2020-03

Influence of cattle movement, ecological and risk factors on trypanosome infections of cattle in Simanjiro and Monduli districts, Tanzania

Ngongolo, Kelvin

NM-AIST

INFLUENCE OF CATTLE MOVEMENT, ECOLOGICAL AND RISK FACTORS ON TRYPANOSOME INFECTIONS OF CATTLE IN SIMANJIRO AND MONDULI DISTRICTS, TANZANIA

Kelvin Ngongolo

A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

March, 2020

ABSTRACT

African animal trypanosomiasis is a neglected tropical disease with significant impacts on pastoral community livelihoods. This study sought to determine the influence of seasonal cattle movements and risk factors including age, sex, and herd size and treatment intervention, in conjunction with studying ecological factors on trypanosome infections in cattle in of the Maasai steppe in northern Tanzania. To identify potential hotspots areas of trypanosome transmission and associated ecological factors, I worked with five pastoralist households in each of three study villages in the Maasai steppe. Consecutively, 10 individual cattle were sampled in three periods, covering both wet and dry months grazing ranges (July, 2017 to January, 2018). Each time a blood sample was collected from the cattle, a prophylactic dose of diminazene aceturate was administered to clear any trypanosome infections acquired in the previous three months. Participatory mapping techniques were then used to identify the areas in which each pastoralist had grazed their herds since the last sampling period. Herders were also interviewed about the presence of cultivated farms and other habitat types observed in grazing areas, the abundance of wild animals per visit, risk factors related to the cattle, previously infected cattle and any disease control methods they had practiced since my last visit. Trypanosome infections in the blood sample were detected using nested polymerase chain reaction with ITS-1 primers. The overall prevalence of trypanosome infections across all sampling periods and villages was 13.1%. Prevalence in July, 2017 (19.3%) was significantly greater than prevalence in October, 2017 (2%) ($P<0.05$). Cattle acquired trypanosome infection in 21 out of 45 grazing areas identified. Herd sizes between 51-100 cattle were observed to be positively associated with the prevalence of trypanosome infections in different villages. Wild animal abundance particularly buffaloes together with their habitat types, notably woodland, were observed to be positively associated with trypanosome infection while cultivation was negatively associated with trypanosome infections. Targeted awareness on influence of seasonality, hotspot areas, risk factors, ecological factors and cultivation of trypanosome infections will help Maasai pastoralists to plan movement of their cattle strategically, properly controlling and avoiding disease risks.

DECLARATION

I, **Kelvin Ngongolo** do hereby declare that this thesis is full representation of my own work and that its contents is only for this original work submitted by me to the Nelson Mandela African Institution of Science and Technology (NM-AIST) for the degree of Doctor of Philosophy (PhD) in Life Sciences (Biomedical Health Science and Engineering) and that it has not been submitted for consideration of a similar degree award in any other University.

Date.. Signature...

Kelvin Ngongolo (Candidate)

The above declaration is confirmed by

Supervisors

1. Date…………………………Signature………….. ………………

   Prof. Paul Gwakisa (Supervisor 1)

2. Date ……………… …..…….Signature…………….. …………….

   Prof. Peter Hudson (Supervisor 2)

3. Date……… ………..…….Signature…………………………………………..

   Dr. Anna Estes (Supervisor 3)

COPYRIGHT

This dissertation is copyright material protected under the Bern Convention, the Copyright act of 1999 and other international and national in enactments, in that behalf, on intellectual property. It must not be reproduced by any means, in full or in part, except for short extract in fair dealing; for researcher private study critical scholar review or discourse with an acknowledgement, without the written permission of the office of Deputy Vice Chancellor of Academics, Research and Innovations, on behalf of both the author and the Nelson Mandela African Institution of Science and Technology.

CERTIFICATION

This is to certify that the accompanying thesis titled "**Influence of Cattle Movement, Ecological and Risk Factors on Trypanosome Infections of Cattle in Simanjiro and Monduli Districts, Tanzania**" is written by Kelvin Ngongolo under the supervision of Prof. Paul Gwakisa, Prof. Peter Hudson and Dr. Anna Estes at the Nelson Mandela African Institution of Science and Technology. The undersigned certify that they have read and hereby recommend the thesis to the NM-AIST Senate for award of the Degree of Philosophy in Life Sciences (Biomedical Health Science and Engineering) of the Nelson Mandela African Institution of Science and Technology.

………..   Date……………………………….

Prof. Paul Gwakisa (Supervisor 1)

…………………   ……………..Date………..……….. ……………

 Prof. Peter Hudson (Supervisor 2)

……………………………………………...Date……………………………….…..

Dr. Anna Estes (Supervisor 3)

ACKNOWLEDGEMENT

DEDICATION

I am dedicating this work to my wife Atuhombye Ayubu Sigala and my son Given Kelvin Ngongolo. They have been patient during my study period and were supportive to me by providing moral support and encouragement during hard times. A further dedication extends to my mother (Tumain Mahenge) for providing encouragement and moral support during my study period.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

AAT	Animal African Trypanosomiasis
AVOVA	Analysis of Variance
ITS	Internal Transcriber Spacer
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization (United Nations)
GLMM	Generalized Linear Mixed Effect Model
HAT	Human African Trypanosomiasis
NM-AIST	Nelson Mandela African Institution of Science and Technology
PCR	Polymerized Reaction Chain
QGIS	Quantum Geographical Information System
WHO	World Health Organization
TTB	Tanzania Tourists Board
TANAPA	Tanzania National Parks
TAWA	Tanzania Wildlife Authority
WMAs	Wildlife Management Areas
CBNRM	Community Based Natural Resource Managements
CNS	Central Nervous System
ADI	Acceptable Daily Intake
OR	Odd Ratio
IR	Infection Rate
GLMM	Generalized Linear Mixed Effect Modal
MCF	Malignant Catarrhal Fever
EDTA	Ethylene Diaminetetraacetic Acid

CHAPTER ONE

INTRODUCTION

1.1.   Background of the problem

Tsetse flies are vectors of the parasitic protozoans, the *Trypanosoma* spp., which cause the disease trypanosomiasis in animals, including cattle and sleeping sickness in humans (World Health Organization, 2013). About 30 species and subspecies of tsetse fly belonging to the genus *Glossina* under the three genera known as Austenia (currently referred as fusca group) found in forest, Nemorhina (*G. palpalis*) found in savannah and Glossina (*G. morsitans* group) from riverine habitats. The species of economic importance include *G. pallidipes, G. brevipalpis, G. morsitans* and *G. swynnertoni* since they transmit trypanosomes to both cattle and humans (Salekwa *et al*., 2014). Despite of what is known about on the effects of season, habitat and host species on the prevalence of the parasite in the vectors (Ngonyoka *et al*., 2017; Nnko *et al*., 2017) yet the influence of livestock movement patterns influence on trypanosomes infection in cattle required further investigation which prompted this study.

Trypasoma congolense, T. brucei and *T. vivax* are known trypanosome species that infect cattle which cause a reduction in the productivity of cattle and mortality. In addition, T. *brucei rhodesiense* (common in East Africa) and *T. brucei gambianse* (common in West Africa) cause Human African Trypanosomiasis (HAT) in humans (sleeping sickness) (Muhanguzi *et al*., 2014; Salekwa *et al*., 2014). Depending on the severity of the diseases, the negative impacts on cattle productivity may include lowered fertility, high rates of abortion, low calf birth weight, reduced calf growth rate, reduced milk yield and poor beef quality, thus leading to poor livestock off-take and reduced revenue (Chanie, Adula & Bogale, 2013). Apart from cattle, trypanosomiasis also affects other domestic animals including pigs, sheep and dogs (Ezeani *et al*., 2008). Naturally, livestock keepers have a negative attitude towards the vectors and have developed control strategies including the application of natural and artificial chemotherapy to targets and livestock (Kimaro, Toribia, Gwakisa & Mor., 2018). An additional and focused approach is to identify the transmission hot spots, where cattle become infected.

Livestock productivity in many parts of the tropics, including Tanzania, is limited by vector-borne diseases including trypanosomiasis, an infection transmitted by tsetse flies (*Glossina* spp) (Chanie *et al.*, 2013). High tsetse fly densities limits livestock production in many parts

of Tanzania including the Maasai steppe of northern Tanzania (Malele, 2011; Salekwa *et al.*, 2014). Trypanosomiasis in the Maasai steppe is further exacerbated by high human-livestock-wildlife interaction (Simwango *et al.*, 2016). The association of the three components (human-livestock-wildlife) is believed to maintain the circulation of tsetse flies and trypanosomes among hosts (Miller, Leslie & McCabe, 2017). For instance, a study done by Ruiz, Nyingilili, Mbata and Malele (2015) in Ngorongoro Conservation Area, showed that, the domestic animals, in particular sheep, are reservoirs of *Trypanosome brucei rhodensiense* which causes sleeping sickness in humans. There is scarce information regarding the whether the interactions between humans, animals and the ecosystems influence prevalence of animal infective trypanosomes in cattle of the Maasai steppe. The primary aim of this study was to understand the impact of animal movement patterns, human activities and ecological factors on trypanosome infection.

Most wild animals, like buffaloes (*Syncerus caffer*), are reservoirs for trypanosomes that can be passed by vectors to livestock and human beings (Baticados, Castro & Baticados, 2012). A study in northern Tanzania showed higher prevalence of trypanosomes in cattle in Mswaki Juu village than Oltukai village due to wildlife abundance variation (Swai & Kaaya, 2012). Furthermore, Malele, Nyingilili and Msangi (2011) noted that traps set near a protected area rich in wildlife (wildlife conservation area) in Mtwara, Tanzania, yielded a higher abundance of tsetse flies than an area not occupied by wildlife. Similarly a work done by Ngonyoka *et al.* (2017) also showed that seasonal, host species, habitats and distance from wildlife reserves played (Tarangire National Park) a significant role in the abundance of tsetse flies and the infection of trypanosome in the vectors. However the influence of the protected area and animal movement on trypanosome infections in livestock such as cattle was not considered in these studies. In addition, the influence of control interventions such as treatment and environmental variables like habitat type and human activities such as cultivation were also not taken into consideration.

1.2. Statement of the problem

Tsetse flies are vectors of trypanosomes that infect animals as well as humans. Although numerous studies have been conducted on tsetse fly abundance and trypanosome prevalence in Tanzania, only a few studies have examined the vectors and trypanosome transmission in an intensive human-livestock-wildlife interface. This study will be helpful in developing effective evidence-based control strategies for trypanosomiasis by further understanding the

influence of human activities (e.g. cultivation), cattle movement patterns and ecological factors on trypanosome infections in cattle. The Maasai, who comprise the majority of the pastoralists occupying the Maasai Steppe, are vulnerable community of smallholder livestock keepers. The smallholder livestock keepers, including the Maasai, live and carry out their daily livestock activities in the proximity of reservoir animals (wildlife) where vectors are also abundant. Maasai communities, together with their cattle, are vulnerable to trypanosomiasis and other vector borne diseases and this can increase through climate change (Nnko *et al*., 2017). Assessing vulnerability of smallholder livestock keepers to trypanosomiasis and how their livestock are impaired in this complex interface scenario is important.

Trypanosomiasis has socio-economic impacts on pastoral communities because it reduces quality and quantity of meat and milk, cause food insecurity, loss of income, poverty and health risk. For instance, in Simanjiro District 24 244 cattle were diseased while 10.5% between 2011 and 2013 died equivalent to 892 150 USD loss of income (Mndolwa, 2014). The economic losses is likely to be higher when morbidity taken into account. In this case proper understanding of the epidemiology of the diseases and how ecological factor, cultivation, cattle movement influence the infection in cattle is needed. This will help in developing proper control strategies of trypanosomiasis so as to lessen the economic loss caused.

1.3.   Rationale of the study

Maasai steppe is an important area which comprises mainly Maasai communities who practices small holder livestock keeping. The livelihood of Maasai like other pastoralists mainly depend on livestock keeping (Reda, 2012). Any challenging factor on the production of their livestock will have directly or indirectly negative impact on pastoralist communities' livelihood. Among the important challenge threatening livestock productivity in pastoralists communities is trypanosomiasis disease to cattle (Nnko *et al*., 2017). Previous studies in this area have shown that trypanosome infection is having an impact on pastoralists (Simwango *et al*., 2017). However there was a lack of knowledge on the influence of the risk factors, movement and ecological factors on trypanosome infection in cattle which was   addressed in this study.

1.4. Objectives

1.4.1. General objective

To examine the influence of cattle movement, management interventions, risk factors and their interaction with ecological factors on the infection of cattle by trypanosome in the Maasai steppe, Tanzania.

1.4.2. Specific objectives

The following were the specific objective for this study:

(i)   To determine the influence of seasonal grazing patterns on infection of cattle with trypanosomes.

(ii)   To assess the influence of land cover and wildlife reservoirs on infection of cattle with trypanosomes.

(iii)   To assess the influence of risk factors and treatment interventions on trypanosome infections of cattle.

1.5. Hypothesis

(i)   Seasonal grazing patterns are the primary factor determining trypanosome infection of cattle on the Maasai steppe.

(ii)   Specific land cover (i.e. woodland, grassland, cultivated land) and wildlife host species significantly influence the risk of trypanosome infections in cattle.

(iii)   Interventions and risk factors significantly reduce rates of trypanosome infection in cattle of the Maasai steppe.

1.6. Significance of the study

The findings accrued from the study will contribute significantly to the existing foundation of knowledge on the control of trypanosomes in cattle. This is of socio-economic importance to small livestock keepers in the Maasai steppe. The result will provide baseline information on the impacts of land cover, animal movement and human activities on the prevalence of trypanosomes. The results from this study might also be helpful to various stakeholders,

including the small livestock keepers, involved in controlling tsetse flies and trypanosomes across the whole region since the factors will help to predict hotspots of prevalence. Furthermore, the policy makers might find the information useful for developing control strategies policy and priorities for this neglected yet economical important disease (trypanosomiasis) to livestock keepers. The findings from this study will guide the policy maker to prioritize the area for the control if they know where the disease is causing more economic loss.

1.7.   Delineation of the study

The time interval involved for sampling during this study was short. This study took place in less than one year (July, 2017, October, 2017 and January, 2018). Also, only three villages with 15 herders were involved in the study which was few. The few villages and little time involved were due to limited resources and available time for the study. As results, this would be having impact on the sample size to reach a strong conclusive recommendation. It would be worth to cover more villages and longtime of at least one year.

Generally this study focused on generating knowledge on how the prevalence of trypanosome infections in cattle of the Maasai steppe are determined by human activities such as cultivation, livestock movement and the ecological factors of wildlife presence and habitat types at the human-livestock-wildlife interface (Fig. 1).
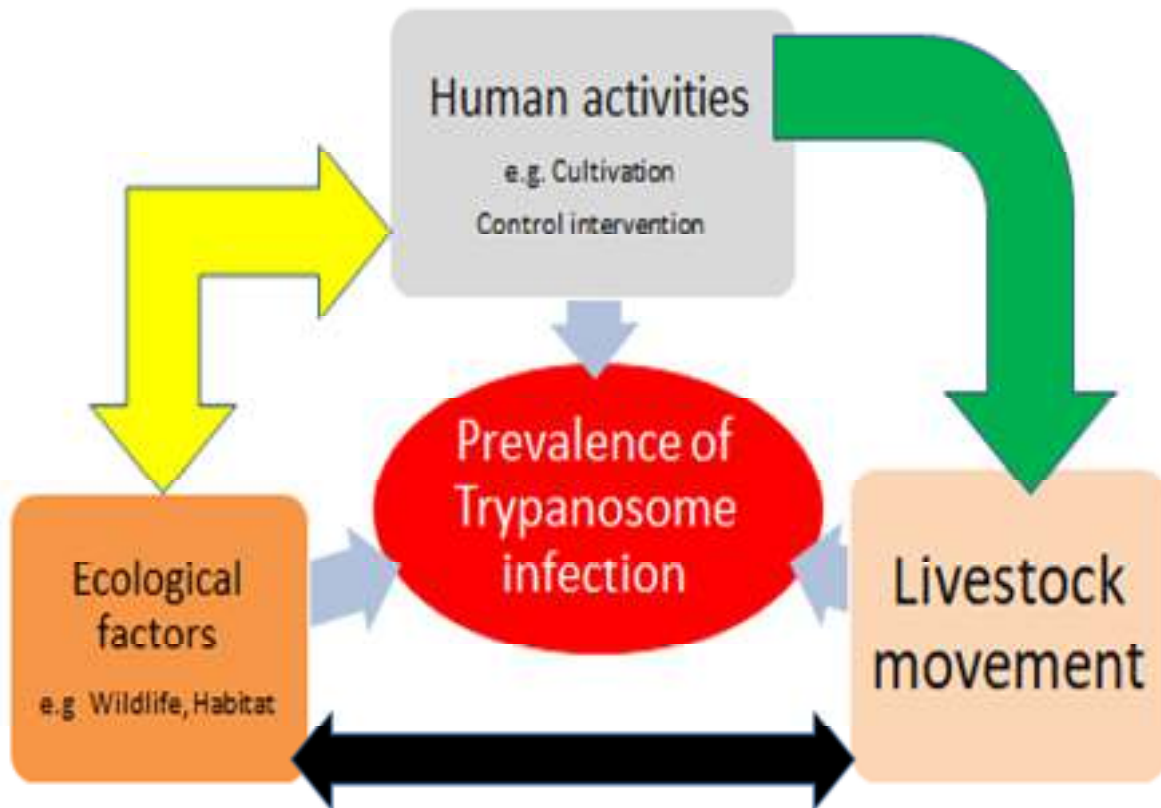
Figure 1: A conceptual framework on human activities, ecological factors and animal movement influencing the prevalence trypanosomes and tsetse fly infestation

CHAPTER TWO

LITERATURE REVIEW

2.1. Maasai steppe, people and livelihood

The landscape of the Maasai steppe is an ecosystem with wildlife and livestock interaction. It spreads across two regions of northern Tanzania, namely Arusha and Manyara, and stretches over eight districts in Tanzania and some parts of Kenya (Nelson, 2012). The landscape is semi-arid with grassland, *Acacia*-dominated woodland in the low-lying areas, and Miombo woodlands in the hills rising in the southwest towards. The area is occupied mainly by Maasai pastoralists who keep livestock such as cattle, goats, sheep and chickens for subsistence and commercial purposes. The mode of livestock keeping by pastoralists is a nomadic form of life, however due to scarcity of land and environmental change they are moving toward a sedentary mode of livestock keeping (Msoffe *et al*., 2011). In addition, the pastoralist also practice small scale cultivation of crops like maize, beans and sesame are grown (Msoffe *et al*., 2011).

2.2. Ecological perspective

The Maasai steppe is an ecosystem with a high diversity of wildlife species. The area is occupied by diverse habitats which support livestock, humans, wildlife and tsetse flies, the vectors of trypanosomes (Cecchi, Mattioli, Slingenbergh, De la Rocque., 2008; Ngonyoka *et al.*, 2017; Nnko *et al*., 2017). The habitats in this area include grassland, woodland, forest, seasonally water-logged habitats (known as Engusero).

Conservation biology is the management of flora and fauna (biodiversity) with the purpose of protecting species, their habitats, and ecosystems from excessive rates of extinction and the erosion of biotic interactions. In Tanzania, there are several ways to which the biodiversity resources are managed such as Conservation area, National parks, Wildlife management area (WMA) and game reserve. A good example of a conservation area in Tanzania is the Ngorongoro Conservation Area which covers about 8292 km^2 (Tanzania tourist board, 2017). A conservation area is a location which receives protection because of their recognized natural, ecological and/or cultural values.

A National Park is the highest level of resource protection in Tanzania where only non-consumptive resource utilization is allowed and it's primarily role is conservation to secure a functional ecosystem. National parks in Tanzania are managed by TANAPA (Tanzania National Parks). Example of National park in Tanzania includes: Ruaha, Serengeti, Gombe, Katavi, Lake Manyara and Tarangire (Tanzania tourist board, 2017).

Game Reserves, are managed by the Tanzania Department of Wildlife under Tanzania Wildlife Authority (TAWA). Under this management regime both consumptive and non-consumptive utilization is allowed and includes game viewing, hunting, research and education. The best examples in Tanzania are: Selous, Moyowosi, Kigosi, Mkomazi, Usangu, Lukwila-Lumesule, Msangesi and Ugalla (Tanzania tourist board, 2017).

Wildlife Management Areas (WMA) are a form of wildlife management in Tanzania where communal land is set aside exclusively as habitat for wildlife by member villages. Under this management approach, it is believed that, the local communities will benefit from the wildlife resources when the land is conserved. This approach is commonly known as community-based natural resource management (CBNRM). It assumes that, the local communities will provide support in the conservation of biodiversity resources if they are receiving benefits from the conserved natural resources (Tanzania tourist board, 2017).

2.3.   Conservation and wildlife in the Maasai steppe

There are different levels of protected area in the Maasai steppe, which form the core of the wildlife habitat. These include Tarangire and Lake Manyara National Parks (Msoffe *et al*., 2011). Wildlife Management Areas (WMAs) in Maasai steppe like other part in Tanzania are being used as conservation strategies for wildlife and natural resources found outside the national parks. Even areas without formal protection in the Maasai steppe ecosystem host wildlife that migrate outside Tarangire National Park in the wet season, and graze alongside livestock (Nelson, 2012). The area has habitats which support diverse species of wildlife such as elephant (*Loxodonta africana*), lion (*Panthera leo*), wildebeest (*Connochaetes taurinus*), zebra (*Equus quagga*), giraffe (*Giraffa camelopardalis*), buffalo (*Syncerus caffer*), and oryx (*Oryx beisa*), although these areas are increasingly under threat from agricultural conversion (Msoffe *et al*., 2011) .

2.4.   Human activities

The core socio-economic activity for the Maasai communities in the Maasai steppe is pastoralism. However to boost their income and livelihood, the communities have adopted more income earning activities; for instance they cultivate crops, get involved with tourism and work with other small business activities, including small-scale mining (Homewood, Trench & Brockington, 2012). Agriculture has been observed to disrupt the ecosystem and the wildlife and livestock associated with this area (Msoffe *et al*., 2011). The crops grown in this area are primarily maize, beans, sesame and sunflower.

The engagement of pastoralists in agriculture has been associated with the increase in human population density, decreasing the land available for livestock grazing, and the national policy of "Kilimo kwanza" (Farming first) which promoted agriculture to Tanzanians (Conroy, 1999; Lankester, Davis & Allen, 2016). Cultivation is anticipated to have impacts on the habitat for wildlife hosts and the tsetse vectors for trypanosomes which could impact prevalence of trypanosome infection. However, limited studies have been undertaken to determine the influence of cultivation on the prevalence of trypanosome infections in cattle.

Expansion of farmland in the Maasai steppe has made many pastoralists to switch from nomadic lifestyle to semi-sedentary or sedentary mode of life. The reason for the changes could be due to a decline of land supporting nomadism; most of the land being converted to protected area and other demands for social services like school and health centers which encourage pastoralists to become sedentary. In addition, adaptation to climate change has made pastoralist adapt to a sedentary lifestyle where they can engage in other economic activities to support their life such as agriculture, business and tourism sectors (Bonfoh *et al*., 2011; Reda, 2012). This has also been noted in other studies where cultivation has made pastoralists shift from nomadism to being sedentary or semi-sedentary (Majekodunmi *et al*., 2013). The majority of suitable pasture for livestock is found close to protected areas where there is intense wildlife-livestock–vector interaction (Ngonyoka *et al*., 2017; Nhamitambo, 2017; Nnko *et al*., 2017; Simwango *et al*., 2017) that subsequently increases the risk of exposure of cattle trypanosome infection. Other human activities which are known to have a negative association with the prevalence of trypanosomes include hunting of wildlife and fire burning. These activities decrease available habitat for tsetse flies, hosts for the parasite, exposure of cattle to infection and thus the prevalence of trypanosomes (Guerrini *et al*., 2008; Munangandu, Siamudaala, Munyeme & Nalubamba., 2012).

2.5.   Animal movement

Pastoralists in this area engage in seasonal movement of livestock (such as cattle) from one grazing area to another, in search of fresh pasture and water (Sidiropoulou, Karatassiou, Galideki & Sklavou., 2015).   This is sometime defined as the seasonal transfer of herds and flocks, and occurs along well-defined routes, repeated each year and always accompanied by the cattle owners (Lankester *et al.*, 2016). This kind of movement is also referred to as transhumance, which comes from the Latin Trans, meaning across or over, and humus, meaning ground or soil. In some places this practice has posed serious problems by increasing exposure of cattle to diseases, dangerous wild animals and other catastrophic events (Abiola, Biaou & Niang, 2005). However, the extent to which seasonal cattle movement in Maasai steppe is impacting the prevalence of trypanosomes is not known.

Other factors which have been observed to stimulate movement of pastoralists with their cattle include avoidance of diseases transmission during a given season, drought and avoidance of conflicts between the livestock keepers and crop farmers (Abiola *et al.*, 2005). Although animal movement can be strategic for avoiding transmission of infection in one place, it can still be the means of acquiring new infections from the destination area. This has been observed in cases of foot and mouth disease, rabies, and bovine tuberculosis (Fèvre *et al*., 2006). A study done by Lankester *et al.* (2016) in the Simanjiro plains of the Maasai steppe revealed that during wildebeest calving seasons, cattle tended to move away from the plains into denser bush to avoid the risk of getting malignant catarrhal fever disease, which is transmitted by wildebeest afterbirth. Movement into the denser bush, particularly areas with stable populations of host wildlife species, can increase the risk of cattle exposure to tsetse vectors and the chances of acquiring trypanosome infections.   The impact of animal movement on the transmission of trypanosome infections to cattle was considered during this study and used to identify potential hot spots of infection.

2.6. The interface driving vector, wildlife and livestock

The ecosystem of the Maasai steppe has common resources which drive the abundance of vectors, wild animals and livestock in unison. Migration of wildlife from Tarangire national park to adjacent area increases the interaction of wildlife, livestock and tsetse flies. The pastoralists are anticipated to encounter the migrating wildlife and also tsetse flies when grazing their cattle.

A study by Nnko et al. (2017) revealed that wildlife and tsetse fly abundance tend to vary seasonally in the Maasai steppe. The study showed that the variation in the abundance of wildlife and tsetse flies was due to habitat type, conditions (greening of vegetation) and rainfall. Assessing the impact these factors have on the prevalence of trypanosome infections in not just the vector, but also the cattle hosts and it is important because proper control strategies need proper understanding of associating factors.

2.7. Economic implication of trypanosomiasis

Trypanosomiasis is a disease of socio-economic importance to pastoral communities. For instance, in Tanzania about 4.4 million livestock and 4 million people are at risk of contracting tsetse-borne trypanosomiasis (Malele et al., 2011). Furthermore, a report from Simanjiro District showed a total loss of 2 549 cattle over 3 years between 2011 and 2013, representing about 10.5% of the total cattle population (n=24 244), equivalent to nearly 900 000 USD (Mndolwa, 2014). The economic loss due to trypanosomiasis can only be lessened with mitigation strategies based on thorough evidence and understanding of the factors contributing to infection risk, which include the influence of cattle movement patterns, habitats, and interventions.

2.8. Trypanosome infection prevalence

Unlike incidence, which is the number of new cases per unit time, prevalence of trypanosomes is the number of active infections observed in a particular population at a specific time (Dicker, 2006). The prevalence can be categorized as point prevalence which occurs at a specific point in time in contrast to period prevalence which occurs during a given time interval like 12 months. Also lifetime prevalence (LTP) is the proportion of all animals that become infected at some point during their life (Dicker, 2006). Trypanosome prevalence in different locations is influenced by various factors. For instance, a study by

Simwango *et al*. (2017) in Maasai steppe revealed that, season and location plays significant role in the prevalence of trypanosome in tsetse flies and cattle. Study by Salekwa *et al*. (2014) showed that distance from Tarangire National park, grazing zone, residential areas had effects on the infection of trypanosome in tsetse. This study looked on how ecological factors, cultivation and the movement influence the prevalence of trypanosome infection in cattle.

In many parts of Africa including Tanzania, trypanosome infection in small livestock holders and their livestock has been limited by influenced by several factors. Among other factors responsible for the prevalence of trypanosome infection includes human activities, ecological factors, animal movement partners and Livestock management regime (Munangandu *et al.*, 2012). However, little has been done in the Maasai steppes to determine the impacts of different factors to the prevalence of Trypanosomiasis in cattle.

2.9.   Transmission of the trypanosome parasites

Trypanosomiasis is a disease of socio-economic impact to both human and livestock (cattle). The causative agent for human trypanosomiasis is *T. b. rhodesiense* (East African sleeping sickness) for eastern and southeastern Africa such as Tanzania, Uganda, Malawi, and Zambia where over 95% cases are reported (World Health Organization, 2015). *T. b. gambiense* (West African sleeping sickness) is mainly responsible for human trypanosomiasis in central Africa and West Africa (World Health Organization, 2015). Animal trypanosomiasis in cattle is mainly caused by *Trypanosoma congolense, T. vivax and T. brucei.*

The parasites responsible for both human and animal trypanosomiasis are mainly transmitted through the bite of an infected tsetse fly (Firesbhat & Desalegn, 2013) although other modes such as mechanical, pregnant woman passing the infection to her unborn baby, blood transfusion or sexual contact have been reported (World Health Organization, 2015). For instance, *Trypanosoma vivax* is mechanically transmitted through biting insects such as *Stomoxys calcitrans* and Tabanid flies (Lukes, 2009) while *Trypanosoma equipedum* causes a disease called Dourine in horses is sexually (venereal) transmitted (Food and Agriculture Organization, 1998). *Trypanosoma cruzi* and *T. vivax* has been reported to be transmitted congenitally from mother to child by crossing through the placenta or by bleeding of the mother during birth of an offspring (Lyons, 1993; Food and Agriculture Organization, 1998).

Also some studies have shown that, travellers can carry the parasite to areas where the diseases or parasite was absent formally (Burgess, 2001).

The transmission of trypanosome parasites from the vectors is associated with several factors such vectors abundance, seasonal change, location and weather patterns (Leak *et al*., 1993; Ngonyoka *et al.*, 2017; Nnko *et al*., 2017). For instance, a study by Leak *et al*. (1993) in the

Ghibe valley, southwest Ethiopia showed a significant correlation between tsetse abundance (G*lossina pallidipes)* and the prevalence of trypanosome infections in cattle. The tsetse density was observed to be related to monthly rainfall (Leak *et al*., 1993).

2.10. Life cycle of the trypanosome in vector and host

The flow of trypanosome circulation in tsetse flies and the host is shown in Fig. 2. In this case the definitive host is a human. An infected tsetse fly (genus *Glossina*) injects metacyclic trypomastigotes into skin tissue of the host during a blood meal and this enterers the lymphatic system and passes into the bloodstream (1). Inside the host, they transform into bloodstream trypomastigotes, are taken to other parts of the body fluid such as lymph, spinal fluid (2) (Ferluga, Nayak & Kishore, 2014). Inside the body replication continues by binary fission (3). The entire life cycle of African trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host (4, 5). Inside the tsetse fly's midgut, the parasites transform into procyclic trypomastigotes and multiply by binary fission (6). The procyclic trypomastigotes leave the midgut, and transform into epimastigotes (7). The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission. The cycle in the fly takes approximately 3 weeks. Rarely, *Trypanosoma brucei gambiense* may be acquired congenitally if the mother is infected during pregnancy.
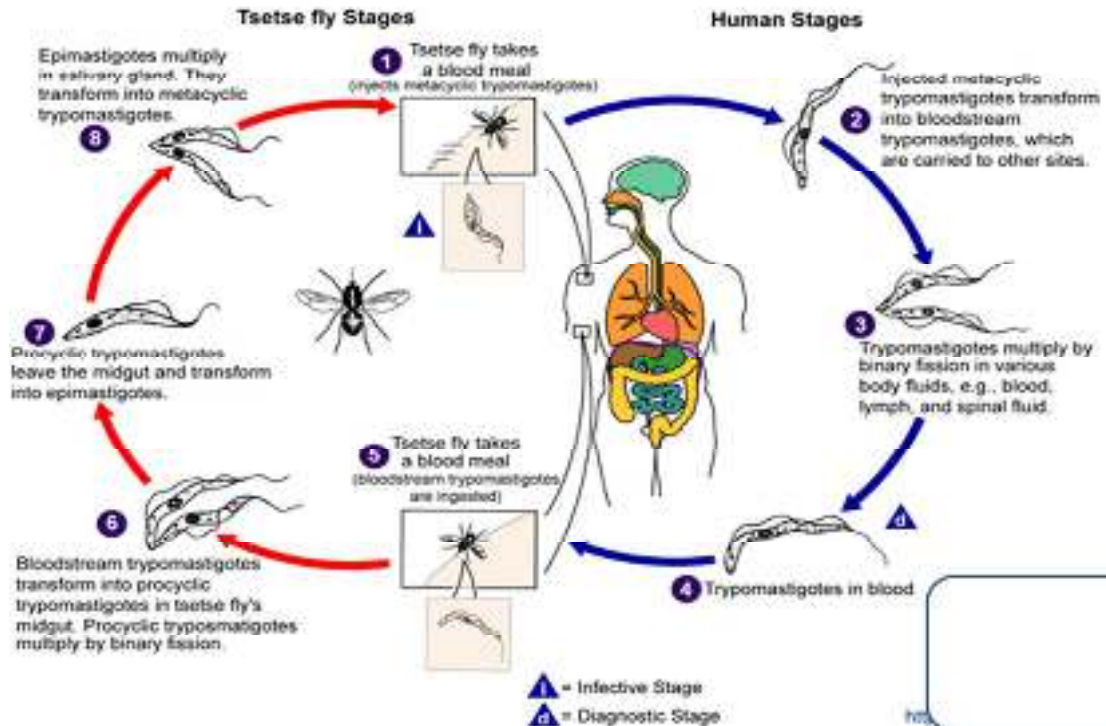
Figure 2: The life cycle of trypanosome parasite in host (e.g. man, cattle) and the vector (tsetse flies) (Sources CDC, 2019, https://www.cdc.gov/parasites/sleepingsickness/ biology. html)

2.11. Epidemiology of trypanosomes

The presence of a susceptible host, suitable environmental factors increases the risk of infect ion of vector borne diseases like trypanosomiasis (Munangandu *et al.*, 2012). The Maasai steppe is one of the ecosystem in Tanzania which provides an environment for the interface of the reservoir hosts, vectors, and susceptible hosts and has made trypanosomiasis endemic in the ecosystem (Auty *et al*., 2012).

2.12. Trypanosome intrinsic factor

Intrinsic factors in the parasite, including gastric intrinsic factors enables the parasite to survive in the host smoothly. Through gastric intrinsic factor, glycoprotein is produced by the parietal cells of the stomach essential for the absorption of vitamin B12 later in the ileum of the small intestine (Fedosov *et al*., 2003). Intrinsic factors play a key role in the survival of trypanosomes in the mammalian hosts. This is achieved when Intrinsic factors of trypanosome parasites leads to antigenic factors/antigenic shift which cause alteration of

proteins on its surface and thus avoiding host immune response (Skraban & Lutley, 2002; Vincendeau & Bouteille, 2006). Also the intrinsic factor helps the trypanosome to produce enzymes in the host which helps in the multiplication. In this case, Intrinsic factors are important in the pathogenicity of trypanosome parasite in host like cattle. For instance, intrinsic behavior of *Trypanosoma brucei gambiense* has enabled it to cross the blood-brain obstacle and attack the central nervous system (CNS) and cause neurological symptoms (Bucheton, Macleod & Jamonneau, 2014). Different species vary in level of intrinsic behavior which could have implication on the virulence of parasite to host. For instance, species like *Trypanosoma congolense* and *T. vivax* are more virulent than others like *Trypanosoma theileri* (Beziel, Girma, Dagnachew & Tadesses., 2014).

2.13. Host factors

The common reservoirs hosts for trypanosome species which cause trypanosomiasis in human and cattle are wildlife (Munangandu *et al.*, 2012). The presence of wildlife in terms of diversity and abundance has a great influence on the availability and circulation of the *Trypanosome* parasite. Different wildlife species are favored and affected by the different tsetse species (as Vector) (Munangandu *et al.*, 2012). Studies have shown that some wildlife species are more affected than others. A study done in Luangwa valley Zambia, showed variation in prevalence of *Trypanosome congolense, T. brucei* and *T. vivax* in wildlife which are of economic importance to cattle owners (Auty *et al.*, 2012).

Wildlife such as cape buffalo (*Syncerus caffer*), warthogs (*Phacochoerus africanus*), the tape bushbuck or impala (*Tragelaphus sylvaticus*) and waterbuck (*Kobus ellipsiprymnus*) were detected to have the trypanosome parasite while species like elephant (*Loxodonta africana*), spotted hyena (*Crocuta crocuta*), giraffe (*Giraffa* sp) and duikers (*Cephalophus* sp) were observed to have less impact on harboring the trypanosome parasite (Anderson *et al.*, 2011; Auty *et al.*, 2012; Auty *et al.*, 2015). Possibly meal preference for tsetse flies and quantity of sampled animals can explain this. Regardless of the detected parasites in these wildlife species, several studies have showed that, wildlife are resistant to the parasites and they do not always exhibit clinical signs (Baticados, Castro & Baticados, 2012).

The presence of wildlife drives the circulation of trypanosomes in the steppe ecosystem. Regardless of the control strategies carried out by livestock keepers and other persons (Nhamitambo, 2017), the presence of the wildlife hosts ensures persistence of the trypanosomes species that then spread to other hosts like human being and livestock. Most

wildlife species are found in protected area where tsetse flies control strategies are rarely implemented. For instance, in most pastoral communities found outside protected areas, control is done but never inside the protected areas. A study done in North Vietnam revealed that, there were higher cases of *Trypanosome avansi* in buffalos than in cattle. Ecological factors which include wildlife species were identified amongst the influencing factors (Lang, 2001), although, control practices, including treatment of cattle can also count. Also resistance of wildlife such as buffaloes to trypanosomes infection could lead to low mortality rates in infected buffaloes than infected cattle (Baticados *et al*., 2012).

2.14. Environmental factors

Environmental factors have impact on the prevalence of trypanosome infection also. The variation in environmental components, including climate change and habitat alteration can direct influence the diversity and abundance of vectors (tsetse flies) and the wildlife reservoirs. Humans also contribute directly to the change of environmental components through land use and human activities through deforestation, agriculture, population growth, urbanization and environmental pollution (Anderson *et al*., 2015). Trypanosomiasis is an environmentally sensitive vector-borne disease, variations in environment factors can influence various components of the life cycle by altering development and passage from vector to host and back again (Msoffe *et al*., 2011). An environment where there is high wildlife/livestock/vector/human interface has higher contact rates and implications on the dynamics of trypanosome infection when the pastoralist practices on the movements of cattle from one habitation to another in search for food and water (Anderson *et al*., 2015). Availability of a conducive environment with favorable vegetation, humidity, temperature, rainfall and forestry in wildlife/livestock/human interface areas favors the abundance of tsetse flies which drive the transmission of trypanosomes (Ngonyoka *et al*., 2017). The change in environment factors such as climate change have a great influence on the density, distribution and diversity of vectors which affect transmission patterns of vector borne disease causing agents (Simwango, 2016).

2.15. Control of trypanosomiasis

Trypanosomiasis has been a disease of socio-economic important particularly for pastoral communities. Due to its negative implication to cattle different control strategies have been adopted by various stakeholders. Regardless of expense, eradication has been successful in some parts like Zanzibar but in most African countries like Tanzania mainland, the diseases are still a challenge (Food and Agriculture Organization, 1998; Bourn *et al*., 2005). Several methods have been used in control strategies for trypanosome and tsetse flies in most African countries. These methods among others include; chemical control, biological control and mechanical control.

2.15.1. Chemical control

Chemical control involves the use of chemicals that are usually insecticidal in controlling the tsetse flies and also the use of drugs mostly trypanocidal for treating the infected animals. Chemical control is widely used although more education on application is needed for user to minimize environmental pollution due to improper usage. The common chemicals used for tsetse flies control include pyrethroids and deltamethrin for sprayings (Maudlin, 2006). The spray of insecticides can be direct on the animals to kill the vector on the surface of skin or on to the habitat of the vector. The previously used method of spraying the habitat of tsetse flies was usually through aerial spraying although this also has a negative impact to the ecosystem since is not selective and kills many invertebrates (Malele, 2011).

Another chemical means of tsetse fly control is the use of baited traps to capture the tsetse flies. This method is effective where traps can be maintained in small areas, otherwise it requires prior training before setting (Adamu *et al*., 2011). This method has other limitations since they can be destroyed by wind, wild animals, fire and flood, depending on where they are set (Adamu *et al*., 2011). This method involves the use of visual and odor attractants. Visual attractant can be a cloth of either blue or black color while odor attractant involves the use of chemicals, such as carbon dioxide, acetone or octanol. All these modes of attractants mimic the smell of mammalian hosts (Malele, 2011).

The other commonly used chemical method practiced by the livestock keeper is treatment to the sick animals. Those animals which show clinical signs of trypanosomiasis are injected with trypanocidal. There are various trypanocidal chemicals used for treatment and prophylaxis. including: Diminazene aceturate a curative drug against *Trypanosoma vivax, T.*

congolense and T. brucei, Homidium bromide and Homidium chloride which are used in both cattle and equids while Isometamedium is used in cattle against *Trypanosoma congolense* and *T. vivax* as a curative and prophylactic drug (Giordani, Morrison, Rowan, Koning & Barrect., 2016). Other drugs used for treatments are; Quinapyramine and Suramin effective in horses, camels, pigs and dogs against (Tuntasuvan & Viseshakul, 2002; Tuntasuvan *et al.*, 2003) while Melarsomine dichlorhydrate is both curative and prophylactic drug in camels against *Trypanososma evansi* (Giordani *et al.*, 2016).

2.15.2. Biological control

Biological Control of tsetse flies involves using other organisms such as parasites and predators, to control or eradicate the tsetse flies and improve the economic productivity of livestock industry (Oluwafemi, 2008). There are different ways of undertaking the biological control of tsetse flies. These include; use of trypanotolerant breeds, sterilization of male tsetse flies and use of symbiotic bacteria to overcome trypanosomiasis challenges.

The use of sterile tsetse males is based on the copulation behavior of female tsetse flies. Female tsetse flies copulate only once during their life time so if the male involved in the copulation process is sterile, then the female tsetse flies will not be fertilized and produce no offspring (Adamu *et al.*, 2011). Sterilization of male tsetse flies can be done by treating them with mutagenic agents such as gamma rays, and chemosterilants (Oluwafemi, 2008; Adamu *et al.*, 2011). The use of sterile males can also significantly reduce the population of tsetse, however it is very expensive and sophisticated for field based control (Adamu *et al.*, 2011). The success of this method depends on the integration with other methods such as spraying and trapping. Zanzibar is a good example in which eradication of tsetse flies has been made possible through integration approach (Vreysen *et al.*, 2012).

Trypano-tolerant breeds are those animal breeds which are able to tolerate or resist the stress caused by the parasite and are able to survive infection. The method can be achieved by encouraging the farmer to practice breeding of animals which are strong with high survival traits in the herd (Adamu *et al.*, 2011). In west Africa for instance, N'Dama and West African shorthorn cattle, their sheep (Djallonke) and goat (Dwarf West African) have traits which enable them to survive and be productive in tsetse-infested areas (Adamu *et al.*, 2011).

2.15.3. Use of symbiotic bacteria

The use of symbiotic bacteria is the association of bacteria with the vector where within the symbiotic relation, the bacteria cause genetic modification to produce anti-trypanasome molecule. The good example is in the triatominae bugs, the genetic modification with symbiotic Rhodococcus bacteria leads to production of anti-trypanosome molecules (Sassera *et al.*, 2013). This is also called, paratransgenesis where symbiotic microorganism is engineered in order to reduce the vector competence to the host (Sassera *et al.*, 2013). The efficient of this method on tsetse flies control needs to be more evaluated.

2.15.4. Mechanical methods

Mechanical methods involved physical involvement in the control process of the tsetse fly vector. Normally it encompasses bush clearing to destroy the habitat of tsetse flies and removal of game to reduce the reservoir of the parasite (Bouyer, Carter, Balavia & Nelson., 2019). Campaigns for eradicating tsetse flies has been developed and implemented regularly (Malele, 2011). However other stakeholders advocate that, eliminating tsetse flies will have benefit to human and livestock but harm wildlife species due to unbalanced ecosystem (Bouyer *et al.*, 2019). Game removal as a control method of tsetse flies can have important ramifications for change the whole ecosystem, has legal implications and is not ecologically friendly.

2.16. Ecological factors

Ecological factors are naturally among the factors that influence the transmission process and influence the abundance of the vectors, their biting rate, the prevalence of trypanosomes in the vector and the availability and prevalence of infection on reservoir hosts (Anderson *et al.*, 2011; Anderson *et al.*, 2015). A study undertaken in the Luangwa and Zambezi Valley Ecosystems of Zambia, showed that the habitats provide refuge sites for tsetse flies and other factors such as climate, vegetation and rainfall allowed favorable interaction between wild reservoir hosts and vector tsetse flies (Munangandu *et al.*, 2012). In addition, the wildlife species in these protected areas were observed to be reservoirs of the parasite. Potential wildlife species which were reservoirs to trypanosomes included the following: warthogs, lion and bovidae species such as waterbuck, bushbuck, eland and kudu (Munangandu *et al.*, 2012).

In East Africa, it has been observed that wild animals provide 80–90% of tsetse feeds which suggests that the wild animal populations is very important in influencing both the abundance of the tsetse flies and the prevalence of trypanosome infections (Ngonyoka *et al.*, 2017; Nhamitambo, 2017). Similar findings have come out of Nigeria, which showed that vector-host contact, density of tsetse flies, ecological and anthropogenic factors were key factors determining the prevalence of trypanosomes (Ohaeri, 2010) and illustrates the importance of understanding the influence of ecological factors in controlling tsetse flies and trypanosomiasis and retaining conservation objectives. This study therefore focused on the influence of wildlife abundance and land cover types on the prevalence of trypanosome infections in cattle in the Maasai steppe, as these factors were shown to be key drivers for trypanosome prevalence in the tsetse vectors (Ngonyoka *et al.*, 2017; Nnko *et al.*, 2017) .

2.17. Diminazene aceturate

Diminazene aceturate is a chemical with the molecular formula of $C_{22}H_{29}N_9O_6$ and a molecular weight of 515.5 g/mol (Miller, 2005). Diminazene aceturate has been used for treatment of protozoans' parasites including trypanosome parasite in cattle and other domestic animals by livestock keeper stakeholders for many years (Mamman, Aliu & Peregrine, 1993). For instance, the drug has been used for treatment against *Trypanosoma congolense, T. vivax* and less active on *T. brucei brucei* and *T. brucei evansi* in cattle, sheep, goats and dogs however it can be toxic to some animals such horses, donkeys, dogs and camels (Giordani *et al.*, 2016). The dosage for diminazene aceturate is 3·5 mg/kg administered intramuscularly (up to 8 mg/kg for resistant trypanosomes, 5–10 for *Trypanosoma evans*) (Giordani *et al.*, 2016). The drug contains about 40 parts per million (ppm) after 7 days in river. The recommended withdraws period in cattle and sheep is 20 days after administration because under this period the level is lower than the acceptable daily intake value (ADI) (Food and Agriculture Organization/World Health Organization, 1989). Its mode of action for instance in *Babesia* parasites involves interfering with aerobic glycolysis, as well as with synthesis of DNA in the parasite (Miller, 2005). In immunocompromised mice, high levels of resistance to the drug are observed in *Trypanosoma brucei evansi*. Resistance development appears to be favored by immunosuppression thus explaining the importance of linking between immunity and chemotherapy. This shows that, efficacy of trypanocidal appears to be reduced by immunosuppression (Giordani *et al.*, 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study areas

The study was conducted in three villages of the Maasai steppe: Sukuro, Kimotorok and Oltukai (Fig. 3). The three villages were selected to accommodate variation in distances to nearby protected areas, which are considered the main reservoirs of African Animal Trypanosomiasis (AAT) (Miller, Leslie & McCabe., 2014). Pastoralists in the study area keep cattle under an extensive management style which forces them to move from one location to another in pursuit of pasture and water. These seasonal movements often push the livestock to potential infection hot spot areas which may be, in or near protected areas where cattle encounter infected tsetse flies. The Maasai steppe ecosystem covers the area of about 35 000 km^2 (9700 sq mi) (Nelson, 2012), spanning from northern Tanzania into southern Kenya (from 3°40' and 4°35' S, to 35°50' and 36°20' E) (Salekwa *et al.,* 2014; Ngonyoka *et al*., 2017) found at the attitude of 1440-1515 m. The area is a home to important wildlife populations that also move seasonally from protected areas into surrounding community lands, increasing wildlife-livestock interactions. Dominant vegetation types include: Acacia-Commiphora woodland, open grassland, seasonal swamps and often dense bushland with dominant tree species including *Erythrina burtii, Combretum spp*., *Albizia spp.,* and *Cordia spp* (Ngonyoka *et al*., 2017). The area is semi-arid, receiving approximately 542 mm of rain per year, with a prolonged dry season from June to October, and a short rainy season from November to January and a long rainy season from March to May (Nnko *et al*., 2017). The Average monthly temperature varies between 18 °C and 30 °C (Simwango, 2016).
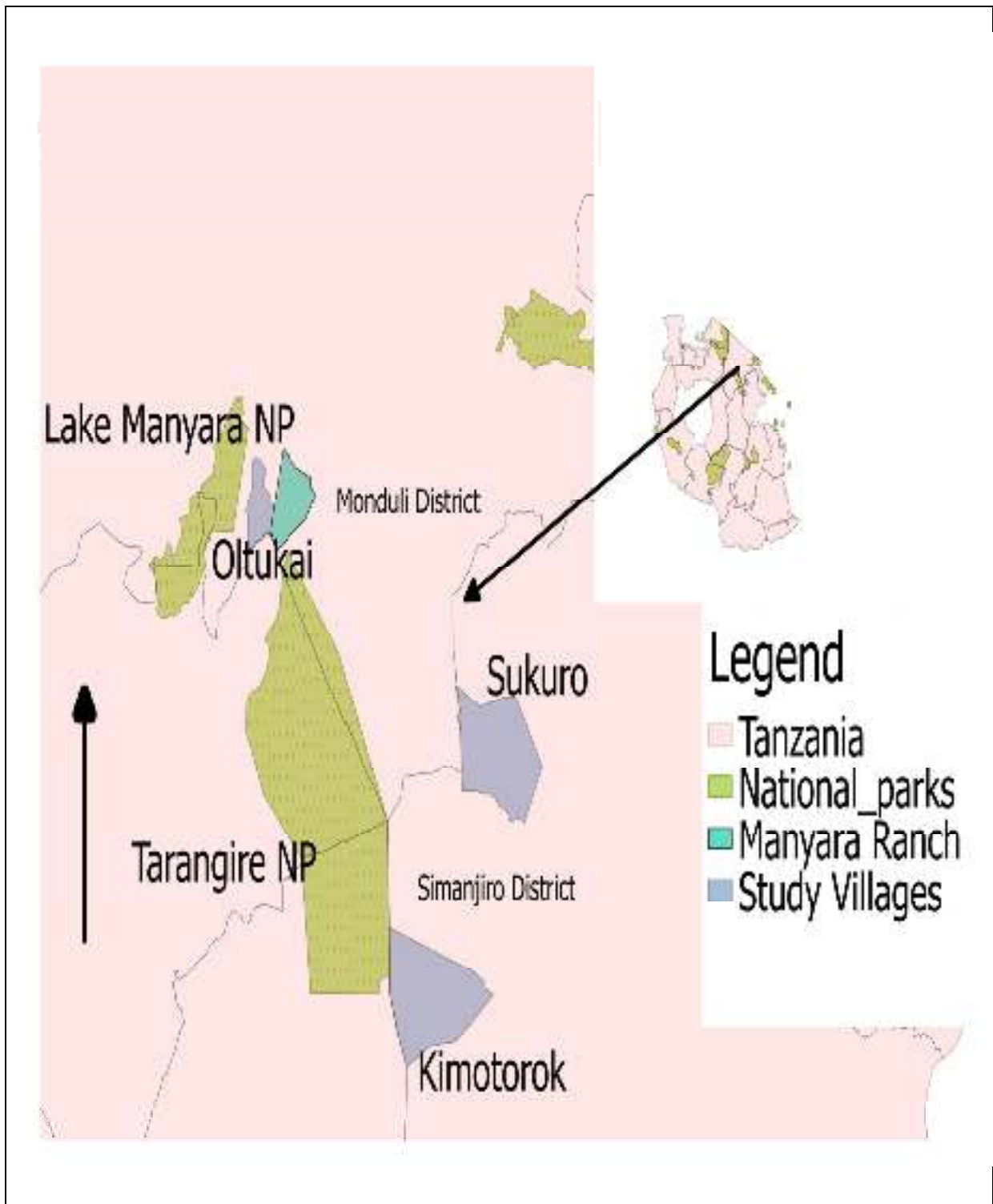
Figure 3: Map showing the three study villages: Sukuro and Kimotorok from Simanjiro District and Oltukai from Monduli District in relation to nearby protected area

3.2. Sample size determination (power analysis)

Sample size for assessing the influence of seasonal grazing on infection in cattle was estimated based on prevalence (p1) levels in cattle of 17.2% from previous studies in this area (Simwango *et al*., 2017). The sample size for assessing influence of risk factors, land cover and wildlife host was based on the prevalence (p2) of 5.8% in cattle (Kimaro *et al*., 2018). Sample size was estimated from the equation: $n = (Z^2pq)/e^2$, where n = is required sample size Z =1.96 at 95% Confidence level, standard error of the mean, p1 =0.172 and p2=0.058, the previously reported prevalence (Simwango *et al*., 2017; (Kimaro *et al*., 2018), q =1-p, e=0.05 at 5% absolute error, Using the formula above, this study required at least 219 samples for assess the influence of seasonal grazing on infection in cattle while requiring at least 84 samples for assessing influence of risk factors, land cover and wildlife host .

There were three villages for sampling whereby in each village there were 50 cattle. Each cattle was sampled in three sessions (July, 2017, October, 2017 and January, 2018). In each session sampled was considered as sample. In this case, 150 samples from each village were to be obtained and making anticipation total of 450 samples for the three villages.

3.3. Blood sampling in cattle

The sampling frame of all livestock keepers (boma) in each village was obtained from the local government leaders. All names of households (boma) known to keep cattle were entered in Microsoft excel spreadsheets and simple random sampling was performed to select sampling units using computer. Five bomas were selected from each village for blood sampling. A cross-sectional study was then conducted to collect blood in the selected bomas. Heads of household were approached and informed consent sought to bleed and treat their cattle. A list of cattle in each boma was sought with the assistance of the herder and each animal was numbered. The lists of animals were entered in excel and random selection performed in a computer to obtained the required ten cattle for sampling per boma. Blood samples were taken in July, 2017, October, 2017 and January, 2018 from 10 selected and tagged cattle in each boma. During the first sampling period (July, 2017), blood samples were collected from tagged animals that were then treated using diminazene aceturate to clear any potential trypanosome infections. Blood re-sampling was done after an interval of three months (i.e. October, 2017 and January, 2018). Cattle were treated using diminazene aceturate after each sample taken, to clear infections so that we could tie any subsequent

infections to the areas they had grazed in the subsequent months. Diminazene aceturate becomes ineffective 20-21 days after treatment (Auty *et al*., 2012; Miller, 2003), after which it was assumed that the animals could acquire new infections. At each sampling period, 3 milliliters of blood were collected from the jugular vein by venipuncture into an Ethylenediaminetetraacetic acid (EDTA)-containing vacutainer tube from all 150 cattle (50 blood samples from each village). The blood was kept in ice boxes and transported to the laboratory at NM-AIST where it was kept at -20 ^{o}C until DNA extraction.

In case the tagged animals became sick between sampling periods, and the farmer wanted to treat them before data collection, proper communication was established by the pastoralist to ensure blood was collected before treating the animal. Some cattle were not re-sampled during subsequent sessions in October, 2017 (n=25) and January, 2018 (n=14) due to different reasons including death. These cattle were therefore replaced to maintain the sample size of 150. The cattle for replacement were selected randomly as explained previously, although the formerly selected cattle were excluded in the selection procedures. The treatment status was requested from the pastoralists prior to selection of replacements. Priority was given to those animals which were not treated within the last two months. For example, two cattle from two different herds which were confirmed to have trypanosome infections in July, 2017 were not found in the herds during October, 2017.

3.4. Tracking cattle movements between sampling periods

Information pertaining to livestock movement in the previous two months (the period during which they were able to acquire new infections) was gathered from each of the households/bomas in which cattle were sampled. A semi-structured questionnaire (Appendix 1) was discussed with each cattle owner to extract information on patterns and reasons for cattle movement. Pastoralists were requested to indicate on a scale of 1 (most important) to 10 (least important) their reasons for moving their livestock. Detailed information on where the cattle were grazed in the previous two months was then acquired using participatory mapping methods. This involved using high resolution satellite imagery from Google Earth Pro (version 7.3.1.4507). The pastoralists first familiarized themselves with known landscape features around their bomas and villages using maps printed from the high resolution images. Google Earth Pro on an Apple Ipad, Version 9.3.5 (13 G36) was used to allow the pastoralists to zoom in and out to better identify known areas, and I used this technique to map where they grazed their cattle in the past two months prior to the day of

blood sampling. Polygons of these grazing areas were digitized in Google Earth and exported as shape files into Quantum GIS (QGIS Development Team, 2014) for further analysis and creation of maps. After delineating the grazing areas with participatory mapping, each identified area was subsequently visited with the herder for physical validation. In addition the herders were asked to note and rank reasons for moving their cattle herds from one area to another.

3.5. Ecological factors, human activities and risk factors for trypanosome infection

Along with the cattle blood samples, pastoralists were requested to record the habitats (i.e. Woodland, grassland, water logged areas, ecotone and riverine) and the wildlife animal species observed in the grazing areas. All 15 herders were interviewed each time cattle were bled and treated, totaling 45 responses on the ranked presence of wildlife in the previous months' grazing areas that could be correlated with infections found in the blood of the sampled cattle. They were asked to estimate the abundance of the wildlife species observed in the grazing areas 2 months prior to the sampling period and ranked them based on the abundance. The most abundant wildlife species observed in the grazing area scored 1 during ranking. In chronological order the followed wildlife species in abundance score other number in ascending order as 2, 3, 4, ...nth. Because agricultural activities could influence prevalence, the areas shown by pastoralists as being used for grazing which were observed to have farms/cultivation were classified as cultivated area, while those areas without farms or cultivation were classified as uncultivated areas. In addition, polygons for grazing areas were created using google earth. Visualization during polygon creation was done by zooming in and out to identify the target areas. After interviewing the pastoralists, a field visit was undertaken to validate the information given by the pastoralists. The field visit was guided by the interviewed herder to show those areas where they have been grazing. In the field the information on habitats, wildlife and cultivation were collected and compared with those provided by the pastoralists.

 Percentage cover of cultivated areas and habitat types within polygon were calculated in relationship to the identified grazing areas:

Percentage of cultivated area or Habitat type = [size of cultivated area or habitat type (km^2) within grazing area polygon/Size of Grazing area Polygon (km^2)] x100%.

Furthermore, risk factors which could be associated with the prevalence of trypanosome infections were recorded during the survey. The risk factors included in this study were cattle age, sex, herd size, and treatment intervention. Age groups were classified as proposed by Sobhy, Barghash, Bahaur and Razin (2017) with minor modification (Table 1).

All the pastoralists owning the sampled cattle were observed to use diminazene aceturate for treatment of trypanosome infections. During sample collection, the pastoralists were asked to identify the last day they treated their cattle. Since diminazene aceturate is active in the blood for 21 days, cattle administered with diminazene aceturate within 21 days of sampling were considered treated animals while others were considered untreated (Food and Agriculture Organization/ World Health Organization, 1989; Miller, 2005). The main reason for including treatment intervention in this study was to understand if treatment would have negative or positive impact on trypanosome infection. To make profound and easy data analysis, these factors were classified as shown in Table 1.

Table 1: Classification of risk factors for prevalence of trypanosome infection in cattle

C/N	Factor	Grouping	Classification
1.	Age (years)	Age≤1Year 1< age ≤2 age > 2	Calf Young Adult
2.	Treatment (days)	Treated < 21 Not treated Treated >21	Treated Untreated
3.	Herd size(n)	1-50 51-100 Above 100	Small Medium Large
4.	Sex (M/F)	Male Female	
5.	Previous infection (yes/no)	Previously infected Previously not infected	

3.6. DNA extraction from cattle blood

Deoxyribonucleic acid (DNA) was extracted using specific extraction kits for genomic DNA (Zymo Research) and by following kit instructions as provided by the manufacturer. The extracted DNA was kept at 4 ^{o}C until when the PCR was performed.

3.7. Detection of trypanosomes using PCR

ProFlex™ PCR System Thermocycler (Applied Biosystems®, Life technologies) was used to run Nested ITS-PCR for detecting infection to identify trypanosome species according to band size (Table 2) Eluate (2.5 μL) with extracted DNA was added to make a total of 12.5 μL of PCR master mix. This master mix contained the outer ITS primers ITS1 (5' - GAT TAC GTC CCT GCC ATT TG - 3') and ITS2 (5' - TTG TTC GCT ATC GGT CTT CC - 3') for the first round. The second round of PCR contained the inner ITS primers ITS3 (5' - GGA AGC AAA AGT CGT AAC AAG G - 3') and ITS4 (5' - TGT TTT CTT TTC CTC CGC TG - 3') (Inqaba Biotec) seeded with PCR products (0.5 μL) from the first round. *Trypanosoma vivax* was used as positive control while water as negative control.

Electrophoresis for ITS-PCR products: a 1.5 % (w/v) agarose gels for electrophoresis were prepared in 1 x TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0) (AMRESCO®) and stained in 5 μL GR Green (Excellgen). The gel was run for 40 minutes at 80 volt along with 100 bp molecular weight Quick-Load DNA Ladder (Quick-Load®, New England

Biolabs, Inc). For identification of band size the Fluorescent gel images were visualized in the E-Box imaging system (E-Box C X 5, Vilber Lourmat). Species of trypanosome were identified and classified based on the band size as shown in the Table 2.

Table 2: Cycling conditions for nested ITS-PCR and expected band sizes for each trypanosoma species

Trypanosoma species	Band size (bp)	Cycling conditions
T. congolense (Forest)	1513 bp	Two rounds, 35 cycles,
T. congolense (Kilifi)	1422 bp	each. One cycle:
T. congolense (Savannah)	1413 bp	95 °C for 7 mins 94 °C for
T. brucei s.i.	1207-1224 bp	1 mins 55 °C for 1 mins
T. theileri	988 bp	72 °C for 2 mins.
T. simiae (Tsavo)	954 bp	
T. simiae	850 bp	
T. vivax	611 bp	

Source: Ahmed, Picozzi, Welburn and MacLeod (2013), Cox *et al.*, (2005) and Nhamitambo, (2017)

3.8. Data analysis

To test for significant differences in prevalence related to sampling period and grazing areas, I used analysis of variance (ANOVA) when data were normally-distributed (parametric), or Kruskall Wallis and Mann-Whitney U test when data showed non-parametric patterns. Analyses were performed in R statistical software (R Development Core Team, 2011). Generalized Linear mixed models (GLMM) were used to determine the association existing between different factors such as season, sex, age, habitat, wildlife, cultivation, treatment intervention, herd size (independent variables) with prevalence of trypanosome infection (dependent variable) in different study periods in the Maasai steppe, considering maximum likelihood with binomial as family.

The Odds Ratio (OR) values were estimated from the coefficient values from the model to predict the direction of effect if it was negative or positive. In this case, if OR>1 the impact was considered positive while OR<1 showed negative effect of the risk factors. Trypanosome infection rate was calculated at two time intervals, t1 and t2. The two time intervals were July, 2017 to October, 2017 (t1) and November, 2017 to January, 2018 (t2). The infection

rate was calculated using the following formula: IR=1-(Log (N-n) N), where IR=infection rate, N=Original number not infected, n= Number that become infected.

During data analysis the abundance of each of wildlife species were classified into three categories which are high abundance (101 and above), moderate abundance (51-100) and Low abundance (50 and below). Data were analyzed using generalized linear mixed models (GLMM) and considered all species, villages and time intervals.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1.   Results

4.1.1. Prevalence of trypanosome infection in cattle

The overall prevalence of trypanosome infections in cattle in the three study villages was 13.14%. Highest prevalence of 20% was observed in Sukuro village, followed by Oltukai (12.1%) and Kimotorok (8.3%). The difference in prevalence between the three villages was statistically significant (Kruskal-Wallis Test Statistic=8.456, P=0.015, df=2). Post Hoc test (Tukey) showed that, significant difference exist between Oltukai and Sukuro (p=0.016). The difference in prevalence among the three villages varied with season. For instance, in Kimotorok and Oltukai highest prevalence was observed in January while for Sukuro highest prevalence was observed in July (Table 3).

Table 3: Prevalence (%) of trypanosomes infection in each village for July, 2017, October, 2017 and January, 2018

Months	Kimotorok (%)	n	Oltukai (%)	n	Sukuro (%)	n	P
July, 2017	10	50	10	50	38	50	<0.001
October, 2017	0	44	2.04	49	4	44	0.347
January, 2018	22.58	43	12.5	40	14.63	41	0.245
P-value	0.001		0.164		0.001		

Note: n = the number of cows sampled, P=probability where if p<0.05 is significant while when p>0.05 is insignificant.

The prevalence of trypanosome also varied among sampling seasons. The highest prevalence of 19.33% (n=29 infections) was observed in July, 2017 followed by January, 2018 with a prevalence of 17.74% (n=22), while the lowest prevalence of 2.19% (n=3) was observed in October, 2017. Kruskal-Wallis Test Statistic at Kurtosis of 7.34 (>3) showed significant variation of prevalence among sampling seasons (Kruskal-Wallis Test Statistic=19.18, P<0.001).

 Generalized Linear Mixed effect Model revealed that, the influence of season varied among study villages where in July and October the infection was significantly low in Sukuro while in

July, low infection in Kimotorok was observed with no effect of season in Sukuro village (Table 4).

Table 4: The association of season and prevalence of trypanosome infection in cattle for each village family=binomial, intercept=January, 2018

Variables	Kimotorok		Sukuro		Oltukai	
	Coeff±S.E	P	Coeff±S.E	P	Coeff±S.E	P
Intercept	-<0.001±0.35	0.002	-1.64±0.58	0.0043	-2.06±0.01	<2e-16
July, 2017	-1.375±6.28	0.03	0.89±0.68	0.19	-0.51±0.01	<2e-16
October, 2017	-3.433±<0.01	1	-1.83±0.96	0.056	-1.95±0.01	<2e-16
Random effects; Boma	Variance=3.677e-17		0.709		0.1782	
	Std=6.064e-09		0.842		0.4221	
AIC	84.8		117.2		75.7	
logLik	-38.4		-54.6		-33.8	

4.1.2. Identification of individual trypanosome species

Overall, eight trypanosome species were detected using the specific nested PCR. *Tryapanosoma simiae* had the highest prevalence (5.33%) followed by *T. vivax* (2.89%), *T. simiae tsavo* (2.22%), *T. congolence savanna* (0.44%) and *T. congolense kilifi* (0.44%) whereas the lowest prevalence was shown by *T. c. savannah* (0.22%), *T. congolense forest* (0.22%) and *T. brucei* (0.22%), each of which were detected in a single animal (Table 5). Five co-infections were detected in five cattle, three being from Kimotorok village and two from Sukuro. The three co-infections in Kimotorok village were as follows: The first was the co-infection between *Trypanosma vivax* and *T. theileri*, the second was between *T. vivax* and *T. simiae* while the last co-infection was between *T.vivax* and *T. congolense savannah*. The two co-infections detected in two cattle in Sukuro village were between *T. simiae* and *T. congolense* while the second was between *T. simiae* and *T. vivax*. All co-infections were detected in female cattle. Seasonal variability was documented for the number of trypanosome species detected, either individually or as co-infections. Thus, five trypanosome species, *T. simiae, T. simiae tsavo, T. vivax, T. theilleri* and *T. brucei* were detected in July, 2017 while only two species which are *T. simiae* and *T. vivax* were detected in October, 2017. Furthermore, six trypanosome species (*T. simiae, T. simiae tsavo, T. vivax, T. congolense*

savannah, T. congolense forest and *T. congolense kilifi*) were detected in January, 2018 (Table 5). The number of detected infections was mostly due to those two species which were *T. simiae* and *T. vivax.*

Table 5: The number of trypanosome species detected in each of three sampling periods

Trypanosoma species	Jul-2017	Oct-2017	Jan-2018	Total
	n=150	n=150	n=150	n=450
T.simiae	16	2	6	24
T.s.tsavo	3	0	7	10
T.vivax	5	1	7	13
T.theilleri	2	0	0	2
T.c. savanna	0	0	1	1
T.brucei	1	0	0	1
T.c forest	0	0	1	1
T.c.kilifi	0	0	2	2

4.1.3. Trypanosome infections in cattle in relation to their use of grazing areas

A total of 45 grazing areas were identified. Of all identified grazing areas, cattle acquired new trypanosome infections in 21 (46.67%) of these, as indicated by the presence of at least one trypanosome infection in the sampled cows. Overall, 13 of the 15 mapped herds were infected with trypanosomes at least once during the study, with variable distribution of the infections across herds (Fig. 4, 5, 6 and 7).

There was a variation in the distribution of infections across the identified cattle grazing areas identified by participatory mapping using the Maasai herders' information. Thus, in July, 2017, Trypanosome infections were detected in cattle which were grazing in seven different areas, three of which were in Kimotorok and two were in Sukuro and Oltukai villages (Fig. 5 and 6). There were fewer grazing areas (n=3) where parasite infections were detected in October, 2017 compared to July, 2017, and their distribution was shown in two overlapping grazing areas in Kimotorok village and one area in Oltukai village. No parasite infections were detected in Sukuro village in October, 2017. In January, 2018, Kimotorok village was again

shown to have more grazing areas with infected cattle (n=5) in comparison to Sukuro and Oltukai villages, where three grazing areas each were scored with infections. While variations were observed in infection prevalence between the 13 herds and across the three periods, infections were consistently present in cattle sampled from two herds in Kimotorok village (Herds 1 and 5) as well as in one herd in Oltukai village (herd 7). Furthermore, GLMM analysis revealed that, the number of infection increased in July, 2017, followed by January, 2018 if were to be compared with October (Table 6).

Table 6: The association of trypanosome prevalence with month of sampling, where July is the intercept

	Months	Estimate	Std Error	z value	Pr (>|z|)	AIC
1.	(Intercept)	3.80	0.09	40.55	<2e-16 ***	
2.	October	-0.004	0.02	-0.27	0.79	328.54
3.	January	-0.06	0.01	-9.63	<2e-16 ***	
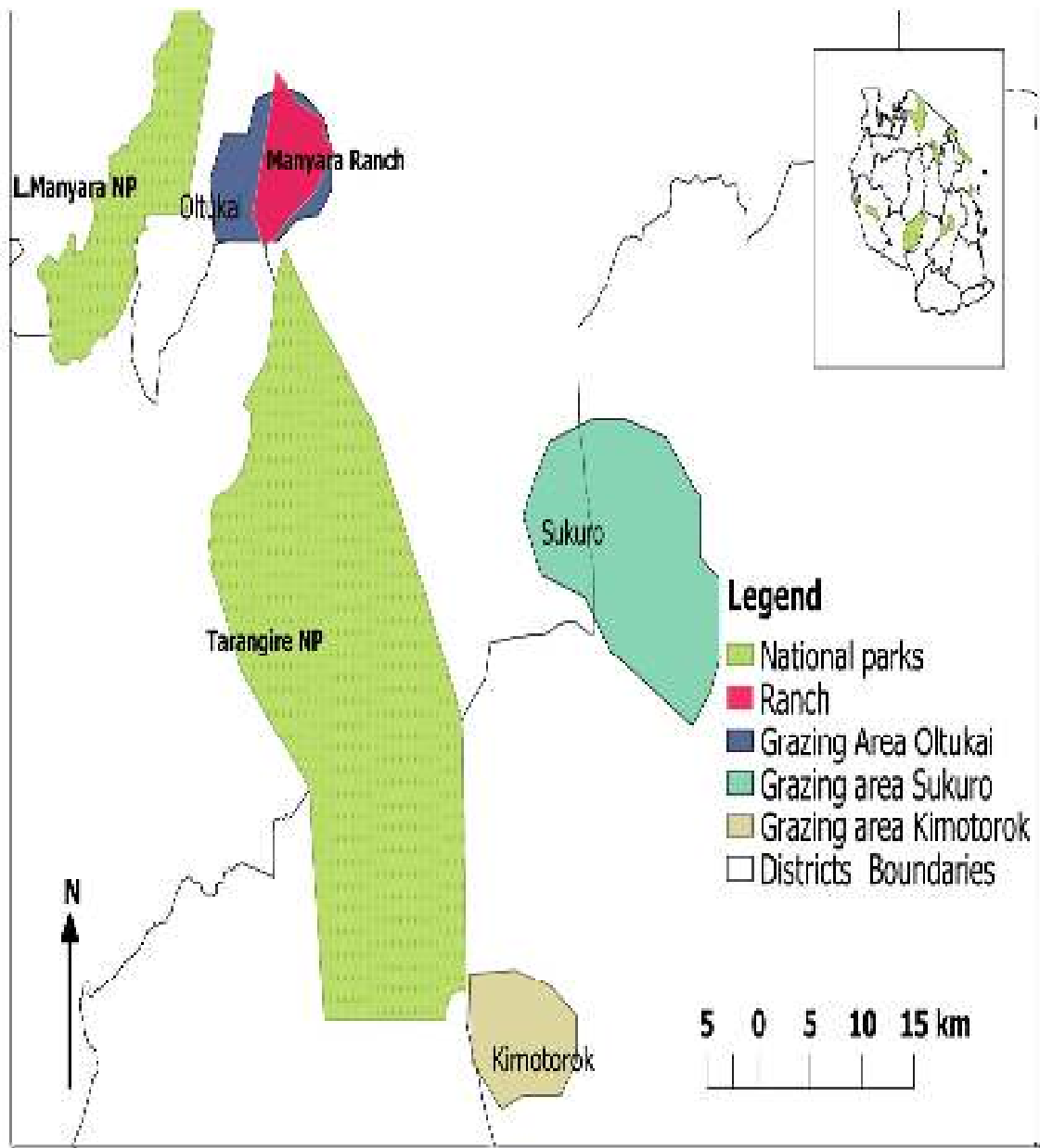
Figure 4: Map showing the study sites and overall grazing areas in the 3 villages
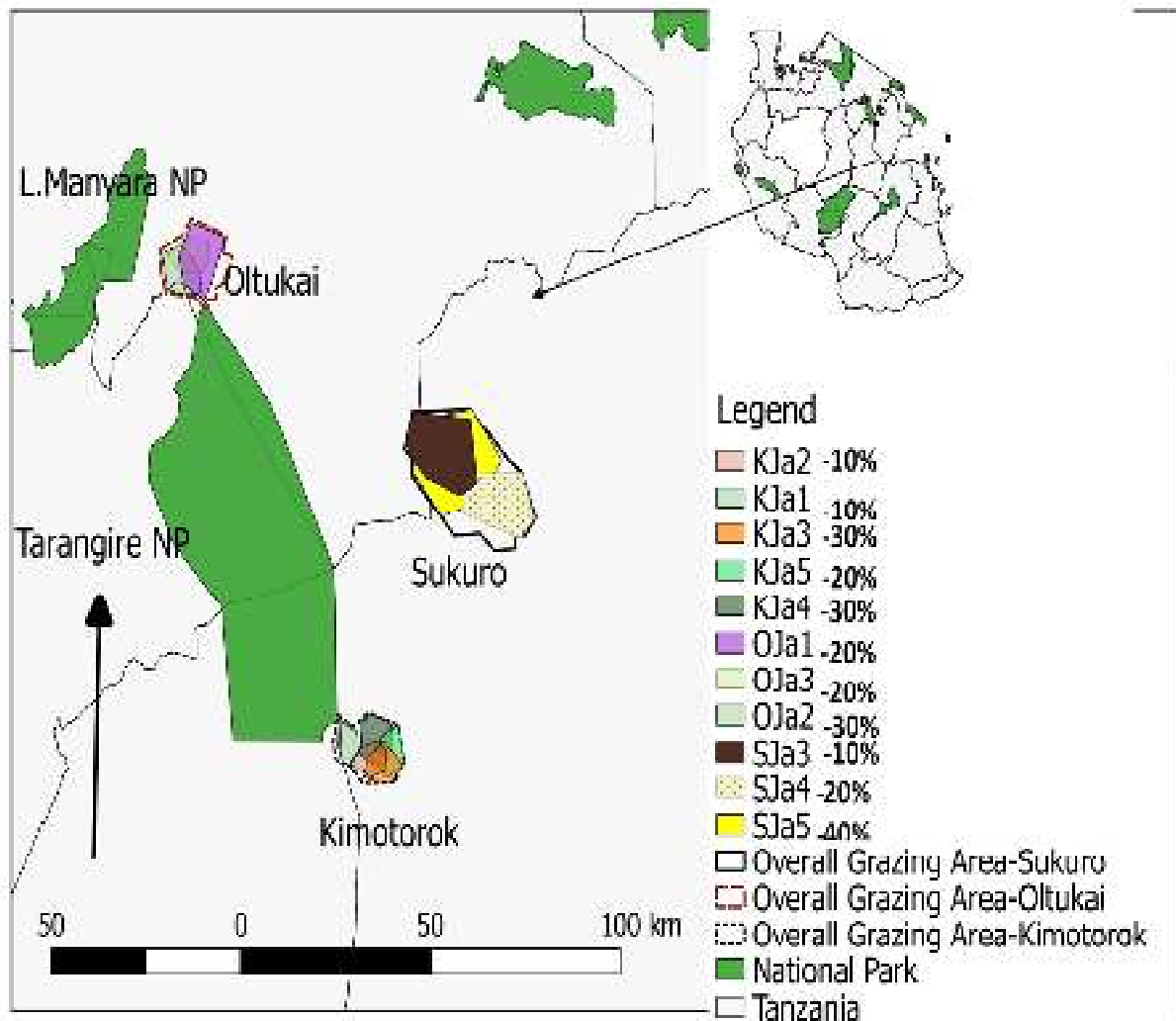
Figure 5: Map showing grazing areas (plots) in the month of January, 2018 for the three villages

Note: Refer legend for the corresponding prevalence (%) (n=10 in each herd during each sampling period). Key for names of grazing area as shown in legend: OJa=Oltukai January, SJa-Sukuro January, KJa-Kimotorok January.
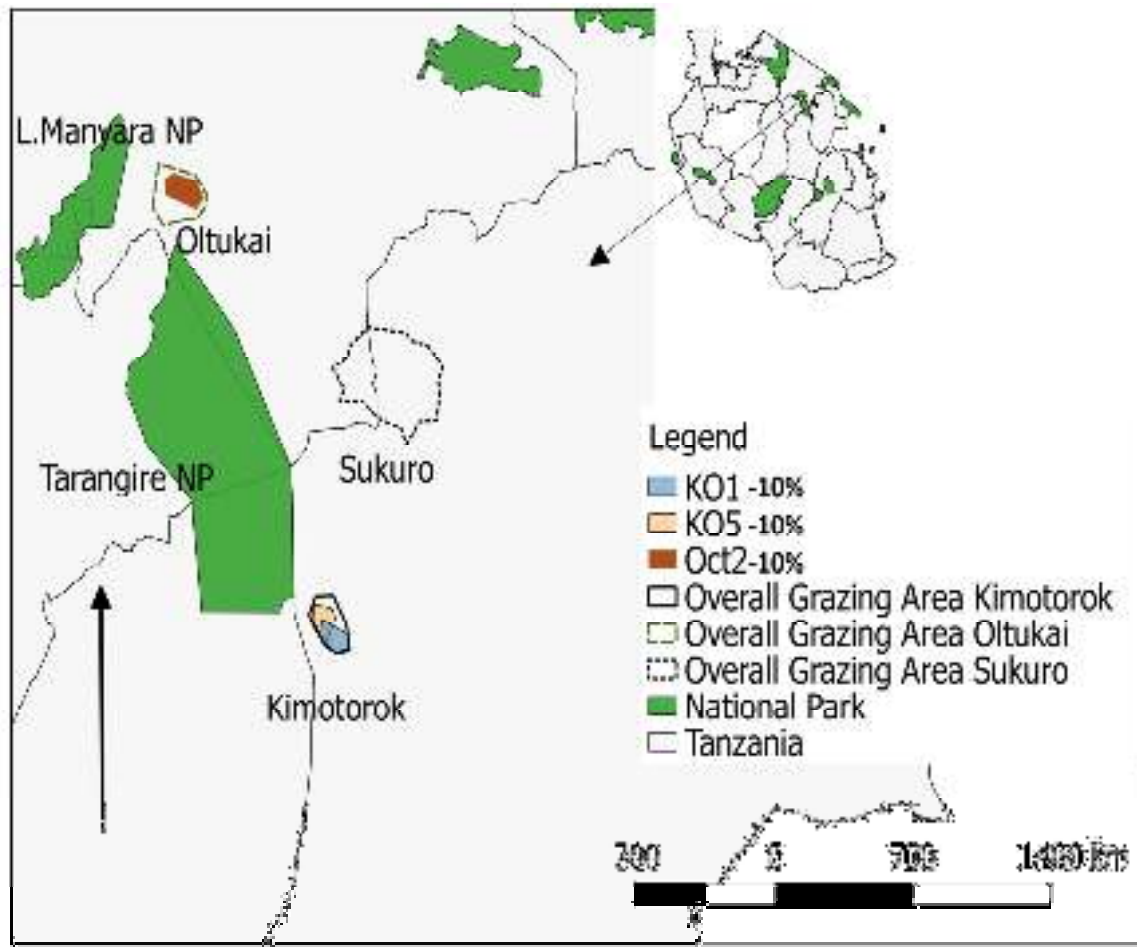
Figure 6: Map showing grazing areas (plots) in the month of October, 2017 for the two study
villages

Note: Refer legend for the corresponding prevalence (%) (n=10 in each herd during each
sampling period). However, no positive infection was observed in October in
Sukuro village (Key for names of grazing area as shown in legend: Oct-Oltukai
October, and KO-Kimotorok October).
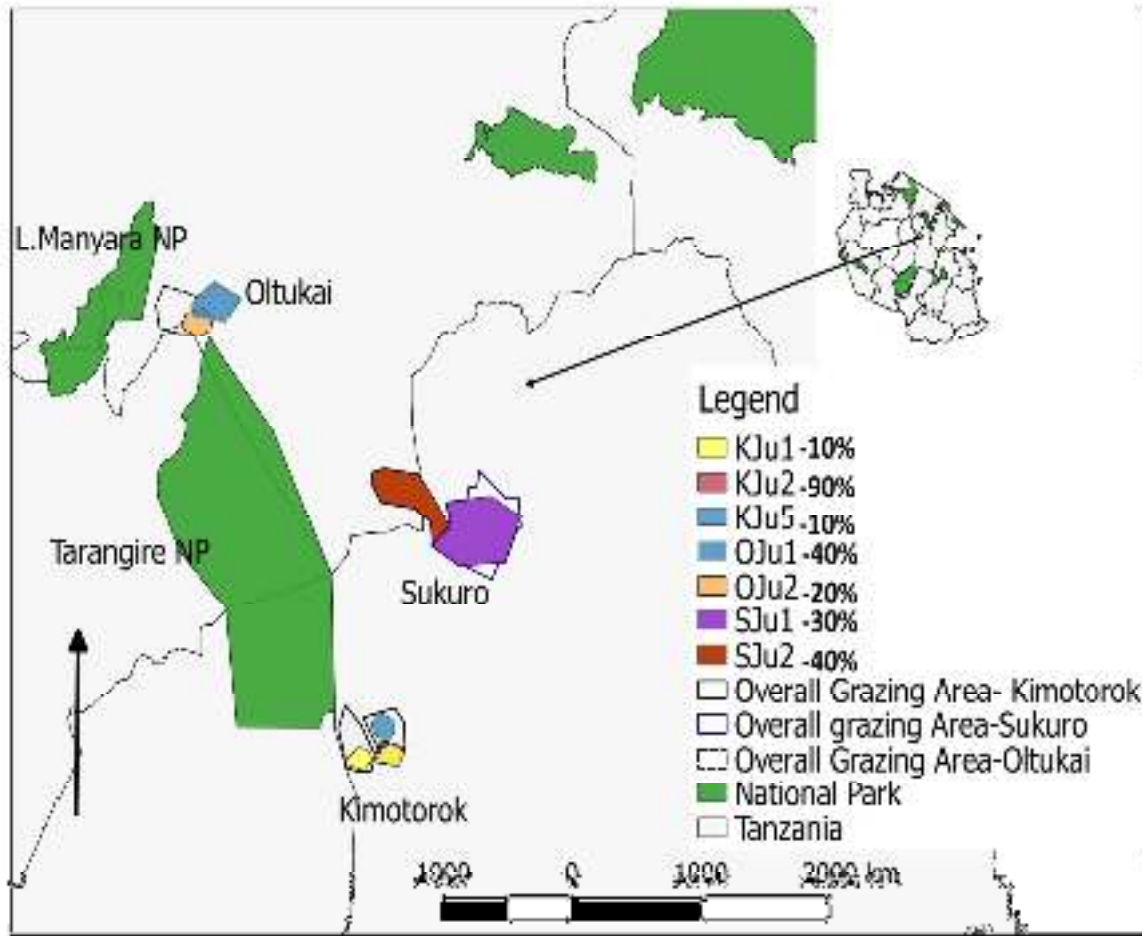
Figure 7: Map showing grazing areas (plots) in the month of July, 2017 for the three study villages

Note: The colored polygon shows the area which were infected with trypanosome parasites while the colorless shows the overall areas in each village. Refer legend for the corresponding prevalence (%) (n=10 in each herd during each sampling period). Key for names of grazing area as shown in legend; OJu-Oltukai July, SJu-Sukuro July and KJu-Kimotork July.

4.1.4. Assessment of herders' responses for herd movement

Search for pasture and water during the dry season was the most frequently mentioned driver for cattle movement, whereas avoidance of invasive plant species and ownership of a large number of cattle were least mentioned by the livestock owners (Table 7). Note that reasons unknown to pastoralists or considered as part of Maasai cultural norms were ranked as 0. Some variables were equally scored by respondents across the three villages. For example, searching for pasture and water were scored highly and the scores were homogeneous by respondents across the three villages. However, opinions of individual herders differed when responding to some questions about disease avoidance, predation avoidance and ownership of large cattle herds. The overall variation of the mean scores between the three villages was insignificant (Kruskall Wallis=0.62, p=0.2, df=2) although scores for individual variables such as herd size, predation avoidance and avoidance of invasive plant species varied significantly across villages.

Further analysis was done to examine the difference in prevalence when the pastoralists practiced diseases avoidance compared with those that did not. The disease avoidance is done by moving the cattle away from the plains areas during wildebeest calving season to avoid contact with Malignant Catarrhal Fever (MCF). Out of the 15 herders sampled, 6 (40%) herders practiced diseases avoidance while 9 (60%) of them did not practice diseases avoidance. For the herders who practiced diseases avoidance, 4 (66.67%) herders were from Sukuro while in Kimotorok and Oltukai each had 1 (16.67%) herders who are practicing diseases avoidance. The study found that there was a statistical significant higher prevalence of trypanosome infection when disease avoidance is practiced (Mann-Whitney U test statistic = 17 559.500, P<0.0001, df=1) (Fig. 8).
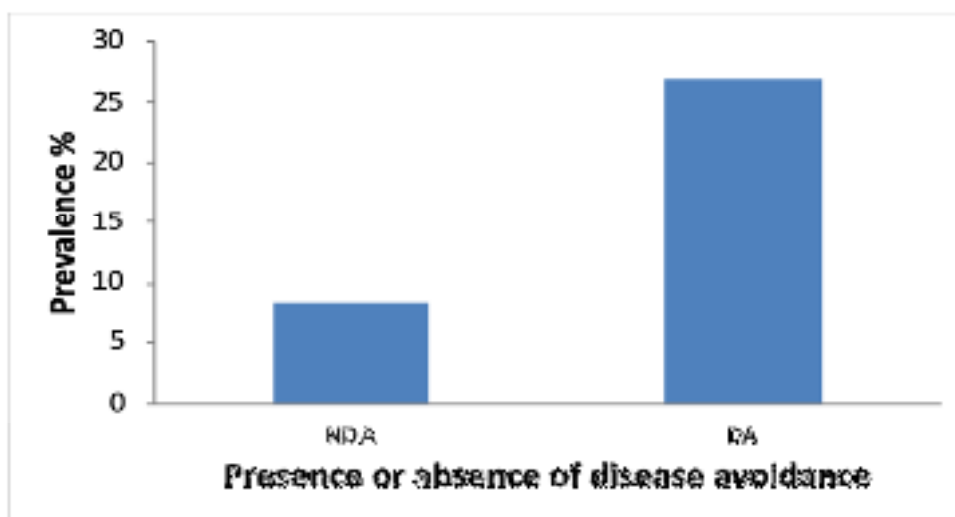
Figure 8: Prevalence of trypanosome infections when the pastoralists were practicing or not practicing malignant catarrhal fever avoidance

Note: NDA=Not practicing disease avoidance, DA=Practicing disease avoidance.

Table 7: The mean rank scores of drivers for cattle movement in the three villages in Maasai steppe

	Villages			
Variables	Kimotorok	Sukuro	Oltukai	Overall rank
Searching for pasture	1	1	1	1
Searching for water	2 .4	2.5	2.75	2
Disease avoidance from wildlife	5	3	2.5	2
Avoid cultivated areas	4.5	3.5	2.5	3
Avoiding water logged area	3	5.5	4.3	4
Predation avoidance	5	4	0	5
Large cattle herd size	5.5	4.5	5	6
Pasture conservation	6	6.5	4.75	7
Avoid invasive plants	0	7	4.5	8
Culture practices and norms	0	0	0	9

Note: The ranking is based on a sample size of 45 respondents, 15 in each village. Ranking score 1–9, whereby, 1= highest rank while 9=lowest rank while unknown reasons by pastoralists such as Maasai cultural norms were ranked as 0.

4.1.5. Assessment of risk factors for trypanosome infections in cattle

(i)   Trypanosome infection rates in cattle at the herd level

The herd with the highest infection rate had three out of nine cattle while two herds showed no infections for both periods t1 and t2 (Table 8). The rate of infection was higher in the time interval between November and January (t2) than the time interval between July and October (t1) (Fig. 9). The Mann-Whitney U test statistic revealed this difference to be statistically significant (U = 7.5, P< 0.0001, df=1).

Table 8: The number of infected cattle in each boma for the three study villages

Village	Herd id	Herd size	Cattle sampled	Infected at t1	Infected at t2	Total infected
Kimotorok	K1	Large	9	1	2	3 (33.3%)
Kimotorok	K2	Large	10	0	1	1 (10%)
Kimotorok	K3	Large	9	0	2	2 (22.2%)
Kimotorok	K4	Large	10	0	3	3 (30%)
Kimotorok	K5	Medium	8	0	3	3 (37.5%)
Oltukai	O1	Small	10	0	1	1 (10%)
Oltukai	O2	Medium	9	0	2	2 (22.2%)
Oltukai	O3	Medium	10	1	0	1 (10%)
Oltukai	O4	Medium	10	0	1	1 (10%)
Oltukai	O5	Medium	10	0	1	1 (10%)
Sukuro	S1	Small	10	1	1	2 (20%)
Sukuro	S2	Small	8	0	0	0 (0%)
Sukuro	S3	Small	9	1	2	3 (33.3%)
Sukuro	S4	Medium	9	0	1	1 (11.1%)
Sukuro	S5	Small	10	0	0	0 (0%)
			141	4	20	24 (17.02%)

Note: Large herd size=100 and above cattle, Medium herd size=51-99 cattle and small herd
        size=1-50cattle
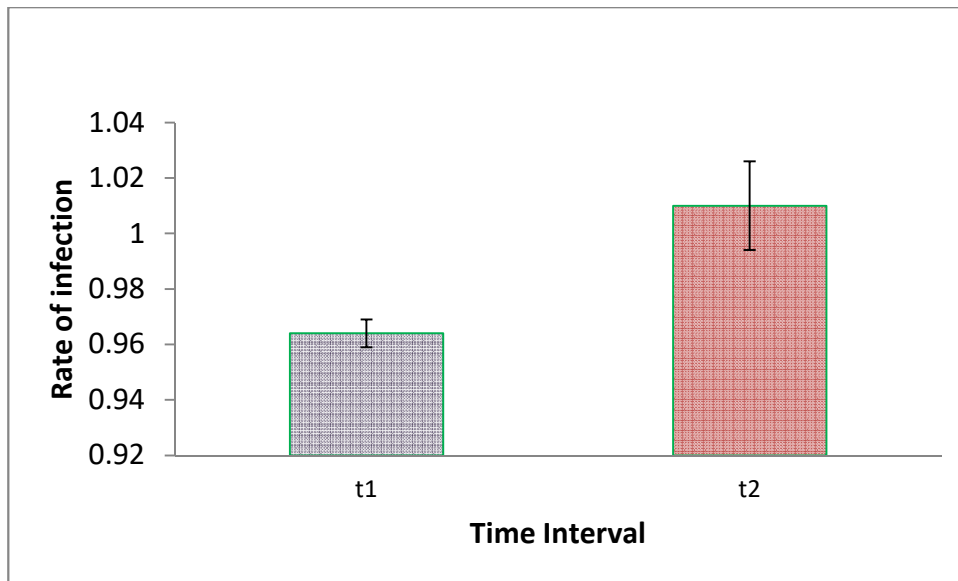
Figure 9: Infection rate of cattle herds with trypanosomes compared between July-October, 2017(t1) and November, 2017 –January, 2018 (t2).

Comparison of infection rates between the three study villages indicated that Kimotorok village had the highest infection rate during time intervals, t1 and t2. The lowest infection rate during ti (July, 2017 to October, 2017) was observed in Sukuro village, while in Oltukai village the lowest infection rate during t2 (November, 2017 to January, 2018) (Fig.10 and 11). However, the variation in infection rate among villages was not statistically significant in either of the two time intervals; July to October, 2017 (Kruskal-Wallis statistical test (H)=3.70, P=0.157 df=2) and November, 2017 to January, 2018 (Kruskal-Wallis statistical test (H)=4.90, P=0.086, df=2).
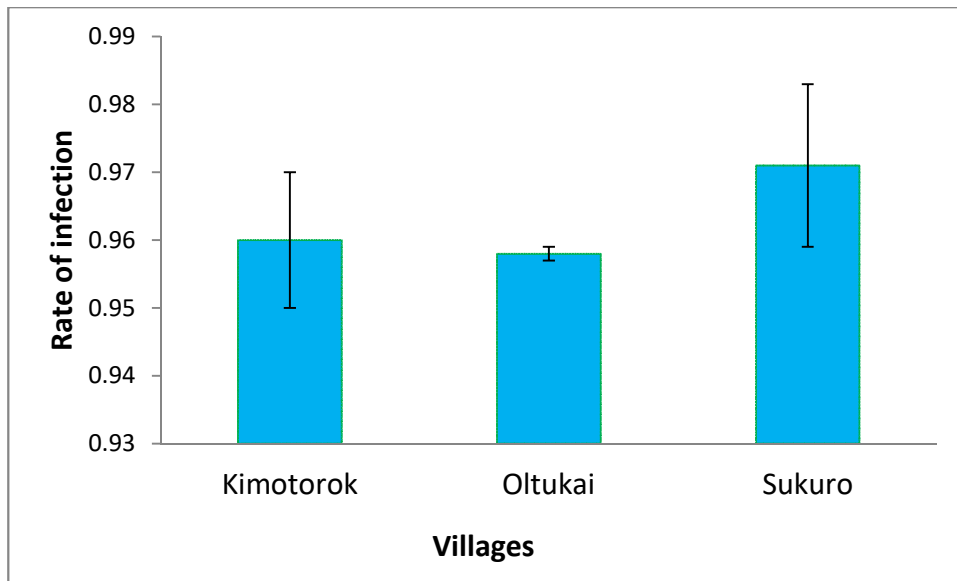
Figure 10: Trypanosome infection rates (M±S.E) in cattle in the three study villages for the time interval of July, 2017-October, 2017 at herd level
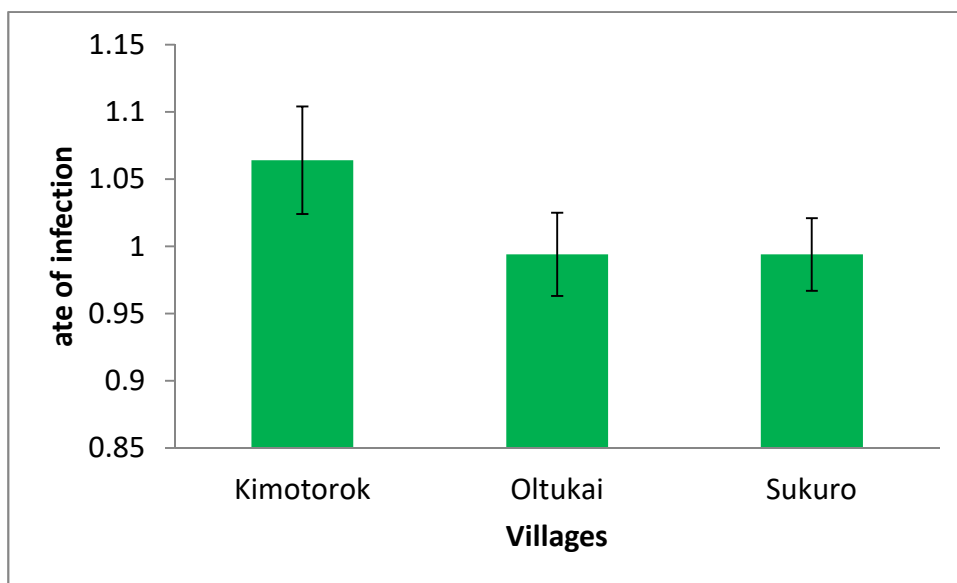
Figure 11: Trypanosome infection rates (M±S.E) in cattle in the three study villages for the time interval of November, 2017 to January, 2018

(ii)   Sample size and the risk factors

A total of 141 cattle in 15 herds which generated a sum of a total of 423 for all the sampling periods and 282 for the two time intervals were included in the final analyses (after removal of individuals not present at all sampling periods), of which 24 (17%) were observed to be infected with trypanosomes across all three time periods. The number of individuals infected by trypanosome parasites varied in each category. For instance, the adult cattle age group had the highest number of infected individuals compared to the calves' age group (Table 9).

Table 9: The number of individuals infected with trypanosome species in each category

Factor	Grouping	Classification	Infected cattle (t1)	Infected cattle (t2)	Total infected cattle
Age (years)	Age≤1Year	Calf	0	4	4 (40%)
	1< age ≤2	Young	1	2	3 (15.8%)
	age > 2	Adult	3	14	17 (15.2%)
Treatment (yes/no)	Treated < 21	Treated	1	6	7 (26.9%)
	Not treated Treated>21days	Untreated	3	14	17 (14.8%)
Herd size (n)	1-50	Small	2	3	5 (13.2%)
	51-100	Medium	1	11	12 (18.5%)
	Above 100	Large	1	6	7 (18.4%)
Sex (m/f)	Male		1	4	5 (33.3%)
	Female		3	16	19 (15.1%)
Previous infection (y/n)	Previously infected		25	11	36 (12.7%)
	Previously not infected		116	130	246 (86.6%)

Note: t1 is time interval between July and October while t2 time interval between November to January

(iii)   The influence of different risk factors on trypanosome infections in the individual cattle

Cattle that had been previously infected, younger individuals, males, and cattle from medium and small herds were all more likely to have trypanosome infections (Table 10). Their effect varied among the study villages (Table 11).

(iv)   Individual risk factors by village

Small herd size and male sex in Oltukai show the likelihood of being infected by trypanosome parasite while in Kimotorok male sex, Medium herd size and previous infected individuals had a positive effect on trypanosome infection (Table 11).

Table 10: The association of risk factors and trypanosome infections in cattle

Variables	Estimate	S.E	OR	p	Random effect
Intercept	-2.6146	0.2827		<2e-16 ***	Random effect: Vi
Previous infection	1.2125	0.5068	3.01	0.016 *	llage
Young	1.2788	0.6163	3.10	0.038 *	AIC=165.0
Male	3.071	1.224	1.55	0.012 *	logLik=-79.5
Medium	1.9554	0.8578	1.72	0.023 *	df= 279
Small	2.3636	1.0904	1.13	0.030 *	
Influence of interacting variables					
Variables	Estimate	S.E	OR	p	Random effect:
Intercept	-3.323	9.090e-01		0.0003	Village
Medium:Male	-4.416	1.790	1.42	0.0136	AIC=165.2
					logLik=60.6
					df= 260

Note: Only those risk factors which showed a significant association with trypanosome infection of cattle were presented in this table. Intercepts represents female for sex, large for herd size, adult for age and treated for treatment. Binomial was the family used with maximum likelihood. Where Odd Ratio (OR)<1 indicate negative direction while OR>1 indicate positive effect.

Table 11: Linear mixed effect models among risk factors of trypanosome infection which showed significant association in Kimotorok and Oltukai villages

	Oltukai			Kimotorok		
Variable	Coeff±SE	P	Variable	Coeff±SE	P
Intercept	3.8989±1.1572	0.999	Intercept	-3.3685±0.816	3.66e-05
Male	4.369±1.419	0.0021	Male	3.1109±1.204	0.0098
Small herd size	4.369±1.419	0.0021	Medium	4.369±1.419	0.0008
			Previous infection	3.0629±1.114	0.0060
Random effect=sampling period			Random effect=sampling period		
AIC=. 169.1, logLik=-75.5, df=273			AIC=165.0, Loglik=-79.5, df=159.0		

Note: None of the risk factors showed significant effects in Sukuro. Sampling period is considered as a random factor and the standard deviation (S.D) are reported. Intercepts represents female for sex, large for herd size, adult for age and treated for treatment.

4.1.6. Ecological Factors and the trypanosome infections

(i) Wild animal Abundance and prevalence of trypanosome infections in cattle

A total of 14 wildlife species including wildebeest, buffalo, eland, zebra, elephant, impala, ostrich, giraffe, hyena, lion, warthog, leopard, and cheetah and vervet monkey were observed by pastoralists in the grazing areas. All 14 species were observed in grazing areas in Kimotorok, 13 species in Sukuro, and six species in Oltukai. Ten of the 14 species were used for analysis because they were frequently noted by pastoralists, known as reservoirs for trypanosome and have influence on the abundance of tsetse flies (Auty *et al.*, 2012; Ngonyoka *et al.*, 2017). Overall highest abundance was reported for wildebeest followed by zebra and buffalo while least abundance was observed for warthogs (Fig. 12). High peaks of relative wildlife abundance were observed in July, 2017 and January, 2018 with clear distinction for wildebeests, zebra and buffalo in Kimotorok village (Fig. 12). Kruskal-Wallis Test Statistic (H) showed that the variation in relative abundance among wildlife species was statistically significant (H = 2846.401, P<0.0001).

The result revealed that, overall abundance of wildlife species combined (that is overall number of individuals for all species) showed significant effect on the prevalence of trypanosome in cattle. The likelihood of cattle being infected with trypanosome infection

was observed in low and moderated abundance of wildebeest, zebra and buffaloes. The interaction of zebra and buffaloes was observed to be positively associated with the prevalence of trypanosome infection (Table 12).
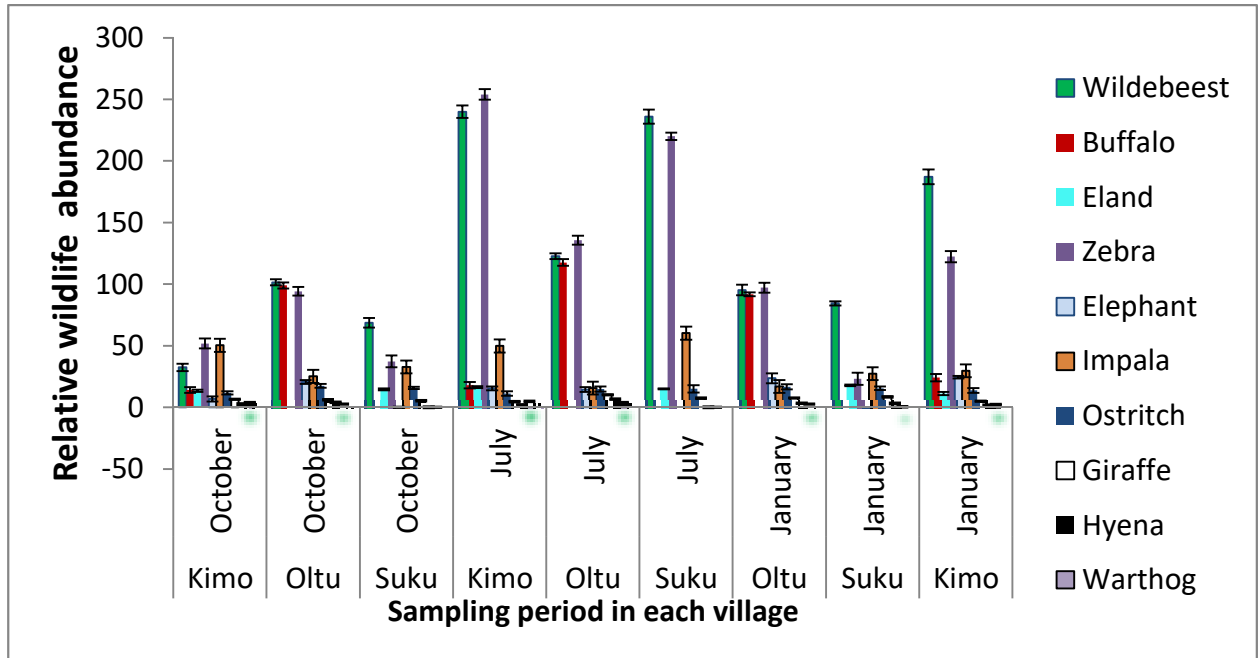
Figure 12: The average abundance of wild animal for the three sampling period of July, 2017, October, 2017 and January, 2018 for each of the three study villages

Table 12: Generalized linear mixed model on the overall association between abundance of wildlife species and the prevalence of trypanosome infections in cattle

Variables		Estimate	Std. error	OR	p
	(Intercept)	-1.647	2.051		0.6096
Wildebeest	Moderate	-1.145	5.958e-01	0.41	0.0546
	Low	-1.522	7.702e-01	0.86	0.0481 *
Buffalo	Moderate	-1.145	7.385e-01	1.04	0.4438
	Low	1.319	6.344e-01	2.93	0.0375 *
Zebra	Moderate	3.0038	1.2857	0.33	0.0195 *
	Low	-1.871	8.369e-01	0.91	0.0253 *

AIC=302.1, logLik=-138.0

Interaction of wildlife species which showed significant effect of trypanosome infection

Interacting species		Estimate	Std. error	OR	p
	(Intercept)	-5.43	1.65		0.0010 ***
Bufallo*Zebra	Moderate	-3.70	1.52	2.1	0.0149 *
	Low	-1.21	4676.88	1.3	0.1000

AIC=297.3, logLik=-130.7

Influence of overall wildlife abundance on of the trypanosomes infection

		Estimate	Std. Error	OR	p
	(Intercept)	-3.0801	0.4947		4.79e-10 ***
Wildlife	Abundance	0.0026	0.0009	2.85	0.0043 **

AIC=299.8, logLik=-146

Note: Only those wildlife which showed significant effects were presented in this table. Model used binomial errors where p=probability value, intercept represent high abundance. OR=Odd ration where OR>1 shows positive effect while OR<1 shows negative effect

(ii)   Influence of habitat types

The overall estimated size of the grazing area using Google earth Pro was 1102 km^2 of which 667 km^2 were in Sukuro, 227 km^2 in Kimotorok and 208 km^2 in Oltukai.

Five habitat types were identified in the grazing areas for the three study villages: woodland, grassland, water logged areas and riverine. The grazing areas were dominated with grassland and woodland while being least for riverine (Fig. 13). The percentage cover of different types of habitats varied among the three villages where by woodland dominated in Kimotorok, grassland dominated in Sukuro and Oltukai while least percentage cover of riverine in all the three villages (Fig. 14).

The likelihood of cattle being positively infected with trypanosome infection was observed when cattle are grazing in woodland unlike grassland which showed negative association with trypanosome infection (Table 13). However variation in impact was observed when considering the three villages separately. For instance, both grassland and woodland revealed positives association with trypanosome infection in Kimotorok (Table 14).

(iii)   Association between human activities (agricultural activities) and trypanosome infection in cattle

Highest percent of cultivation in   grazing areas was observed to occur in Sukuro and Kimotorok while Oltukai had least (Fig. 12). The difference in percentage of cultivated areas in the grazing areas between the three villages varied significantly (KWS=243.788, P<0.001, df=2).

In addition, cultivation was observed to have impact on the trypanosome infection. Overall, the impact was negative with OR<1 (Table 14).   In considering each study village, a significant positive effect was observed in Kimotorok (Table 15).
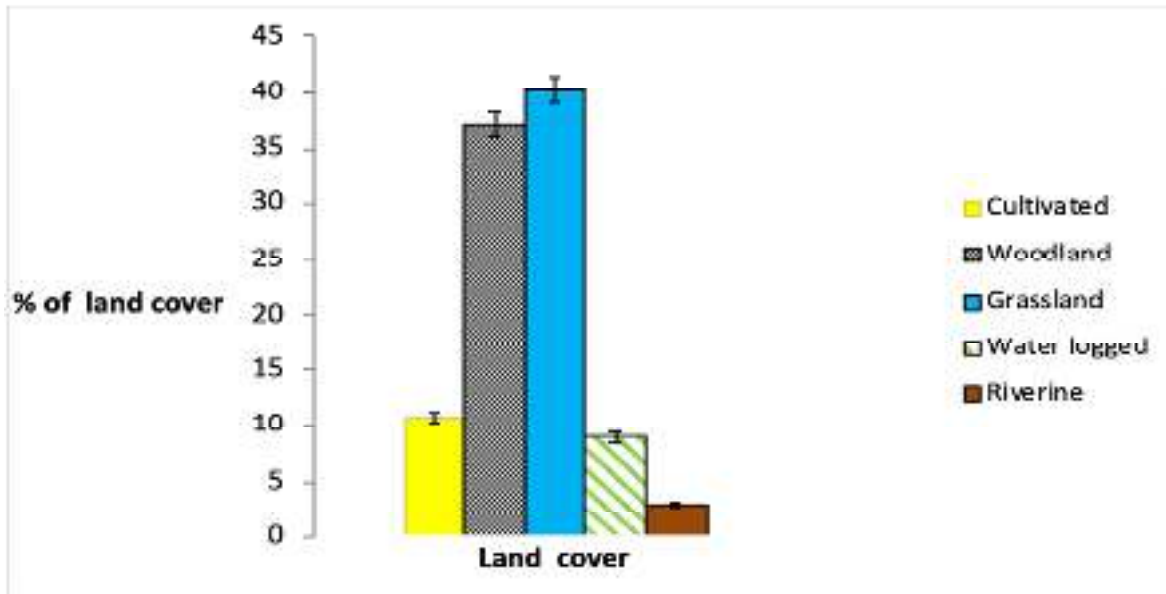
Figure 13: The overall percentages of land cover for habitats and cultivated area in grazing areas in the study areas

Note: Woodland is a low-density forest forming open habitats with plenty of sunlight and limited shade which may support an understory of shrubs and herbaceous plants including grasses Grassland is dominated by grasses with no or only sparse tree or shrub cover. Waterlogged area is a land which its soil is saturated with water forming muddy specifically in the Maasai steppe. Riverine is a stream resembling river relating to a system of inland wetlands and deep-water habitats associated with non-tidal flowing water
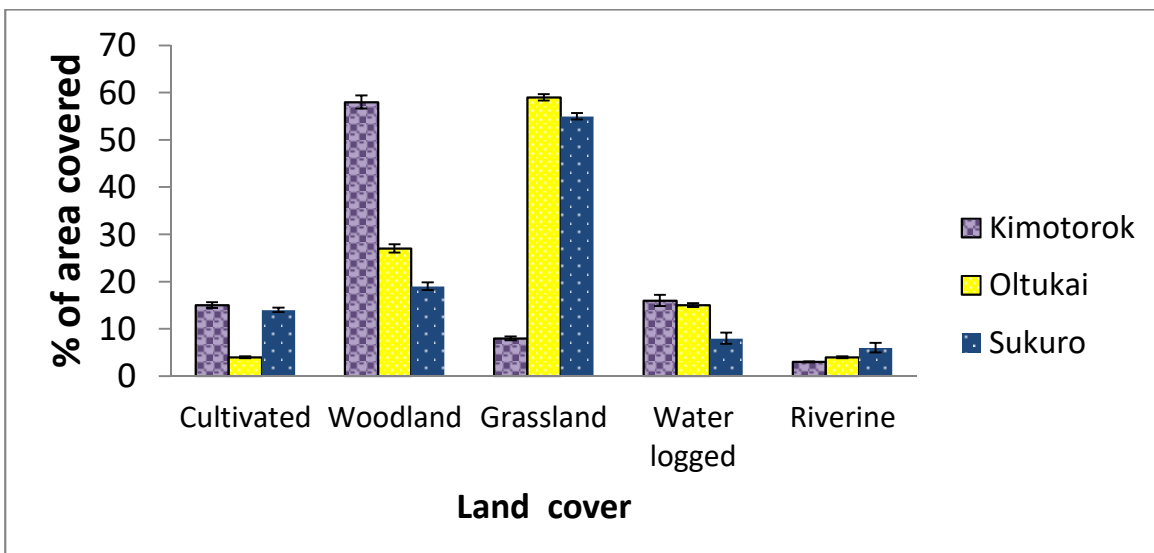
Figure 14: Land covers for habitats and cultivated area in grazing areas in each of the three village during the study period

Table 13: Table showing the association of land covers (habitat type and cultivation) with the prevalence of trypanosome infections in cattle of the Maasai steppe

	Fixed Effect	Estimate	Std. Error	OR	p
	(Intercept)	-1.51	0.58		0.08
1.	Cultivation	-12.02	0.04	0.92	<0.001**
2.	Woodland	-0.08	0.03	1.02	0.01 *
3.	Grassland	-0.08	0.03	0.98	0.009 **
4.	Water logged	-0.04	0.03	0.93	0.16
5.	Riverine	0.07	0.09	0.92	0.47
6.	Random effects (Villages)	Variance=0.73, AIC=277.4, LogLik= -131.7			

Note: Season of sampling was considered as a random effect. The effects of the habitats were compared to the intercepts.

Table 14: The association of land covers (habitat type and cultivation) with trypanosome infection in cattle of the Maasai steppe for each of the three villages

	Kimotorok			Sukuro			Oltukai		
Variables	Coeff	OR	P	Coeff	OR	P	Coeff	OR	P
Intercept	-0.29		<2e-16	12.43		0.03	28.70		0.051
Cultivation	0.03	1.04	<2e-16	-0.12	0.88	0.02	0.27	1.31	0.6
Woodland	-0.01	1.01	<0.001	-0.13	0.86	0.09	-0.27	0.76	0.09
Grassland	-0.012	1.02	0.002	-0.14	1.01	0.01	-0.40	0.66	0.01
Water logged	-0.03	1.05	0.533	0.005	0.87	0.9	-0.03	0.71	0.81
Riverine	-0.85	1.10	<2e-16	-0.17	0.84	0.52	-0.18	0.84	0.56
Random effects; Months	Variance=2.54			-			0.0341		
	Std=1.594			-			0.1847		
AIC	88.3			104.6			71.8		
logLik	-37.1			-45.3			-45.0		

Note: Months of sampling were considered as random effect. The effects of the habitats were compared to the intercepts. Family=Binomial. OR=Odd ration where OR>1 shows positive effect while OR<1 shows negative effect

4.2. Discussion

The findings from this study have shown that exposure of cattle to trypanosome infections varies both by season and grazing area. In general, associated with the proximity of Tarangire National Park, higher prevalence in cattle was recorded in grazing areas in Kimotorok than in Sukuro and Oltukai villages, with the highest number of infections observed in July. Although herders can graze their cattle away from Tarangire National Park the overall proximity of the village's land area to the park could lead to elevated risk. In fact, Salekwa *et al.* (2014) found that abundance of the tsetse vector declined dramatically with increasing distance from the park, and was negligible outside an 8 km distance. Furthermore, areas close to the park have traditionally been used as extreme dry season grazing reserves, further corroborating increased risk at this time of year.

4.2.1. Prevalence of trypanosome infection

The prevalence of trypanosome infections in cattle in the three study villages in the Maasai steppe observed during this study period was 13.14%, which was comparable to the result of 17.2% revealed in Emboreet, Loiborsiret , Kimotorok, Loiborsoit and Ortukai villages of the Maasai steppe in similar months of the year (Simwango *et al.*, 2017). However, the prevalence was much higher than the 5.8%-4.2% prevalence found by Kimaro *et al.* (2018) in Monduli District area of the Maasai steppe. Also, the obtained prevalence was much lower than the results obtained by Nhamitambo (2017) who recorded a prevalence of 51.47%. The difference between this study and previous could be due to variation of sampling period, location, year and ecological factors. For instance, Kimaro *et al.* (2018) focused her study in Monduli district only and Nhamitambo (2017) focused in Mikumi National Park while this study took place in both Monduli and Simanjiro District which could exhibit variation in terms of wildlife abundance, climatic conditions and human impact.

This study revealed *Trypanosoma simiae* was the most prevalent trypanosome species. A study by Nhamitambo (2017) also reported this species to be the most prevalent in an area with wildlife-livestock interface in eastern Tanzania. Besides high prevalence of *T. simiae*, the presence of *T. vivax* (2.89%) and *T. congolense* (0.22%) is of more interest in this study considering that these species are pathogenic to cattle (Mamoudou, Suh & Ebene, 2015; Kimaro *et al.*, 2018), regardless of their lower prevalence. The finding of pathogenic species of trypanosomes in the study area is of the greatest relevance to pastoralists, as it confirms

risk and vulnerability of their cattle to the disease AAT. Irrespective of the prevalence of these species in this area, one infected tsetse fly can bite multiple cattle, and therefore even low levels of infection can carry significant risk for the disease.

This study showed that prevalence of trypanosome infections varied between months of the year, being highest in July, followed by January. These results suggest the presence of at least two peaks of infection prevalence in a year: July and January. These months of the year correspond to the dry period in the Maasai steppe when there is a shortage of pastures on grazing lands and drought. This is expected to lead to nutritional stress and decreased immunity of the cattle, making them vulnerable to Trypanosomiasis as was noted by other researchers (Mungube *et al*., 2012; Hamill, Kaare, Welburn & Piccozi., 2013; Emmanuel, 2015; Dagnachew *et al*., 2017). Concurrently, lower trypanosome prevalence was indicated in the month of October. A plausible explanation to this result relates to previous study done in the same villages, whereby it was showed that prevalence of trypanosomes in tsetse flies increased from January to November with some monthly fluctuations (Nnko *et al*., 2017).

The presence of trypanosomes in cattle throughout the year in this study area could be associated with several factors including: the year-round presence of wild animals as "trypanosome reservoirs",   infestation of tsetse flies (vectors) (Sindato *et al*., 2007; Ngonyoka *et al*., 2017) and the frequent cattle-wildlife interaction in common grazing area, thus allowing the chance of year-round trypanosome circulation in the area (Ngonyoka *et al*., 2017). The low trypanosome prevalence recorded in October was unexpected but this was possibly due to the fact that October coincides with a period of short rains when livestock graze on plots close to homesteads, thus reducing rates of contact between cattle and vectors while increasing rate of wildlife-vector contact.   Also, low cattle-wildlife interactions shairing similar grazing areas during this season due to most of wildlife being back into Tarangire National Park.

Others studies have shown that, higher mortality during the dry season (October) occurs due to prolonged drought which results in starvation, stress and can motivate cattle herders to move into higher risk areas such as grazing areas near wildlife reserves and water sources with high tsetse flies infestation as reported by Emmanuel (2015). The vulnerability of cattle to trypanosomiasis diseases in dry season is thought to be due to starvation and other stress caused by prolonged drought which lowers their immunity (Nnko *et al*., 2017). Also a study done by Emmanuel (2015) showed that the presence of wildlife hosts influenced the

abundance of vectors which had implications for the prevalence of trypanosome infections cattle. The cattle-wildlife interaction and common grazing areas used by cattle and wildlife in the study area are thought to have a major influence on the prevalence of trypanosome infections (Ngonyoka *et al.*, 2017).

4.2.2. Risk factors for trypanosome infections

The results from this study show the association of risk factors with trypanosome infections in cattle in the Maasai steppe. The study found that cattle aged less than one year were more likely to have trypanosome infections compared to older cattle. This is in agreement with a study done in western Kenya which found that, trypanosome infection is also more prominent in young aged animals (Von Wissmann *et al.*, 2011). This may be explained by different management practices observed during data collection. Young calves were normally grazed near the homesteads, and received infrequent treatments unlike adult cattle. For instance, out of all animals that were treated by the herders in-between sampling periods, only 10% and 12.5% were from the calf and young age groups, respectively, while 86.5% were adult cattle. The more infections detected in calves and younger age groups could also be related to the development of the immune system at this age, as well as lesser exposure to of these age groups to frequent treatment, as practiced by the Maasai herders. Previous studies on bovine babesiosis showed that calves have less immunity compared to adult cattle, thus making them more susceptible to new infection (Levy, Clabaugh & Ristic, 1982). In contrast, a study by Muhanguzi *et al.* (2014) in Tororo District, south-eastern Uganda showed that cattle above 3 years of age were more likely to be infected, counter to my findings.

The discrepancies between my findings and the other studies can be explained either by cattle breed differences or by the varying management practices in the Maasai steppe compared to the study sites in Uganda. For instance, the breeds of cattle involved in Uganda reported by Muhanguzi *et al.* (2014) were Boran × short horn Zebu cross, Boran × Holstein Friesian cross and African short horn Zebu (Nkedi) under either zero grazing or semi-zero grazing while in my study only Zebu (*Bos indicus*) cattle were sampled. Furthermore, in the Maasai steppe, older cattle are trekked long distances for pasture and water and become exposed to tsetse-infested areas, close to wildlife, like the Tarangire National Park. However results under this section should be explained with caution because the sample size for different age group varied and could impair the conclusions.

Herd size was also observed to have a significant impact on trypanosome infections in cattle. Medium (51-100 cows) and small herds (≤50 cows) were observed to be positively associated with the infection of trypanosomes in cattle. A study done by Bhutto *et al*. (2010) found similar trend were highest infection rates were reported in smaller and medium size herds than larger size herds. However, my finding could have been influenced by the distribution of herd sizes in the different study villages. Farmers from Kimotorok village were observed to have larger herd sizes (>100 cows) with high rates of treatment against trypanosome infections. The majority of farmers with medium (51-100) and small herd size (≤50 cows) were from Sukuro and Oltukai, which had lower treatment frequency against trypanosomiasis. As the number of cattle is strongly related to wealth in Maasai communities, the higher prevalence of infections in smaller herd sizes could be explained by lack of funds to purchase drugs for treatment (Garnett & Lewis, 2007).

Infection rate between two sampling time intervals, which were July-October, 2017 and October 2017 to January 2018, varied significantly. Highest infection rate was observed between time intervals of October, 2017-January, 2018. The variation in rate of infection between the two time intervals might be explained by the change vegetation cover such as loss of leave through falling off to woody plants which support tsetse flies availability during dry season with no/little rainfall but higher temperature. In addition, the change from low interaction in July-October, 2017 to high interaction in October, 2017-January, 2018 between cattle with wildlife reservoirs and tsetse flies vector for parasites can positively explain the results. This is supported by the study done in this area and showed that tsetse fly abundance peaks in December, which is within the time interval of October to January, due to favorable weather conditions (Nnko *et al*., 2017). Also during this time interval (t2), wildlife including reservoirs for trypanosomes was observed to come out from the Tarangire National Park thus increasing the cattle-wildlife interaction and increasing exposure. For instance, in Sukuro, I saw high interaction of wildlife and cattle and reported by herder during time interval of October to January.

Unlike the studies done with camels by Bhutto *et al*. (2010) in Pakistan and by Sobhy *et al*. (2017) in North-West Egypt, that showed that female hosts had significantly higher prevalence than males, due to stress of lactation and successive pregnancies, this study found a significant influence of male sex on the rate of trypanosome infections in cattle. The findings of this study agrees with a study done by Bilando *et al*. (2015) who found high cases

of trypanosome infection in male humans in the Democratic Republic of the Congo and Anderson *et al.* (2011) who found a positive influence of male sex on trypanosome infection rate in wildlife reservoirs in Zambia. I ascribe my observations to behavior of Maasai pastoralists who value and give more care to cows than bulls when it comes to animal health care, and this includes treatment and vaccination. In my study, the male cattle which were found to have trypanosome infection were also shown to have received less treatment attention compared to female cattle. The pastoralists give higher value to cows as capital required for herd perpetuation. Further contrast was observed for a study done in Kenya which showed that sex did not influence trypanosome infections apart from grazing habit, management practices, species of animals involved in the study, location where sample were taken, cattle condition, cattle age and interaction between species and cattle age (Von Wissmann *et al.*, 2011). The divergence of these studies with the current study could be due to differences in geography and methods used in data analysis.

Understanding the risk factors for trypanosomiasis is essential for developing proper control strategies of the infection. In my study I focused on the influence of age, sex, treatment intervention, and herd size as potential risk factors. I also examined the influence of season and land cover in grazing areas. However, further studies need to be done to address more on the factors for the prevalence of trypanosome infection in the Maasai steppe. For instance, factors reported by other researchers to influence the prevalence of trypanosome infection in cattle were body condition score of cattle (Kimaro *et al.*, 2018), location, tsetse flies abundance (Sobhy *et al.*, 2017), immunity and drug resistance (Dagnachew *et al.*, 2017).

4.2.3. Wildlife abundance and prevalence of trypanosome infections in cattle

Overall wildlife abundance, regardless of species, revealed a positive likelihood of cattle being infected with trypanosome infection in a wildlife-cattle co-existence. Resident wildlife species such buffaloes showed positive association with the trypanosome infection in cattle. The positive association can be attributed to these species since they are known to be the reservoirs of trypanosome parasite hence their presence in the grazing areas promoted the circulation of trypanosome infection in cattle. Other studies have also indicated that, buffaloes are infected with *Trypanosoma congolense, T. brucei brucei and T. vivax* (Moloo *et al.*, 1999). Furthermore, it has been observed by other studies that existence of wildlife species and tsetse flies in the supporting habitat facilitated the existence of trypanosome infection in the ecosystem (Auty *et al.*, 2012; Munangandu *et al.*, 2012). Although, buffalos

showed positive association with trypanosome infection, still the presence of other wildlife species such as Impala, Eland, Giraffe and Ostrich in the ecosystem need not to be underestimated on the influence of trypanosome infection to cattle in Maasai steppe, because they have been observed in other places to harbor trypanosome parasites (Auty *et al*., 2012; Munangandu *et al.*, 2012). However the seasonal migratory behavior of wildlife between Tarangire National Park and the adjacent areas is important for explaining more the important of wildlife. A study by Ngonyoka *et al*. (2017) showed that, host species like wildlife play significant role in the abundance of tsetse flies with greater impacts in dry season.

4.2.4. Land cover and the prevalence of trypanosome infections

Although five categories of habitats were identified in the study area, woodland showed a positive and significant effect on trypanosome infection while grassland had a negative effect. The positive association of woodland on trypanosome infection can be tied to the vectors existing in this area supported by this habitat type. Previous study in this area have shown that the most abundance species for tsetse flies in this area which could utilize the woodland habitats are *Glossina morstans, G. swynnertoni* and *G. pallidipes* which are vector for important trypanosome species in this area like *Trypanosoma brucei Trypanosoma vivax* and *Trypanosoma congolense* (Nnko *et al.,* 2017; Salekwa *et al*., 2014). In addition, the influence of habitats is tied with season and dominated habitat type. The habitat in this study area was dominated with woodland. Physiognomy of habitats changes between the dry season and wet season. A study by Ngonyoka *et al*. (2017) showed that, habitats type like ecotone zone between woodland and grassland play significant role in the abundance of tsetse flies with greater impacts in dry season.

Cultivation activities exhibited a negative association with the trypanosome infections in cattle with discrepancy among villages, with a larger positive effect in Kimotorok. Human activities such as agriculture (Cultivation) played a significant role on the abundance and distribution of tsetse flies vectors and wildlife hosts species for trypanosome infection. The negative association between the cultivation and prevalence of trypanosome infection can be explained by habitats being destroyed and driving wildlife reservoirs and tsetse flies away from this area. This is supported by findings in another study done in Kenya, the results showed cultivation as means of human development in a growing population destroys the habitats for tsetse flies (Lewis, 1942).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

Seasonal cattle movements have a significant impact on the prevalence of trypanosome infections in cattle in the Maasai steppe. Time of the year and grazing in areas close to the wildlife interface has been shown to influence prevalence of trypanosome infections in cattle. Based on farmers' opinions, the major driver for movement of their livestock is search for pasture and water, and this is exacerbated during the dry season.

Trypanosomiasis in cattle will continue to be a challenge among the pastoralist communities in Tanzania due to ecological and other risk factors. For instance, in this study risk factors such as herd size of 51-100 cattle was observed to positive correlate with prevalence in Maasai steppe which could be due to low drug purchasing power to handle all diseased animals, resulting into no or improper treatment for affected animals. However, when different villages were considered, age of 0-1 years in Oltukai and age between 1-2 years in Kimotorok were positively correlated with prevalence of trypanosome infection probably due to low immunity and less treatment priority in this age.

Woodland and grassland was observed to be positively and negatively respectively associated with the prevalence of trypanosome infection. This is explained by the association existing between this habitat types and tsetse flies vector in this area.

5.2. Recommendations

Based on the results of this study coordinated planning of grazing patterns is recommended to reduce the risk of infection in hot spot areas at certain period of the year. Proper treatment and other control strategies such as controlled grazing patterns, tsetse fly control, dipping/spraying should be emphasized when animals are seasonally moving and grazing in the identified trypanosome hotspots areas. Furthermore, creation of awareness on existing hot spot areas and control strategies for trypanosome infection should be provided to the pastoral communities in the Maasai steppe.

It is important and I recommend more studies to be done in this area at large scale and longer period so as to generate enough data which can capture the significant impacts of factors

under the study on the trypanosome infection in cattle which could lead to a strong conclusive comment. This study will help to reveal and clarify how the hot spots areas for trypanosome infections can actually be translated into trypanosomiasis as a diseases in cattle and its socio-economic important to pastoralists of Maasai steppe. Information accrued from such studies will provides precaution measures for controlling trypanosome infection when cattle are grazing in such areas. Understanding cattle movement trajectories and also the hotspot areas in the ecosystem will help the pastoralists and other stakeholder to properly deal with the transmission of trypanosome infection in cattle at village as well as at National level. This can be achieved by changing patterns to reduce risk in areas when infection is mostly likely.

Trypanosome control efforts should be emphasized during July and into November (wet seasons), the months with higher prevalence of trypanosome infection and abundance of tsetse Also, more education should be provided to the pastoralists on, treatment skill, how to overcome the risk factor of trypanosome infection, the acquiring amount of cattle which can be supported by the carrying capacity in their grassing areas to reduce stress which can lead to animals becoming prone to trypanosome infection. The government through the Ministry of livestock and fisheries should provide veterinary technical support to pastoralists in controlling the diseases. Much emphasis should be focused on the treatment intervention as control strategy against animal trypanosomiasis and reduce drug misuse which is practiced by the pastoralists in the study area.

It is recommended that, the control strategies for trypanosomiasis in this livestock-wildlife co-existing ecosystem to consider wildlife as key factor for trypanosomes circulation in the ecosystem without forgetting other risk factors such as age, herd size, habitat type of grazing and the existing human activities like agriculture. To achieve the best control goals, it is important to have integrated trypanosome control strategies where different stakeholders like conservationists, livestock keeper and agricultural sector are involved.

REFERENCES

Abiola, F. A., Biaou, C., & Niang, M. (2005). Socio-Economic and Animal Health Impact of Transhumance. *Conference by OIE World Organization for Animal Health,* 105–119. Retrieved from https://www.oie.int/doc/ged/d3248.pdf

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

Anderson, N. E., Mubanga, J., Fevre, E. M., Picozzi, K., Eisler, M. C., Thomas, R., … Welburn, S. C. (2011). Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. *PLoS Neglected Tropical Diseases,* 5(6), e1211. https://doi.org/10.1371/journal.pntd.0001211

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

Auty, H., Anderson, N. E., Picozzi, K., Lembo, T., Mubanga, J., Hoare, R., … Welburn, S. C. (2012). Trypanosome Diversity in Wildlife Species from the Serengeti and Luangwa Valley Ecosystems. *PLoS Neglected Tropical Diseases,* 6(10), e1828. https://doi.org/10.137 1/journal.pntd.000182

Auty, H., Torr, S. J., Michoel, T., Jayaraman, S., & Morrison, L. J. (2015). Cattle trypanosomosis : the diversity of trypanosomes and implications for disease epidemiology and control Trypanosome species of relevance to cattle. *Revue Scientifique et Technique, International Office of Epizootics,* 34, 587–598

Baticados, W. N., Castro, D. L., & Baticados, A. M. (2012). Parasitological and PCR detection of Trypanosoma evansi in buffaloes from Luzon, Philippines. *Ceylon Journal of Science,* 40, 141–146

Beziel, M., Girma, M., Dagnachew, S., Tadesse, D., & Tadesse, G. (2014). African Trypanosomes : Virulence Factors, Pathogenicity And Host Responses, *Journal of Veterinary Advances,* 4(2), 732–745

Bhutto, B., Gadahi, J. A., Shah, G., Dewani, P., & Arijo, A. G. (2010). Field investigation on the prevalence of trypanosomiasis in camels in relation to sex, age, breed and herd size. *Ceylon Journal of Science (Biological Sciences),* 30(2), 175–177. http://doi.org/10.4038/cjsbs.v40 i2.3930

Bonfoh, B., Zinsstag, J., Fokou, G., Weibel, D., Ould Taleb, M., Maselli, D., … Marcel, T. (2011). Pastoralism at the Crossroads: New Avenues for Sustainable Livelihoods in Semi-arid Regions. In *Research on Sustainanable Development,* 6, 549–570

Bourn, D., Grant, I., Shaw, A., Torr, S., Protection, C. E., House, O., … Mej, K. (2005). Cheap and safe tsetse control for livestock production and mixed farming in Africa. *Aspects of Applied Biology,* 75, 1–12

Bouyer, J., Carter, N. H., Batavia, C., & Nelson, M. P. (2019). The Ethics of Eliminating Harmful Species: The Case of the Tsetse Fly. *Bioscience,* 69(2), 125–135

Bucheton, B., Macleod, A., & Jamonneau, V. (2014). Human host determinants influencing the outcome of Trypanosoma brucei gambiense infections Human host determinants influencing the outcome of Trypanosoma brucei gambiense infections. *Parasite Immunology,* 33, 438–447

Burgess, T. (2001). Guide to the Design of Questionnaires. A general introduction to the design of questionnaires, Topic 2, University of Leeds, Edition 1, 1-21. Retrieved from http://158.132.155.107/posh97/private/Questionnaire/design.pdf

Cecchi, G., Mattioli, R. C., Slingenbergh, J., & De La Rocque, S. (2008). Land cover and tsetse fly distributions in sub-Saharan Africa. *Medical Veterinary Entomology,* 22, 364–373

Chanie, M., Adula, D., & Bogale, B. (2013). Socio-Economic Assessment of the Impacts of Trypanosomiasis on Cattle in Girja District, Southern Oromia Region, Southern Ethiopia. *Acta Parasitology Global,* 4, 80–85

Conroy, A. (1999). Maasai agriculture and land use change. *Land Degradation.* Retrived from http://www.fao.org/fileadmin/templates/lead/pdf/05_article01_en.pdf

Dagnachew, S., Tsegaye, B., Awukew, A., Tilahun, M., Ashenafi, H., Rowan, T., … Goddeeris, B. M. (2017). Prevalence of bovine trypanosomos is and assessment of trypanocidal drug resistance in tsetse infested and non-tsetse infested areas of Northwest Ethiopia. *Parasite Epidemiology Control,* 2, 40–49

Dicker, R. C. (2006). Principles of Epidemiology in Public Health Practice. *Cdc* Glossary of Epidemiology Terms. *Center for Diseases Control and Prevention.* Retrieved from https://www .cdc.gov/careerpaths/k12teacherroadmap/classroom/principlesofepi.html

Emmanuel, N. (2015). *Prevalence and Risk Factors Associated With Trypanosomosis in Cattle Herds in Kilwa District Lindi Region of Tanzania* (Master's dissertation). Retrieved from https://pdfs.s emanticscholar .org/20de/7da92a0b7f2fa465df3c1444f2 abdd1457be.pdf

Ezeani, M. C., Okoro, H., Anosa, V. O., Onyenekwe, C. C., Meludu, S. C., Dioka, C. E., … Azikiwe, C. C. (2008). Immunodiagnosis of bovine trypanosomiasis in Anambra and Imo states, Nigeria, using enzyme-linked immunosorbent assay: Zoonotic implications to human health. *Journal of Vector Borne Diseases,* 45, 292–300

Food and Agriculture Organization/ World Health Organization (FAO/WHO). (1989). Residues of some Residues of some veterinary drugs in animals and foods.Retrieved from http://www.fao.org/3/y5612e/y5612e00.htm

Food and Agriculture Organization (FAO). (1998). A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis. *FAO Corpotation Document Repostory,* 1, 1–170. Retrived from http://www.fao.org/3/X0 413E/X0413E00.htm

Fedosov, S. N., Laursen, N. B., Nexø, E., Moestrup, S. K., Petersen, T. E., Jensen, E., … Berglund, L. (2003). Human intrinsic factor expressed in the plant Arabidopsis thaliana. *Europian Journal of Biochemistry,* 270, 3362–3367

Ferluga, J., Nayak, A., & Kishore, U. (2014). Host-pathogen interaction : Plasmodium and Trypanosomes Chapter 9 Host - pathogen interaction in Malaria and Trypanosomiasis. *Microbial Pathogenesis: Infection and Immunity Springer,* 9, 168-200. Retrived from

Fèvre, E. M., Bronsvoort, B. M. D. C., Hamilton, K. A., & Cleaveland, S. (2006). Animal movements and the spread of infectious diseases. *Trends Microbiology,* 14, 125–131

Firesbhat, A., & Desalegn, C. (2015). Epidemiology and Impacts of Trypanosomiasis in Cattle. *Europian Journal Appied Science,* 7, 220–225

Garnett, G. P., & Lewis, J. J. C. (2007). The Impact of population growth on the epidemiology and evolution of infectious diseases, 6. Springer, Dordrecht. Retrived from https://link. springer.com/chapter/10.1007/978-1-4020-6174-5_2

Giordani, F., Morrison, L. J., Rowan, T. G., De Koning, H. P., & Barrett, M. P. (2016). The animal trypanosomiases and their chemotherapy: A review. *Parasitology,* 143, 1862–1889

Guerrini, L., Bord, J. P., Ducheyne, E., Bouyer, J., Aubreville, A., Bouyer, J., … Swallow, B. M. (2008). Fragmentation analysis for prediction of suitable habitat for vectors: Exampl e of riverine tsetse flies in Burkina Faso. *Journal of Medical Entomology,* 45, 1180–6

Hamill, L. C., Kaare, M. T., Welburn, S. C., & Picozzi, K. (2013). Domestic pigs as potential reservoirs of human and animal trypanosomiasis in Northern Tanzania. *Parasit. Vectors,* 6 (322), (2013). https://doi.org/10.1186/1756-3305-6-322

Homewood, K. M., Trench, P., & Brockington, D. (2012). Pastoralist livelihoods and wildlife revenues in East Africa: a case for coexistence. *Pastoralism,* 2(19), (2012). https://doi.org/ 10.1186/2041-7136-2-19

Kimaro, E. G., Toribio, J. A., Gwakisa, P., & Mor, S. M. (2018). Occurrence of trypanosome infections in cattle in relation to season, livestock movement and management practices of Maasai pastoralists in Northern Tanzania. *Veterinary Parasitology and Region Stududies Reports,* 12, 91–98

Lang, S. (2001). Studies on incidence and control of Trypanosomiasis in buffalos caused by Trypanosome avansi steel 1885 in North Vietnam. *In Proceedings of Buffalo Workshop,* Hanoi, Vietnam

Lankester, F., Davis, A., & Allen, P. G. (2016). Pastoralism and wildlife: historical and current perspectives in the East African rangelands of Kenya and Tanzania Pastoralism in East Africa: a brief overview. *Revue Scientifique et Technique (International Office of Epizootics)*, 35(2), 473-484. (doi:10.20506/rst.35.2.2536) (PMID:27917978)

Lankester, F., Russell, G. C., Lugelo, A., Ndabigaye, A., Mnyambwa, N., Keyyu, J., … Cleaveland, S. (2016). A field vaccine trial in Tanzania demonstrates partial protection against malignant catarrhal fever in cattle. *Vaccine,* 34(6), 831–838

Leak, S. G. A., Mulatu, W., Authié, E., d'Ieteren, G. D. M., Peregrine, A. S., Rowlands, G. J., … Trail, J. C. M. (1993). Epidemiology of bovine trypanosomiasis in the Ghibe valley, southwest Ethiopia 1. Tsetse challenge and its relationship to trypanosome prevalence in cattle. *Acta Tropical,* 53(1993), 121–134

Levy, M. G., Clabaugh, G., & Ristic, M. (1982). Age resistance in bovine babesiosis: role of blood factors in resistance to Babesia bovis. *Infection and Immunity,* 37(3), 1127–113

Lewis, E. A. (1942). Tsetse-flies and Development in Kenya Colony, *East African Agricultural Journal,* 1, 9–14

Lukes, J. (2009). Trypanosoma Gruby 1843. *Version 02 January, 2009.* Retrieved from. *http//tolweb.org/Trypanosoma/98034/2009.01.02 Tree Life Web Proj. http//tolweb.org/*

Lyons, M. (1993). African trypanosomosis sleeping sickness. Fact Sheet. *Releve Epidemiologique Hebdomadaire,* 79(32):297-300. https://apps.who.int/iris/bitstream /handle /10665/232553/WER7932_297-300.PDF

Majekodunmi, A. O., Fajinmi, A., Dongkum, C., Picozzi, K., Thrusfield, M. V., & Welburn, S. C. (2013). A longitudinal survey of African animal trypanosomiasis in domestic cattle on the Jos Plateau, Nigeria: Prevalence, distribution and risk factors. *Parasites & Vectors,* 6, 239. https://doi.org/10.1186/1756-3305-6-239

Malele, I. I. (2011). Fifty years of tsetse control in Tanzania: challenges and prospects for the future. *Tanzania Journal of Health Research,* 13(1), 399–406

Malele, I., Nyingilili, H., & Msangi, A. (2011). Factors defining the distribution limit of tsetse infestation and the implication for livestock sector in Tanzania. *African Journal of Agricultural Research,* 6(10), 2341–2347

Mamman, M., Aliu, Y. O., & Peregrine, A. S. (1993). Comparative pharmacokinetics of diminazene in noninfected Boran (Bos indicus) cattle and Boran cattle infected with Trypanosoma congolense. *Antimicrobial Agents Chemother,* 37(5), 1050–1055

Mamoudou, A., Suh, P., & Ebene, J. (2015). Impact of Tick Infestation and Trypanosomiasis in Cattle in the Sudano-Sahelian Zone of Cameroon. *Journal of Veterinary Advances, 5*(5), 919-924 DOI: 10.5455/jva.20150513122211

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

Miller, B. W., Leslie, P. W., & McCabe., J. T. (2014). Coping with natural hazards in a conservation context: resource-use decisions of Maasai households during recent and historical droughts. *Humun Ecology Interdisplinary Journal,* 42(5), 753–768

Miller, D. (2005). *The pharmacokinetic of diminazene aceturate after intramuscular and intravenous administration in the healthy dog* (Unpublished master's disertation) University of Pretoria, South Africa

Miller, R. S., Sweeney, S. J., Slootmaker, C., Grear, D. A., Di Salvo, P. A., Kiser, D., & Shwiff, S. A. (2017). Cross-species transmission potential between wild pigs, livestock, poultry, wildlife, and humans: implications for disease risk management in North America. *Scientific Report,* 7, 7821. doi: 10.1038/s41598-017-07336-z

Mndolwa, N. D. (2014). The status of tsetse and Trypanosomiasis in Simanjiro District. Monduli. *District Veterinary Technical Report,* 1, 1-5

Moloo, S. K., Orinda, G. O., Sabwa, C. L., Minja, S. H., & Masake, R. A. (1999). Study on the sequential tsetse-transmitted Trypanosoma congolense, T. brucei brucei and T. vivax infections to African buffalo, eland, waterbuck, N'Dama and Boran cattle. *Veterinary Parasitology,* 80(3), 197-213

Mpiana, B., Mpinga, K. E., Bukasa, T. J. C., Chastonay, P., Kyhi, I., Ilunga, M., … Kandolo, T. (2015). Risk Factors of Human African Trypanosomiasis in Mbuji Mayi, Eastern Kasai Province, Democratic Republic of the Congo. *International Journal of Tropical Diseaseases and Health,* 5(3), 190-208

Msoffe, F. U., Said, M. Y., Ogutu, J. O., Kifugo, S. C., De, J., Gardingen, P. Van, & 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 Conservation,* 3(7), 280–290

Muhanguzi, D., Picozzi, K., Hattendorf, J., Thrusfield, M., Kabasa, J. D., Waiswa, C., & Welburn, S. C. (2014). The burden and spatial distribution of bovine African trypanosomes in small holder croplivestock production systems in Tororo District, south-eastern Uganda. *Parasites and Vectors,* 7, 1–10. https://doi.org/10.1186/s13071-014-0603-6

Munangandu, H. M., Siamudaala, V., Munyeme, M., & Nalubamba, K. S. (2012). A review of ecological factors associated with the epidemiology of wildlife trypanosomiasis in the Luangwa and Zambezi valley ecosystems of Zambia. *Interdisciplinary Perspectives on Infectious Diseases*, 2012(13). https://doi.org/10.1155/2012/372523

Mungube, E. O., Vitouley, H. S., Allegye-Cudjoe, E., Diall, O., Boucoum, Z., Diarra, B., … Clausen, P. H. (2012). Detection of multiple drug-resistant Trypanosoma congolense populations in village cattle of south-east Mali. *Parasites and Vectors,* 5, 155. https://doi.org/10.1186/1756-3305-5-155 1–9

Nelson, F. (2012). Natural conservationists. Evaluating the impact of pastoralist land use practices on Tanzania's wildlife economy. *Pastoralism Research, Policy and Pracactic es.,* 2, 15. https://doi.org /10.1186/2041-7136-2-15

Ngonyoka, A., Gwakisa, P. S., Estes, A. B., Nnko, H. J., Hudson, P. J., & Cattadori, I. M. (2017). Variation of tsetse fly abundance in relation to habitat and host presence in the Maasai steppe, Tanzania. *Journal of Vector Ecology,* 42(1), 40--43

Nhamitambo, N. L. (2017). Molecular Identification of Trypanosome Species in Cattle of the Mikumi Human/Livestock/Wildlife Interface Areas, Tanzania. *Journal of Infectious Diseases and Epidemiology,* 3(2), 1–10. doi: 10.23937/2474-3658/1510029

Nnko, H. J., Ngonyoka, A., Salekwa, L., Estes, A. B., Hudson, P. J., Gwakisa, P. S., & Cattadori, I. M. (2017). Seasonal variation of tsetse fly species abundance and prevalen ce of trypanosomes in the Maasai steppe, Tanzania. *Journal of Vector Ecology,* 42(1), 24–33

Nnko, H. J., Gwakisa, P. S., Ngonyoka, A., Saigilu, M., Ole-Neselle, M., Kisoka, W., … Estes, A. (2017). Pastoralists' Vulnerability to Trypanosomiasis in Maasai steppe. *Ecohealth,* 14(7), 718–731

Ohaeri, C. (2010). Prevalence of Trypanosomiasis in Ruminants in Parts of Abia State, Nigeria. *Journal of Animal and Veterinary Advances,* 9(18), 2422–2426

Oluwafemi, R. (2008). Biological Control of Tsetse fly Project (BICOT) in Lafia Local Govenrment Area of Nasarawa State, Nigeria, 1984. *The Internet Journal of Veterinary Medicine,* 7(1), 1–5

Reda, K. T. (2012). Pastoralism at Crossroads: Changing Features of Climate, Livelihood and Social Organization in East Africa. *Global Journal of Human Social Science Sociology, Economics & Political Science*, 12(9), 1–6

Ruiz, J. P., Nyingilili, H. S., Mbata, G. H., & Malele, I. I. (2015). The role of domestic animals in the epidemiology of human african trypanosomiasis in Ngorongoro conservation area, Tanzania. *Parasites & Vectors,* 8, 4–9. doi: 10.1186/s13071-015-1125-6

Salekwa, L., Nnko, H., Ngonyoka, A., Estes, A. B., Agaba, M., & Gwakisa, P. S. (2014). Relative abundance of tsetse fly species and their infection rates in Simanjiro, Northern Tanzania. *Livestock Research for Rural Development,* 26(12), 1–8

Sassera, D., Epis, S., Pajoro, M., & Bandi, C. (2013). Microbial symbiosis and the control of vector-borne pathogens in tsetse flies, human lice, and triatomine bugs. *Pathogens and Global Health,* 107(6*),* 285-92

Sidiropoulou, A., Karatassiou, M., Galidaki, G., & Sklavou, P. (2015). Landscape pattern changes in response to transhumance abandonment on mountain vermio (North Greece). *Sustainability,* 7, 15652–15673. doi:10.3390/su71115652

Simwango, M. (2016). *Molecular prevalence of trypanosome infections in cattle* (Unpublished master's disertation). Sokoine University of Agriculture. Morogoro, Tanzania

Simwango, M., Ngonyoka, A., Nnko, H. J., Salekwa, L. P., Ole-Neselle, M., Kimera, S. I., & Gwakisa, P. S. (2017). Molecular prevalence of trypanosome infections in cattle and tsetse flies in the Maasai steppe, northern Tanzania. *Parasites and Vectors,* 10(507), 1–11. https://doi.org/10.1186/s13071-017-2411-2

Sindato, C., Malele, I. I., Mwalimu, C. D., Nyingilili, H. S., Kaboya, S., Kombe, E., … Mañoza, A. (2007). Seasonal variation in human African trypanosomiasis in Tarangire National Park in Babati district, Tanzania. *Tanzania Health Research Bulletin,* 9(2), 136-139

Skraban, R., & Lutley, R. (2002). Selection of antigenic variants in maedi – visna virus infection. *Journal of General Virology,* 83, 2543–2551

Sobhy, H. M., Barghash, S. M., Behour, T. S., & Razin, E. A. (2017). Seasonal fluctuation of trypanosomiasis in camels in North-West Egypt and effect of age, sex, location, health status and vector abundance on the prevalence. *Beni-Suef University Journal of Basic and Applied Sciences,* 6(1), 64–68

Swai, E. S., & Kaaya, J. E. (2012). A parasitological survey for bovine trypanosomosis in the livestock/wildlife ecozone of Northern Tanzania. *Veterinary World,* 5(8), 459–464

TTB (Tanzania Tourist Board). (2017). Tanzania, Africa's Best Safari Country. Dar-Es-Salaam: Tanzania Tourist Board.Retrived from https://tanzaniatourism.go.tz/en/

Tuntasuvan, D., & Viseshakul, N. (2002). Chemotherapy of surra in horses and mules with diminazene aceturate. *Vaterinary Parasitology,* 110(3), 227–233.https://doi.org/10.1016/S0 304-4017(02)00304-7

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

von Wissmann, B., Machila, N., Picozzi, K., Fèvre, E. M., dec Bronsvoort, B. M., Handel, I. G., & Welburn, S. C. (2011). Factors associated with acquisition of human infective and animal infective trypanosome infections in domestic livestock in Western Kenya. *PLoS Neglected Tropical Diseases,* 5(1), e941. https://doi.org/10.1371/journal.pntd.0000941

Vreysen, M. J. B., Talla, M., Sall, B., & Bouyer, J. (2012). Tsetse flies : Their biology and control using area-wide integrated pest management approaches Tsetse flies : Their biology and control using area-wide integrated pest management approaches. *Journal of Invertebrate and Pathology,* 112(1), S15–S25 doi: 10.1016/j.jip.2012.07.026

World Health Organization (WHO). (2015). Trypanosomiasis, human African (sleeping sickness). Retrived from https://www.who.int/news-room/fact-sheets/detail/ trypano somiasis-human-african-(sleeping -sickness)

World Health Organization (WHO). (2013). Control and surveillance of human African trypanosomiasis. *World Health Organization Technical Report.* Retrived from https://www.who.int/sorry/en/

APPENDICES

Appendix 1: Questionnaire on tsetse flies and trypanosomiasis in Maasai steppe

On the progress of study on the Vulnerability of smallholder livestock farmers to vector-borne diseases specifically trypanosomiasis to Maasai steppe, community participation on this study is vital. The study involves gathering views from interested parties. You are kindly requested to fill the attached questionnaire to avail your knowledge excluding you name. The confidentiality of the answers will be treated by the researcher.

Questions asked to pastoralist during blood collection

1. What is the age of an animal?……………...
2. When was the last day of treatment against Trypanosomiasis?…………………..
3. Do you spray these animals? ………………………………………………………..
4. If Yes, When was the last spray?…………………………………………………...
5. Do you apply dipping?…………………………………………………………...
6. If Yes, When was the last dipping?………………………………………………...

7. Which among the following methods below did you use to control Trypanosomiasis in the past 2 months? (Mark the appropriate and in the order 1,2,3,4….. where 1 is the most significant

C/N	Control methods	Tick the applied	Rank
1	Spraying by using insecticides		
2	Trapping using different types of traps		
3	Bush clearing		
4	The use of sterilized male tsetse flies		
5	Fire burning to manage habitats and control tsetse flies		
6	Game/Wildlife killing		
7	Grazing far from wildlife		
8	Treating sick animal		
9	Vaccinating cattle/Livestock		

8. Where have you been grazing your animals in the last two months…………………
9. Which among the following are the reasons for you to move with animals in the past 2 months? (Tick the appropriate, give the estimate of wildlife abundance for each of the observed species and rank them according to their abundance in order of 1, 2, 3, 4, where 1 is the most significant).

C/N	Reason for animal movement	Tick the applied	Rank
1	Searching Pasture for livestock		
2	Searching water for livestock		
3	Diseases avoidance strategies from wildlife		
4	Large Livestock population		
5	Predation avoidance		
6	Cultural practices		
7	To avoid the cultivated area during wet season		
8	Invasive weeds (Ndelemeti)		
9	Avoid wetland (Water logged areas-engusero) area during wet season		

10. Give other reasons…………………………………………………………………….

11. Which of the following statement are true about availability of tsetse flies and prevalence of trypanosomes in the area grazed last 2 months? (Tick the appropriate and rank them in order of 1,2,3,4,… where 1 is the most significant)

C/N	Ecological factors and human activities	Tick the applied	Rank
1	Cultivated areas have low tsetse flies than intact habitat		
2	Wooded area (Woodland) have more tsetse flies and prevalence of trypanosomiasis than grassland		
3	Fire burning to pastures reduce tsetse flies abundance		
4	Human control like spraying and trapping reduce tsetse flies abundance		
5	Area with wildlife has high abundance of tsetse flies and prevalence of trypanosomiasis		
6	Vegetation type have significant influence to Tsetse flies abundance and prevalence of trypanosomiasis		
7	Water sources during dry season harbor tsetse flies for livestock		

12. Which habitat have you been grazing your animal in the past 2 months (Tick the appropriate and rank them in order of 1,2,3,4,… where 1 is the most significant)

C/N	Habitat type	Tick the applied	Rank
1	Forest		
2	Woodland		
3	Savannah/Glassland		
4	Riverine		
5	Thickets		

13. Name others……………………………………………………………………

14. Which of the following wild animal species did you observe in your grazing area in the past 2 months? (Tick the appropriate and rank them in order of 1, 2, 3, 4, where 1 is the most significant).

C/N	Wild animal species	Tick the applied	Rank	Number
1	Wildebeests			
2	buffalo			
3	Eland			
4	Zebra			
5	Elephants			
6	Impala			
7	Ostrich			
8	Giraffe			
9	Hyena			
10	Lion			
11	Warthog			
12	Leopard			
13	Cheetah			
14	Pongo			
15	Others			

15. What were the human activities observed in your grazing area in the last 2 months?

C/N	Human activities	Tick the applied	Rank
1	Cultivation		
2	Hunting		
3	Fishing		
4	Tourism activities		
5	Tsetse fly control/traps		
6	Marketing/Mnada		
7	Vegetation Burning		

16. Name other human activities observed………………………………………………
…………………….

Thank you Very Much for your positive co-operation

Appendix 2: Data collection sheet to the field in a grazing area

1. Village…………………….. Date………………………

2. Pastoralist grazing in the area……………………………………………………………
   ………………………………………………

GPS	Habitat	Wildlife Species	Human Activities	Burning status