax; T.c = Trypanosoma congolense*

4.3 DETERMINATION OF RISK FACTORS

Multivariable Logistic Regression analysis was carried out to determine significant predictors of cattle being positive for AAT. Because the samples used in the present study were pooled and anlysed at herd level, variables such as animal age, sex and breed were not included as factors which may have had an influence on the prevalence of AAT. As such, it was only possible to determine AAT prevalence at herd level.

The Hosmer and Lemeshow test was not-statistically significant ($p = 0.888$) and the Omnibus Test for Model Coefficients was significant ($p \leq 0.001$), suggesting that the model fitted the data. However, the confidence intervals were very wide, indicating the uncertainty in the estimates. The results of the analysis are summarized in table 7. Herds that sourced water from the river were 7.6 (95% CI= 1.734-33.587) times more likely to be positve for AAT than those that sourced water from dams/Ponds. Herds that originated from Mbarali were 0.343 (95% CI = 0.022-5.283) times less likely to be positive for AAT than those that were from Kilwa. Urambo: 3.213, (95% CI = 1.116-9.285), Dodoma: 0.081, (95% CI = 0.006-1.010), Rufiji: 0.045, (95% CI = 0.004-0.479) also were less likely to be positive for AAT than those that were from Kilwa district. Grazed in game reserve Yes Vs No: were 13.915, (95% CI = 2.485-77.959) more likely to be positive for AAT than those not grazed in game reserve (Table 7). Season of increased vector dry season Vs rainy season 0.204, (95% CI = 0.049-0.829). Affordability of drugs Yes Vs No: were 0.152, (95% CI = 0.023-0.988) less likely to be positive than those from araeas where ant-trypanosoma drugs were not affordable. Form of watering point Perennial Vs Seasonal were 6.860 (95% CI = 1.070-43.9880) times more likely to test positive to AAT than those gatting water from seasonal source. (Table 7). These data

suggest that District of origin, type of watering point, grazing in reserve, season with increased vectors, and form of watering points are good variables to predict occurrence of AAT in the study area

Table 7: Logistic regression for significant factors associated with cattle trypanosomosis by LAMP test

VARIABLES	n	ODD RATIO	95% C.I. for OR		Sig
			Lower	Upper	
Type_watering_point:	21	7.632	1.734	33.587	0.007
River Vs Ponds or Charcoal dam					
Original_District					0.019
Mbarali Vs Kilwa	34	0.343	0.022	5.283	0.044
Urambo Vs Kilwa	11	3.218	1.116	9.285	0.031
Dodoma Vs Kilwa	2	0.081	0.006	1.010	0.050
Rufiji Vs Kilwa	3	0.045	0.004	0.479	0.010
Grazed_in_reserve:	45	13.915	2.484	77.959	0.003
Yes/No					
Season_ with increased_ vector:	27	0.202	0.049	0.829	0.026
Dry season Vs Rain season					
Affordabilityof drugs:	81	0.152	0.023	0.988	0.049
Yes/No					
Form_of_watering _point Perennial/Seasonal	24	6.860	1.070	43.988	0.042

Using information gathered by administering questionnaire, the LAMP results, analyzed according to the district of origin (District from which pastoralist and their livestocks are shifting from before they come to settle in Kilwa district) as shown in table 8. Accordingly, the prevalence of *T. vivax* infections was highest in Kilwa district (60.0%, 95% CI = 17.1-102.9) and lowest in Mbarali (14.7%, 95% CI = 2.8-26.6). Of note, all the herds from Dodoma were positive for *T. congolense*. This parasite had the lowest prevalence in herds from Bunda 14.3% (95% CI = 0-40.2). Furthermore, herds from Dodoma were all positive for *T. brucei*, whereas herds that originated from Morogoro had the lowest prevalence of 12.5% (95% CI = 0-25.7) of *T.brucei* (Table 4.5). There was a significant difference in *T. brucei* prevalence among the districts (p = 0.040).

Table 8: Prevalence of trypanosome species by District of origin according to LAMP test

District of origin	n	Prevalence (95% Confidence interval)		
		T. v	***T. c***	***T.b***
Kilwa	5	60.0 (17.1-102.9)	60.0 (17.1-102.9)	60.0 (17.1-102.9)
Mbarali	34	14.7(2.8-26.6)	32.4(16.8-48.1)	26.5(11.7-41.3)
Urambo	11	36.4 (8.0-64.8)	27.3(1-53.6)	18.5(0-41.5)
Doadoma	2	0.0	100 (1000)	100 (100)
Morogoro	24	16.7 (1.8-31.6)	25.0 (7.8-42.3)	12.5 (0-25.7)
Rufiji	3	33.3(0-86.6)	0.0	0.0
Bunda	7	28.6(0-62.1)	14.3(0-40.2)	25.6(0-57.9)
p-value		**0.440**	**0.218**	**0.040***

Key: * significant P-value, (***T. b =Trypanasoma brucei; T. v = Trypanasoma vivax; T. c = Trypanasoma congolense*****)**

The LAMP results according to watering point are summarized in table 9. No significant difference was observed in AAT prevalence except for the mixed infection of *T. congolense* and *T. vivax* which were recorded to be higher in those herds that obtained water from perenial sources than those that obtained water from seasonal watering points (p = 0.049) .

Table 9: Prevalence of trypanosome species by form of watering points according to LAMP test.

Form of watering point	n	Prevalence (95% Confidence interval)			
		T. v	***T. c***	***T.b***	***T.C+T.v***
Seasonal	62	19.4(9.6-29.2)	32.3(20.7-43.9)	29.0(17.7-40.3)	3.2(0-7.6)
Perennial	24	25.0(7.68-42.3)	25.0(7.7-42.3)	16.7(1.8-31.6)	16.7(1.8-31.6)
P-value.		**0.380**	**0.606**	**0.283**	**0.049***

Key: * significant P-value. (*T. b =Trypanasoma brucei; T. v* = T*rypanasoma vivax; T. c = Trypanasoma congolense*)

The results of the LAMP analysis according to whether the herd grazed in Selouse Game Reserve or not are shown in table 10. The prevalence of *T. congolense* and *T. brucei* were significantly higher in herds that grazed in the Game reserve than in those that did not (p = 0.004 and 0.046, respectively). The prevalence of cattle infected with all the three trypanosomes in herds that grazed in the game reserve was signifiantry high (p = 0.027) indicating that those herds which were grazing in game reserve were 13.3% (95% CI =3.4-23.2) times infected than those which

were not, while none of the herds were co-infected with all the three *trypanosome spp*, among those that did not graze in the game reserve (Table 10). There was no significant difference in the prevalence of *T. vivax* between those herds that grazed in Selouse Game Reserve and those that did not (p = 0.195).

Table 10: Prevalence of trypasome species according to whether cattle graze in game reserve or not.

Grazing in game reserve	n	Prevalence (95% Confidence interval)			
		T. c	***T. v***	***T.b***	***T. v+ T. c+ T.b***
Yes	45	44.4(29.9-59.0)	26.7(13.8-39.6)	35.6(21.6-49.6)	13.3(3.4-23.2)
No	41	14.6 (3.7-25.0)	14.6(3.8-25.4)	14.6(3.8-25.4)	0.0
P-value.		**0.004***	**0.195**	**0.046***	**0.027***

Key: * significant P-value. (*T. b =T. brucei; T. v = T. vivax; T. c = T. congolense*)

The prevalence of *Trypanosome spp* among those herds whose owners could afford to buy drugs for treatment of ant-trypanosomes was low compared to that in the herds whose owners could not afford to buy those drugs. However, the prevalence of mixed infections of all the three parasites was significantly lower in those herds whose owners could afford to buy drugs than in those whose owners could not afford (p = 0.037). (Table 11).

Table 11: Prevalence of trypanosome species by affordability of drugs according to LAMP test

Do you afford to treat your animals?	n	Prevalence (95% Confidence interval)			
		T. c	***T. v***	***T.b***	***T. v + T. c + T.b***
Yes	81	28.4(18.6-38.2)	18.5(10.0-27)	24.7(15.3-34.1)	4.9(0.2-9.6)
No	5	60.0(17.1-100)	60.0(17.1-100)	40.0(0-82.9)	40.0 (0-82.9)
P-value.		**0.160**	**0.060**	**0.599**	**0.037***

Key: * significant P-value. (*T. b = Trypanasoma brucei*; *T. v = Trypanasoma vivax; T. c = Trypanasoma congolense*)

CHAPTER FIVE

5.0 DISCUSSION

Kilwa District is one of the five districts of Lindi region in southern zone of Tanzania with human population of about 171,057 (National Bureau of Statistics, 2002). The original people of this district were not culturally pastoralist, earning their living through growing of crops and fishing. The current movement of pastoralist with their livestock from different parts of the country to Kilwa has increased the chance of disease transmission including trypanosomosis (Lea Berrang-Ford *et al.,* 2006). This study has found that AAT is prevalent in Kilwa District.

Animal African trypanosomosis has continuously been reported from different parts of Tanzania using different diagnostic techniques including microscopy, PCR and LAMP (Connor, Halliwell, 1987; Karimuribo *et al*., 2011; Nonga and Kambarage 2009; Haji *et al*., 2015). In the present study, a low herd prevalence (9.3%) of AAT was obtained by microscopy. However, this prevalence was more than four times higher (41.9%) when the samples were analysed by LAMP. As such, the rest of the analysis used in this study was based on the more sentive LAMP test. This finding is in agreement with previous reports (Chappuis, 2005; Thekisoe *et al*., 2007; 2008; Matovu *et al.,* 2010*;* Bukowa *et al*., 2012; Namangala *et al.*, 2013). LAMP is a novel diagnostic technique that rapidly amplifies the target DNA under isothermal condition (Notomi *et al* 2000). It is relatively simple, rapid, sensitive and specific nucleic acid amplification method (Tomito *et al*., 2008). Basic reactions use four primers, namely forward and backward inner primers (FIP and BIP); forward and backward outer primers (F3 and B3), additional forward and backward loop primers (LF and LB) significantly accelerate reaction time of LAMP to less than half an hour (Notomi *et al*.,

2000; Nagamine *et al.,* 2002; Iwasak *et al.,* 2003; Njiru *et al.,* 2007). It was able to differentiate trypanosomes to species levels (*T. vivax, T. congolense, T. brucei)* which indicate that this is a more reliable technique for diagnosis of AAT in cattle herds. LAMP has also been effective in diagnosis of other diseases such as Babesiosis, Theileriosis, Tuberculosis and Malaria (Ikadai *et al*., 2004, Thekisoe *et al*., 2010, Iwamoto *et al*., 2004, Poon *et al*., 2006).

Microscopy, on the other hand, is used in the field as the routine screening diagnostic test for AAT. It is used as a gold standard for direct detection of trypanosomes on wet or dry blood smears (Woo, 1970). However, it has low sensitivity and specificity. Concentration techniques such as the mini-hematocrit centrifugation techniques increases its sensitivity (Woo, 1970). Despite its limitations in terms of sensitivity and specificity, it is the most commonly used technique in the field in sub-Saharan Africa where AAT is endemic, especially in rural areas of poor countries because it is cost effective, simple, and has ability to diagnose many haemoparasitic species at once (Mitashi *et al.,* 2012). Direct observation of parasites through microscopy in blood is particularly important to prevent unnecessary treatment for false positives (Mitashi *et al.,* 2012)

Results from this study suggest that cattle in Kilwa District are infected by mainly three species of trypanosomes, namely *T. congolense, T. vivax* and *T. brucei*. *Trypanosoma congolense* as well as *T. vivax* are highly virulent and cause debilitation, loss of productivity and death in cattle (Moloo *et al.,* 2008). According to the obtained data, *T. congolense* recorded the highest prevalence, followed by *T. brucei*. The high proportion of *T. congolense*

infections in the Kilwa cattle is in agreement with previous studies in various parts of Africa (Connor and Hallowell, 1987; Leak, 1999; Van den Bossche, 2001; McDermott *et al*., 2003; Abebe and Wolde, 2010). Even at village level, *T. congolense* was the most prevalent species, most of which was recorded from Miteja whereas Mavuji and Makangaga villages recorded no *T. congolense* infections. Such variations between herds of cattle are attributed mainly to differences in disease management practices whereby in some villages they use dipping while others use hand pump sprayer for insecticides (pyrethroides) application and possibly differences in the virulence of circulating trypanosome strains (Masumu *et al*., 2006).

According to data obtained from the present study, *T. vivax* was more prevalent among cattle herds from Hotel Tatu village while Mavuji and Somanga villages recorded no *T. vivax* infections. Similar results were previously reported from Busia, Kenya (Karanja, 2005); and in Tororo, South East Uganda (McOdimba, 2006). It is suspected that the transmission in those areas is mechanical since most infections are *T. vivax* (transmitted both by tsetse flies and mechanically by other biting flies including *Stomoxys calcitrans*, *Tabanus spp*. and *Haematopota spp* (Van den Bossche *et al*., 1999)). It is tempting to speculate that Hotel Tatu village could be inhabited by more of these biting flies than other villages.

Data obtained from this study suggest that cattle grazing in game reserve have 13.9 times more chance of getting infected with trypanosomes than those which do not (Table 7). *Trypanosoma brucei* was highly prevalent at Miteja which is closer to game reserve and lowest in those villages which do not graze in the game reserve including Hotel Tatu, Kiwawa, Matandu and Mavuji, which did not record any infection with this trypanosome

species. This variation may be attributed to differences in environmental conditions which favor occurrence and activity of the tsetse vectors (*Glossina spp*) as well as the presence of reservoirs. Thus, in agreement with Majekodunmi *et al.* (2013), factors such as microhabitat, land use patterns and husbandry practice may have a significant impact on the epidemiology of AAT in cattle. Although no human infective *T. b. rhodesiense* parasites were detected in this study, those villages should not be considered to be completely free from HAT in view of the reservoir role of cattle for *T. b. rhodesiense,* coupled with the increased cattle movements from different parts of Tanzania where *T. b. rhodesiense* has previously been reported (Kaare *et al.,* 2006; 2011; Haji *et al.*, 2015).

We could detect simultaneous infection of two or three trypanosome species in a single herd. In agreement with Connor and Halliwell (1987), that seems to be a common phenomenon in southern Tanzania and elsewhere (Masiga *et al*., 1996; Lehane *et al*., 2000, Van den Bossche *et al*., 2004, Konnai *et al*., 2008). Furthermore, in agreement with Haji *et al.*, (2015), this study also reports co-infections of trypanosomes and other haemoparasites such as *Anapalsma marginale*. Taken together, the simultaneous occurrence of two or more parasites in a host enhances the severity of the disease (Ngailo *et al.,* 2011).

This study reports high herd prevalence of AAT (41.9%) by LAMP analysis. Similar high prevalence of AAT in cattle was reported in Nigeria 46.8% (Majekodunmi *et al.,* 2013) and Uganda 38.0% (Anosike *et al.,* 2003). Migration may increase cattle susceptibility by reducing immunity condition due to stress resulting from treking, exposure to other vector borne diseases which also increase susceptibility of cattle to AAT due to stress and immune depression (Anosike *et al.,* 2003). Similarly, increased livestock movement has been behind

the spread of HAT in many areas of Uganda (Hoppe *et al.,* 2003 Fèvre *et al* 2004; Berrang-Ford *et al.,* 2006; Welburn *et al.,* 2006; Batchelor *et al.,* 2009; Endfield *et al.,* 2009; Selby *et al.,* 2013). Following increased movements of animals from Northern and Western parts of Tanzania through tsetse infested trypanosomes active foci, the likelihood of animal and human infective trypanosome species being introduced or increased in Kilwa cannot be ignored.

The risk factors that were associated with transmission of AAT in this study were grazing in Selous Game Reserve, district of origin, form of watering point, season of increased vector and affordability of the drugs (Table 7). In agreement with Kaare *et al.,* (2006), the prevalence of AAT was relatively higher in animals which had challenges of tsetse fly in their grazing area. AAT is maintained in ecological system by tsetse flies, woody vegetation and game animals. It is only when livestock are introduced into this system that tsetse fly will use livestock as their source of food and infect them with trypanosomes (Ogochukwu Emma Ikenna, 2008).

In agreement with previous studies (Van den Bossche and Staak, 1997; Van den Bossche *et al.,* 2000; Van den Bossche and De Deken, 2002;), animals tend to have higher chance of getting infected with trypanosomes during the rainy season when there is an increase of vectors than during dry season (Table 7). On the other hand, circumstances such as prolonged drought and low plane of nutrition during dry season stress the animals resulting in lowered immunity and increased vulnerability to diseases including AAT (Elamin *et al.,* 1998). Furthermore, this study reports the frequency of using trypanocides to inversely influence the prevalence of AAT.

CHAPTER SIX:

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSIONS

Based on this study, the following conclusions were made:

1. According to the more sensitive LAMP test, there is high herd prevalence (41.9%) of AAT among cattle herds in Kilwa district, southern Tanzania.

2. Data from the present study suggest that various trypanosome species, dominated by *T. congolense*, circulate in cattle herds within Kilwa district, southern Tanzania.

3. Although *T. brucei* was detected, no human-infective *T. b. rhodesiense* was recorded in the present study.

4. Grazing in Selous Game Reserve, affordability of the antitrypanosomal drugs, district of origin, form of watering point and seasonality were found to be risk factors associated with AAT in Kilwa district.

6.2 RECOMMENDATIONS

The following recommendations are made based on the findings of the present studies:

1. Continuous AAT surveillance and monitoring, using sensitive and specific molecular techniques should be encouraged.

2. Regular screening of cattle and treatment of all infected animals should be encouraged to reduce the parasite circulating in the community.

3. Settlement within or closer to and also grazing in Selous Game Reserve should be discouraged through community education on its effect in relation to high possibility of contracting trypanosome infections.

4. The use of tsetse fly traps and insecticide-impregnated targets should be encouraged especially at the wildlife-domestic animal-human interface area in order to control trypanosome infections.

6.2 FUTURE STUDIES SHOULD FOCUS ON:

1. Determining the prevalence and risk factors associated with *T .b. rhodesiense* in domestic animals, using sensitive and specific molecular tests, in the study area at different times of the year to establish the possible influence of seasonality on prevalence.

2. Determining the prevalence of HAT among people living in the wildlife- livestock-human interface areas in the study area.

3. Determining the prevalence of trypanosomes in domestic animals-wildlife -human and tsetse flies in all district boudering Selous Game Reserve.

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APPENDIX

APPENDIX A

Questionnaire for collection of data for Determination of risk factors for trypanosomosis in Kilwa district.

S/N…….

QUESTIONNAIRE

Questionnaire format to interview Selected farmers individually Kilwa District Lindi Region

Name of farmer_____Age ___sex_____ Village_____

District _____

Region_____ Zone_____ Country _____

I. Head of the family

1. When did you start Livestock keeping in Kilwa District? Specify----------
2. In which district did you come from----------?
3. How many animals were you having at the beginning (cattle -------Goats -----)?

3. Which livestock species are you keeping?

a) Cattle
b) Sheep and goat
c) Donkey
d) Dogs
e) Others

4. What major types of crop are you producing?

a) Maize
b) Sorghum
c) Sweet potatoes.
d) Simsim
d) Others

5. Which livestock species used for draught power?

a) Cattle
b) Equines
c) Others

6. What is the management system of cattle?

a) Communal and free grazing; live at the outside of the farmer's house but communally
b) Private and free grazing
c) Tether
d) Stall feed

7. If the cattle managed in communal and free grazing type, are they in larger or small herds?

8. Where is the grazing and watering point?
a) Around the locality of farm
b) Long distance away from the farm
9. Are the watering points perennial or seasonal?
10.Are the watering system natural or artificial?
If it is perennial; Rivers, ponds or charcoal dam
If it is seasonal; Rivers, ponds or charcoal dams
11. Do you experience water scarcity? Yes /No
12. If yes in which season does water scarcity occurs?
a) After rainy season
b) During dry season
c) During the rainy season
13. Do you experience forage scarcity? Yes /No
14. If yes in which season does forage scarcity occurs?
a) After rainy season
b) During dry season
c) During the rainy season
15. What are major constraints of livestock keeping?
a) Feed shortage
b) Lack of grazing land
c) Drought
d) Burning of forage
e) Livestock diseases
f) Others (Specify).....
16. What is the distance between the grazing area and Selous Game Reserve? Km
17. Is there a time of the year when livestock are grazed in the game reserve?
Yes No
If yes at what time of the year……
Why?.....
.....
.....
18. Is there any problem of grazing livestock in the game reserve?
Yes No
If yes mention them
1
2
3
4
5
19. What are the most common livestock diseases affecting your cattle?

1
2
3
4
5

20. Do you know the disease called trypanosomosis?

Yes No

21. What do you call it by local name?

22. Does trypanosomosis occur in this area?

a) Yes

b) No

c) I don't know exactly

23 What is the importance of this disease compared to other diseases……..……..

24. Which livestock species is affected most by trypanosomosis?

a) Cattle

b) Small ruminants

c) Equines

d) Dogs

25. What are the main clinical signs observed when an animal is affected by trypanosomosis?

1……

2……

3……

4……

5……

6……

26. At what time does the disease commonly occur?

a) During rainy season

b) During dry season

c) At the beginning of rain season

d) At the end of rain season

27. Since when did you know the problem of trypanosomosis in this area?

a. Just when you started to live here

b. after 5 years of settlement

c. after 10 years of settlement

d. after 20 years of settlement

f. before settlement

28. What is the disease's situation of this disease since you arrived in this area?

a) It is getting better

b) It is getting worse

c) Nothing is changed

d) I don’t know.

Why? .....

29. What transmits (vector) this disease?

a) Flies

b) Ticks

c) Others

d) I don’t know

30. What is the local name of the fly that transmitter of trypanosomosis?.....

31. In which season are these flies most abundant?

a) During rainy season

b) During dry season

c) At the beginning of rain season

d) At the end of rain season

32. Where this fly population is very high?

a. In grass land areas

b. In cultivated land

c. In bush land

d. In savanna areas

e. In areas close to river and watering points

33. What are the methods of controlling these flies?

a) Dipping

b) Spraying

c) Use of targets

d) Bush clearing

34. What insecticide do you use for dipping or spraying your animals?

1

2

3

4

35. How many times do you spray or dip your animals?

a) Twice in a week

b) Once in a week

c) Twice in a month

d) Once in month

36. Do you think this method and regime is effective

Yes

No

Why? …….

37. What are the main control measures of trypanosomosis?

a) Treatment of affected animals

b) Feeding well of affected animals
c) Injecting drugs for prophylaxis
38. What are the main control measures of tsetse flies?
a) Use of tsetse flies targets
b) Use of acaricide by dipping
c) Use of acaricide by spraying
d) Use of animal bait
39. What are the types of drugs used for treatment and prophylaxis?
a) 1
b) 2
c) 3
d) 4
40. How often do you use these drugs?
a) Every week
b) After two weeks
c) Every month
d) After six month
41. Who treats your animal when they are sick?
a) Vet. Officer
b) Livestock field officer
c) Assistant livestock field officer
d) Owner of the animal
42. Where do you sources the drugs from?
a) Veterinary clinics
b) Private legal and
c) Illegal drug shops
d) Others
43. Do you think those drugs are effective? Yes/No
44. If it is not effective what is the reason behind that?
45. How far is the drug source?
a) 1km
b) 10km
c) 50km
d) 100km
e) >100km
46. Do you think the cost of treating trypanosomosis is affordable?
Yes /No
47. Are there traditional method of treatment and management practices for controlling and prevention of trypanosomosis?

Thank you!
Name of interviewer_____
Signature_____
Date_____

APPENDIX B:

CONSENT FORM

We want to conduct a research of livestock disease in kilwa District, in order to do this; we need the help of livestock keepers who agree to take part in a research study. This form tells you about this research study. We are asking you to take part in a research study that is titled: **PREVALENCE AND RISK FACTORS ASSOCIATED WITH TRYPANOSOMOSIS IN CATTLE IN KILWA DISTRICT –LINDI REGION OF TANZANIA**.
Name of the interviewer in the area……

This study is sponsored by-INTRA-ACP MOBILITY SCHEME

Purpose of the Study is: To determines the prevalence and risk factors of trypanosomosis in cattle in Kilwa District, Lindi Region of Tanzania.

Study Procedures
This study will involve asking you few questions and collecting blood from maximum of five cattle from your farm for laboratory analysis.

Compensation This research is considered to be minimal risk. We will not pay you for the time you volunteer while being in this study

I confirm that I have read and understand the information sheet for the above research and I have had the opportunity to ask questions ( )

I appreciate that my participation is helpful and that I am free to leave at any time with no reason ( )

I freely give my consent to take part in this study. ( )

❖

I am 18 years of age or older ( )

Publication: We will publish what we learn from this study, but we will not let anyone know your name. We will not publish anything else that would let people know who you are.

Contact Information: If you have questions regarding the research, please contact the Principal Investigator **Dr. EMMANUEL NICHOLAUS KASSIAN**
MOBILE NO +255788012301 EMAIL ekassian@gmail.com

Name of Participant	Date	Signature

Name of investigator at the area	Date	Signature

APPENDIX C:

ARTICLE

A manu script for the artile is in the final stages for publication:

Title: - PREVALENCE AND RISK FACTORS ASSOCIATED WITH TRYPANOSOMOSIS IN CATTLE HERDS IN KILWA DISTRICT - LINDI REGION OF TANZANIA.

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