# MOLECULAR PREVALENCE OF TRYPANOSOME INFECTIONS IN CATTLE AND TSETSE FLIES IN SIMANJIRO AND MONDULI DISTRICTS, MAASAI STEPPE OF NORTHERN TANZANIA

**MARY SIMWANGO**

**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER IN EPIDEMIOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.**

**2016**

# ABSTRACT

Livelihoods of the Maasai people in Northern Tanzania is sustained by Agriculture, specialising mostly in pastoral livestock production which is threatened by vector-borne diseases of which trypanosomosis is one of them. The vulnerability of these communities to trypanosomosis is enhanced by their interaction with wild animals due to their proximity to wildlife interface areas which are highly infested with ticks and tsetse flies. A study was carried out from June 2015 to March 2016 to quantify trypanosome infections in cattle and tsetse flies using PCR based molecular techniques that amplify the Internal Transcribed Spacer (ITS) 1gene of the trypanosome ribosomal DNA. This study revealed an overall prevalence of trypanosome infections in cattle to be 17.2% (At 95% CI (14.91-19.68)) and a prevalence of 6.9% in tsetse flies. The highest prevalence of trypanosome infections in cattle was observed at the end of wet season. Out of the five study villages, the highest prevalence of trypanosome infections in cattle was observed in Loibor-soit-A (35%) and the lowest prevalence was found in Emboreet village (12.1%). The nPCR identified five *Trypanosoma species*which by order of abundance are*T. vivax* (41.8%), *T. brucei* (20.9%), *T. simiae* (19.6%), *T. theileri* (10.0%), *T. congolense* (7.5%), and 2 yet to be identified putative species in 15.1% of the positive cattle. Mixed trypanosome infections occurred in 41.8% of the infected cattle and *T. vivax*/*T. simiae*co-infection was most dominant (26.4%). This study also revealedthat ECF vaccinated cattle were 43% less likely to carry trypanosome infections and moreover, the proportion of cattle carrying three infections of trypanosome,*T. parva* and *E. ruminantium*was low.This study confirms prevalenttrypanosome infections in both cattle and tsetse flies and suggests the importance of comprehensive control regimes targeting ecological, biological and socio-cultural interventions in the Maasai communities of northern Tanzania.

# DECLARATION

I, Mary Simwango, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither being concurrently submitted nor submitted in any other Institution.

| | |
|---|---|
| **Mary Simwango** | **Date** |
| (MSc. CANDIDATE) | |

The above declaration has been confirmed by;

| | |
|---|---|
| **Prof. P. S. Gwakisa** | **Date** |
| **(Supervisor)** | |

| | |
|---|---|
| **Prof. S.I. Kimera** | **Date** |
| **(Supervisor)** | |

## COPYRIGHT

# ACKNOWLEDGEMENTS

I would like to thank God for all the blessings and opportunities He has granted me. I would like to appreciate my supervisor Prof. P.S. Gwakisa for his tireless mentorship, guidance, support, and motivation to shape this work. May God bless and grant you the desires of your heart. My sincere gratitude also go to my supervisor Prof. S.I. Kimera for energy and passion to help me during the course of this research.

I would like to acknowledge my sponsors, the Commission for European Union (EU) through EACEA in the project “Intra-ACP Mobility scheme” for the scholarship to study at Sokoine University of Agriculture.I would also like to acknowledge thefinancial support from the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the Canadian International Development Research Centre (IDRC) through the research programme Population Health Vulnerabilities to Vector Borne Diseases: Increasing resilience under climate change conditions in Africa, and specifically, the project “Predicting vulnerability and improving resilience of the Maasai communities to vector borne infections: An Eco-health approach in Maasai steppe ecosystem.

My special thanks to Ms, Linda Salekwa, Ms Happiness Nnko, Mr AnibarikiNgonyoka and Dr.Moses Ole Neselle for helping me with my data collection and laboratory analysis.I am thankful to the VVBDI staff in Tanga for assisting me with the human infective trypanosomes research. I would also like to acknowledge the Maasai people from Simanjiro and Monduli districts for allowing me to conduct this study in their communities.To my friends and colleagues at SUA, it was lovely to work with you.

# DEDICATION

I am extremely grateful to God for the abundance of strength and love that He has shown me during this undertaking. With His unyielding grace and mercy, I have emerged victorious. It is with great honour that I dedicate this dissertation to my lovely parents Constance and Sophia Simwango, my brothers, sisters and the rest of my family for theendurance and support during this study.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF APPENDICES

# LIST OF ABBREVIATIONSAND SYMBOLS

| | |
|---|---|
| AAT | African Animal Trypanosomosis |
| ACP | African, Caribbean and Pacific |
| AFLP | Amplified Fragment length Polymorphism |
| AWF | African Wildlife Foundation |
| B3 | Backward outer primer |
| BIP | Backward Inner Primer |
| Bp | Base pair |
| CFSPH | Centre for Food Security and Public Health |
| CFT | Compliment Fixation Test |
| CNS | Central Nervous System |
| dATP | Deoxyadenosine Triphosphate |
| dCTP | Deoxycytidine Triphosphate |
| dGTP | Deoxyguanosine Triphosphate |
| DNA | Deoxyribonucleic Acid |
| dNTP | Deoxy ribonucleotide Triphosphate |
| dTTP | Deoxythymidine triphosphate |
| EACEA | Education, Audio-visual and Culture Executive Agency |
| ECF | East Coast Fever |
| EDTA | Ethylene Diamine Tetra Acetic Acid |
| ELISA | Enzyme Linked Immunosorbent Assay |
| EU | European Union |
| F3 | Forward Outer Primer |
| FAO | Food and Agriculture Organization |

| | |
|---|---|
| FIP | Forward Inner Primer |
| gDNA | Genomic Deoxyribonucleic acid |
| GSC | Genome Science Centre |
| HAT | Human African Trypanosomosis |
| IFAT | Indirect Fluorescent Antibody Test |
| ITM | Infection and Treatment Method |
| ITS | Internal Transcribed Spacer region |
| KIN | Kinase |
| LAMP | Loop-mediated Isothermal Amplification |
| LB | Loop Backward primer |
| LF | Loop Forward primer |
| ml | Millilitre |
| mm | Millimetres |
| mM | Milimoles |
| nPCR | Nested Polymerase Chain Reaction |
| OIE | World health organization for Animal Health |
| OR | Odds Ratio |
| p104 | Microneme-rhoptry protein |
| PCR | Polymerase Chain Reaction |
| Pmol | Pico moles |
| RAPD | Random Amplified Polymorphic DNA |
| rDNA | Ribosomal DNA |
| RFLP | Restricted Fragment Length Polymorphism |
| RIME | Repetitive Insertion Mobile Element |
| rpm | Revolution per Minute |

| | |
|---|---|
| RT-PCR | Real Time Polymerase Chain Reaction |
| SRA | Serum Resistance Associated gene |
| SSUrRNA | Small Subunit Ribosomal Ribonucleic Acid |
| SUA | Sokoine University of Agriculture |
| TAE | Tris-acetate-Ethylene Diamine Tetra Acetic Acid |
| Taq | *Thermus aquaticus* |
| TBE | Tris-Borate-Ethylenediaminetetraacetic acid |
| TDR | Tropical Disease Research |
| TM | Trademark |
| TMA | Tanzania Meteorological Agency |
| UPGMA | Unweighted Pair Group Method with Arithmetic Mean |
| USA | United States of America |
| VSG | Variable Surface Glycoprotein |
| VVBDI | Vectors and Vector borne Disease Institute |
| WHO | World Health Organization |
| ZR | Zymo Research |
| µl | Microlitre |
| µM | Micromoles |

# CHAPTER ONE

## 1 INTRODUCTION

### 1.1 Background

Over the years, people have evolved from the traditional conservative lifestyle to a more modern way of living due to increased knowledge, population growth, environmentalchanges and exposure to other cultures(Sutherst, 2004; Malele *et al*., 2011b). These changes have increased the mechanisms to sustain their livelihoods. These mechanisms include land use activities such as agricultural production and urbanization(Livingstone, 2010). The increase in land use has forced the natural habitat barrier between game areas and human settlement areas in the Maasai steppe to reduce, thereby increasing the wildlife, livestock and human interaction (Patz*et al*., 2005).Wild animals act as reservoir hosts of many disease causing agents. The interaction between wild animals, domestic animals and humans exposes them to various vector borne infectious diseases such as African trypanosomosis(Munangandu *et al*., 2012).African trypanosomosis is a parasitic protozoal disease that affects humans and animals. The disease affecting wild and domestic animals is called African Animal Trypanosomosis (AAT) and is commonly called Nagana while in humans, it is called Human African Trypanosomosis (HAT) and is commonly called sleeping sickness (WHO, 2004).

African animal trypanosomosis (AAT) is one of the important vector borne diseases that prevents economic growth of the Maasai people who depend mostly on livestock production for their livelihoods.Livestock production contributes to the Agricultural economy by more than 30% in developing countries and about 350 million people depend on it for their livelihoods (Otte and Knips, 2005; Hassane, 2013). The Maasai people in particular depend on animal agriculture as their main source of income. The pastoralists

generate their income mostly through livestock, milk and beef sales. By doing this, they develop both economically and socially. However, the livelihood of the Maasai people is threatened by the vulnerability of their livestock to AAT. This threat is mainly due to the ecological nature of the area which supports breeding and multiplication of tsetse flies thereby increasing the chances of transmitting the disease (Munang'andu*etal*., 2012). The environment in the Maasai steppe also supports development and multiplication of ticks which transmit parasites such as *Theileriaparva*, *Ehrlichia ruminantium*, *Anaplasma* species and *Babesia* species. These infect and co-infect cattle further increasing animal losses (FAO, 1983; Njiri *et al*., 2015). The understanding of these parasites and how they relate to the present control measures is crucial in their successful control strategies.

Tsetse flies are important vectors of African trypanosomosis. There are about 23 species of tsetse flies in sub-Saharan Africa that naturally feed on about 30 species of wild animalsfrom which they transmit several species of trypanosomes to domestic animals and humans (Muturi *et al*., 2011; OIE, 2013). Trypanosome infections in tsetse flies are highly influenced by changes in climate, environment and host availability(Okoh *et al*., 2012). Human and animal hosts become available to tsetse flies as the Maasai people move closer to the wildlife areas and water bodies in search for food and water for their livestock. This exposure to tsetse fly infested areas increases both tsetse fly infection rates and trypanosomoses in animals and humans (Wardrop, 2016).

Human African trypanosomosis (HAT) has a social economic impact due to high cost of surveillance and control of the disease (Kibona*et al*., 2002). The number of reported cases of HAThave reduced significantly in the past seven years due to efforts of controlling the disease (WHO, 2016).Once humans get infected with human-infective trypanosomes, the clinical signs develop quickly and can be diagnosed and controlled promptly (Lumbala*et*

*al*., 2015).Domestic animals such as cattle play a role in transmission of HAT in that they actas reservoir hosts of trypanosomes. This is because cattle can graze in high vegetative areas (AWF, 2015), where tsetse flies hide, they get infected and carry the infections (Swai and Kaaya, 2012) back to villages, where humans reside.

In cattle, clinical signs can take some time to develop.This makes them become carriers which facilitate the spread of the trypanosome infections to other animals (OIE, 2013).Thusthe importance of early detection of infections before they manifest into clinical disease (Radostitis*et al*., 2006).Molecular techniques have been developedand used in research to detect disease causing agents before and after the onset of clinical signs. This is useful for long term surveillance and control of infectious diseases. Polymerase chain reaction (PCR) is one of the molecular techniques used for detection and characterization of infectious agents (Mohini and Deshpande, 2011). It has also been used in this study to screen for trypanosome infections in cattle blood and tsetse fly tissues.

The PCR technique uses specific oligonucleotides (primers) to amplify a specific gene of an organism. For trypanosomes, the gene of interest is Internal Transcribed Spacer (ITS) 1 and 2 which is amplified using ITS primers in a single or nestedPCR (Cox *et al*., 2005: Thumbi*et al*., 2008).

Tsetse flies, as vectors of trypanosomes, are known to harbour infections and transmit them to other organisms such as humans and animals either biologically or mechanically(Malele *et al*., 2003). Previous studies in the Maasai steppe have shown a 3-6% prevalence of trypanosomes in tsetse flies which provides a risk to the exposed susceptible hosts (Morlaris *et al*., 1998: Malele *et al*., 2003: Adams *et al*., 2008: Mwandiringana *et al*., 2012: Salekwa*et al*., 2014).

This study focusses on the prevalence of trypanosome species that occur in cattle and tsetse flies in the Maasai steppe. The prevalence in cattle and tsetse flies defines the risk of trypanosomosis in humans which is directly associated with the exposure to the parasites.This study is also focused on the significance of the wildlife/ livestock/ human interface area on the prevalence and distribution of African trypanosomes in the Maasai steppe of Northern Tanzania.

## 1.2 Problem Statement and Justification

### 1.2.1 Problem statement

The interaction of wildlife, livestock and humans in the Maasai steppedue to changing climate, human activities and land use has increased occurrence of African trypanosomosis in cattle and humans leading to reduced animal production and income to the Maasai people(Savić *et al*., 2014).The temperature and rainfall patterns associated with climate change in the pastoral lands of Simanjiro and Monduli districts have influenced the biological development of tsetse flies which are important vectors of trypanosomes. Trypanosome transmission is highly influenced by interaction of tsetse flies with susceptible hosts, in ecosystems that sustain circulation of these parasites. Thus, human activities associated with land use (Agriculture and livestock movements) provide an ecological niche for the spread of trypanosomosis. Livestock movements in the Maasai steppe is a customary activity to protect their livestock from starvation. However, there is insufficient knowledge on the current status of tsetse-borne trypanosomosis and other vector borne diseases, which leads to lack ofsustainable means and non-uniform vector control in the interface areas that do not protect humans and their livestock from trypanosomosis.

### 1.2.2 Justification

Studies have shown that the prevalence of trypanosome infections in both cattle and tsetse flies is dependent on the presence of reservoir hosts, and ecological factors associated with

the life cycle of trypanosomes. However the understanding of current infection status is key to providing the required knowledge of controlling the African trypanosomosesappropriately and preventor reduce future occurrence, which is highly dependent on the prompt diagnosis of the infectious agents. Establishment of coinfections in cattle is essential in the prediction of severity of infections and vulnerability of cattle to other vector borne diseases. Therefore there is need to study and quantify infections existing between the ecological players of the disease. This study will also bridge the gap between trypanosome infections in tsetse flies and their impact in the livelihood of the Maasai people through infection of their cattle in interface areas.

## 1.3 Objectives

### 1.3.1 Main objective

To determine the extent of vulnerability of the Maasai communities and their livestock to tsetse-borne African Trypanosomoses in the wildlife/livestock/human interface areain northern Tanzania.

### 1.3.2 Specific objectives

i. To determine the molecular prevalence of trypanosome infections in cattle.
ii. To determine the trypanosome infection rates in tsetse flies.
iii. To establish the influence of ECF vaccination on trypanosome and other concurrent infections.

### 1.3.3 Research questions

i. What is the prevalence of trypanosome species in cattle?
ii. What is the prevalence of trypanosome infections in tsetse flies?
iii. What is the influence of ECF vaccination on the prevalence of trypanosomes and other concurrent infections in cattle?

# CHAPTER TWO

## 2 LITERATURE REVIEW

### 2.1 Trypanosomosis

There are two types of trypanosomoses, namely Americanand African trypanosomoses. American trypanosomosis(Chagas' disease) is caused by *Trypanosoma cruzi* and occurs in about 21 Latin American countries (Lyons, 1993). African Trypanosomosis is a protozoal zoonotic disease of humans and animals.There are two forms of the disease(Steverding, 2008).There is Human African trypanosomosis (HAT) which is commonly known as 'sleeping sickness' and African animal trypanosomosis (AAT) commonly known as 'Nagana'(WHO, 2013).

HAT is a disease of public health importance that affect humans.There are two forms of the disease; the chronic form (*T. b. gambiense* form) which is accounts for about 98% of reported cases and the acute form (*T. b. rhodesiense* form) accounting for the remaining2% of reported cases of sleeping sickness(Lyons, 1993). The disease is characterized by intermittent fevers and neurological symptoms such as, abnormal reflexes, ataxia, paraesthesia, convulsions, coma and death occurring in the late stages of the disease (WHO, 2013).

African Animal Trypanosomosis (AAT) is a disease of both domestic and wild animals. The disease causes serious losses in cattle, sheep, goats, pigs and camels(Finelle*et al*., 1983). The diseasecan be acute or chronic and ischaracterized by intermittent fevers, anaemia, oedema, abortion, listlessness, progressive emaciation and cachexia, hair loss, ocular discharge and death(OIE, 2013:WHO, 2016). Animals do not develop lasting immunity against trypanosome parasites due to the antigenic variation of the glycoprotein

that covers the trypanosome parasite. This leads to increased use of body proteins by the animals leading to weight loss (Pathak, 2009). Because of this the vaccine production industry has not been successful in producing a vaccine against trypanosomes. (Matthews *et al*., 2015). Trypanosome species cause severe disease in domestic animals while mild disease occurs in wild animals.

## 2.2 Aetiology of the Trypanosomosis

All forms of trypanosomoses are caused by protozoan parasites called trypanosomes. These are pathogenic parasites that live and multiply extracellularly in the blood, and tissue fluids of their mammalian hosts, (OIE, 2013:Steverding, 2008). Tsetse-borne trypanosomes of production and economic importance include *Trypanosoma congolense, Trypanosoma brucei, Trypanosoma vivax* and*Trypanosoma simiae.* Other trypanosomes that cause AAT are *Trypanosoma suis, Trypanosoma evansi*and *Trypanosoma godfreyi*(Steverding, 2008: CFSPH, 2009).

Human infective trypanosomes that cause disease in Africa are*Trypanosoma brucei gambiense* which cause the chronic form found mainly in central and West Africa while *Trypanosoma brucei rhodesiense* causes acute disease mainly found in East and Southern Africa (OIE, 2013). Trypanosomes inhabit invertebrate host which can be vectors during transmission while they cause disease in vertebrate hosts.

## 2.3 Classification of Trypanosomes

Trypanosomes are classified based on their morphological and molecular characteristics as belonging to the genus *Trypanosoma* of the family Trypanosomatidae which belongs to the order Kinetoplastida, class Zoomastigophoraof the phylum Sarcomastigophora, subkingdom Protozoa, which belongs to the kingdom Protista. Three subgenera which

make up a group of trypanosomes of importance in domestic animals and humans include *Duttonella*, *Trypanozoon* and *Nannomonas*. The *Nannomonas* group is made up of *T. congolense*, *T. godfreyi* and *T. simiae*. The *Trypanozoon* group consists of all members of *T. brucei*, *T. equipedum* and *T.evansi*. *Trypanosomavivax* and *T. uniforme* belong to the Duttonella sub-genus (Morlais *et al*., 1998; Ulienberg, 1998; Eyob and Matios, 2013; Taioe, 2013; Bezie *et al*., 2014).

## 2.4 Classification of Tsetse Flies

Tsetse flies belong to the genus *Glossina* of the family *Glossinidae*, superfamily *Hippoboscida*e, Order *Diptera*, Class *insecta*, phylum Arthropoda of the kingdom Animalia.The three subgenera include *Austenia* (fusca group), *Nemorhina* (Palpalis group) and *Glossina* (Morsitans group). Tsetse flies can also be classified based on their preferred habits as Savannah (Morsitans group), forest (Fusca group) and riverine (Palpalis group) flies (CFSPH, 2009). Savannah flies are mostly found in grasslands areas where livestock keepers graze their animals and therefore are more likely to transmit trypanosomes to domestic animals and humans. These include *Glossina morsitans*, *Glossina pallidipes*, *Glossina swynnertoni*, *Glossina austeni,* and*Glossinalongipalpis*. The riverine group mainly transmit trypanosomes that infect cattle, pigs and humans. These include *Glossina fuscipes*, *Glossina palpalis* and *Glossina tachynoides*. The forest tsetse flies include*Glossina longipennis*, *Glossina brevipalpis.* These prefer feeding on pigs and ruminants (Radostitis*et al.,* 2006).

## 2.5 Lifecycle and Pathogenesis of Trypanosomes

Tsetse transmitted trypanosomesrequire vertebrate (mammalian) and invertebrate (tsetse fly) hosts to complete their life cycle, as a result they cause disease of varying severity in the different hosts such as wildlife, livestock and humans that they infect (Auld

andTinsley, 2015). Tsetse flies usually rest in sheds of forests near potential food areas such as forests, water collection points, and vegetation near villages. They feed on blood and get attracted by moving objects, blue objects, carbon dioxide and animal scents. The life cycle of a trypanosome begins when a tsetse fly picks up the trypanosome parasite by feeding from an infected host. Tsetse flies pick up trypomastigote forms of trypanosomes, which lose their glycoprotein surface coats, elongate and multiply in the midgut of a tsetse fly thereby transforming intoelongated procyclic trypomastigotes which transform into epimastigote forms in salivary glands or proboscis of tsetse fly. These epimastigotes produce the infective metacyclic trypomastigotes.*Trypanosoma vivax*develop in the proboscis while *T. congolense*develops in the midgut and proboscis of the tsetse fly.*Trypanosoma brucei*migrates from the midgut to the salivary glands within the tsetse fly host. Thus trypanosome species can also be identified based on their location.The epimastigotes then multiply and give rise to small infective metacyclic forms of trypanosomes which acquire a glycoprotein surface coat. At this point the tsetse fly becomes infective to the new host it feeds on (Bezie *et al*., 2014; Firesbhat and Desalegn, 2015). This process has been illustrated in Fig. 1.

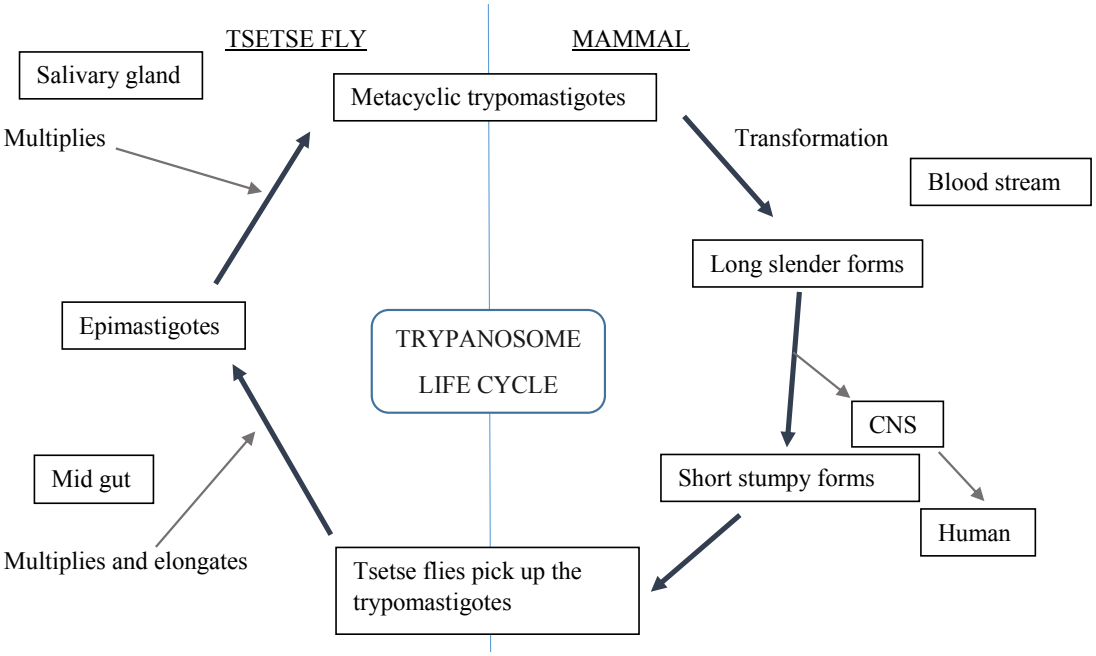



**Figure 1: Illustration of the life cycle of *Trypanosoma species* in tsetse fly and mammalian hosts**

When the metacyclic trypanosomes are injected into a susceptible mammalian host, they develop and multiply locally at a site of infection causing skin chancre. The mature trypomastigotes are then released into the blood stream through lymph vessels, broken capillaries and tissues where they multiply further by binary fission and can be detected by parasitological examinations. *Trypanosoma congolense* localize in capillaries and small blood vessels where they attach to vascular endothelial cells. This is enabled by their small size. *Trypanosoma brucei* and *T. vivax* invade the tissues of various organs where they cause damage. The immune response that occurs causes the clinical signs exhibited by the vertebrate hosts. This occurs within 2 to 3 weeks of infection (Radostitis*et al*., 2006; Bezie *et al*., 2014).

## 2.6 Transmission of Trypanosomes

Trypanosomes can be transmitted either by vector (tsetse-borne) transmission, mechanical or sexual transmission. Vector-transmitted African trypanosomes are primarily transmitted

by *Glossina* spp which are also known as tsetse flies(Ulienberg, 1998). Trypanosomes undergo morphological,development and multiplication inside the tsetse fly and are transmitted to susceptible animals and humans through saliva during feeding(Finelle*et al.*, 1983).There are several species of *Glossina* that transmit trypanosomes in Africa. These include; *Glossina morsitans*, *Glossina palpalis, Glossina fuscipes*, *Glossina swynnertonni*and*Glossina palidipes*(CFSPH, 2009).Mechanical transmission of trypanosomes can occur through biting insects or iatrogenically through blood transfusion and organ transplant. An example of a mechanically transmitted trypanosome is*Trypanosoma vivax*which can be transmittedby both tsetse flies and mechanically by biting flies such as *Stomoxys calcitrans* and Tabanid flies (OIE, 2013).*Trypanosoma evansi* which causes a disease called Surra is transmitted mechanically by biting flies and occurs mostly in tropical and subtropical regions of Africa.Another route of transmission of trypanosome infections is by stercoraria (faecal transmission) method. This type of transmission is enabled by Tabanid, Triatomine and Hippoboscid flies that excrete metacyclictrypomastigotes. When stercorarian transmitted trypanosomes infect mammalian hosts, they multiply as amastigotes or epimastigotes. Examples of such trypanosomes are *T. cruzi*, *T. theileri* (infects cattle) and *T. melophagium* (infects sheep) (Lukes, 2009).Sexually (venereal) transmitted trypanosomes, *Trypanosoma equipedum* causes a disease called Dourine in horses (Ulienberg, 1998; CFSPH, 2009).Once infected with trypanosomes, clinical disease may develop within 4 days to 6 weeks depending on the trypanosome species involved. Trypanosomes can also be transmitted congenitally from mother to child by crossing through the placenta or by bleeding of the mother during birth of an offspring. Examples of trypanosomes transmitted this way are *T. cruzi* and *T. vivax*. Incidental transmission of trypanosomes can occur by iatrogenic means through transfusion of infected blood, use of needles and surgical instruments that have been used on infected blood and infected organ transplant (Lyons, 1993; Ulienberg, 1998; WHO, 2012).

## 2.7 Distribution of Tsetse Flies and Trypanosomes

Tsetse flies are found in Africa where they are widely distributed across the tropical and sub-Sahara regions between latitude 14$^{o}$North and 29$^{o}$ South (Firesbhat and Desalegn, 2015). The distribution of trypanosomes correspond to the distribution of the tsetse flies in Africa.*T. vivax* is also found in central and south America (Maré, 2004; Radostitis*et al*., 2006). Tsetse flies inhabit about 37% of Africa's total land. They are found mostly in areas that are close to wildlife due to the conduciveness of the environment for their survival and accessibility to food.

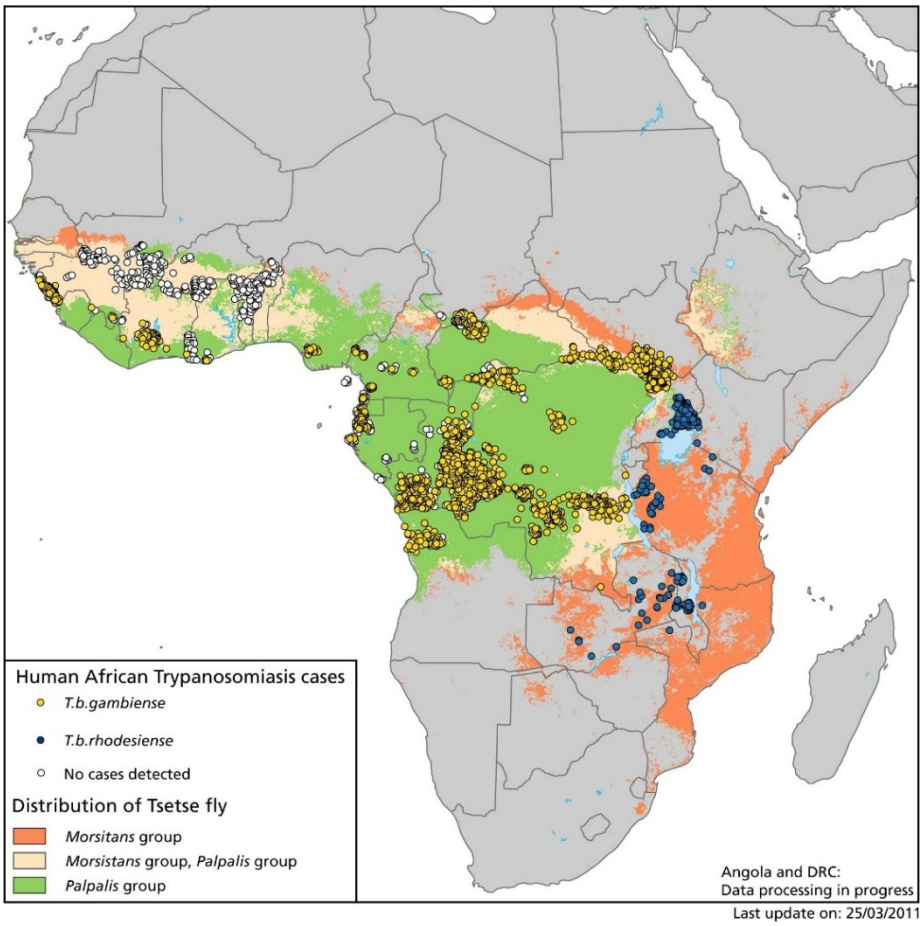



**Figure 2: Distribution of Tsetse flies and African trypanosomosis cases**

**Note:** The map in Fig. shows the similar distribution of tsetse flies species and the two forms of African trypanosomosis (gambiense and rhodesiense form). It also shows the high density of *G. morsitans* associated with the distribution of *T. b. rhodesiense* in east and central Africa.Taken from the Atlas of Human Infectious Diseases (Wertheim *et al*., 2012).

In Tanzania, *Glossina* spp are widely distributed across the country especially in protected areas, game reserves, National parks and wildlife corridors such asthe Maasai steppe.Tsetse density has been shown to be high in Manyara, Mara and Tanga regions.The distribution of tsetse has changed over time due to environmental changes by human activities such as agriculture and extensive land use (Malele *et al*., 2011). Studies have shown that the most widely distributed tsetse fly species in Tanzania is *G. morsitans* (also shown in Fig. 2). Others species reported are sub- species of *G. morsitans* (*G. m. morsitans* and *G. m. centralis*) and *G. fuscipes* (*G. f. fuscipes* and *G. f. martini*) found in the western part of Tanzania (Malele *et al*., 2011a). *Glossina pallidipes* and *G. swynnertoni* were reported to be most abundant in Northern Tanzania (Malele *et al*., 2011a; Salekwa *et al*., 2014).

Trypanosomes are also widely distributed in animal and human hosts. They cause disease in a wide range of domestic animals including cattle, pigs, camels, goats, sheep, dogs, cats, horses and monkeys (FAO, 1992). Rats, mice and guinea pigs can be used as laboratory animals for trypanosome research. A wide range of wild animals can be infected with trypanosomes making them carriers and maintenance hosts of the disease(Bengis *et al*., 2002). Cattle are important reservoir hosts of trypanosomes aiding in transmission of infections to other animals and humans.

## 2.8 Ecology and Epidemiology of Trypanosomes

The ecology and epidemiology of vector borne diseases is driven by factors such as the presence of a suitable vector, susceptible hosts, the parasite and environment (Thrusfield, 2005). The interaction of these factors leads to the occurrence of the diseases. The Maasai steppe ecosystem provides an environment for interaction of reservoir hosts, vectors, and susceptible hosts(Auty *et al*., 2012). This makes trypanosomosis one of the most occurring vector borne diseases in the Maasai steppe.

### 2.8.1 Factors influencing the occurrence of trypanosomes

#### 2.8.1.1 Trypanosome intrinsic factors

Trypanosomes survive in mammalian hosts due intrinsic factors that support their survival. One of the factors isantigenic shift. This aids trypanosomes overcome the mammalian immune response by changing the variant surface glycoprotein (VSG) coat (Vincendeau and Bouteille, 2006). A trypanosome infection causes extreme form of antigenic variation in the host that manifests into series of parasitaemia occurring with a single infection. Other factors are presence of enzymes and host derived receptors that aid in multiplication of trypanosomes. The pathogenicity and virulence of the parasite (WHO, 2012) are major factors that lead to disease manifestation and harmin susceptible hosts (Mathews *et al*., 2015). These factors influence the behaviour of the parasite in the mammalian hosts (Harrus and Baneth, 2005).An example of a pathogenic trypanosome is *T. brucei*. For example, *T. b. gambiense*has properties that enable it to cross the blood-brain barrier and invade the central nervous system (CNS) and cause neurological symptoms (Bucheton *et al*., 2011). The virulence of the trypanosome depends on the trypanosome species involved. Some species such as *T. congolense*and *T. vivax*are more virulent than others like *T. theileri* (Bezie *et al*., 2014).

#### 2.8.1.2 Host factors

Wild animals are the main reservoir hosts of trypanosomosis, which play a major role in the epidemiology of the disease in bothhumans and animals. Wild animals associated with transmission of trypanosomes include buffalos, elands, impalas, bushbucks. Wildlife species from the family Bovidae are most significant in harbouring pathogens of importance in cattle (Kock, 2005). These maintain low persistent levels of trypanosome infections without showing clinical signs. However, humans and domestic animals are highly susceptible to trypanosomes and can suffer serious illness depending on the species

of trypanosome involved (Berrang-Ford *et al*., 2005). Thus, interaction between humans, domestic and wild animals in interface areas influence the spread of trypanosomes(Dantas-Torres *et al*., 2012). The major host factor that contributes to the survival of the trypanosomes is the susceptibility to the disease. Examples of host susceptibility scenarios are the susceptibility of horses to dourine (caused by *T. equipedum*) and cattle to Surra (caused by *T. evansi*), pigs to *T. suis*and humans to *T. b. rhodesiense*.Another example is the susceptibility of pigs to pathological effects of *T. simiae* compared to cattle. Trypanotolerant breeds of cattle such as the N'dama, (West African short-horn Bos taurusfound in West and Central Africa), the Maasai zebu and Sheko breed (found in eastern Africa) are less susceptible to trypanosomosis than other breeds.They have the ability to control the development of pathological effects of the trypanosomes (Radostits *et al*., 2006); Bezie *et al*., 2014). Trypanotolerant cattle are resistant to trypanosomosis due to features like skin coat, (that reduce tsetse infestations on the animal), ability to fight anaemia (due to a stronger haematopoietic system), and a high complement immune response that is effective against trypanosomes (Murray, 1990; Dolan, 1998; d'Ieteren *et al*., 1998).

#### 2.8.1.3 Environmental factors

The environment has changed over time due to natural and human based factors (Ellis and Wilcox, 2009). Natural factors related to climate change are temperature, rainfall and humidity. These have been reported to affect the development of trypanosomes and the ability of tsetse flies to feed on the required host (Macleod *et al*., 2007). Human based factorsthat have altered the environment are closely related to land use and human activities, includingdeforestation, agriculture, population growth, urbanization and environmental pollution (Anderson *et al.*, 2015). Thesefactors have changed the natural habitats and survival of disease vectors including tsetse flies (Harrus and Baneth,

2005).Trypanosomosis is one of the environment-sensitive vector borne diseases,and is highly affected by changes that alter the conditions of the lifecycle of trypanosomes (Patz *et al*., 2005; Auld and Tinsley, 2015). These alterations to the existing ecosystem increase the vulnerability of the Maasai people and their domestic animals to tsetse fly infestation and trypanosome infections (Mboera *et al*., 2011).

#### 2.8.1.4 Social and cultural factors

Human comfort play a major role in the epidemiology of African trypanosomosis. Social factors influencing the spread of vector-borne diseases are trade, Agriculture, tourism, international travel and the nomadic movement of pastoralists and agro-pastoralist (Harrus and Baneth, 2005). Human activities such as cultivation, clearing of land have the ability to change the ecosystem which in turn can alter the behaviour of tsetse flies and trypanosomes. Other social factors responsible for the spread of trypanosomosis are management regimes that fail to protect humans and animals against the disease(Ellis and Wilcox, 2009; Majekodunmi *et al*., 2013). Livestock farmers may not fully comply with the necessary requirements for prevention of and control of trypanosomes. Pastoralist practices on the movements of cattle from one place to another in search for food may also have an implication on the dynamics of trypanosomes especially in the wildlife/ Livestock/ human interface areas (Anderson *et al*., 2015).

### 2.8.2 Tsetse fly abundance

Vectors play a crucial role in the epidemiology of trypanosomes causing disease in domestic animals and humans (Sahinduran, 2012). The abundance of vectors and vector-borne diseases is highly affected by globalization, land use and climate change which can be observed through surveillance systems such as geological information systems, knowledge of the ecology, remote sensing techniques and the use of satellite to detect

climatic variables (Madder *et al.*, 2013). Climate change also influence the density, distribution and diversity of vectors which affect transmission patterns of vector borne disease causing agents. Factors favouring the abundance of tsetse flies in wildlife/ livestock / human interface areas are availability of hosts and conducive environmental conditions such as rainfall, temperature, humidity, vegetation type and forestry. Tsetse flies are important in the transmission of trypanosomes and they pose as a major factor contributing to the occurrence of trypanosomosis. There are several factors that support successful transmission of trypanosomes by tsetse flies.

These are vector competency which is the ability of tsetse flies to maintain and sustain trypanosomes during their biological developmental stage within the vector (Franco *etal*., 2014), dispersion of tsetse fly species in an area which in the Maasai steppe ecosystem depends highly on the presence of hosts (blood meals), density of vegetation, rainfall, and temperature (WHO, 2004).

## 2.9 Importance of African Trypanosomosis

The interaction of humans, livestock and wildlife expose the Maasai people to tsetse infestations and trypanosomosis. African trypanosomoses pose as a disease of public health importance in these areas due to its zoonotic nature. As a result humans and animals who live in wildlife interface areas such as the Maasai steppe are at risk of significant impact of the disease. The importance of African trypanosomosis can be looked at based on the individual diseases such as HAT and AAT (CFSPH, 2009).

### 2.9.1 Importance of HAT

Human African trypanosomosis is caused by *T. b. gambiense* and *T. b. rhodesiense*. These parasites are of major public health importance in Africa(Simarro*etal*., 2008).The two

parasite species that cause HAT have distinct pathological effects on humans. The gambiense HAT (*T. b. gambiense*) causes the chronic form of trypanosomosis in west and central Africa. This marks a long term disease circulation in the human reservoir hosts (Franco *et al*., 2014). The rhodesiense form (due to *T. b. rhodesiense*) causes an acute disease leading to severe damages within a short period of time. The two forms of HAT can cause serious neurological damages, organ failure, comatose and death with case fatality close to 100% if they are not treated.Production losses in humans with clinical HAT occurs when they cannot perform their usual duties to their full capabilities due to their compromised health (Lutumba *et al*., 2007; WHO, 2002). Economic losses of HAT are due to costs of diagnosis, treatment and surveillance.

### 2.9.2 Importance of AAT

Most wildlife are tolerant to AAT due to the long coexistence of game animals and tsetse flies. This tolerance however does not extend to domestic animals like cattle, goats, sheep and pigs. This makes domestic animals highly susceptible to not only trypanosome infections but also AAT(Connor, 1994) thereby limiting livestock productivity in sub-Saharan Africa (Odeyemi, 2015). The disease has an impact on the health of domestic animals by causinginfertility, anaemia, weakness and weight loss, leading to reduced production and productivity. African Animal Trypanosomosis in cattle causes serious economic loss in the Maasai communities due to animal losses, production losses, nutritional losses and cost of treatment (Connor, 1994; CFSPH, 2009; Chanie *et al*., 2013). Production losses in cattle occurs when animals are clinically sick. This reduces and compromises their milk yield. Death may occur if the clinically sick animals are not treated. This reduced the number of animals in a herd thereby reducing the resources (Swallow, 1999).

The impact of AAT in the Maasai steppe highly depends on the tsetse fly density, the distribution of the trypanosomes, susceptibility and availability of alternative hosts,

vegetation, livestock production practices, control strategies and exposure to tsetse infested areas (Desta *et al*., 2013). The Maasai people in the Maasai steppe also live within the tsetse fly belt and are exposed to the same tsetse flies domestic animals are exposed to (WHO, 2013).

## 2.10 Concurrent Trypanosome Infections

Vector borne diseases are one of the major threats to Agricultural development and food security in tropical and subtropical region of the world. They affect about 80% of the world's cattle population through high morbidity and mortality rates (Demessie and Derso, 2015). In Africa they are a major set-back to the livestock industry. These diseases are enhanced by presence of suitable vectors, susceptible hosts, disease causing agents, presence of reservoir hosts and suitable climatic conditions (Gubler, 2009; Savić*et al*., 2014). Trypanosomes are one of the major vector borne diseases affecting the livestock industry in Africa. Their occurrence alone cause serious economic losses in the Maasai steppe. The disease has been known to deplete proteins and immunity of infected animals leading to concurrent infections(Eshetu and Begejo, 2015).

Several diseases have been reported to occur concurrently with trypanosomosis. These include parasitic, bacterial, fungal and viral diseases. Protozoan parasitic diseases have been known to cause similar impact as trypanosomosis leading to more serious economic losses (Demessie and Derso, 2015). These diseases are caused by *Theileria parva*, *Ehrlichia ruminantium*, *Anaplasma marginale*and*Babesia* spp. These parasites are transmitted by ticks which are abundant in the Maasai steppe (Oakgrove *et al*., 2014). Ticks are the most important vectors of cattle diseases and the second, after mosquitoes, most important vectors of human pathogens (Kock, 2005; Ghosh and Nagar, 2014). They are able to transmit vector borne pathogens due to their wide range of hosts to feed from

especially in highly vegetative areas. they are able to survive in harsh conditions for long periods of time due to their hardness and prolonged existence while maintain high population numbers (Kock, 2005).

Concurrent infections are common in the Maasai steppe and their influence on occurrence on trypanosomosis is important in understanding the epidemiology and behaviour of trypanosomes in the mammalian hosts (FAO, 1992). The interaction of parasites with susceptible hosts has a clinical consequence in animals which are immune compromised. The interaction of several parasites in one host may create competition for host's nutrients(Oakgrove *et al*., 2014). The immune response of the host may be able to eliminate other parasites, but they may not be able to eliminate trypanosomes due to the variant surface glycoprotein coating. As a result the animal may die from persistent trypanosomosis and nutrient depletion which is characterized by chronic wasting (FAO, 1992; Wang *et al*., 2003).

### 2.11 Control of Trypanosomosis

Trypanosomosis prevalence has continued to increase in the Maasai steppe due to diversity of the pathogens which have not been targets for control. Understanding the epidemiology of these species is key to controlling the disease (Fe`vre *et al*., 2006). Prevention and control of African trypanosomosis is by breaking the transmission cycle of the trypanosome parasite. This ismainly achieved by controlling tsetse flies and exposure of susceptible hosts to vectors (Eyob and Matios, 2013). There are limited methods of controlling African trypanosomosis due to high costs and inadequate sustainable control programs.

#### 2.11.1 Chemical control

Chemical control is a widely used method that involves the use of insecticides/ trypanocidal drugs to control of both tsetse flies and trypanosomes (Adamu *et al*., 2011).

The method of vector control practiced by the agro pastoral and semi-nomadic communities in the Maasai steppe in mainly chemical control by strategic acaricide and insecticides application (d'Ieteren *et al*., 1998).Pyrethroids are most widely used insecticides for control of tsetse flies and deltamethrin is used as aninsecticide in animal sprays and odour bated targets (Maudlin, 2006). However the knowledge on the application of these chemicals in the communities is crucial in preventing drug resistance and environmental pollution due to over use and under use.

#### 2.11.1.1 Aerial spray

Aerial spray of tsetse flies, bush clearing, wildlife eradication and insecticide spraying methods have been used in the past for control of tsetse flies in larger areas such as National parks and forest areas. This had an ecological implication that could lead to ecosystem imbalance (Malele, 2011).

#### 2.11.1.2 Use of traps baited with attractant

Traps, targets and screens have been used as a method of capturing tsetse flies for research purposes and to control tsetse fly density in the tsetse belt. This method of control is effective where traps can be maintained. The technique involves setting up a device with attractants of visual (blue or black) and odour (carbon dioxide, acetone or octanol) that mimic mammalian hosts (Malele, 2011). This technique requires prior knowledge of the process before setting it up. Some traps have limited areas where they can be set. This limits the density of flies that can be captured in a given area. Another limit to the use of traps is that they can be destroyed by strong wind, wild animals fire or floods (Adamu *et al*., 2011).

#### 2.11.1.3 Use of trypanocidal drugs

The diseases are also controlled through treatment of sick animals. Animals showing clinical signs of AAT are commonly treated with diminazeneaceturate, and to a lesser

extent isometamidium (prophylactic drug). The successful treatment of trypanosomosis is highly dependent on the correct diagnosis of the diseases (Radostitis*et al.,* 2006). Human African Trypanosomosis is mainly treated by Suramin (in the early stages of *T. b. rhodesiense* infections) and Pentamidine (which is used for treatment in the early stages of *T. b. gambiense* infections). Later stage treatment involves the use of Melarsoprol, Eflornithine and a combination of Eflornithine and Nifurtimox (WHO, 2012). These drugs have been used for a long time in the treatment of both African trypanosomoses (Steverding and Tyler, 2005; WHO, 2012).

### 2.11.2 Biological control

#### 2.11.2.1 Use of trypanotolerant Breeds

Trypanotolerant breeds are used to control trypanosomosis in Africa as they have properties that reduce infections and disease(Murray, 1990; Dolan, 1998; d'Ieteren *et al*., 1998). Breeding practices that encourage maintainingstrong animals with high survival traits in the herd, have been known to reduce incidence of trypanosomosis. This is because this method selectively targets animals with strong immunity and trypanotolerant genes that can be passed on to others, with the aim of fighting infections. This method is supported by prevention of inbreeding of animals (Vincendeau and Bouteille, 2006).Although this method is effective in controlling the disease in tsetse infested areas,number of such trypano-tolerant animals is small as it takes years to select or cross breed animals. Thus the method is not appropriate for immediate control of epidemics and outbreaks of trypanosomosis. The use of trypanotolerant breed is best used for long term control coupled with other methods such as trypanocidal drug and vector control (Adamu *et al*., 2011).

#### 2.11.2.2 Sterilisation of male tsetse flies

Release of male tsetse flies is another method of controlling trypanosomosis. This technique controls breeding of tsetse flies thereby reducing their population density

(Scoones, 2014). It involves the systematic release of sterile males into the tsetse fly population. The released sterile males mate with the females which only copulate once in their life time, rendering them non fertile (Adamu *et al*., 2011). The effectiveness of this method would be achieved with other control techniques to reduce the spread of tsetse flies from other areas The limitations to this technique are; high cost of breeding and sterilizing the male tsetse flies, the technique cannot be used by individual farmers as a routine control method in the field and it is more amenable for use by government (Malele, 2011a).

#### 2.11.2.3 Use of Symbiotic bacteria

Another technique researchers have attempted to develop for control of African trypanosomosis is the use of symbiotic bacteria (transgenic *Solidalis*) modified to produce trypanocidal effects and render tsetse flies resistant to trypanosome infections. The effects of this method on the tsetse fly density and trypanosome infections is being evaluated (Gilbert *et al.*, 2016).

### 2.11.3 Effect of Chemotherapy on Trypanosome Prevalence

The prevalence of trypanosome infections have reduced significantly from about 60% to about 10% due to vector control measures applied by the livestock keepers in African countries. The control is usually based on parasitological detection (Radostits*et al*., 2006) which only allows detection of diseased animals. Morbidity, mortality and case fatality rates of trypanosomes vary with the species of trypanosomes involved, the host resistance and the ability of the farmer to control the disease.One of the reasons why trypanosome have not been eradicated in the Maasai steppe is resistance to chemotherapeutic agents due to misuse of drugs through under-dosing, overdosing, misdiagnosis of the disease or wrong use of drugs which creates resistance. In as much as farmers may report frequent

use of acaricide and insecticides, the tick and tsetse fly burden still remains high due to selective application of acaricide to animals that are perceived to be at risk (Goldman, 2011; Swai and Kaaya, 2012).This further, exposes cattle to trypanosome and other vector borne infections which impinge on the economy of the people due to high cost of treatment and animal losses.

### 2.12 Diagnosis of African Trypanosomosis

African trypanosomosis can be noted based on clinical signs and history of the disease especially in endemic areas. Important history for diagnosis of African trypanosomosis includes environmental predisposition associated with tsetse fly bites. Such information can be obtained from sick individuals or livestock farmers with prior knowledge pertaining to animal movements.History of pre-existing trypanosomosis in endemic areas is essential in the diagnosis of the disease (Nakayima, 2016). The disease can also be diagnosed based on prior knowledge of the distribution on the causative *Trypanosoma* spp(Batchelor *et al*., 2009). However, clinical diagnosis of this disease is difficult due to similarity of the clinical signs which are not pathognomonic to African trypanosomoses. Thus, the definitive diagnosis can be obtained by performing parasitological profile,serological or molecular examination (Radostits*et al*., 2006; Nakayima, 2016). The choice of a diagnostic method is limited by sensitivity, specificity, cost, expertise, urgency of required results and the disease or parasite involved. The importance of correct diagnosis in to enable the understanding of the parasite and the control measures to be put in place (Eshetu and Begejo, 2015).

#### 2.12.1 Parasitological diagnosis

There are several techniques for detecting parasitaemia due to trypanosome infections. Microscopical parasitological diagnosis is the most widely used method for diagnosis of

African trypanosomosis. These mainly detect the morphology and motility of the trypanosome species.

#### 2.12.1.1 Blood smears

Wet smearpreparations of infected blood can be performed on a slide and viewed microscopically. This method can easily be performed due to its simplicity, low cost and does not consume much time (Eshetu and Begejo, 2015). The method however has lower sensitivity and does not differentiate between the different species of trypanosomes. Also it has to be performed at the farm.

Thick and thin smearscan demonstrate presence ofpiroplasms and trypanosomes in the blood when the blood smear is stained with Giemsa or Quick-differential stain. Piroplasms of *Anaplasma species*, *Babesia species* and *Theileria species* appear intracellularly whiletrypanosomes appear extracellularly on a blood smear and*Ehrlichia ruminantium* appear as a morula in endothelial cells of the brain. Parasites can be identified based on their morphological characteristics.*Trypanosoma congolense* normally appears as a short and small parasite with a marginal kinetoplast, no prominent undulating membrane and no free flagella. *Trypanosoma brucei* can occur as a large parasite occurring in short, long slender or intermediate forms on a stained slide. The parasite has varying sizes of free flagella, sub-terminal kinetoplast and a well-developed undulating membrane.Thin and thick smears can be made in the field and can provide temporal storage before getting the blood sample to a laboratory centre.It is a challenge to differentiate other species of trypanosomes using parasitological analyses. These can be correctly identified and characterised using molecular diagnostic tools (Moody and Chliodini, 2000).

#### 2.12.1.2 Concentration techniques

Haematocrit centrifugation techniqueis used for parasitological detection of trypanosomes. It involves concentrating the parasite in the blood in a capillary tube. It is a reliable and

effective method of diagnosing trypanosomes in the blood by parasitological method (Bezie *et al*., 2014). The technique however, cannot be used in the field because it requires electricity (Nantulya ,1990). Another technique used for purification and detection of trypanosomes is the Anion exchange (woo's) technique. This technique is achieved by the use of ionic charges of the parasite and the host's blood (Zillmann *et al*., 1996; Camara *et al*., 2010).

### 2.12.2 Serological diagnosis

Serological tests for parasitic diseases detect specific antibodies elicited against parasite antigens that infect animals. Detection of the antibodies suggests exposure to parasite even though there is no proof of active infection. Serological tests can detect past infection and in some cases vaccination as in ECF (Eshetu and Begejo, 2015). Two serological techniques used to detect presence of trypanosome infections include Enzyme-Linked Immunosorbent Assay (ELISA) and Indirect Fluorescent Antibody Test (IFAT). Other tests include Rapid agglutination and compliment fixation test.

#### 2.12.2.1 Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-Linked Immunosorbent Assay (ELISA)can be used for detection of parasite specific antibody (Antibody ELISA), immune complexes or antigen (Antigen ELISA) detection. Sensitivity and specificity is usually obtained through the use of purified antigens. ELISA does not require sophisticated equipment and can therefore be performed in a basic laboratory (Nantulya, 1990). However, the lysate used as an enzyme for ELISA reaction is difficult to standardise.

#### 2.12.2.2 Indirect Fluorescent Antibody Test (IFAT)

Indirect Fluorescent Antibody Test (IFAT)involves fixing of infected blood smears on a slide using a mixture of acetone and formaldehyde and stored at cool temperatures. This

method requires sophisticated equipment and cannot be used in the field (Nantulya, 1990).This method is mostly used for detection of African trypanosomes in herds (Connor, 1994; Radostits *et al*., 2006; Nakayima, 2016). This technique is sensitive and specific in the detection of trypanosomes in animals and humans. However, the technique tends to cross react making comparison of results for different trypanosome species difficult (Nakayima, 2016).

#### 2.12.2.3 Compliment Fixation test (CFT)

Another method useful in the diagnosis of Equine trypanosomosis is Compliment Fixation test. This is a technique that was used for diagnosis of *T. evansi* in domestic animals and for eradication of *T. equipedum* in horses in Canada and South Africa (Nantulya, 1990). The test in however difficult to perform and not practical at field level (Nantulya, 1990).

### 2.12.3 Laboratory animal inoculation

Infected blood can be inoculated into laboratory animals such as mice or rats. This method enables multiplication of the trypanosomes such that they can be detected easily by parasitological methods. The technique is highly sensitive and can be used by researchers for long term observation of results (OIE, 2012; Eshetu and Begejo, 2015). However, it is expensive due to maintenance costs of experimental animals, requires laboratory animals and it takes time to yield results and requires other diagnostic techniques such as wet smear examination(Nantulya, 1990).

### 2.12.4 Molecular detection techniques

Clinical diagnosis of trypanosomes in the Masai steppe gives unreliable picture of the trypanosome parasites circulating in the area due to epidemiological and ecological characteristics of the disease. Early detection of any disease is key to its successful treatment and control. To achieve this, nucleic acid based techniques have been developed

for a number of parasites. These detect infections prior to clinical manifestation of the disease.Thus determination of the prevalence of trypanosomes in cattle requires more sensitive tests (Picozzi*et al*., 2002; Nakayima, 2016).

There are a number of molecular diagnostic techniques that can be used to detect parasitic infections. These include conventional PCR, reverse transcriptase PCR, real time PCR Amplified fragment length polymorphism (AFLP), Microsatellite marker method, multiplex PCR, RFLP PCR, micro-arrays, PCR- ELISA, and Loop-mediated isothermal Amplification (LAMP). These amplification methods have advantages of being sensitive, specific, easy to use, can analyse a large number of samples at once early in infective stage of the parasites(Tang *et a*l., 1997; Ulienberg, 1998; Deborggraeve *et al*., 2010; Tavares *et al*., 2011; Osman *et al*., 2016).

#### 2.12.4.1 Polymerase Chain Reaction (PCR)

Polymerase Chain Reactiondeveloped by Kary Mullis and colleagues in the 1980's is the most widely used nucleic acid amplification technique (Valones *et al*., 2009). It is used for amplification of specific sequences from a large mixture of DNA thereby detectinga wide range of genes including those of parasitic infections. This is because of its high sensitivity, specificity, relative low cost and flexibility to detection of variable genes(Tang *et al*., 1997; Cox *et al*., 2010). The parasite genes can be detected in samples from both the host and the vector. It accomplishes this using a pair of oligonucleotide primers complementary to the Deoxyribonucleic Acid (DNA) sequence of specific genes(Netto *et al*., 2003;Malele *et al*., 2003). The basic PCR involves the mixture of reagents such as PCR Buffer, $MgCl_2$, dNTPs, water,forward and reverse Primer, and the target DNA polymerase. These undergo repeated cycles of denaturation (separation of the DNA strands at high temperature), annealing (synthetic oligonucleotides attaching to their

complimentary strands of DNA at a lower temperature) and extension (sequences between the oligonucleotides are extended by DNA polymerase) steps. The primers determine the specificity of the PCR by targeting only a specific segment of a genome from a wide range of sources. These include generic, species- specific and nested PCR primers. These are all based on the amplification of a specific fragment of a gene. An example of parasite genes that undergo this kind of amplification include a 1098-bp fragment of species-specific variations in small subunit ribosomal ribonucleic acid(SSUrRNA) gene of *Theileria* spp, in which a 277-bp internal fragment located between 2784 and 3061 bases of the p104 gene of *T. parva*(Ariyaratne *et al*., 2014). Another example is a 279-bpfragment within open reading frame 2 of the 1306-bp pCS20 sequence of *E. ruminantium* DNA (Peter *et al*., 2000; Wagner *et al*., 2004) and an Internal Transcribed spacer 1 and 2 regions of ribosomal DNA of trypanosomes.There are a different types of primers (Oligonucleotides) that can be used for amplification of trypanosome DNA (Morlais *et al*., 1998).The Internal Transcribed Spacer 1 region of ribosomal DNA is commonly used as a target gene in the amplification of trypanosome DNA in a single Primer-PCR (single PCR) or in a PCR that uses 2 sets of primers (nested PCR).The nested ITS primers amplify both ITS 1 and ITS 2 regions of the trypanosome DNA giving it a higher sensitivity and specificity (Cox *et al.,* 2005; Thumbi *et al*., 2008; Njiru *et al*., 2008a).

#### 2.12.4.2 Nested PCR

This method involves a double process of amplification of a target gene of DNA using two sets of primers that increase the sensitivity and specificity of the reaction (Hernández-rodríguez and Ramirez, 2012). The nested ITS PCR has been proven to be accurate, cost effective, robust, simple, quick, reliable and can be used to screen a large number of samples (Cox *et al*., 2005). The nested PCR method detects a diversity of trypanosome species that can be considered in the knowledge and understanding of the epidemiology of AAT in wildlife/ livestock interface areas (Desquesnes *et al*., 2001; Njuri *et al*., 2005;Osman *et al*., 2016).

#### 2.12.4.3 Loop-mediate Isothermal Amplification method (LAMP)

LAMP is technique used for gene amplification by using a set of six primers to displace the DNA strands with synthesized DNA polymerase under isothermal conditions that range from $62^{o}$ to $65^{o}$C. Gene amplification by this method occurs rapidly and efficiently due to the sensitivity and specificity of the method (Notomi *et al*., 2015). This technique can be used to detect trypanosome gene through the use of specific markers that can be specific to a group of species such as Repetitive Insertion Mobile Element (RIME-LAMP) specific for *Trypanozoon* and Serum Resistant associated gene (SRA-LAMP) for *T. brucei rhodesiense* (Njiru *et al*., 2008a). The reaction uses a set of outer and inner primers complimentary to a chain of region to be amplified in a sequentialrepetitive manner to elongate the displaced strand. LAMP has a similar sensitivity to nested PCR and the reaction takes a shorter time to complete (one hour) due to its amplification efficiency and can be visualised by naked eye (Kuboki *et al*., 2003; Notomi *et al*., 2015). The limit to this technique is that it cannot be used as a diagnostic tool under filed conditions (Notomi *et al*., 2015).

# CHAPTER THREE

## 3 MATERIALS AND METHODS

### 3.1 Description of the Study Area

The study was conducted in the Maasai steppe of Northern Tanzania which ismade up of Simanjiro plains, Tarangire National park and Lake Manyara National park (AWF, 2015). It stretches over eight districts of Arusha and Manyara regions of Tanzania. It lies between $3^{o}52'$ and $4^{o}24'$ south and $36^{o}05$ and $36^{o}39$east. The area has two rainfall seasons of spatial and temporal variation, comprising of the short rains from October to December and the long rains from February to May.The Average temperature in the area is between $18^{o}C$ and $30^{o}C$. The Maasai steppe is made up of natural ecosystem consisting of a variety of animal species, vegetation, conducive temperature variations and rainfall, all of which support a natural habitat for living organisms, including predators, vectors and various parasitic organisms (Patz *et al*., 2005). Such an ecosystem regulates the density of organisms found in the area. The area is semi-arid with the national parks providing a home for wildlife. Livestock and crop production are the main sources of livelihood in the Maasai steppe with cattle production being the major activity (Msoffe *et al*., 2011). This area also consists of a wildlife corridor within the tsetse fly belt which is bordered by Monduli district and Manyara ranch located in the north and Tarangire National park on the South-western side. Due to its proximity to the National park, the study area has high interaction of wild, domestic animals and humans which increases circulation of trypanosomes through bites from tsetse flies, making trypanosomosis endemic in the area.

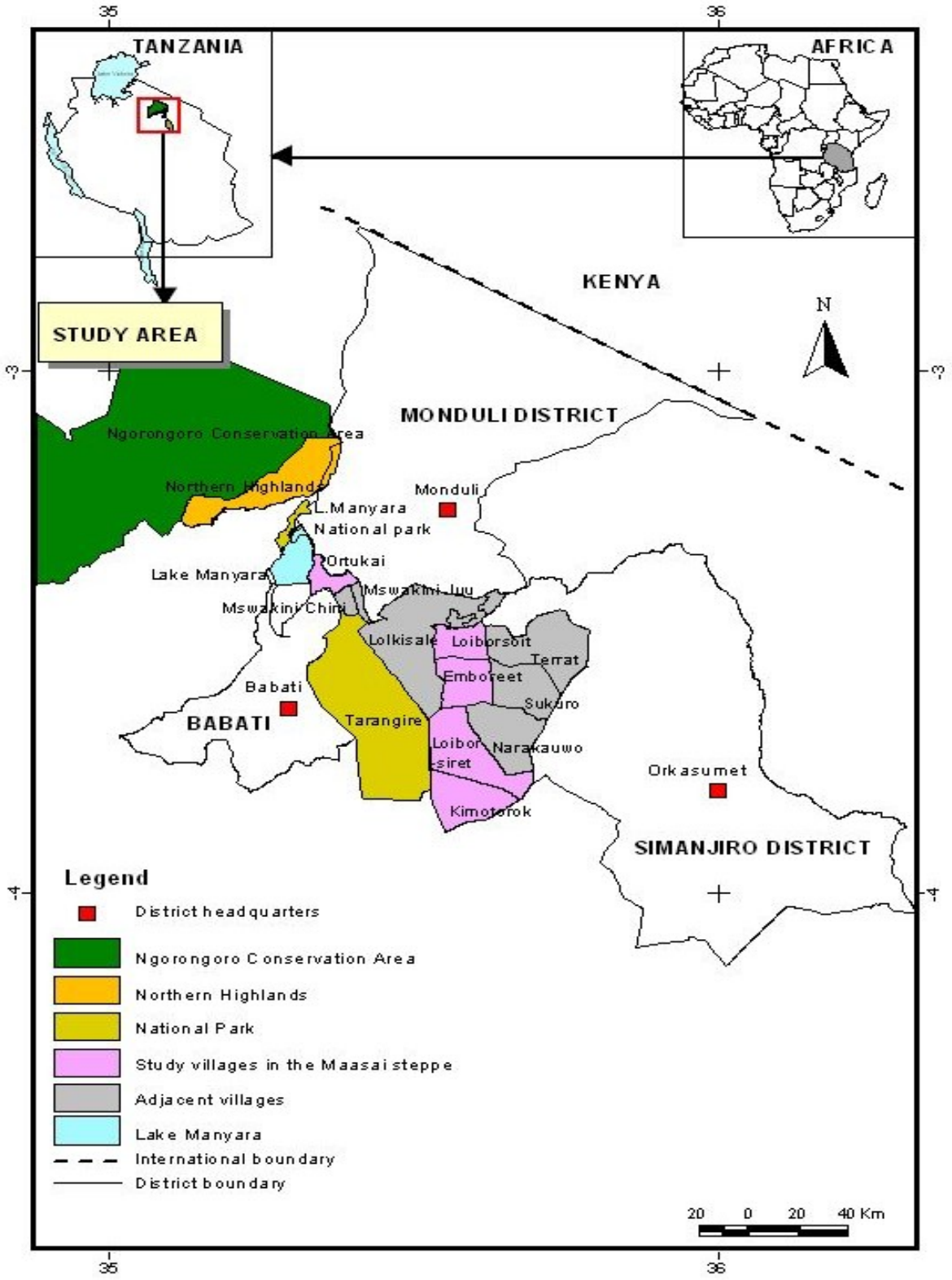



**Figure 3: Map showing the study area in Simanjiro and Monduli districts, Northern Tanzania**

### 3.2 Study Design

A repeated cross sectional, observational study design was used to obtain the required results in which two samplings were conducted in 4 villages including an additional third sampling in one village. This was done as a comparative study of the magnitude of trypanosome infections between wet and dry seasons. Bomas (traditional Maasai homesteads, usually consisting of a number of huts surrounding an enclosure for livestock, especially cattle) sampled were not necessarily the same in each sampling. Tsetse fly sampling also was done in a repeated cross sectional manner.

### 3.3 Sample Size Determination

Sample size determination was based on a simple random sampling (Thrusfield, 2005) with modifications according to cluster sampling methods. Sample size was calculated using the following formula;

$$N = \frac{(Z\alpha)^2\ (pq)}{d^2} \times D_{effect}$$

Where N is the sample size, Zα =1.96 at 95% Confidence level, standard error of the mean, p =0.278, the previously reported prevalence for Monduli district (Haji *et al*., 2015), q =1-p, d=0.05 at 5% absolute error, $D_{effect}$=1.2 (design effect).

The calculated sample size was 389. However 400 animals were sampled in each sampling due to the mark up value that was added to prevent field and laboratory analytical errors.

### 3.4 Sampling

A multistage cluster sampling technique was used to sample cattle in which a tsetse fly corridor was used as land marks for sampling.Based on this, five villages were selected purposively due to their proximity to Tarangire National park, in the same areas where

tsetse flies werecaptured. In these villages, 50% of the sub-villages were selected at random in which 50% of the Bomaswere selected systematically. A bomawas a sampling unit in which animals were selected at random.To determine the prevalence of trypanosomes in cattle, 1002 blood samples were collected in June 2015 (end of wet season), August 2015 (dry season) and February 2016 (wet season) from 5 villages: Emboreet, Loiborsiret, Kimotorok, Loiborsoit and Ortukai. The average number of cattle collected per Maasai boma was sevenand a total of 106 bomaswere sampled in Monduli and Simanjiro districts. 100 cattle were sampled from each village except for Loibor-soit-A village (where 60 blood samples were collected on the basis of ECF-vaccination status) and Emboreet village (where 340 blood samples were collected three times in order to capture seasonalityof trypanosome prevalence).

### 3.4.1 Blood collection

Cattle blood was drawn from the jugular vein using vacutainer needles into EDTA vacutainer tubes and stored in cool boxes for overnight while in the field and later at -20$^{o}$Cin the Genome Science Centre (GSC), Faculty of Veterinary Medicine in Sokoine University of Agriculture (SUA) until processing time.Tables 1 and 2 show details of the blood samples.

**Table 1:Villages and number of samples collected**

| SN | Name of Village | Number of animals sampled | |
|---|---|---|---|
| | | **Wet season** | **Dry season** |
| 1 | Emboreet | 100 | 100 |
| 2 | Loiborsiret | 100 | 100 |
| 3 | Kimotorok | 100 | 100 |
| 4 | Ortukai | 100 | 100 |
| | **Total** | **400** | **400** |

**Table 2: Cattle blood samples collected based onECF-vaccination status**

| | Vaccination status | Number of samples |
|---|---|---|
| 1 | Vaccinated | 100 |
| 2 | Unvaccinated | 100 |
| | **Total** | **200** |

### 3.4.2 Tsetse Fly Sampling and Trapping

Tsetse fly trapping was an activity in a WHO-TDR-funded project, to which this dissertation is part of. The project, 'Predicting vulnerability and improving resilience of the Maasai communities to vector-borne infections: an eco-health approach in the Maasai Steppe ecosystem' deployed trapsin sites which were selected through stratified random sampling of the major vegetation types in four villages of Loibor-siret, Emboreet, Kimotorok and Ortukai located along the protected areas of the Maasai steppe. About 4421 tsetse flies were trapped.Individual tsetse flies were dried, ground, stored in Eppendorf tubes and transported to SUA, for DNA extraction.

## 3.5 Laboratory Analysis

Laboratory analyses were conducted in the laboratory of the Genome Science Centre at the Faculty of Veterinary Medicine in Sokoine University of Agriculture in Morogoro and at Vectors and Vector borne Disease Research Institute (VVBDRI) in Tanga, Tanzania.

### 3.5.1 DNA extraction

#### 3.5.1.1 DNA extraction from cattle blood

DNA was extracted from cattle blood using the, Quick-gDNA blood mini prep kit (D3017, Zymo research, USA). Blood was left to thaw at room temperature for three hours before DNA extraction. One hundred µlof blood was put into a 1.5ml micro-centrifuge tube, followed by 400µl of genomic lysis buffer (containing 0.5% beta-mercaptoethanol). The

contents were mixed thoroughly at 20 Hertz for five Seconds on a vortex (VELP Scientifica)and incubated at room temperature for 10 minutes. The mixture was then transferred to a Zymo-spin™ IICcolumn² in a collection tube and centrifuged at 10000rpm for one minute (Eppendorf 5417R, USA). The collection tube was then discarded with the flow- through solution. The Zymo-spin IIC™ column was then transferred to a new collection tube and 200µl of DNA Pre-Wash Buffer was added and centrifuged at 10000rpm for one minute. Up to 500µl of g-DNA Wash Buffer was added to the spin column and centrifuged at10000rpm for one minute. The spin column was then transferred to a clean and sterile micro-centrifuge tube where DNA was eluted by adding 50µl of DNA Elution Buffer and incubating for five minutes at room temperature. Then the spin column was discarded and DNA collected in a micro-centrifuge tube. The eluted DNA was checked for quality and quantity using spectrophotometry (JENWAY Genova) before storage at -20$^{o}$C.

#### 3.5.1.2 DNA extraction from whole tsetse fly

Ground tsetse flies from amicro-centrifuge tube were individuallyadded into a ZR BashingBead™Lysis Tube (ZR Tissue and Insect DNA MiniPrep, D6016, Zymo Research). Up to 750µl of Lysis solution was added to the tube containing the sample and vortexed for 15 minutes. The ZR BashingBead™Lysis Tube was centrifuged at 10000rpm for one minute. Four hundred µl of supernatant was transferred to a Zymo-spin™ IV spin filter in a collection tube and centrifuged at 7000rpm for one minute. One thousand two hundred µl of Genomic Lysis buffer was then added to the filtrate and 800µl of the mixture was transferred to a Zymo-spin™ IIC column in a collection tube and centrifuged at 10000rpm for one minute. The flow through solution wasdiscarded from the collection tube and the previous step was repeated to finish the remaining amount of Genomic Lysis Buffer. Two hundred µl of DNA Pre-wash Buffer was added to the Zymo-spin™ IIC

column in a new collection tube and centrifuged at 10 000rpm for one minute. Five hundredmicrolitresof g-DNA wash Buffer was then added to the Zymo-spin™ IIC column and centrifuged at 10000rpm for one minute. The Zymo-spin™ IIC column was then transferred to a clean 1.5ml micro-centrifuge tube where 50µl of DNA Elution Buffer was added directly to the column matrix and centrifuged at 10 000rpm for 30 seconds. The eluted DNA was checked for quality and quantity using spectrophotometry (JENWAY Genova) before storage at -20$^{o}$C.

### 3.5.2 Molecular detection of the ITS 1 trypanosome gene from cattle and tsetse flies using Polymerase Chain Reaction (PCR)

Stock solutions of ITS1 forward and reverse oligonucleotides (primers) that amplify an Internal Transcribed Spacer 1 gene were diluted from 100µM to 10µM working solution which was diluted further such that 0.2µM of each primer was used forthe PCR reaction. Sequences of the primers are shown in Table 3.

PCR was performed in 25µl reaction volume containing12.5µl of mastermix (Quick-Load Taq 2X Master Mix, New England BioLabs Inc.) which contains DreamTaq DNA polymerase supplied in 2X DreamTaq buffer, 0.4mM of each of the dATP,dCTP,dGTP and dTTP, and 4mM $MgCl_2$, 0.2µM of each of the forward and reverse primers, 6.3µl nuclease free water and five µl DNA template. PCR was conducted on a thermocycler (ProFlex PCR system, Applied Biosystems) with an initial denaturation step of 94$^{o}$C for three minutes, followed by 30 cycles of 94$^{o}$C for 30 seconds, annealing step at 55$^{o}$C for 30 seconds, 72$^{o}$C for 30 seconds and final extension step at 72$^{o}$ C for 10 minutes (Silbermayr *et al*., 2013).

### 3.5.3 Nested PCR for detection of trypanosomes

Nested PCR (nPCR) reaction was conducted in 12.5µl reaction comprising of 6.25µlMastermix (Quick-Load Taq 2X Master Mix, New England BioLabs Inc.), 2.5µl

DNA template, 3.25µlnuclease free water and 0.2µM of each ITS 1 and ITS 2 primers in the first round. The second round amplification was performed using ITS 3 and ITS 4 primers at the same concentration as the first round, oneµl of the PCR products from the first round as a template and 4.75µl of nuclease free water. Amplification conditions involved an initial denaturation step of 95°C for seven minutes followed by 35 cycles of denaturation at 94°C for one min, annealing step of 55°C for one minute, then extension step of 72°C for two minutes and final extension at 72°C for 10 minutes (Cox *et al*., 2005; Ahmed *et al*., 2013; Tran *et al*., 2014).

**Table 3:Sequences of PCR primers used in this study**

| | Parasite | Target gene | Primer sequence | Expected size | Reference |
|---|---|---|---|---|---|
| 1. | Trypanosomes | ITS 1 | F: 5'-CCG GAA GTT CAC CGA TAT TG-3'<br>R: 5'-TTG CTG CGT TCT TCA ACG AA-3' | Variable | (Thumbi*et al*., 2008; Silbermayr*e t al*., 2013) |
| | | ITS 1<br>ITS 2 | ITS1: 5'-GAT TAC GTC CCT GCCATT TG-3'<br>ITS2: 5'-TTG TTC GCT ATC GGTCTT CC-3'<br>ITS3: 5'-GGA AGC AAA AGT CGT AACAAG G-3'<br>ITS4: 5'-TGT TTT CTT TTC CTCCGC TG-3' | Variable | (Cox *et al*., 2010 (; Ahmed *et al*., 2013) |
| 2. | *Ehrlichia ruminantium* | pCS20 | AB128: 5'-ACTAGTAGAAATTGCACAATC TAT-3'<br>AB129: 5'-TGATAACTTGGTGCGGGAAAT CCTT-3' | 279 bp | (Peter *et al*., 2000) |
| 3. | *Theileria parva* | P104 | 5-ATT TAAGGA ACC TGA CGT GAC TGC-3<br>5-TAA GAT GCC GAC TAT TAAT-GACAC C-3<br>5-GGC CAA GGT CTCCTT CAG AAT ACG-3<br>5-TGG GTG TGT TTC CTC GTC ATC TGC-3 | 277 bp | (Konnai*et al*., 2006; Kabi*et al*., 2014; Kazungu*et al*., 2015) |

The variable expected sizes of trypanosomes vary with the species and primers used

**Table 4: Expected band sizes of trypanosome species**

| SN | Trypanosome species | ITS 1 PCR (Bp) | Nested ITS PCR (Bp) |
|---|---|---|---|
| 1. | *T. congolense Forest* | 710 | 1513 |
| 2 | *T. congolense savannah* | 700 | 1413 |
| 3. | *T. congolense kilifi* | 620 | 1422 |
| 4. | *T. brucei* | 480 | 1207-1224 |
| 5 | *T. simiae* | 400 | 850 |
| 6 | *T. vivax* | 250 | 611 |
| 7 | *T. godfreyi* | 300 | |
| 8 | *T. theileri* | | 988 |

### 3.5.4 SRA-LAMP for Detection of Human Infective Trypanosomes

All *T. brucei* positive DNA from cattle and tsetse flies were subjected to SRA-LAMP technique in order to detect human infective trypanosomes (*T. b. rhodesiense*) (Njiru *et al.,* 2008b).SRA-LAMP was conducted in 25µl reaction volume containing 2.5µl of 1X Thermopol buffer, five µl of 0.8M betaine,one µl of 200µM dNTPs, one µl of 0.2µM F3 and B3 primers,one µl of two µM FIP and BIP primers,one µl of 0.8µM loop primers LB and LF, one µl of 8U Bst DNA polymerase, twoµl of DNA template and 7.5µl of nuclease free water. Reaction condition for the SRA-LAMP were set at 62$^{o}$C for one hour in a GeneAmp® PCR system 9700 (Applied Biosystems™), USA. The reaction was terminated by increasing the temperature to 80$^{o}$C for five minutes. The SRA-LAMP products were visualised through colour change after addition of two µl SYBR Green I. An orange colour indicated negative results while colour change to green signified positive results. The SRA-LAMP products were confirmed by gel electrophoresis.

**Table 5:SRA-LAMP primer sequences**

| Primer | Primer type | Primer sequence |
|---|---|---|
| SRA-F3 | F3 | GCGGAAGCAAGAATGACC |
| SRA-B3 | B3 | TCTTACCTTGTGACGCCTG |
| SRA-FIP | FIP | GGACTGCGTTGAGTACGCATCCGCAAGCACAGACCACAGC |
| SRA-BIP | BIP | CGCTCTTACAAGTCTTGCGCCCTTCTGAGATGTGCCCACTG |
| SRA-LF | LF | CGCGGCATAAAGCGCTGAG |
| SRA-LB | LB | GCAGCGACCAACGGAGCC |

### 3.5.5 Molecular detection of *Ehrlichia ruminantium*

PCR amplification of pCS20 gene of *Ehrlichia ruminantium*was performed in 12.5µl reaction containing 6.25µl of mastermix (Quick-Load Taq 2X Master Mix, New England BioLabs Inc.), nuclease free water, 2.5µl of the DNA template and 0.125µl of AB 128 and AB 129 primers that flank a 279-bp fragment within open reading frame two of the 1306-bp pCS20 sequence of *C. ruminantium*. Amplification conditions involved an initial denaturation step of 95°C for 10 minutes, followed by 45 cycles of 94°C for one minute, then 55°C for one minute, 72°C for two minutes and a final extension step of 72$^{o}$C for 10 minutes (Peter *et al*., 2000).

### 3.5.6 Detection of p104 gene of *Theileria parva*by PCR

Amplification of *T. parva*-specific104-kDa antigen gene(p104) was performed in 10µl reaction volume that included 6.25µl mastermix (Quick-Load Taq 2X Master Mix, New England BioLabs Inc.), ten pmol of each forward and reverse primers, 2.5µl DNA template and nuclease free water. The second round was amplified using 0.25µl of the post PCR product from the first round. Amplification conditions involved an initial denaturation step of 94$^{o}$C for five minutes, followed by 35 cycles of denaturation at 94$^{o}$C

for 60 seconds, annealing step at 60$^{o}$C for 60 seconds and extension at 72$^{o}$C for 60 seconds in the first round. The second round amplification conditions were the same as the first round but the annealing temperature was reduced to 50$^{o}$C (Konnai *et al*., 2006; Kabi *et al*., 2014; Kazungu *et al*., 2015).

### 3.5.7 Agarose gel electrophoresis

PCR products stained with GRGreen Nucleic Acid Stain (Excellgen) were loaded in 1.5% agarose gelsprepared in TAE buffer.The products were separatedat 80 volts for 40 minutes before visualization and documentation on a Gel Doc$^{TM}$ (Bio Rad, USA). SRA-LAMP products were loaded on 1.5% agarose gel prepared in 1X TBE and stained with oneµl 10mg/ml ethidium bromide. The products were separated at 120 volts for 30 minutes and visualised using Ultra violet trans-illuminator.

### 3.5.8 Data analysis

The prevalence of infections was estimated using frequency and contingency tables in Excel and Epi info™ version 7.0 (CDC, Georgia, Atlanta, USA) statistical software. Comparative location and seasonal prevalence was estimated using contingency tables and logistic regression where the Chi-square expected and p-values were obtained. The Chi-square values were obtained by test of independence and homogeneity of proportions between dependent (outcome) and independent (Exposure) variables using Pearson uncorrected test for two-way tables. These values were used to test the strength of association between exposure and outcome variables whereby the larger the chi-square value, the stronger the association. To compare the prevalence of trypanosome infections in cattle and tsetse flies, Mid-P was used to obtain the p-values in Epi info using Statcalc 2 by 2 tables. The p-values of the results were analysed (using p=0.05 as a cut off value) at 95% confidence Intervals to indicate the level of uncertainty around the obtained

values.Odds ratio (OR) was used to measure the effect of ECF vaccination on trypanosome, *E. ruminantium* and *T. parva* infections in which the value equal to 1 (OR=1) indicated that there was no difference between vaccinated and unvaccinated cattle while a value greater (OR>1) or less (OR<1) than 1 implied that there was a difference between them.The DNA fragments of different trypanosome species were compared and confirmedfor genetic similarity using GelCompar II Gel electrophoresis software version 6.5.This was achieved by using Pearson similarity coefficient and a dendrogram was drawn with unweighted pair group (UPGMA) method.

# CHAPTER FOUR

## 4 RESULTS

### 4.1 Prevalence of Trypanosomes in the Study Area

The overall prevalence of trypanosome infections in the study area was 17.2% (172/1002; 95% CI (14.91-19.68)). This valuewas obtained when the 1002 cattle of different age, sex and location were analysed for presence of trypanosome infections.The data are presented inTable 6.

**Table 6:Prevalence of trypanosome infections in the study population by age, sex and village**

| SN | Category | Sub-category | N | Overall prevalence (%) | $\chi^2$ | p-value |
|---|---|---|---|---|---|---|
| 1. | Age (Years) | <2 | 301 | 16.9 | 32.91 | 0.0631 |
| | | 2-6 | 484 | 16.3 | | |
| | | 6-10 | 212 | 18.9 | | |
| | | >10 | 5 | 40.0 | | |
| | | **Total** | **1002** | **17.2** | | |
| 2. | Sex | Female | 716 | 17.5 | 0.15 | 0.6977 |
| | | Male | 286 | 16.4 | | |
| | | **Total** | **1002** | **17.2** | | |
| 3. | Village | Emboreet | 340 | 12.1 | 21.42 | 0.0003 |
| | | Kimotorok | 200 | 20.5 | | |
| | | Loiborsiret | 200 | 18.0 | | |
| | | Loibor-soit-A | 60 | 35.0 | | |
| | | Ortukai | 202 | 16.3 | | |
| | | **Total** | **1002** | **17.2** | | |

N= total number of cattle analysed, $\chi^2$= Chi-square

The results in Table 6 show that prevalence of trypanosome infections varied in cattle of different age categories, although the difference between age groups was not statistically significant based on the p-value obtained. However, the chi-square value shows a strong association between trypanosome prevalence and age of the animal. Thus, highest prevalence (40%) was obtained in cattle aged more than 10 years, whereas lowest trypanosome prevalence (16.3%) was shown in cattle aged between 2and 6 years. The results also show that there was a high strength of association ($\chi^2$ =32.9) between trypanosome infections and age of cattle. The average prevalence of trypanosomes was higher (17.5%) in female cattle than in males (16.4%) but there was no significant difference between the two subcategories.

Table 6 further shows significant variation of trypanosome infections between the 5 villages (P=0.0003). Thus, highest prevalence was found in Loibor-soit-A village (35%) followed by Kimotorok (20.5%), whereaslowest prevalence (12.1%)was shown in Emboreet village.The differencesof prevalence between Kimotorok and Emboreet (p=0.009)andLoibor-soit-A and Emboreet (p=0.000) werehighly significant.

### 4.2 Seasonal Distribution of Trypanosome Infections in the Study Area

The seasonal distribution of trypanosome infections is shown in Table 7 and Fig.4.

**Table 7: Distribution of Trypanosome infections in different seasons**

| **Season** | **N** | **Prevalence** (%) | $\chi^2$ | **p- values** |
|---|---|---|---|---|
| Wet | 200 | 33 (16.5%) | 1.0731 | 0.5848 |
| End of Wet | 402 | 75 (18.7%) | | |
| Dry | 400 | 64 (16.0%) | | |
| Total | 1002 | 172 (17.2%) | | |

N= Number of cattle analysed, $\chi^2$= chi-square

The results show that the highest prevalence of trypanosome infections was 18.7% (75/402) at the end of wet season (June 2015) followed by 16.5% (33/200) during the wet season (February 2016) and the lowest prevalence being 16.0% (64/400) during the dry season (August 2015). However, no significant differences were established between the 3 sampling seasons.

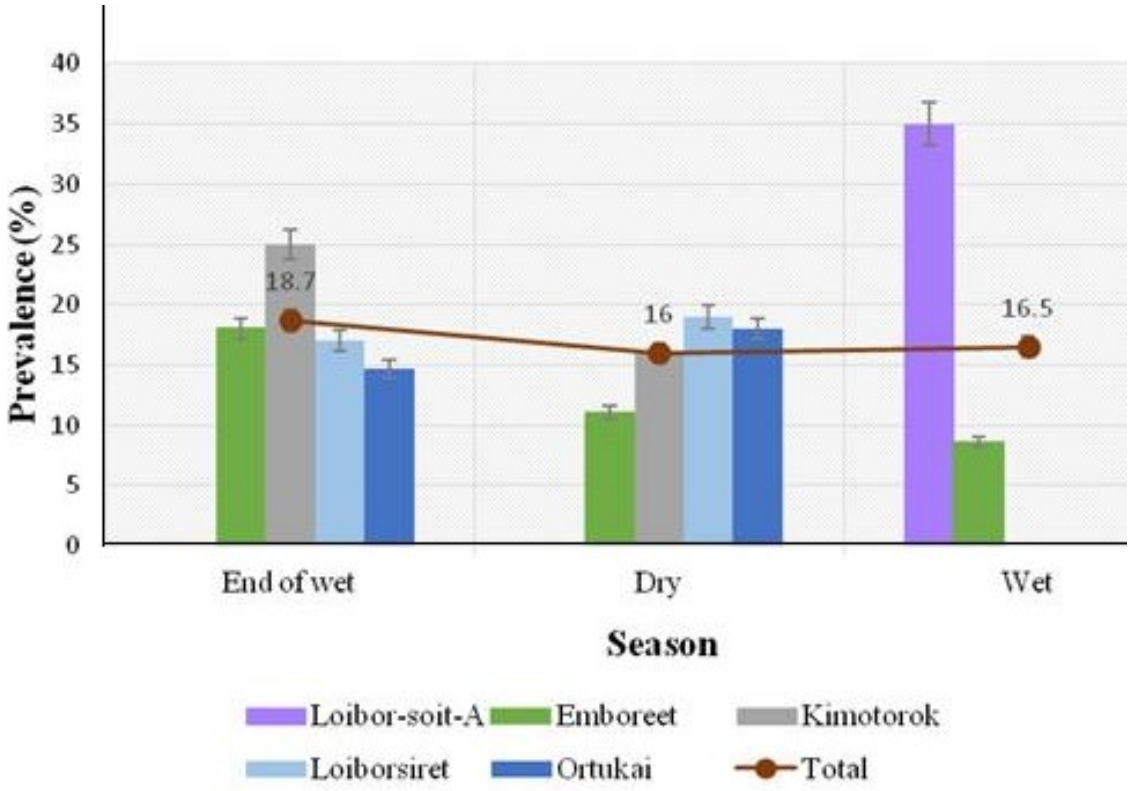



**Figure 4: Spatial distribution of trypanosome infections by season in cattle**

**Note**: Fig. 4 shows prevalence of trypanosome infections in cattle blood collected in June (End of we season) and August (Dry season) in all villages. In February (wet season), cattle were sampled only in Emboreet and Loibor-soit-A villages.

Analysis of seasonality overall trypanosome infection data by village is depicted in Fig. 4.Infection prevalence was highest inLoibor-soit-A during the wet season (35%), whereas it was lowest in Emboretduring the wet season (8.6%). Kimotorok had intermediate prevalence value (25%) at the end of wet season.

### 4.3 Abundance and Mixed Trypanosome Infections in Cattle

Overall, five trypanosome species were detected in cattle; *T. vivax*, (100/261), *T. brucei* (50/261), *T. simiae* (47/261), *T. theileri* (24/261) and *T. congolense* (18/261), in that order of abundance across all villages.Fig.5 shows a gel image and fragment sizes of various trypanosome species found in this study.

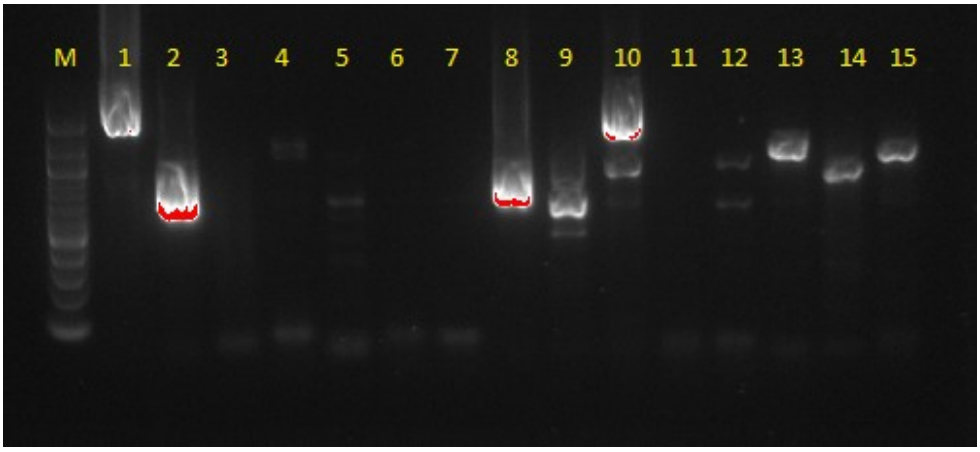



**Figure 5: A gel picture of trypanosomes amplified using nPCR**

M is a 100 bp DNA marker, lane 1 shows a *T. congolense* positive DNA control, lane 2 is *T. vivax* positive DNA control, lane 3 is a negative control. Lanes 4, 13 and 15 are *T. brucei*, 5 and 8 are *T. simiae*, lane 9 is a putative mixed infection of *T. vivax* and an unidentified 500-bp fragment, while 10 is a mixed infection of *T. congolense*, *T. simiae* and *T. theileri*, 12 is a mixed infection of *T. brucei* and *T. vivax*, and lane 14 is *T. theileri*.

Similarity of DNA fragments of the same size shown in Fig. 5 was confirmed using GelcomparII electrophoresis software (Applied Maths, Sint-Martens-lanten, Belgium).This was used to identify the trypanosome species detected by the nPCR. The analysis results are shown in Fig. 6.

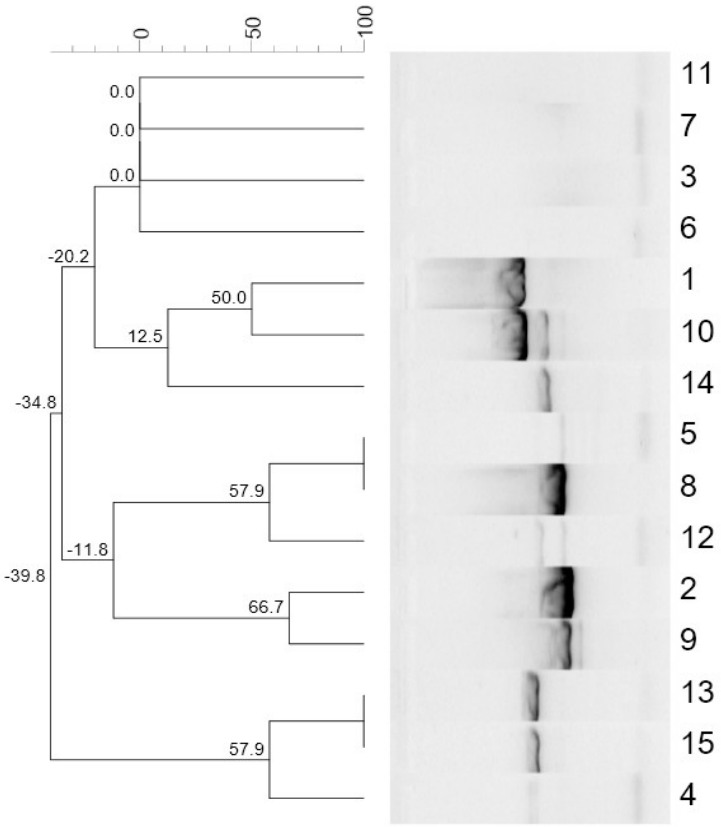



**Figure 6: Genetic similarities of trypanosome species**

The dendrogram displays the detected trypanosome species arranged according to their fragment size similarity. The values above each branch represent extent of similarity of the respective fragments used to identify distinct trypanosome species. The Zero value between any two branches indicates negative samples.Negative values between branches indicate a wide dissimilarity between the fragments representing respective trypanosomespecies. For example, samples 5 and 8 show 100% similarity between two*T. simiae*positive samples. Similarly, samples 13 and 15 show 100% genetic similarity of two*T. brucei* positive samples.

The nPCR detected 5 trypanosome species of which 100 out of 239 *T. vivax* infections, 50/239 were *T. brucei*, 47/239 *T. simiae*, 24/239 *T. theileri* and 18/239 were *T. congolense* infections in that order of abundance across all villages in the 3 seasons.. Additionally, two

unidentified fragments with 300 and 500 bp lengths were also found across villages (Table 8 and Fig. 7).

**Table 8: Distribution of trypanosome infections, by species, across study villages**

| Village | *T. congolense* | *T. vivax* | *T. simiae* | *T. brucei* | *T. theileri* | Total |
|---|---|---|---|---|---|---|
| Emboreet | 4 | 25 | 17 | 9 | 6 | **61** |
| Kimotorok | 4 | 30 | 11 | 12 | 11 | **68** |
| Loiborsiret | 5 | 22 | 5 | 10 | 3 | **45** |
| Loibor-soit-A | 3 | 4 | 4 | 13 | 2 | **26** |
| Ortukai | 2 | 19 | 10 | 6 | 2 | **39** |
| **Total** | **18** | **100** | **47** | **50** | **24** | **239** |

This table describes the number of trypanosome species found, regardless of the number of cattle analysed.

The overall abundance of trypanosome infections was highest in Kimotorok (68/239) and lowest in Loibor-soit-A (26/239). When different species were taken into account, *T. vivax* was the most occurring species in all villages except for Loibor-soit-A where *T. brucei* was most abundant as shown in Table 8 and Fig. 7. Additionally, two unidentified fragments with 300 and 500 bp lengths were also found across villages in 15.1% of trypanosome positive cattle.

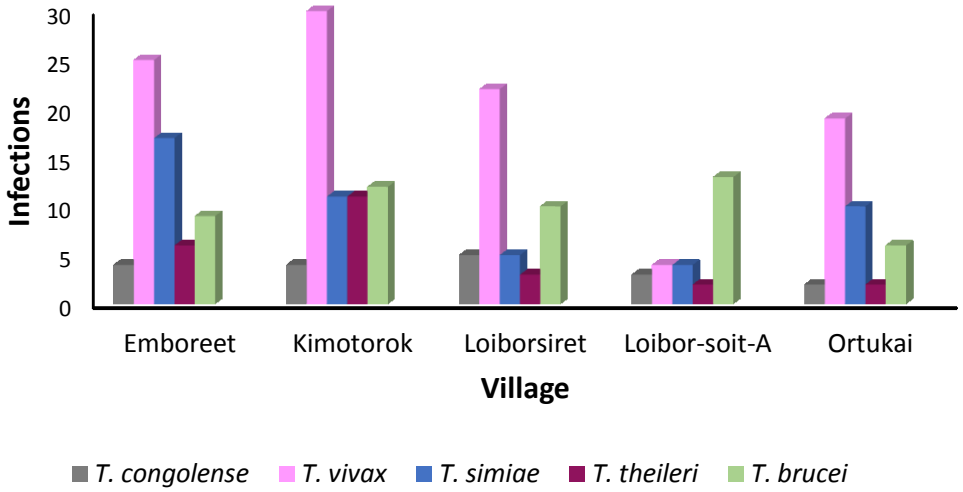



**Figure 7: Distribution of trypanosome infections, by species, across study villages**

Prevalence of the different trypanosomes was then studied in relation to season. Findings of the present study also reveal that prevalence of individual species varied across seasons.However the variation was only significant between seasons for *T. brucei* and *T. theileri* (P=0.025). Strength of association (Chi-square) between species prevalence and season was high for the two species (Table 9).

**Table 9: Seasonal prevalence of individual trypanosome species**

| Species | End of wet | Dry | Wet | $\chi^2$ | p-value |
|---|---|---|---|---|---|
| *T. vivax* | 11.9% | 10% | 6% | 5.25 | 0.072 |
| *T. congolense* | 2.49% | 1% | 2% | 2.58 | 0.275 |
| *T. brucei* | 5.47% | 3% | 8% | 7.36 | 0.025 |
| *T. simiae* | 6.22% | 4% | 3% | 3.81 | 0.149 |
| *T. theileri* | 3.98% | 1.5% | 1% | 7.36 | 0.025 |

A total 239 infections were detected in the 172 trypanosome positive cattle. Only 100 out of 172 (58.1%) cattle carried infections with single trypanosome species. Interestingly, 44 out of 172 cattle (25.6%) carried multiple infections with 2 trypanosome species, 12 cattle

(7.0%) carried 3 trypanosomes and only 1 animal (0.6%) was infected with 4 trypanosome species. The most abundant multiple infections were *T. vivax*/*T. simiae* occurring in 26.4 % of cattle followed by *T.brucei*/*T. vivax* (12.0%). The most abundant multiple infections were *T. vivax*/*T. simiae* (25.3 %) and *T. brucei*/*T. vivax* (12.0%). The most abundant multiple infections were *T. vivax*/*T. simia*e (25.3 %) and *T. brucei*/*T. vivax* (12.0%). Appendix 2 shows details of multiple trypanosome infections in the cattle. None of the *T. brucei* positive DNA were positive for human infective trypanosome species (*T. b. rhodesiense*, *T. b. gambiense*).

### 4.4 Prevalence of Trypanosomes in Cattle and Tsetse Flies

In order to understand transmission of trypanosome infections between tsetse flies and cattle, this study compared infection rates in 1584 tsetse flies and 942 cattle in November 2014 and June and August 2015 in the same study area.

**Table 10: Comparison of trypanosome prevalence between cattle and tsetse flies**

| | Cattle | | Tsetse flies | | |
|---|---|---|---|---|---|
| **Village** | **N** | **Prevalence** | **N** | **Prevalence** | **p-value** |
| Emboreet | 340 | 30(8.8%) | 169 | 22(13.0%) | 0.1413 |
| Kimotorok | 200 | 30(15.0%) | 22 | 0(0%) | 0.03443 |
| Loiborsiret | 200 | 26(13.0%) | 1230 | 86(7.0%) | 0.003357 |
| Ortukai | 202 | 26(12.9%) | 163 | 2(1.2%) | 0.0000324 |
| **Total** | **942** | **112(11.9%)** | **1584** | **110(6.9%)** | **0.00002186** |

The total prevalence had a chi-square value of 18.02

The results in Table 10 show that the overall prevalence of trypanosomes in cattle was significantly higher (11.9%) than in tsetse flies (6.9%) (P=0.000021862). As reported for cattle,prevalenceof *T. vivax*in tsetse flies was found to be higher (100/110; 90.9%) than

that of *T. brucei* (6/110; 5.5%) and *T. congolense* (5/110; 4.5%).Additionally one tsetse fly was detected with a *T. vivax/T. congolense* mixed infection(1/110; 0.9%). When distribution of trypanosome infections in cattle and tsetse flies was studied across the 4 villages, a variation in the spatial prevalence was notable. For example, inEmboreet village the prevalence of trypanosomes was high in tsetse flies (13.0%) but low in cattle (8.8%). Conversely, in Kimotorok village no trypanosome infections were detected in tsetse flies but a high prevalence of the parasites was found in cattle (15.0%).

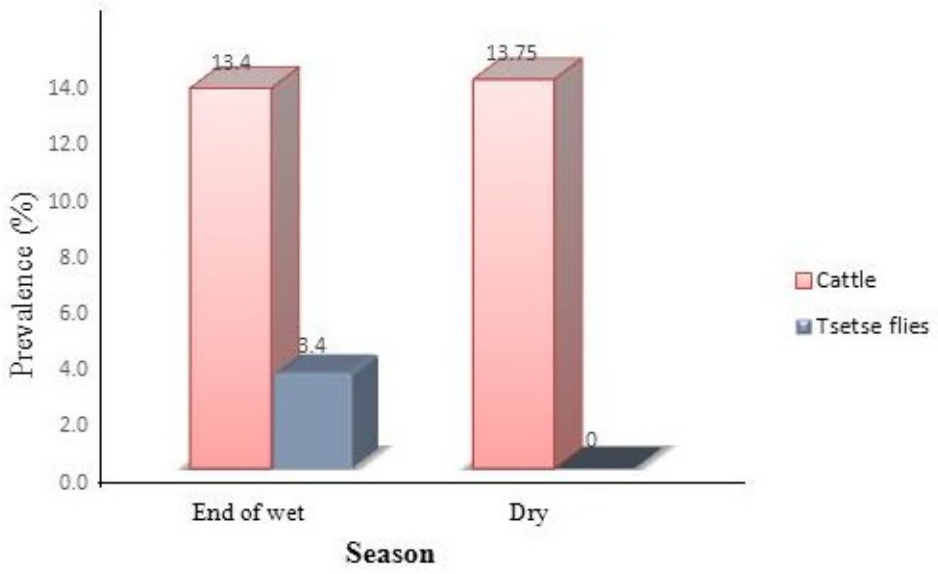



**Figure 8: Seasonality of trypanosome infections in cattle and tsetse flies**

Furthermore,data shown in Fig.8 portrays a confluent pattern of seasonality of trypanosome infections in tsetse flies and cattle. Hence, higher infection rates were shown in June 2015 (end of wet season) whereas lower prevalence was indicated in August2015 (dry season) for both, cattle and tsetse flies.

### 4.5 Prevalence of Trypanosomes and Concurrent Vector-borne Haemoparasitic Infections in ECF-Vaccinated and Non-vaccinated Cattle

In order to understand the variations of prevalence of trypanosomes in cattle, the data was categorized based on ECF vaccination status. Prevalence of two concurrent infections

by*Theileria parva* and *Ehrlichia ruminantium* was determined. The results are shown in Table 11.

**Table 11:Prevalence of trypanosome and concurrent infections in ECF-vaccinated and non-vaccinated cattle**

| Infection | N | ECF-Vaccinated (%) | ECF-Non-vaccinated (%) | OR | P-value | CI |
|---|---|---|---|---|---|---|
| Trypanosomes | 33(16.5%) | 11 | 22 | 0.43 | 0.03 | 0.19-0.96 |
| *T. parva* | 25 (12.5%) | 17 | 8 | 2.35 | 0.05 | 0.96-5.74 |
| *E. ruminantium* | 8(4%) | 4 | 4 | 1.00 | 1.00 | 0.24-4.11 |
| Negative to all | 134 | 68 | 66 | | | |
| **Total** | **200** | **100** | **100** | | | |

**Note**: Table 11: Two hundred cattle split into ECF vaccinated (N=100) and ECF non-vaccinated (N=100) were studied to determine prevalence of concurrent infections. P values and Odds ratio (OR) compare between ECF-vaccinated and ECFnon-vaccinated cattle.

Out of the 200 cattle screened for three infections, prevalence of trypanosomes, *T. parva* and *E. ruminantium* was 16.5%, 12.5% and 4%, respectively. There were significantly fewer trypanosome-positives among ECF-vaccinated than ECF non-vaccinated cattle (P=0.03). This finding was further corroborated by an OR value of 0.43. As expected higher proportion of *T. parva* carriers was detected among ECF-vaccinated cattle (n=17) as compared to ECF non-vaccinated cattle (n=8) and this difference was shown to be statistically significant (P=0.05; OR 2.35). The proportion of *E. ruminantium* (4/8) was not variable and there was no difference (P=1.00) between ECF vaccinated and non-vaccinated cattle.

Five trypanosome/*T. parva* and two trypanosome/*E. ruminantium* co-infections were confirmed among the 33 trypanosome positive cattle. Out of the five trypanosome/*T.*

*parva* co-infected cattle three had been ECF-vaccinated whereas two were non-vaccinated. One calf which was ECF-vaccinated carried all the three infections and one animal carried *T. parva* and *E. ruminantium* infections. However, there was no significant difference between the ECF vaccination status and the co-infections (p=0.684 at 95% CI (0.2205, 12.950)).

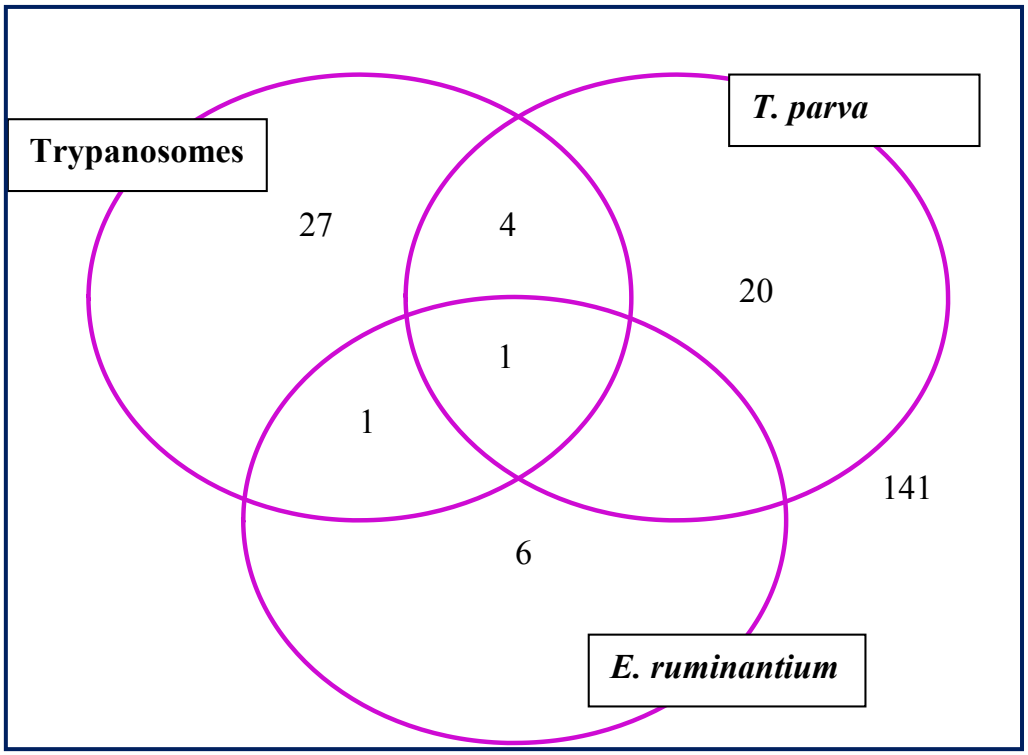



**Figure 9: Distribution of trypanosomes, *T. parva* and *E. ruminantium* infections in cattle**

Fig.9represents Venn diagram depicting the abundance of the three infections occurring singularly or as a co-infection (N=200). The number of cattle infected or co-infected with either, trypanosomes, *E. ruminantium* or *T. parva* is indicated inside each circle, whereas number 141 outside the circles represent negative cattle.

# CHAPTER FIVE

## 5 DISCUSSION

This study investigated prevalence of trypanosome infections in cattle and tsetse flies in five villages in Simanjiro and Monduli districts within the Maasai steppe of northern Tanzania. The study revealed anoverall prevalence of trypanosome infections in cattle to be 17.2%. This value was higher than an 11% prevalence found by Nzalawahe (2010) using ITS primers and a 5.4% prevalence obtained by Laohasinnarong *et al*. (2011). The difference in prevalence may be ascribed to ecological and other associated conditions, which favour tsetse fly biology in different areas where cattle were sampled. Whilethis study sampled cattle in a livestock-wildlife interface area bordering Tarangire National park, the study by Nzalawahe (2010) sampled cattle in Kigoma, western Tanzania and that by Laohasinnarong *et al*. (2011) was conducted in Serengeti, Tanzania. The prevalence of trypanosome infections established in this studywas close to that found in Tororo district (15.3%) of Uganda (Muhanguzi *et al*., 2014). Another reason for the prevalence differences could be sensitivity of PCR methods applied by the different studies.Laohasinnarong *et al*. (2011) applied KIN- PCR which is less sensitive in detection of *T. vivax* east African subtype than ITS- PCR, used in the present work. The 17.2% prevalence of trypanosome infections in cattle in this study could also be due to the high interaction of livestockwith wildlife in the interface areas of Simanjiro and Monduli districts, where tsetse flies are also highly abundant. Cattle are also highly preferable hosts of tsetse flies compared to other domestic animals (CFSPH, 2009). The prevalence of trypanosome infections obtained in this study is an indication of the magnitude of infection that does not manifest into clinical disease, suggesting an imaginable risk of cattle population that could suffer from trypanosomosis. Previous studies showed that the prevalence of the disease using parasitological examination methods in Monduli district

(which is part of the current study area) was 5% (Swai and Kaaya, 2012). It should be stressed that the 17.2% reported in the current study reflects the proportion of infected cattle regardless of their health status.These results provide an insight of the proportion of subclinical infections, which eventually may manifest into clinical disease. The proportion of infected cattle (17.2%) accounts for both pathogenic and non-pathogenic trypanosomes. Only cattle infected with pathogenic trypanosomes would be able to show clinical signs depending on the virulence of the infecting species involved. This explains the difference in the proportion of cattle that get infected with trypanosomes and those that develop in clinical disease. For example, cattle infected with *T. congolense* are more likely to show clinical signs than cattle infected with *T. theileri* because *T. congolense* is more pathogenic in cattle than *T. theileri* (Goossens *et al*., 2006; CFSPH, 2009; Auty*et al*., 2015).

The present study compared prevalence of trypanosome infections in different age groups of cattle.Cattle older than two years were shown to have higher prevalence (40%) of trypanosome infections compared to younger cattle. This was expected since this group of cattle were grazed in tsetse fly infested areas whereas younger animals (less than two years of age) grazed close to homesteads, hence their lower exposure to tsetse flies. These findings are supported by a study by Ikenna (2008), who found a lower prevalence of trypanosome infections in younger cattle than older ones. Likewise, this study has shown that trypanosome prevalence did not significantly differ by sex, although there were more females than males in the study population. The greater number of female animals in the study area is also a reflection of herd partitioning, a practice by the Maasai farmers to facilitate breeding and prevent inbreeding.

When spatial prevalence of trypanosomes was studied a significant variation was established between villages.The differences in the prevalence between villages could also

be due to land cover changes, which are common due to seasonal variation within the study area. The changes in the vegetation enhance traditional movement of the animals by the Maasai people from one village to another in search of pasture and water. Such movements are likely to influence transmission dynamics of trypanosome infections. Furthermore, the movements of herds increase interaction with wildlife and hence the potential for disease transmission between wild and domestic hosts. One of the reasons behind movement of livestock herders within the Maasai steppe is their recent need for land for agriculture(Majekodunmi *et al*., 2013). This has contributed to bush encroachment, reduced pasture and water availability to livestock and wildlife, overgrazing and hence facilitating greater contact with infectious vectors and hosts.

This study also compared the prevalence of trypanosome infections in cattle sampled during the wet, dry and end of wet season.There was no statistically significant variation of trypanosome prevalence between the different seasons although the prevalence was higher at the end of wet season (18.7%) than during the dry and wet seasons. The non-significant overall variation of trypanosome infections could be due to the chronicity of the parasites. The study could have detected the same trypanosome infections in more than one season (Franco *et al*., 2014). The findings of this study are comparable to those of Rundassa *et al*. (2013) and Majekodunmi *et al*. (2013) who reported a higher incidence of trypanosomosis at the end of wet season than during the dry season. This is partly due to changes in vegetation cover and cattle movement which happens mostly at the beginning of the dry season. Thistransition may alert the farmers to prepare their animals for tsetse fly exposure by treating the herds with acaricides that kill the vectors and reduce parasite infections. The infections begin to increase when acaricides begin to wear off in the wet season and become highest during the transition from wet to dry season. This suggests a change in transmission dynamics of the parasite which may be directly related to climate

change (Naicker, 2011). In order to gain a better understanding of the relationship between trypanosome infections and seasonality, monthly maximum temperature and rainfall data from Tanzania MeteorologicalAgency (appendix 3) were used to explain the variations. The findings indicated that higher prevalence of trypanosome infections at the end of the rainy season correlated with the beginning of the dry months and lower temperatures of the year.This variation could also be due to the ecology and biology of tsetse flies and trypanosomes, which suggests that transmission of trypanosomes depends on the ability of the tsetse fly to successfully transmit the parasite to the susceptible host. This is directly related to the environmental conditions that allow tsetse fly survival such as temperature of about 16$^{o}$C to 38$^{o}$Cand a relative humidity of 50% to 80% (Childs, 2014; Franco *et al*., 2014).

This study also analysed seasonality of individual trypanosome species, which exhibited a variation between the wet, end of wet and dry seasons. All trypanosome species showed a similar pattern of higher prevalence at the end of wet season except for *T. brucei* which occurred with higher prevalence in the wet season. These findings are similar to those found by Majekodunmi *et al*. (2010). There was also a significant variation of *T. brucei* and *T. theileri* infections across the seasons. This variation could be due to the vector competency, biology and epidemiology of the tsetse flies that transmit these trypanosomes. For example, *Glossina palpalis* is the main vector transmitting *T. b. gambiense* while *Glossina morsitans* is a good vector for *T. congolense* (Geiger *et al*., 2005) while*G. pallidipes,G. morsitans* and *G. swynnertoni* are efficient vectors for *T. brucei* (Dennis *et al*., 2014; Salekwa*et al*., 2014). *Trypanosoma theileri* is not dependent on the tsetse fly distribution but that of Tabanid flies (FAO, 1992). Cattle may come into close contact with the vectors either during normal grazing or movement due to seasonal changes in pasture availability. The seasonal variation of trypanosome species could also

be attributed to the different adaptable ecosystems by the various genetically divergent subgroups of *Trypanosoma* spp(Auty *et al*., 2015). For example, *T. congolense* forest subtype might have different ecological requirements based on its distribution mainly in the western African forest habitats with *Glossina palpalis* being the main vector, while*T. congolense kilifi* is found in the East and southern part of Africa (Auty *et al*., 2015). These environments may have different seasonal implications that may affect the distribution of the subgroups which explains the various seasonal patterns of trypanosomes. Another reason explaining the seasonal variation of the different trypanosome species is the mechanical transmission component of *T. congolense* and *T. vivax.* These do not depend solely of the biology and epidemiology of tsetse flies but also of Tabanid flies. Some Tabanid flies are more abundant in the wet than the dry season while others circulate all year round (Ahmed *et al*., 2005). This could correlate with the distribution of mechanically transmitted trypanosomes.

Detection of *Trypanosoma* spprevealed a diversity of both pathogenic and non-pathogenic infections identified as *T. congolense, T. brucei, T. vivax, T. theileri, and T. simiae*. These species were also identified by Cox *et al*. (2005) by nested ITS- PCR. These species were identified based on their fragment sizes. GelCompar II software rated single fragments more precisely than mixed trypanosome infection fragments. The most prevalent trypanosome species was *T. vivax* which was identified in 41.8% of the infections. This finding did not correlate with Majekodunmi *et al*. (2010, 2013) and Simukoko *et al*. (2007) who found *T. congolense*and Karimuribo *et al*., (2011)who reported *T. brucei*to be the most abundant. The differences between the previously reported most abundant trypanosome species and that of the current study are the ecological and environmental factors associated with the study areas. However, the findings of this study were comparable to studies bySilbermayr *et al*. (2013)and Nabulime*et al*. (2014) who found the

highest prevalence of trypanosome infections to be due to *T. vivax*. The abundance of *T. vivax* could be attributed to its transmission patterns being not only through tsetse fly bite but also through mechanical transmission by other biting flies such as Tabanids and Stomoxys. This gives the *T. vivax* parasite more chances of infection into an animal. *Trypanosoma brucei* (20.9%) and *T. simiae* (19.6%) were also abundant.The abundance of *T.simiae* shows the risk of disease in pigs where as in cattle, the parasite in non-pathogenic, even though it has been reported with low abundance level (CFSPH, 2009; Nimpaye *et al*., 2011). The primers used in the present study also revealed 2 DNA fragments of 350 and 500 base pairs which occurred in 15.1% of the positive cattle. The DNA fragments could potentially represent variants of already existing species such as *T. vivax* or *T. simiae*, or they could be new *Trypanosoma* spp or unidentified non Trypanosomatid organisms (Auty *et al*., 2012). The identity of these species by DNA sequencing could yield more results. In total, 41.8% (72/172) of trypanosome positive cattle harboured mixed infections of two up to four trypanosome infections. This could be due to infection and reinfection of cattle with different trypanosome species during the long period of persistent or chronic infections. Indigenous cattle breeds can live with low doses of trypanosomes in their blood without showing any signs of infection. This does not exempt them from getting re-infected with the same or different species of trypanosomes, which could lead to multiple infections. Another reason behind the occurrence of mixed trypanosome infections could be the ability of tsetse flies to carry and develop more than one species of trypanosomes. This is attributed to the different developmental predilection sites of trypanosome species. For example *T. congolense* develops in the midgut while *T. vivax* develops entirely in the proboscis. This means that one tsetse fly can transmit more than one trypanosome species (Mwandiringana*et al*., 2012). Mixed infections in mammalian hosts increase activities that utilise nutrients and immune components due to competition for space and resources by the parasites involved

(Rynkiewicz *et al*., 2015), this has clinical implications on the mammalian host involve (for example cattle). However, this concept only applies to trypanosome species that develop in the same tsetse fly organ. Trypanosomes that develop in different organs of the tsetse flies require different conditions for survival and no clinical implications inside the vector. Mixed infections in tsetse flies were not as abundant as in cattle. This could be due to self-regulation of trypanosome species vector by secretion of certain factors such as Lectins (produced by trypanosomes) and tsetse EP protein (produced by tsetse flies) that inhibit the establishment of other trypanosome species in the same vector to reduce trypanosome numbers (Ahmed *et al*., 2015). Kubi *et al*. (2005) suggested that age of a tsetse fly also has an influence on development of mixed infections in tsetse flies in that older flies are more susceptible to mixed infections than younger ones. The most occurring mixed infection was found to be *T. vivax*/*T. simiae* which appeared in 26.4% of the positive samples in all the five study villages. *Trypanosoma simiae* is not pathogenic in cattle. This give animals co-infected with this species a higher chance of survival compared to those co -infected with *T. vivax* /*T. congolense* or *T. congolense* /*T. brucei* due to competition by strain specific immune responses to fight these parasites (Balmer *et al*., 2009). Mixed infections by pathogenic strains would lead to manifestation of clinical signs in an infected host while infection by non-pathogenic species would result in chronicity of sub-clinical infections (Balmer *et al*., 2009). The mixed trypanosome infections were comparable to the ones found in tsetse flies by Malele *et al*. (2011b), who found the most abundant mixed infections to be that of *T. vivax*/ *T. simiae*/ *T. brucei*. The number of individual trypanosome species also varied in the studied villages. Kimotorok village had the most abundant trypanosome species (68/239) whereasLoibor-soit-A had less (26/239). This could be due to the difference in the sample size of the two villages, especially that Loibor-soit-A had the most infected cattle. However, the two villages had a similar distribution of the trypanosome species.

Comparison of prevalence of trypanosome infections in cattle and tsetse flies revealed a significantly higher prevalence of trypanosome infections in cattle (11.9%) than in tsetse flies (6.9%). This could be due to the fact that the density of caught tsetse flies is comprised of mostly unfedflies from different habitats that have low trypanosome infections (Mohamed-Ahmed and Odulaja, 1997; Okoh*et al*., 2011). These flies may not have been exposed to any susceptible hosts. Thus the unfed flies may have a lower chance of picking up trypanosome infections from infected hosts and transmitting it to other susceptible hosts. The Epsilon traps used in this study have been documented to be selectively specific to savannah tsetse flies (*G. pallidipes* and *G. morsitans*), and as a result, the traps may leave out other tsetse flies and biting flies that may be responsible for transmitting certain species of trypanosomes (Hargrove and langley, 1990; Mhindurwa, 1994; Torr and Hargrove, 1999; Malele*et al*., 2016). This technique could have left out other tsetse flies that are responsible for transmitting other trypanosomes. Cattle, however, are infected with trypanosomes that have already been transmitted from infected tsetse flies giving them a higher prevalence of infections. Thefindings of the current study can be compared with the previously reported results (Nakayima *etal*., 2012).The prevalence of trypanosome infections in tsetse flies found by this study was higher than 3.2% found by Sindato *et al*. (2007) and 3% found by Salekwa*et al*. (2014) in the same study area. However, the trypanosome infection rate found in tsetse flies was the same as the 6.93% found by Desta *et al*. (2013) in a study conducted in Ethiopia. The prevalence trypanosome infections in cattle depend highly on presence of tsetse flies transmitting the infections and the ability of cattle owners to protect their animals from diseases. These findings entail that infections vary with time and location. The variation of trypanosome infections between cattle and tsetse flies could guide the Maasai pastoral communities living in tsetse fly infested areas when to strategically control the disease. This study also revealed an inverse relationship in the spatial prevalence of trypanosome infections in

cattle and tsetse flies (p=0.000245). This was also demonstrated by the pattern of infections in individual villages. For example, Kimotorok village had the highest (15.0%) trypanosome infections in cattle while it had the lowest (0%) infections in tsetse flies. Similarly Emboreet village had the highest (13.0%) prevalence of trypanosome infections in tsetse flies while it had the lowest (8.8%) prevalence of infections in cattle.This was attributed to human activities associated with the ecology of tsetse flies in the two villages and the fact that tsetse flies are found where there are animal hosts. Emboreet village has an environment that allows wildlife to graze in livestock grazing areas. This brings tsetse flies closer to human settlements and therefore more tsetse fly catches. In Kimotorok village, cattle graze inside Tarangire National park where tsetse flies feed and transmit trypanosomes to them.This study also demonstrated similar trend of trypanosome infections by season in both, cattle and tsetse flies, what also explains the dependency theory of trypanosome infections in cattle with the vectors. This could have a major implication to the transmission dynamics of trypanosomes in the Maasai steppe. The current study also found no human infective trypanosomes from both cattle and tsetse flies. These results are comparable to Sindato *et al*. (2007), Auty *etal*. (2012) Salekwa *et al*. (2014) who found no human infective trypanosomes in northern Tanzania. The absence of circulating human infective trypanosomes in the study area does not call for relaxed control and surveillance, the presence of reservoir host signifies an existing risk of the human infective trypanosomes appearing.

In order to investigate whether cattle infected with trypanosomes also carry other concurrent infections, this study also analysed*T. parva* and *E. ruminantium*as concurrent infections. It was revealed that trypanosomes were the most abundant (33/200) infections found. Trypanosome infections were more in ECF non-vaccinated compared to vaccinated cattle. This was demonstrated by probability (P=0.0036) and the odds (OR=0.43) of

infections in two groups, which indicates that vaccinating cattle against ECF reduced the occurrence by 43%. Host factors that can support a higher prevalence of trypanosome infections in non-vaccinated cattle include increased susceptibility to infections due to low immunity (Matthews *et al.,* 2015). The reduced immunity is enhanced by stress and occurrence of chronic and persistent trypanosome infections leading to more concurrent infections (Magona and Mayende, 2002). This could also reduce the general health status of cattle and survival of the sick ones.The high prevalence of *T. parva* infections in vaccinated cattle confirms the development of carrier state following the infection and treatment method using the Muguga vaccine. However, the prevalence of *T. parva*infections (12.5%) was lower than the 37.1% obtained by Kazungu*et al*. (2015) in the same study area.The difference in the magnitude of *T. parva* infections could be due to the timing of the sample collection. Other factors including environmental, ecological and host could be attributed to these findings.The low prevalence of *E. ruminantium* (4%) infections could be attributed to its transmission pattern and the predilection site of the parasite, which is the capillary endothelial cells (Lorusso*etal*., 2016). This study also established that concurrent infections occurred in cattle regardless of theirECF vaccination status. One calf carried all three (*T. parva*, *E. ruminantium* and trypanosomes) parasites despite it having been vaccinated against ECF. This could be attributed to other factors such as vector control, introduction of animals from unvaccinated herds or other underlying diseases occurring within the herd. Most coinfections occurred in ECF-vaccinated cattle which could mask expression of clinical signs of other diseases.

# CHAPTER SIX

## 6 CONCLUSION AND RECOMMENDATIONS

### 6.1 Conclusion

The prevalence of trypanosome infections was determined to be 17.2% in Simanjiro and Monduli districts of the Maasai steppe. Risk factors such as age, sex and season did not influence the prevalence of trypanosome infections. However, location of the villages in relation to proximity to wildlife showed positive influence on the prevalence if trypanosome infections. This study demonstrated little seasonal variation in trypanosome prevalence with higher infections occurring at the end of the wet season. The present study also established relationship between prevalence of trypanosomes in cattle and tsetse flies to be related to transmission patterns of tsetse flies and human activities involving livestock movements. The percentage of cattle carrying mixed trypanosome infections was shown to be 43.6(75/172). The most abundant trypanosome species was *T. vivax*, while *T. congolense*, *T. brucei*, *T. simiae* and *T. theileri* were also shown to be abundant. There were no human infective trypanosomes found in both cattle and tsetse flies. This study also demonstrated the occurrence of trypanosomes,*Theileria parva* and *E. ruminantium*concurrent infections,which were possiblyexacerbated by inadequate vector control and ECF vaccination in cattle. These findings could be instrumental in designing disease and vector control programs.

### 6.2 Recommendations

i. This study recommends the sharing of knowledge with the study communities on the impact of cattle movements and management regimes on transmission, magnitude and distribution of trypanosome infections. This could be a positive influence on disease prevention mechanisms in the studyarea.

ii. Farmers in the Maasai steppe protect their cattle from diseases by acaricide application and treatment with un-prescribed anti-trypanosomal drugs. This mainly leads to under-dosing, overdosing and persistent infections. Drug resistance and genetic mutation of trypanosome species are potential outcome that could arise from incorrect use of trypanocidal drugs. Demonstration of these factors in further research could yield results which could contribute to the improved control of trypanosomosis.

iii. Following a variation of trypanosome infections by location, a monthly study of the spatial distribution of infections in livestock could give the required information to understand the seasonal variation of infections in the Maasai steppe. This could explain the location and time of the year when highest peaks of infections occur.Such a study couldprovide useful information for establishing effective strategic vector and disease control measures by both the affected communities and government based policy makers.

iv. African trypanosomosis epidemics have a tendency to recurring when control and surveillance efforts reduce (usually after a successful eradication). This makes vulnerable communities and animals living in potential tsetse fly infested areasremain at risk of trypanosomosisexposure. This suggests a need for long term and active surveillance systems to be in place in order to aggressively control the diseasein endemic areas.

# REFERENCES

Adams, E. R., Hamilton, P. B., Malele, I. I. and Gibson, W. C. (2008). The identification, diversity and prevalence of trypanosomes in field caught tsetse in Tanzania using ITS-1 primers and fluorescent fragment length barcoding. *Infection, Genetics and Evolution,*8:439–444.

Adamu, U. O., Haruna, M. K., Ovbagbedia, R. P., Bizi, R., Benjamin, W., Malala, U. A., Nwezor, F. N. C. and Muhammed, M. (2011). Control of African Trypanosomiasis in Nigeria: Time to Strengthening Integrated Approaches (A Review). *International Journal of Animal and Veterinary Advances*, 3(3): 138-143.

Ahmed, H. A., MacLeod, E. T., Welburn, S. C. and Picozzi, K. (2015).Development of Real Time PCR to Study Experimental Mixed Infections of T. congolense Savannah and T. b. brucei in Glossina morsitans morsitans.*PLoS ONE* 10(3): e0117147. doi:10.1371/journal.pone.0117147.

African Wildlife Foundation (AWF) (2015). The Maasai steppe. [http://www.awf.org/landscape/maasai-steppe] site visited on 06/08/2015.

Ahmed, A.B., Okiwelu, S. N. and Samdi, S.M. (2005). Species Diversity, Abundance and Seasonal Occurrence of Some Biting Flies in Southern Kaduna, Nigeria.*African Journal of Biomedical Research*, Vol. 8: 113 - 118.

Ahmed, H. A., Picozzi, K., Welburn, S. C. and MacLeod, E. T. (2013). A comparative evaluation of PCR- based methods for species- specific determination of African animal trypanosomes in Ugandan cattle. *Parasites and Vectors*, 6(1):316. doi: 10.1186/1756-3305-6-316.

Anderson, N. E., Mubanga, J., Machila, N., Atkinson, P. M., Dzingirai, V. and Welburn, S. C. (2015). Sleeping sickness and its relationship with development and biodiversity conservation in the Luangwa Valley, Zambia. *Parasites and Vectors,* 8(224): 1 - 14.

Ariyaratne, M. E. A. D. S., Gothami, W. S. and Rajapakse, R. V. P. J. (2014). Application of PCR Technique on Confirming Theileria Infection in Cattle and Buffaloes with Determining the Relationship between A nimals' PCV and WBC Count with the Infection. *International Journal of Scientific and Research,* 4(7): 2012–2015.

Auld, S.K.J.R. and Tinsley, M.C. (2015). The evolutionary ecology of complex lifecycle parasites: linking phenomena with mechanisms. *Heredity*, 114: 125-132.

Auty, H., Anderson, N.E., Picozzi, K., Lembo, T., Mubanga, J., Hoare, R., Fyumagwa, R. D., Mable, B., Hamill, L., Cleaveland, S. and Welburn, S. C. (2012). Trypanosome Diversity in Wildlife Species from the Serengeti and Luangwa Valley Ecosystems. *PLoS Negl. Trop. Dis.,* 6(10): 1 - 10.Doi:10.1371/journal.pntd.0001828.

Auty, H., Torr, S.J., Michoel, Jayaraman, T. S. and Morrison, L.J. (2015). Cattle trypanosomosis: the diversity of trypanosomes and implications for disease epidemiology and control.*Rev. Sci. Tech. Off. Int. Epiz.*, 34 (2): 587-598.

Balmer, O., Stearns, S. C., Schötzau, A. and Brun, R. (2009). Intraspecific competition between co-infecting parasite strains enhances host survival in African trypanosomes. *Ecology,* 90(12): 3367-78.

Batchelor, N. A., Atkinson, P. M., Gething, P. W., Picozzi, K., Fèvre, E. M., Kakembo, A.

S. L. and .Welburn S. C. (2009). Spatial Predictions of Rhodesian Human African Trypanosomiasis (Sleeping Sickness) Prevalence in Kaberamaido and Dokolo, Two Newly Affected Districts of Uganda. *PLoS Negl Tropical Disease* 3(12): 1 - 11.

Bengis, R.G., Kock, R.A. and Fischer, J. (2002). Infectious animal diseases: the wildlife/livestock interface. *Revue scientifique et technique International Office of Epizootics,* 21(1): 53–65.

Berrang-Ford, L., Waltner-Toews, D., Charron, D., Odiit, M., McDermott, J. and Smit, B. (2005). Sleeping Sickness in Southeastern Uganda: A Systems Approach. *EcoHealth*, 2: 183–194.

Bezie, M., Girma, M., Dagnachew, S., Tadesse, D. and Tadesse, G. (2014). African Trypanosomes : Virulence Factors , Pathogenicity and Host Responses African Trypanosomes : Virulence Factors, Pathogenicity and Host Responses. *Journal of Veterinary Advances*, 4(11): 732-745 DOI: 10.5455/jva.20141129012406.

Bucheton, B., Macleod, A. and Jamonneau, V. (2011). Human host determinants influencing the outcome of Trypanosoma brucei gambiense infections: *Review Parasite Immunology*, 33:438–447.

Camara, M., Camara, O., Ilboudo, H., Sakande, H., Kabore', J., N'Dri, L., Jamonneau, V. and Bucheton, B. (2010). Sleeping sickness diagnosis: use of buffy coats improves the sensitivity of the mini anion exchange centrifugation test. *TropicalMedicine and International Health*, 15(7):796–799.

Center for Food Security and Public Health (CFSPH) (2009). African Animal

Trypanosomosis; Nagana, Tsetse Disease, Tsetse Fly Disease:1-5 [www.cfsph.jastate.edu] site vited on 14/12/2015.

Chanie, M., Adula, D. and Bogale, B. (2013). Socio-economic assessment of the impacts of trypanosomosis on cattle in girja district, Southern Oromia Region, Southern Ethiopia. *Acta Parasitologica Globalis,*4(3): 80 - 85.

Childs, S. J. (2014). A Model of Teneral Dehydration in Glossina. *Acta Tropica*, 131: 79–91.

Connor, R. J. (1994). 'The impact of nagana. *Onderstepoort Journal of Veterinary Research,*61(4): 379 - 383.

Cox, A., Tilley, A., McOdimba, F., Fyfe, J., Eisler, M., Hide, G. and Welburn, S. (2005). A PCR based assay for detection and differentiation of African trypanosome species in blood. *Experimental Parasitology*, 111(1): 24–29.

Cox, A. P., Tosas, O., Tilley, A., Picozzi, K., Coleman, P., Hide, G. and Welburn, S. C. (2010). Constraints to estimating the prevalence of trypanosome infections in East African zebu cattle.Parasites and Vectors, 3, 82.http://doi.org/10.1186/ 1756-3305-3-82.

d'Ieteren, G. D., Authié, E., Wissocq, N. and Murray, M. (1998). Trypanotolerance, an option for sustainable livestock production in areas at risk from trypanosomosis. *Revue Scientifique et Technique International Office of Epizootics,* 17(1): 154–175.

Dantas-Torres, F., Chomel, B. B. and Otranto, D. (2012). Ticks and tick-borne diseases: A

One Health perspective. *Trends in Parasitology*, 28(10): 437-446.

Deborggraeve, S. and Büscher, P. (2010). 'Molecular diagnostics for sleeping sickness: what is the benefit for the patient?' *Lancet Infectious Diseases,* 10(6): 433 - 439.

Demessie, Y. and Derso, S. (2015). Tick borne hemoparasitic diseases of ruminants: a review. *Advances in Biological Research,* 9(4): 210-224.

Dennis, J. W., Durkin, S. M., Horsley Downie, J. E., Hamill, L. C., Anderson, N. E. and MacLeod, E. T. (2014). Sodalis glossinidius prevalence and trypanosome presence in tsetse from Luambe National Park, Zambia. *Parasites and Vectors,* 7(378): 1 - 11.

Desta, M., Beyene, D. and Haile, S. (2013). Trypanosome infection rate of Glossina pallidipes and trypanosomosis prevalence in cattle in Amaro Special District of Southern Ethiopia. *Journal of Veterinary Medicine and Animal Health,* 5(6): 164 - 170.

Desquesnes, M., McLaughlin, G., Zoungrana, A. and Dávila, A. M. R. (2001). Detection and identification of *Trypanosoma* spp. of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. *Int. J. Parasitol*., 31: 610-614.

Dolan, R. B. (1998). T*he Orma Boran: A trypanotolerant East African Breed. Fifteen Years of Research on Galana Ranch in Kenya*. Kenya Tryapnosomiasis Research Institute, Kikuyu, Kenya. 110pp.

Ellis, B. R. and Wilcox, B. A. (2009). The ecological dimensions of vector-borne disease research and control. *Cadernos de Saúde Pública* 25(1): 155 - 167.

Elmaleck, B.S.A. (2016). Effect of Seasonal Variations on Distribution of Parasites in

Camels at Assiut Locality. *Veterinary Science and Technology,* 7(279): 1–6.

Eshetu, E. and Begejo, B. (2015). The current situation and diagnostic approach of Nagana in Africa: A review. *Journal of Natural Sciences Research,* 5(17):117–125.

Eygelaar, D., Jori Massanas, F.J., Mokopasetso, M., Sibeko-Matjila,K.P., Collins, N.E., Vorster, I., Troskie, M. and Oosthuizen, M.C. (2015). Tick-borne haemoparasites in African buffalo (Syncerus caffer) from two wildlife areas in Northern Botswana. *Parasites andVectors*, 8(1): 1 - 11.

Eyob, E. and Matios, L. (2013). Review on camel trypanosomosis (surra) due to Trypanosoma evansi : Epidemiology and host response.*Journal of Veterinary Medicine and Animal Health*, 5(12): 334-343.

FAO (1983).Tick-Borne Livestock Diseases and their Vectors ticks and tick-borne diseases: Selected articles from the World Animal Review. Rome, Italy. [http://www.fao.org/docrep/004/X6538E/X6538E02.htm] site visited on 12/07/2015.

FAO (1983).*African Animal Trypanosomosis: Selected Articals From the World Animal Review*. Food and Agricultural Organization, Rome, Italy. 130pp.

FAO (1992).*Training Manual for Tsetse Control Personnel*. Food and AgricultureOrganization, Rome, Italy. 274pp.

Finelle, P., Murray, M., Barry, J. D., Morrison, W. I., Williams, R. O., Hirum, H. and Rovis, L. (1983).*African Animal Trypanosomosis, Food and Agriculture Organization of the United Nations.*Rome, Italy. 126pp.

Firesbhat, A. and Desalegn, C. (2015). Epidemiology and Impacts of Trypanosomosis in

Cattle.*European Journal of Applied Sciences*, 7(5): 220-225.

Franco, J. R., Simarro, P. P., Diarra, A. and Jannin, J. G. (2014). Epidemiology of human African trypanosomosis. *Clinical Epidemiology*, 6: 257–275.

Geiger, A., Cuny, G.and Frutos, R. (2005). Two Tsetse Fly Species, Glossina palpalis gambiensis and Glossina morsitans morsitans, Carry Genetically Distinct Populations of the Secondary Symbiont Sodalis glossinidius. *Applied and Environmental Microbiology*, 71(12): 8941–8943.

Ghosh, S. and Nagar, G. (2014). Problem of ticks and tick-borne diseases in india with special emphasis on progress in tick control research: A review. *Journal of Vector Borne Diseases*, 51(4): 259–270.

Gilbert, J. A., Medlock, J., Townsend, J. P., Aksoy, S., Ndeffo Mbah, M. and Galvani, A. P. (2016). Determinants of Human African Trypanosomiasis Elimination via Paratransgenesis. *PLoS Neglected Tropical Disease,*10(3): 1 - 60.

Goldman, M. J. (2011). Strangers in their own land: Maasai and Wildlife Conservation in Northern Tanzania. *Conservation and Society*, 9(1): 65-79.

Goossens, B., Mbwambo, H., Msangi, A., Geysen, D. and Vreysen, M.(2006).Trypanosomosis prevalence in cattle on Mafia Island (Tanzania). *Veterinary Parasitology*, 139: 74–83.

Gubler, D. J. (2009). Vector-borne diseases. *Rev. sci. tech. Off. int. Epiz.*, 28(2): 583-588.

Haji, I. J., Sugimoto, C., Kajino, K., Malele, I., Simukoko, H., Chitambo, H. and

Namangala, B. (2015). Determination of the prevalence of trypanosome species in cattle from Monduli district, northern Tanzania, by loop mediated isothermal amplification. *Tropical Animal Health and Production*, 47(6): 1139-1143.

Hargrove, J.W. and Langley, P.A. (1990). Sterilizing tsetse (Diptera: Glossinidae) in the field: A successful trial. *Bulletin of Entomological Research*, 80(4):397-403.

Harrus, S. and Baneth, G. (2005). Drivers for the emergence and re-emergence of vector-borne protozoal and bacterial diseases. *International Journal for Parasitology,* 35: 1309–1318.

Hassane, H. M. (2013). *The Promotion of the Livestock Industry for Food Security in Africa and Arab Countries.* AU-PATTEC Coordination Office African Union Commission, Ethiopia. 18pp.

Hernández-rodríguez, P. and Ramirez, A. G. (2012). *Polymerase Chain Reaction: Types Utilities and Limitations.* Universidad de La Salle, Bagota, Colombia. 17pp.

Ikenna, E. (2008). Animal Trypanosomosis In Africa : Aetiology and Epidemiology.*Animal Research International*, 5(1): 811 – 815.

Kaare, M. T., Picozzi, K., Mlengeya, T., Fevre, E. M., Mellau, L. S., Mtambo, M. M., Cleaveland, S. & Welburn, S. C., 2007. Sleeping sickness-a re-emerging disease in the Serengeti?*Travel Medicine and Infectious Disease*, 5 (2): 117-124.

Karimuribo, E. D., Morrison, L. J., Black, A. C., Turner, M. R., Kambarage, D. M. and Ballingall, K. T. (2011). Analysis of host genetic factors influencing African trypanosome species infection in a cohort of Tanzanian Bos indicus cattle. *VeterinaryParasitology,* 179(3): 35 – 42.

Kazungu, Y. E. M., Mwega, E., Kimera, S. I. and Gwakisa, P. (2015). Seroprevalence and

carrier state of Theileria parva in cattle under two tick control regimes in small-holder farming systems of Tanzania. *Livestock Research for Rural Development,* 27:111.

Kibona, S. N., Nkya, G. M. and Matemba, L. (2002). Sleeping sickness situation in Tanzania. *Tanzania Health Research Bulletin* 4(2): 1 - 3.

Kock, R. A. (2005). *What is this Infamous "Wildlife/Livestock Disease Interface?" A Review of Current Knowledge for the African Continent*. Conservation Programmes, Regent's Parrk, London. 50pp.

Konnai, S., Imamura, S., Nakajima, C., Witola, W.H., Yamada, S., Simuunza, M., Nambota, A., Yasuda, J., Ohashi, K. and Onuma, M. (2006). Acquisition and transmission of Theileria parva by vector tick , *Rhipicephalus appendiculatus*.*Acta Tropica*, 99(1): 34-41.

Kubi, C., Van Den Abbeele, J., Dorny, P., Coosemans, M., Marcotty, T. and Van Den Bossche, P. (2005). Arthropod/Host Interaction, Immunity: Ability of Trypanosome-Infected Tsetse Flies (Diptera: Glossinidae) to Acquire an Infection with a Second Trypanosome Species. *Journal of Medical Entomology,* 42(6): 1035-1038.

Kuboki, N., Inoue, N., Sakurai, T., Di Cello, F., Grab, D. J., Suzuki, H., Sugimoto, C. and Igarashi, I. (2003). Loop-Mediated Isothermal Amplification for Detection of African Trypanosomes.*Journal of Clinical Microbiology*, 41(12): 5517–5524.

Laohasinnarong, D., Thekisoe, O.M., Malele, I., Namangala, B., Ishii, A., Goto, Y.,

Kawazu, S., Sugimoto, C. and Inoue, N. (2011). Prevalence of *Trypanosoma* spp. in cattle from Tanzania estimated by conventional PCR and loop-mediated isothermal amplification (LAMP). *Parasitology Research*, 109(6):1735–1739.

Livingstone, D. (2010). *Exploring Disease in Africa: AIDS, Sleeping Sickness Small Pox A Curriculum for Advanced High School+College Students*. African Studies Center, Boston University, USA. 13pp.

Lorusso, V., Wijnveld, M., Majekodunmi, A. O., Dongkum, C., Fajinmi, A., Dogo, A. G., Thrusfield, M., Mugenyi, A., Vaumourin, E., Igweh, A. C., Jongejan, F., Welburn,S. C. and Picozzi, K. (2016). Tick-borne pathogens of zoonotic and veterinary importance in Nigerian cattle. *Parasites and Vectors,* 9(217): 1 - 13.

Lumbala, C., Simarro, P. P., Cecchi, G., Paone, M., Franco, J. R., Betu, V. K., Mesu, K., Makabuza, J., Diarra, A., Chansy, S., Priotto, G., Mattioli, R. C. and Jannin, J. G. (2015). Human African trypanosomosis in the Democratic Republic of the Congo: disease distribution and risk. *International Journal of Health Geographics* 14(20): 1 - 40.

Lukes, J. (2009). Trypanosoma Gruby 1843. under construction. [http://tolweb.org/ Trypanosoma/98034/2009.01.02inTheTreeofLifeWebProject] site visited on 12/04/2016.

Lutumba, P., Makieya, E., Shaw, A., Meheus, F. and Boelaert, M. (2007).Human African Trypanosomiasis in a Rural Community, Democratic Republic of Congo.*Emerging Infectious Diseases*, 13(2): 1-7.

Lyons, M. (1993). African trypanosomosis sleeping sickness. *Fact Sheet,*259: 552–561.

Macleod, E. T., Darby, A. C., Maudlin, I. and Welburn, S. C. (2007).Factors affecting trypanosome maturation in tsetse flies.*PLoS One,* 2(2): 1 - 5.

Madder, M., Horak, I. and Stoltsz, H. (2013). Ticks: Tick Identification. Creative Commons Attribution license. [http://www.afrivip.org/sites/default/files/identification] site visited on 12/04/2016.

Magona, J.W. and Mayende, J.S.P. (2002). Occurrence of concurrent trypanosomosis, theileriosis, anaplasmosis and helminthosis in Friesian, Zebu and Sahiwal cattle in Uganda. *Onderstepoort Journal of Veterinary Research*, 69: 133-140.

Majekodunmi, A., Picozzi, K., Thrusfield, M., Fajinmi, A. and Welburn, S. (2010). Seasonal Variation and the Effect of Land use patterns and on the epidemiology of Trypanosomosis in a previously tsetse free area - the Jos Plateau, Nigeria . *Thirteenth Association of Institutions for Tropical Veterinary Medicine Conference* 23 - 26 August 2010, Bangkok,Thailand. pp. 1 - 27.

Majekodunmi, A. O., Fajinmi, A., Dongkum, C., Picozzi, K., MacLeod, E., Thrusfield, M. V. and Welburn, S. C. (2013). Social factors affecting seasonal variation in bovine trypanosomosis on the Jos Plateau, Nigeria. *Parasites annd Vectors,* 6(293): 1 - 9.

Malele, I., Craske, L., Knight, C., Ferris, V., Njiru, Z., Hamilton, P., Lehane, S., Lehane, M. and Gibson, W. (2003). The use of specific and generic primers to identify trypanosome infections of wild tsetse flies in Tanzania by PCR. *Infection, Genetics and Evolution*, 3(4): 271–279.

Malele, I. I. (2011). Fifty years of tsetse control in Tanzania: challenges and prospects for

the future. *Tanzania Journal of Health Research,* 13(1): 1-10.

Malele, I., Nyingilili, H. and Msangi, A. (2011a). Factors defining the distribution limit of tsetse infestation and the implication for livestock sector in Tanzania. *African Journal of Agricultural Research*, 6: 2341 – 2347.

Malele, I. I. Magwisha, H. B., Nyingilili, H. S., Mamiro, K. A., Rukambile, E. J., Daffa, J. W., Lyaruu, E. A., Kapange, L. A., Kasilagila, G. K., Lwitiko, N. K., Msami, H. M. and Kimbita, E. N. (2011b). Multiple Trypanosoma infections are common amongst Glossina species in the new farming areas of Rufiji district, Tanzania. *Parasites and Vectors*, 4(1):217:1-8. DOI: 10.1186/1756-3305-4-217·

Malele, I. I., Ouma, J. O., Nyingilili, H. S., Kitwika, W.A., Malulu, D. J., Magwisha, H. B. and Kweka, E. J.(2016). Comparative performance of traps in catching tsetse flies (Diptera: Glossinidae) in Tanzania. *Onderstepoort Journal of Veterinary Research*, 83(1):1-7.

Maré, C. J. (2004). African Animal Trypanosomiasis **(**Nagana, Tsetse Disease, Tsetse Fly Disease), Veterinary Science/Microbiology, University of Arizona, Tuson, Arizona, USA.1-12pp.

Matthews, K. R., McCulloch, R. and Morrison, L. J. (2015). *The Within-Host Dynamics of African Trypanosome Infections.* The Royal Society, London. 10pp.

Maudlin, I. (2006). Centennial Review: African trypanosomosis. *Annals of Tropical Medicine and Parasitology*, 100 (8): 679–701.

Mboera, L. E. G., Mayala, B. K., Kweka, E. J. and Mazigo, H.D. (2011). Impact of

climate change on human health and health systems in Tanzania: A review. Tanzania *Journal of Health*, 13(1): 1-10.

Mohamed-Ahmed, M. M. and Odulaja, A. (1997). Diel activity patterns and host preferences of Glossina fiscipes fuscipes (Diptera: Glossinidae) along the shores of lake Victoria, Kenya. *Bulletin of Entomological Research,* 87:179 -186.

Mhindurwa, A. (1994). Field observation of tsetse flies (Glossina spp.) (Diptera: (Glossinidae) with new odour baited trapping devices. *Bull. Entomol. Res*. 84: 529-532.

Mohini, J. Deshpande, J. D. (2011). Polymerase Chain Reaction: Methods, Principles and Application. *International Journal of Biomedical Research*, 1(5):81-97.

Moody, A. H. and Chiodini, P. L. Review: Methods for the detection of blood parasites. *Clinical and Laboratory Haemertology,* 22: 189 - 202

Morlais, I., Grebaut, P., Bodo, J.M., Djoha, S., Cuny, G. and Herder, S. (1998). Detection and identification of trypanosomes by polymerase chain reaction in wild tsetse flies in Cameroon. *Acta Tropica*, 70(1): 109–117.

Msoffe, F. U., Said, M. Y., Ogutu, J. O., Kifugo, S. C., Leeuw, J., Gardingen, P. and Reid, R. S. (2011). Spatial correlates of land-use changes in the Maasai- Steppe of Tanzania: Implications for conservation and environmental planning. *International Journal of Biodiversity and Conservation,* 3(7): 280 - 290.

Muhanguzi, D., Picozzi, K., Hattendorf, J., Thrusfield, M., Kabasa, J., Waiswa, C. and Welburn, S. (2014). The burden and spatial distribution of bovine African trypanosomes in small holder crop-livestock production systems in Tororo District, south-eastern Uganda. *Parasites and Vectors*, 7(1): 603.

Munang'andu, H. M., Siamudaala, V., Munyeme, M. and Nalubamba K. S. (2012). A review of ecological factors associated with the epidemiology of wildlife trypanosomosis in the Luangwa and Zambezi Valley Ecosystems of Zambia. *Interdisciplinary Perspectives on Infectious Diseases,* 7(603): 1 - 10. Doi:10.1155/2012/372523.

Murray, M., Trail, J. C. M. and D'Ieteren, G. D. M. (1990).Trypanotolerance in cattle and prospects for the control of trypanosomiasis by selective breeding.*Rev Sci Tech*, 9: 369-386.

Muturi, C. N., Ouma, J. O., Malele, I. I., Ngure, R. M., Rutto, J. J., Mithöfer, K. M., Enyaru, J. and Masiga, D. K. (2011). Tracking the feeding patterns of tsetse flies (glossina genus) by analysis of bloodmeals using mitochondrial cytochromes genes. *PLoS One* 6(2): 1 - 6. Doi: 10.1371/journal.pone.0017284.

Mwandiringana, E., Gori, E., Nyengerai, T. and Chidzwondo, F. (2012). Polymerase chain reaction (PCR) detection of mixed trypanosome infection and blood meal origin in field-captured tsetse flies from Zambia. *African Journal of Biotechnology*, 11(79): 14490-14497.

Nabulime, L., Mbogua, J., Zirintunda, G., Asiimwe, J.B. and Ekou, J. (2014). Prevalence of Trypanosomosis among Zebu Cattle in Mulanda, Tororo District, Eastern Uganda. *Scholars Journal of Agriculture and Veterinary Sciences,* 1(4): 274 - 278.

Naicker, P.R. (2011). The impact of climate change and other factors on zoonotic diseases. *Archives Of Clinical Microbiology*, 2(4): 1 - 6. Doi: 10:3823/226.

Nakayima, J., Nakao,. R, Alhassan, A., Mahama, C., Afakye, K. and Sugimoto, C. (2012). Molecular epidemiological studies on animal trypanosomoses in Ghana.*Parasites and Vectors*, 5(217): 1 - 7. DOI: 10.1186/1756-3305-5-217.

Nakayima, J. (2016). *Diagnostic Methods for African Trypanosomosis.African Trypanosomosis: Clinical Symptoms, Diagnosis and Treatment*. Nova Science Publishers, Inc., New York, USA. 29pp.

Nantulya, V. M.(1990). Trypanosomosis in domestic animals : the problems of diagnosis. *Review Science Techica*, 9(2):357-67.

Netto, G. J., Saad, R. D. and Dysert, P. A. (2003). Diagnostic molecular pathology: current techniques and clinical applications, part I. *Proceedings (Baylor University. Medical Center)*, 16(4): 379–383.

Nimpaye, H., Njiokou, F., Njine, T., Njitchouang, G. R., Cuny, G., Herder, S. and Simo, G. (2011). Trypanosoma vivax, T. congolense “forest type” and T. simiae: prevalence in domestic animals of sleeping sickness foci of Cameroon. Parasite : *Journal de La SociétéFrançaise de Parasitologie*, 18(2): 171–179. http://doi.org/10.1051/parasite/2011182171.

Njiiri, N. E., Bronsvoort, B. M. deC., Collins, N. E., Steyn, H. C., Troskie, M., Vorster, I. and Toye, P. (2015). The epidemiology of tick-borne haemoparasites as determined by the reverse line blot hybridization assay in an intensively studied cohort of calves in western Kenya.*Veterinary Parasitology*, 210(1-2), 69–76. http://doi.org/10.1016/j.vetpar.2015.02.020.

Njiru, Z.K., Mikosza, A.S., Matovu, E., Enyaru, J.C., Ouma, J.O., Kibona, S.N., Thompson, R.C. and Ndung'u, J.M. (2008a). African trypanosomosis: Sensitive and rapid detection of the sub-genus Trypanozoon by loop-mediated isothermal amplification (LAMP) of parasite DNA. *International Journal for Parasitology*, 38(5): 589–599.

Njiru, Z. K., Mikosza, A. S. J., Armstrong, T., Enyaru, J. C., Ndung'u, J. M. and Thompson, A. R. C. (2008b). Loop-Mediated Isothermal Amplification (LAMP) Method for Rapid Detection of Trypanosoma brucei rhodesiense. *PLoS Neglected Tropical Diseases,* 2(2): 1 - 8.

Notomi, T., Mori, Y., Tomita, N. and Kanda, H. (2015). Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects. *Journal of Microbiology*, 53(1):1-5.

Nzalawahe, J. (2010). Role of domestic animals in the epidemiology of human African trypanosomosis in Kigoma–Tanzania. Dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania. 47pp.

Oakgrove, K.S., Harrigan, R.J., Loiseau. C., Guers, S., Seppi, B. and Sehgal, R.N. (2014). Distribution , diversity and drivers of blood-borne parasite co-infections in Alaskan bird populations. *International Journal for Parasitology*, 44(10): 717–727.

Odeyemi, S.O., Iliyasu, D., Omotosho, K., Omolade, L. O., Ajani, J. A. and Auwal, U. (2015). Hitches of Trypanosomosis in Nigeria From Livestock Production Perspective. *Journal of Animal Production Advances*, 5(9): 747-756.

OIE (2009).*African Animal Trypanosomiasis: Nagana, Tests Disease, Tsetse Fly Disease*. pp.1-5.

OIE (2012).*Trypanosomosis (tsetse-transmitted): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Seventh edition)*. 12, rue de Prony, 75017 Paris, France. 845-856pp.

OIE(2013). *Trypanosomosis (tsetse-transmitted)*. pp.1–5. [www.oie.int/fileadmin/Home/eng/...in.../trypano_tsetse.pdf] sitevisited on 12/04/2016.

Okoh, K. E., Ndams,I.S., Kogi, E. and Vajime, C.G.(2011). Catch Composition of Tsetse Flies (Glossina: Glossinidae).*American Journal of Applied Sciences,* 8 (11): 1067-1072.

Okoh, K.E., Anavhe, A., Ayakpat, H.N., Onotu, C.S., Anchau, R. and Ajakaiye, J.J. (2012). Trypanosomes Infection in Field-Captured Tsetse Flies of the Subgenus : Nemorhina In Southern Guinea Savanna Zone of Nigeria.*Current Research Journal of Biological Sciences*, 4(6): 713-716.

Osman, N. M., Njahira, M., Skilton, R., Ibtisam, A., Goreish, A., Rahman, A.,Goreish, H. and Rahman, A. (2016). Molecular detection of some bovine trypanosome isolates from different areas of Sudan. *Sudan Journal of Veterinary Research,* 31: 11 - 20.

Otte, J. and Knips, V. (2005). Livestock development for sub-saharan africa. pro-poor livestock policy initiative; A Living from Livestock. [http://www.fao.org/;o

nraru/;onraru-home/en] site visited on 20/6/2016.

Pathak, A. K. (2009). Effect of Trypanosoma spp. on Nutritional status and Performance of livestock.*Veterinary World*, Vol. 2(11): 435-438.

Patz, J. A., Confalonieri, U. E. C., Amerasinghe, F. P., Chua, K. B., Daszak, P., Hyatt, A. D., Molyneux, D., Thomson, M., Yameogo, L., Lazaro, M. M., Vasconcelos, P. and Rubio-Palis, Y. (2005). *Human Health: Ecosystem Regulation of Infectious Diseases, Ecosystems and Human Well-being:* Current State and Trend Island Press, Washington DC. 25pp.

Peter, T.F., Barbet, A.F., Alleman, A.R., Simbi, B.H., Burridge, M.J. and Mahan, S.M. (2000). Detection of the Agent of Heartwater, *Cowdria ruminantium*, in Amblyomma Ticks by PCR : Validation and Application of the Assay to Field Ticks. *Journal of Clinical Microbiology*, 38(4): 1539 –1544.

Picozzi, K., Tilley, A., Fèvre, E. M., Coleman, P. G., Magona, J. W. Odiit, M. Eisler, M. C. and Welburn, S. C. (2002)The diagnosis of trypanosome infections: applications of novel technology for reducing disease risk. *African Journal of Biotechnology*, 1(2):39–45.

Radostits, O. M., Gay, C. C., Hinchcliff, K. W. and Constable, P. D. (2006). *Veterinary Medicine: A Textbook of Diseases of Cattle, Horses, Sheep, Pigs, and Goats*. ($10^{th}$ Edn.),Saunders Elsevie Publishers, New York. 2162pp.

Rundassa, M.D., Menkir, S. and Kebede, A. (2013). Prevalence and seasonal incidence of bovine trypanosomosis in Birbir valley, Baro Akobo River system, Western Ethiopia. *Journal of Veterinary Medicine and Animal Health*, 5(5): 138-143.

Rynkiewicz, E. C., Pedersen, A. B. and Fenton, A. (2015). Review: An ecosystem approach to understanding and managing within-host parasite community dynamics. *Trends in Parasitology,* 31(5): 1-10.

Sahinduran, S. (2012). *Protozoan Diseases in Farm Ruminants, A Bird's-Eye View of Veterinary Medicine.*Science, Technology and Medicine Open Access Publisher, China. 500pp.

Salekwa, L. P., Nnko, H. J., Ngonyoka, A., Estes, A. B., Agaba, M. and Gwakisa, P. S. (2014). Relative abundance of tsetse fly species and their infection rates in Simanjiro, Northern Tanzania. *Livestock Research for Rural Development*, 26:213.

Savić, S., Vidić, B., Grgić, Z., Potkonjak, A. and Spasojevic, L. (2014). Emerging Vector-Borne Diseases – Incidence through Vectors. *Frontiers in Public Health*, 2(267): 1-4.

Scoones, I. (2014). *The Politics of Trypanosomosis Control in Africa.* Working Paper No. 57. STEPS Centre, Brighton. 37pp.

Silbermayr, K., Li, F., Soudré, A., Müller, S. and Sölkner, J. (2013). A Novel qPCR Assay for the Detection of African Animal Trypanosomosis in Trypanotolerant and Trypanosusceptible Cattle Breeds. *PLoS Neglected Tropical Diseases,* 7(8): 1 - 9.

Simarro, P. P., Jannin, J. and Cattand, P. (2008). Eliminating human African trypanosomiasis: Where do we stand and what comes next? *PLoS Medicine,*

5(2): 1 - 7.

Simukoko, H., Marcotty, T., Phiri, I., Geysen, D., Vercruysse, J. and Van den Bossche, P. (2007).The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomosis on the plateau of eastern Zambia.*Veterinary Parasitology*, 147(3–4): 231–238.

Sindato, C., Malele, I.I., Mwalimu, C., Nyingilili, H.S., Kaboya, S., Kombe, E., Msumary, C. and Manoza, A.(2007). Seasonal variation in human African trypanosomosis in Tarangire National Park in Babati district, Tanzania.T*anzania Health Research Bulletin*, 9(2): 136-139.

Steverding, D. (2008). The history of African trypanosomosis. *Parasites and Vectors,* 1(3): 1–8.

Steverding, D. and Tyler, K.M. (2005). Novel antitrypanosomal agents.*Expert Opin Investig Drugs*, 14(8):939-955.

Sutherst, R.W. (2004). Global Change and Human Vulnerability to Vector-Borne Diseases *Clinical Microbiology Reviews*, 17(1): 136–173.

Swai, E. S. and Kaaya, J. E. (2012). A parasitological survey for bovine trypanosomosis in thelivestock/wildlife ecozone of Northern Tanzania. *Vet. World*, 5(8): 459-464.

Swallow, B. M. (1999). *Impacts of Trypanosomosis on African Agriculture.* Food and Agriculure Organization, Rome, Italy. 46pp.

Taioe, M. O. (2013). Molecular detection, genetic and phylogenetic analysis of trypanosome species in Umkhanyakude District of Kwazulu-Natal Province, Dissertation for Award of MSc Degree at University of the Free State, South

Africa. 152pp.

Tang, Y.W., Procop, G.W. and Persing, D.H. (1997). Molecular diagnostics of infectious diseases. *Clinical Chemistry*, 43(11): 2021–2038.

TMA (2013). Maproom. [http://maproom.meteo.go.tz/maproom/Climatology/Climate_Analysis/monthly.htm www.meteo.go.tz] site visited on 09/07/2016.

Tavares, R.G., Staggemeier, R., Borges, A.L.P., Rodrigues, M.T., Castelan, L.A., Vasconcelos, J., Anschau, M.E. and Spalding, S. M. (2011). Molecular techniques for the study and diagnosis of parasite infection. *The Journal of Venomous Animals and Toxins including Tropical Diseases*, 17 (3): 239-248.

Thrusfield, M. (2005).*Veterinary Epidemiology*. 2nd Edition, Blackwell Science, Oxford. pp117-198.

Thumbi, S.M., McOdimba, F.A., Mosi, R.O. and Jung'a, J.O. (2008). Comparative evaluation of three PCR base diagnostic assays for the detection of pathogenic trypanosomes in cattle blood. *Parasites and Vectors*, 7: 1–7.

Torr, S. J. and Hargrove, J.W. (1999). Behaviour of tsetse (Diptera: Glossinidae) during the hot season in Zimbabwe: The interaction of micro-climate and reproductive status. *Bulletin of Entomological Research*, 89: 365–379.

Ulienberg, G. (1998). A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis. [http://www.fao.org/docrep/006/x0413e/X04 13E02.htm] site visited on 10/04/2016.

Valones, M. A. A., Guimarães, R. L., Brandão, L. A. C., de Souza, P. R. E., de Albuquerque Tavares Carvalho, A. and Crovela, S. (2009). Principles and applications of polymerase chain reaction in medical diagnostic fields: a review. *Brazilian Journal of Microbiology*, 40(1): 1–11. http://doi.org/10.1590/S1517-83822009000100001.

Vincendeau, P. and Bouteille, B. (2006). Immunology and immunopathology of African trypanosomiasis. *Annals of the Brazilian Academy of Sciences*, 78(4): 645-665.

Wagner, E. R., Bremer, W. G., Rikihisa, Y., Ewing, S. A., Needham, G. R., Unver, A. and Stich, R. W. (2004). Development of a p28-based PCR assay for Ehrlichia chaffeensis. *Molecular and Cellular Probes*, 18(2): 111–116.

Wang, J., Böhme, U. and Cross, G. A. M. (2003). Structural features affecting variant surface glycoprotein expression in Trypanosoma brucei. *Molecular and Biochemical Parasitology* 128: 135–145.

Wardrop, N. A. (2016). Integrated epidemiology for vector-borne zoonoses. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 110: 87–89.

Wertheim, H., Horby, P. and Woodall, J. (2012). *Atlas of Human Infectious Diseases.* Wiley-Blackwell,Oxford; [https://blog.wellcome.ac.uk/2012/03/01/developing-the-atlas-of-human-infectious-diseases/african-trypanosomiasis/] site visited on 26/07/2016.

WHO (2002). Programme to Eliminate Sleeping Sickness Department of Communicable Disease Surveillance and Response. 20, avenue Appia-1211 Geneva 27, Switzerland. 28pp.

WHO (2004). *Strategic Review of Traps and Targets for Tsetse and African Trypanosomosis Control*. World Health Organization, Washington DC. 5pp.

WHO(2012). African trypanosomosis (sleeping sickness). *Fact Sheet,* 259: 552–561.

WHO (2013). *Control and Surveillance of African Trypanosomosi*s. Technical Report Series No. 984. World Health Organization Geneva, Switzerland. 250pp.

WHO (2016). Vector-borne diseases. [http://www.who.int/mediacentre/factsheets/fs387/en/] site visited on 10/04/2016.

Zillmann, U., Konstantinov, S. M., Berger, M. R. and Braun, R. (1996). Short communication: Improved performance of the anion-exchange centrifugation technique for studies with human infective African trypanosome. *Acta Tropica,*62:183-187.

# APPENDICES

## Appendix 1: Biological data collection form

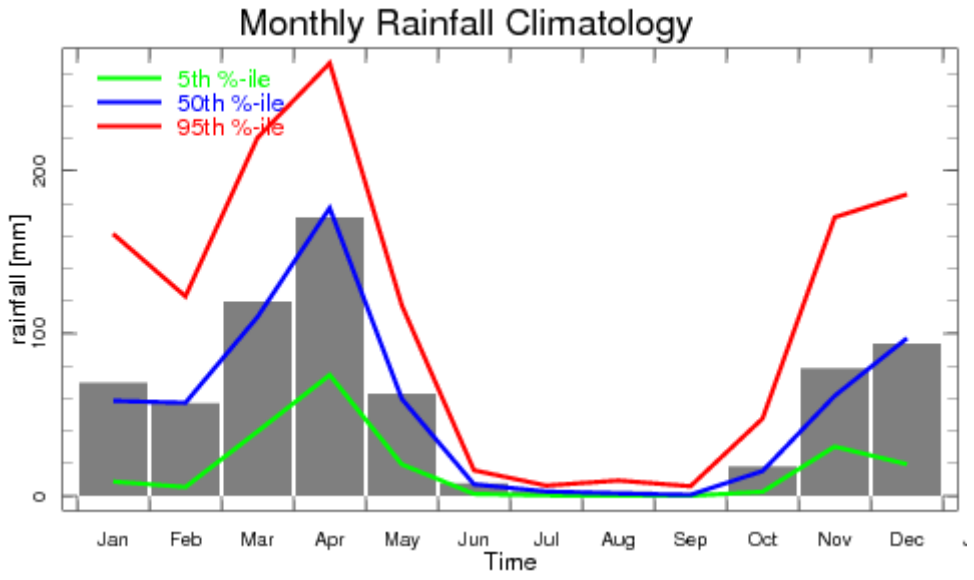
VILLAGE……SUBVILLAGE: …….DATE: …….

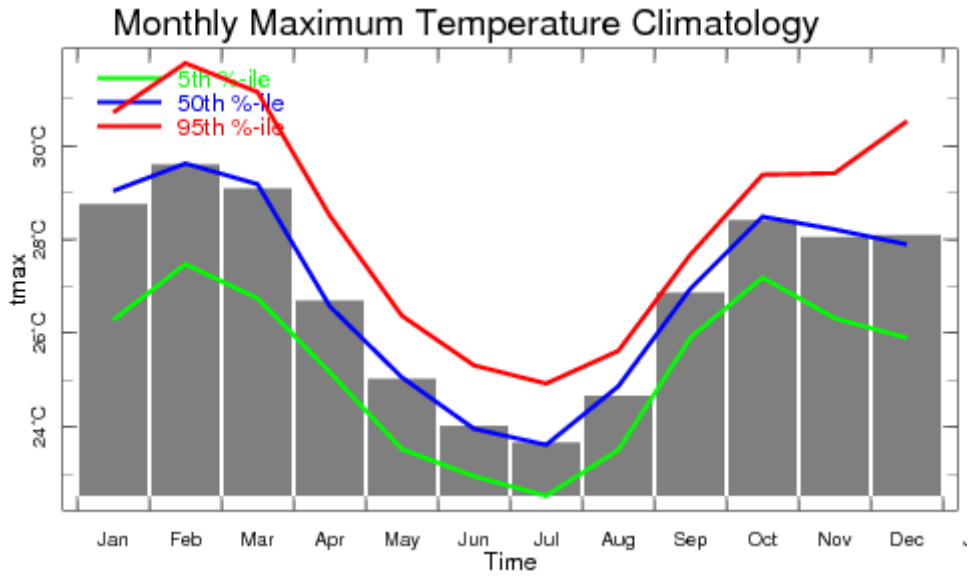
| No | Name of Boma | Animal ID | Sex | Age | Breed | ECF vaccination status | History of vector-borne diseases | Drugs used/when? | Vector control measures applied | Any animals introduced to the herd? /from? |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | |
| 2 | | | | | | | | | | |
| 3 | | | | | | | | | | |
| 4 | | | | | | | | | | |
| 5 | | | | | | | | | | |

**Appendix 2: Mixed trypanosome species**

| Mixed trypanosome species | Frequency |
|---|---|
| *T. simiae/T. theileri* | 1 |
| *T. vivax/*500bp | 9 |
| *T. brucei/T. simiae/*500bp | 2 |
| *T. simiae/T. vivax* | 19 |
| *T. simiae/T.vivax/*300bp | 1 |
| *T. vivax/T. simiae/T. theileri* | 2 |
| *T. vivax/T. brucei* | 9 |
| *T. vivax/*300bp | 3 |
| *T. congolense/T. vivax* | 4 |
| *T. brucei/*300bp | 1 |
| *T. vivax/T. simiae/T. brucei* | 3 |
| *T. vivax/T.simiae/T. theileri/T. brucei* | 1 |
| *T. vivax/T. theileri/T. brucei* | 3 |
| *T. brucei/T. congolense* | 3 |
| *T. congolense/T.vivax/T. theileri* | 2 |
| *T. vivax/T.simiae/*500bp | 2 |
| *T. simiae/*500bp | 2 |
| *T. congolense/T. simiae* | 2 |
| *T. theileri/*300bp | 1 |
| *T. theileri/T. brucei* | 2 |
| *T. congolense/T.brucei/T. theileri* | 1 |
| *T. brucei/T. simiae* | 2 |
| **Total** | **72** |

**Appendix 3: Monthly Rainfall and Maximum Temperature Climatology obtained from TMA**




Created by quality controlled station observations with satellite rainfall and maximum temperature estimates, and downscaled reanalysis product