PREVALENCE OF *THEILERIA PARVA* AND TRYPANOSOME INFECTIONS IN THE DRY SEASON: A CASE OF MONDULI DISTRICT, NORTHERN TANZANIA

MEYIR ZIEKAH YIRYELE

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ABSTRACT

East Coast fever (ECF) caused by *Theileria parva* and African animal trypanosomosis (AAT) caused by some *Trypanosoma* species are the most devastating cattle diseases that affect cattle productivity in Eastern, central and Southern Africa including Tanzania. A study was conducted in Monduli district in the Maasai steppe ecosystem of Tanzania to determine the prevalence of infections during the dry season when there is increased interaction between livestock and wildlife as a result of scarcity of food and water. Blood samples of 480 cattle were randomly selected in 10 villages and analysed using PCR for *T. parva* and trypanosomes targeting their p104 and ITS1 genes respectively. The overall prevalence of *T. parva* was 31.7%. There was a variation in prevalence among villages. Prevalence of trypanosomes was 4.2% varying from 2.1%-14.6%. Three villages had no infections. Mixed species infections occurred in only one village. However, prevalence of co-infections of *T. parva* and trypanosomes was 4.1%. Risk factors associated with the prevalence of *T. parva* and trypanosomes were analysed using χ^2 test in Epi info 7. There was statistical insignificance between Sex and prevalence of *T. parva* ($p>0.05$). Calves less than 6 months had the highest prevalence 33.8% for *T. parva.* The use of oxytetracycline ($p=0.024$) for treatment and village location ($p=0.010$) were statistically significant to *T. parva* infection. For trypanosomes infections, adults had the highest prevalence of 5.1% with no statistical significance ($p>0.05$). However, prevalence of breed and source of animals were significantly associated ($p<0.05$) with trypanosomes. Village location was statistically insignificant ($p=0.0738$) for trypanosomes. Infections by *T. parva* and trypanosomes remain an important constraint to the extensive livestock farming system and development in Monduli since cattle carry the parasites which could be transmitted to the introduced more productive exotic breeds. The knowledge acquired from this present study will inform stakeholders to develop effective and integrated control strategies that could be easily implemented by farmers to control ECF and AAT.

DECLARATION

I, Meyir Ziekah Yiryele, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor currently being submitted in any other institution.

_____   _____

Meyir Ziekah Yiryele   Date

(MSc. Epidemiology Candidate)

The above declaration is confirmed by;

_____   _____

Prof. P. S. Gwakisa   Date

(Supervisor)

_____   _____

Prof. S. I. Kimera   Date

(Supervisor)

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DEDICATION

This work is dedicated to my lovely children Miguel and Cassandra and to my wife Diane for the support, love and prayers and for understanding my two years of absence. To my ever supporting lovely Mum Celestina Lariba Niber and Dad, Yiryele Ziekah who pushed and encouraged me to higher heights. I also dedicate this work to to my sisters Putier and Jelya, to my brothers Emmanuel, Nonetaar, Ziem, Naab and Wura-sey and to the Chiefs and people of Lawra Paramountcy and the entire Kuusiele clan of Ghana and Burkina Faso for the support in diverse ways.

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LIST OF ABBREVIATION, ACRONYM AND SYMBOLS

AAT	Animal African Trypanosomosis
AE	Attached Epimastigotes
AFLP	Amplied Fragment Length Polymorphism
AGDP	Agricultural Gross Domestic Product
CBOs	Community Based Organisations
CCPP	Contagious Caprine Pleuropneumonia
CDC	Centres for Disease Control
CFSPH	Centre for Food Security and Public Health
CI	Confidence Interval
DE	Dividing Epimastigotes
DNA	Deoxyribonucleic acid
ECF	East Coast Fever
EDTA	Ethylene Diamine Tetra Acetic acid
ELISA	Enzyme-linked Immunosorbent Assay
ENSO	El Niño Southern Oscillation
Epi info 7	Epidemiological Package for Information Version 7
et al	and others
ET	Epi-Trypo Dividing Epimastigotes
FAO	Food and Agriculture Organisation
FG	Fore Gut
Fig.	Figure
FMD	Foot and Mouth Disease
GA	Georgia
GALVmed	Global Alliance for Livestock Veterinary Medicines

GSC	Genome Science Centre
HAT	Human African Trypanosomosis
HCT	Haematocrit
HG	Hind Gut
Hx	Hypopharynx
IFAT	Indirect Fluorescent Antibody Technique
IPCC	Intergovernmental Panel on Climate Change
ITM	Infection and Treatment Method
LAMP	Loop Mediated Isothermal Amplification
MG	Mid Gut
MKUKUTA (Swahili)	National Strategy for Growth and Reduction of Poverty
MS	Mesocyclic Trypomastigotes
MT	Metacyclic Trypomastigotes
NARCO	National Ranching Company
NBS	National Bureau of Statistics
NGOs	Non-Governmental Organisations
nPCR	Nested Polymerase Chain Reaction
ºC	Degrees Celsius
OIE	World Organisation of Animal Health
OR	Odds Ratio
p104	microneme- ropthy protein
PATTEC	Pan African Tsetse and Trypanosomiasis Eradication Campaign
PC	Procyclic trypomastigotes
PCR	Polymerase Chain Reaction
PM	peritrophic membrane

pMT	Pre-Metacyclic Trypomastigotes
Pr	Proboscis
Pv	Proventriculus
R	Rectum
RAPD	Randomly Amplified Polymorphic DNA
RVF	Rift Valley Fever
SE	Short Epimastigotes
SG	Salivary Gland
SIT	Sterile Insect Technique
SL	Slender Trypomastigotes
spp	Species
SSA	Sub-Sahara Africa
ST	Stumpy Trypomastigotes
TBDs	Tick Born Diseases
TNRF	Tanzania Natural Resource Forum
TPRI	Tropical Pesticide Research Institute
TSHZ	Tanzania Short Horn Zebu
TVLA	Tanzania Veterinary Laboratory Agency
UN	United Nations
URT	United Republic of Tanzania
USA	United States of America
WHO	World Health Organisation
WWF	World wildlife Fund
χ^2	Chi-square

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Globally, the livestock sector is valued at 1.4 trillion USD and is a source of income, employment, socio-cultural alue and food to about 600 million resource-poor people and creating a market chain that also employs 1.3 billion people (FAO, 2009; Thornton, 2010). Growing trends in urbanization and population growth with increasing income levels are some of the drivers that create high demand for livestock products, and resulting in the high growth of the livestock subsector of Agriculture in developing countries (Godber and Wall, 2014; Thornton, 2010). The United Nations (UN) has estimated the world population to reach 9.6 billion by 2050 with the highest increase occurring in developing countries (UN, 2015). This radical population growth will therefore lead to high demand for livestock products, thereby creating wealth and livelihoods for the rural poor (UN, 2015). For instance, in Tanzania cattle production has increased over the years (Mashingo *et al.,* 2014). Mashingo *et al.* (2014) reported that human population in 1961 was 9 million with 3 million cattle, and in 2012, it increased to 45 million and 28 million respectively.

After Ethiopia and Sudan, Tanzania is one of the largest cattle producers in Africa, which supports about 37% of the rural community (Mashingo *et al*., 2014). The livestock subsector therefore contributes immensely towards the economy of Tanzania with 13% of the Agricultural Gross Domestic Product (AGDP) and 3.8% to the National GDP, helping towards the attainment of the development goals set out in the National Shared Growth and Reduction of Poverty (NSGRP), MKUKUTA of Vision 2025 (URT, 2010).

Tanzanian total cattle production is composed of 94% indigenous breeds (Tanzanian Shorthorn Zebu (TSHZ) and Ankole), out of which agro pastoralist produce 80 per cent and pastoralist 14 per cent. The other 6 per cent is produced by the National Ranching Company (NARCO). These breeds of cattle include; Arbeeden Angus, Boran, Brahaman, Charolais, Chianina, Hereford, Mpwapwa, Santa Gertrudes and Simmental (URT, 2010). However, Mashingo *et al*. (2014) reported that it currently stands at 98 per cent for indigenous cattle and 2 per cent exotic breeds.

Small ruminants (goat and sheep), donkeys and very few chicken are largely kept by farmers in Northern Tanzania where this study was carried out. However cattle rearing dominates Livestock keeping. Covarrubias *et al.* (2012) report that 82% of rural livestock in Tanzania is dominated by cattle ownership. The pastoralist in this part of the country which is arid and semiarid, thrive under serious challenges of climatic conditions to sustain their livestock. The area is not suitable for crop production (Covarrubias *et al.,* 2012).

Despite the significance of the livestock subsector to the economy of Tanzania, high mortality rates due to endemic livestock diseases, low reproductive rates, low quality of livestock products and low performance with low growth rates are challenges that affect the sector (URT, 2010). Vector borne diseases are among the major endemic livestock diseases that affect cattle production with a lot of magnitude. East Coast Fever (ECF) caused by *T. parva* and african animal trypanosomosis (AAT) caused by some *Trypanosoma* species are the most devastating to cattle farmers in East and Southern Africa including Tanzania. ECF alone causes about 70% of losses in the livestock industry (Muhanguzi *et al*., 2014a). Trypanosomosis is second killer of livestock in Tanzania after ECF (Fyumagwa, 2012).

According to Van de Bossche and Coetzer (2008), another major challenge that affects livestock production is climate change. Erratic rainfall patterns, droughts and floods caused by climate change create water and food shortages. Interactions among domestic animals and humans has increased due to the search for scarce pastures, food and water leading to contact with vectors and transmission of vector borne infections. These climatic patterns also create favourable biological conditions for the multiplication and proliferation of vectors and pathogens (Githeko *et al.*, 2000).

It is therefore important to quantify these vector borne infections, especially during the dry season. Livestock and livelihoods of pastoralist are aggravated by climate change-El Niño Southern Oscillation (ENSO) effect (Githeko *et al*., 2000), with scarcity of food and water during this season.

1.2 Statement of the Problem and Justification of Study

1.2.1 Problem statement

One of the consequences of climate change is the scarcity of food and water, which leads to increased interaction between livestock and wildlife. Such interactions escalate the spread of vectors and the chances of transmission of vector-borne infections (Van den Bossche and Coetzer, 2008), such as by *T. parva* and *Trypanosoma,* and other pathogens.

ECF and AAT are known endemic diseases of cattle in most of the extensive livestock farming areas in Northern Tanzania. They affect animal health and productivity, leading to high economic losses to the farmers. Monduli District is home to Maasai pastoralist in this part of Tanzania whose livelihood depends on livestock, especially cattle. They are affected by climate variability and livestock diseases (Mashingo *et al*., 2014) especially during the dry season. However, dynamics of vectors and vector-borne infections such as

T. parva and *Trypanosoma spp* in Monduli district of Northern Tanzania has not been quantified during the dry season. An epidemiological study to determine the prevalence of *T. parva* and *Trypanosoma spp* infections was therefore conducted during the period of August to October 2015. A molecular technique, polymerase chain reaction (PCR) was used to determine the prevalence of the two important vector-borne infections in the study area.

1.2.2 Justification of study

This study will help understand the dynamics of *T. parva* and *Trypanosoma* infections during the dry season and will serve as a guide to identify areas for further research and to design strategies to mitigate effects of ECF and AAT during the dry season. Furthermore, information on prevalence of these major vector borne infections will be updated leading to designing and implementing better control and preventive strategies by the major stakeholders like Government, Community Based Organizations (CBOs), Non-Governmental Organizations (NGOs) and Pastoralist themselves.

1.3 Objectives of the Study

1.3.1 Main objective

To determine the prevalence of *T. parva* and *Trypanosoma spp* in cattle of Maasai pastoral communities of Monduli in Northern Tanzania, during the dry season.

1.3.2 Specific objectives

i.   To determine prevalence of *T. parva* infections in cattle of Monduli district

ii. To establish prevalence of trypanosome infection in cattle of Monduli district

iii. To determine effects of different factors on the prevalence of *T. parva* and trypanosome infection during the dry season.

CHAPTER TWO

2.0 LITERATURE REVIEW

The effects of climate change that worsens rain patterns and creates other unfavourable environmental conditions are a risk to livestock production systems and its management (Mboera *et al.*, 2011). During the dry season, these worsening conditions due to climate change, severely affect the availability of food and water for livestock, wildlife and humans, who dwell, depend on, and share the resources of the Savannah lands of Africa (Keesing *et al.*, 2013).

Changes in climatic conditions also create favourable conditions for the transmission and distribution of vector-borne diseases (Githeko *et al.*, 2000). Moreover, different epidemiological situations of vector borne diseases in endemic areas is induced by climatic and ecological variations (Tarimo, 2013). Warmer climatic temperatures are favourable for vectors' survival, reproduction and development rate, and increased activity and distribution (Githeko *et al*., 2000; Mboera *et al*., 2011).

Disease pathogens and vector populations increase as ecosystems are modified by climate change which affects pastoral livelihoods (Moenga *et al*., 2013). Climate change is as a result of climate variation (FAO, 2009; IPPC, 2013). It causes extreme events of weather conditions, ultimately leading to unreliable rainfall, high temperatures, floods and droughts (IPCC, 2013; WWF, 2006).

The Savannah plains of sub-Saharan Africa (SSA) severely suffer the effects of climate change, but are widely inhabited by pastoralist and their cattle. For example the Northern savannah highlands of Tanzania are inhabited by Maasai pastoralist. This ecological zone

is harshly affected by climate change with its consequential aberrations. In 2009 the area suffered what is described by observers as the worst drought so far (IPPC, 2013).

2.1 The Maasai, Livelihoods, Cattle and Diseases

The Maasai population is estimated to be about 2 million people and are found in the semi-arid zones of Northern Tanzania and in the Rift Valley areas of Kenya. They are known to be a nomadic tribe with a well-organized military-like social organization. Hierarchy and roles are determined by age. It is believed that about 1700 years ago, they relocated to East Africa from Sudan, exploring for better pastures for their cattle (GIAHS, 2012b). They dwell in semi-arid and dry areas not suitable for crop production andLivestock relatively thrives well in this ecosystem (GIAHS, 2012b).

2.1.1 Maasai in Monduli

The population of Monduli is estimated to be about 158 929 people according to NBS (2013). The district is home to the Maasai in Tanzania constituting about 40 per cent of the population (Monduli District council, 2015). Just like the Fulani and Tuareg of western and northern Africa, and the Nuer of Somalia, the Maasai pastoralist settled and practice pastoralism in the Savannah semiarid zones of Africa (Fratkin, 2001).

2.1.2 Livelihoods

Livestock keeping as a livelihood provides milk, meat, fat, hides and blood for the Maasai (Mashingo *et al.*, 2014). The Maasai depend on natural traditional livestock keeping which is estimated to be over 1000 years old. Cattle, sheep, goats and donkeys are animals kept by the Maasai as they practice Transhumance system (mobility of livestock during wet and dry season for pastures) (TNRF, 2011. This is determined by weather patterns and climate change (TNRF, 2011).

The intelligent combination of managing their livestock with water resources, pastures, wildlife and other natural resources at their disposal guarantees a balance in nature (Jacob *et al.*, 2004; GIAHS, 2012a). Current trends of land ownership and tenure systems are making the Maasai lose the communal ownership of land, which affects their main livelihood (McCabe *et al.*, 2011). Conflicts, climate change and modernization have affected them as well and they have therefore diversified their livelihood from strict pastoralist to be involved in crop production alongside (Mccabe *et al.*, 2011).

Dry season: During the dry season months of July to September, most water bodies which hither to serve humans and animals are dry. Maleko and Koipapi, (2015) report of same climatic conditions during the dry seasons. There are either no pastures or the few reserved ones are dry. Most of the land is bare and with very little and sparsely covered with vegetation. Strong dusty wind whirls blow across the plains causing animals to inhale dust which leads to respiratory tract infections (Personal observation). Wildlife such as zebras, buffaloes and some species of antelopes from nearby Parks, share scarce pastures and water resources with livestock, especially in communities near parks with water bodies. The highly productive and healthy animals are selected to relocate to 'Ronjos' (reserved pasture lands in Maa language) several kilometres away. Lactating cows, weak and sick animals and calves less than one year are kept in the Bomas (Kraals where Maasai pastoralist live with their families and livestock). These are made to graze on reserved pastures close to the bomas. Charcos, dams, dug outs and in some villages, boreholes serve as sources of water for the livestock (personal observation).

During the dry season, livestock spend 10-12 hours grazing as against 7-8 hours in the rainy season. A distance of 8-14 km is covered during the dry season to pastures, whiles 1-4 km is covered in the wet season. Animals need 11 hours during the dry season to walk to

and fro watering points and half an hour during the wet season (Maleko and Koipapi, 2015).

Their cattle and diseases: The Maasai are guided by a myth that all cattle is theirs, given to them by God and therefore no other tribe owns cattle (GIAHS, 2012b). This confirms the importance of cattle to them, which is a source of food, income, socio-cultural status maintenance and security.

The Maasai pastoralist recognize vector borne disease such as ECF, Nagana and their respective vectors as major challenges to their cattle. Other diseases such as foot and mouth disease (FMD), black quarter, contagious caprine pleuropneumonia (CCPP) and Rift Valley fever (RVF) are also known (Chengula *et al*., 2013). According to Jacob *et al*. (2004), traditional medicine for preventive and curative purposes have been used by most tribes in human history for both animals and humans. They also report that the Maasai have developed sophisticated methods used to identify disease causing factors, their treatment and prevention. This feat has been achieved through try and error methods as a way of survival. The techniques have been passed from one generation to the other. However, modern veterinary practice has been of immense help to the Maasai pastoralists making their Ethno-veterinary practice to lose prominence (Jacob *et al*., 2004). Oxytetracycline is a drug of choice by most Maasai which for treating most sick animals as first line measure (Chengula *et al*., 2013; Jacob *et al*., 2004).

2.2 Importance of Vector Borne Diseases in the Maasai Pastoral Communities

Diseases caused by vectors are considered very important in pastoralists set ups. For example, Chengula *et al*. (2013) found out that pastoralist consider ECF and Trypanosomosis at 79.8 % and 50% respectively as diseases that cause major loses to their

cattle. Stunted growth, low fertility rate, low milk production, slow recovery of sick animals and paralysis were observed and reported by Maasai pastoralist during this present study (Olwoch *et al.* (2008). direct losses caused by mortality of *T. parva* infected cattle is also a major hindrance for pastoralist (GALVmed, 2015). It is reported that 25 million cattle are at risk of ECF which kills a cow every second (GALVmed, 2015).

2.3 East Coast Fever (ECF)

ECF is a fatal protozoan disease caused by *Theileria parva* (single celled parasite) (Stoltsz, 1989). It is transmitted by the brown ear tick *Rhipicephalus appendiculatus* from infected animals to other domestic and wild animals (Bazarusanga *et al.*, 2008; Olwoch *et al.*, 2008). The African buffalo (*Syncerus caffer*) is a reservoir of the parasites (Gachohi *et al.,* 2012; Grootenhuis, 1989; Pienaar *et al.,* 2011). The most important tick borne disease in Eastern, Central and Southern Africa are caused by *Theileria parva* infections (Muhanguzi *et al.*, 2014b; Stoltsz, 2013). Muhanguzi *et al.* (2014b) asserted that 70% of losses by cattle farmers in East Africa was due to ECF. Infected animals that have recovered from the disease are also carriers and transmit the disease to non-infected animals in the population. The disease causes high morbidity and mortality (Tarimo, 2013).

ECF prevents the introduction of the more productive exotic cattle breeds into endemic areas, as these develop very severe forms of the disease (Olwoch *et al.*, 2008). However the indigenous cattle breeds develop sub-clinical disease (Kazungu *et al.*, 2015b). Mortality of about 90% can occur with naive cattle when introduced into an endemic area (Olwoch *et al.*, 2008). Death may occur after 3 weeks of infection if sick animals are not treated. Cattle that recover from ECF acquire immunity for life to homologous challenge (Tarimo, 2013).

2.3.1 The Parasite *T. parva*

The protozoan parasite, *Theileria parva* (Theiler, 1904) is host and vector specific parasite. Parasites of the genus Theileria which are, *T. parva, T. annulata, T. ovis, T. hirci, T. capreoli, T. equi and T. lestoquadi*. However, *T. annulata and T. parva*, are important parasites for cattle. Tropical theileriosis is caused by *T. annulata* and *T. parva* causes East Coast fever. For example elands can be affected *T. taurotragi* and sheep by *T. ovis* causing deaths. (Jensen *et al.,* 2009). It does not infect man as it is specific to cattle, African buffalo, water buffalo as well as Waterbuck (Githaka *et al.,* 2014); while it develops in *Rhipicephalus appendiculatus, R. pulchelus* and *R. zambeziensis*. Classical ECF occurs when *T. parva* infects the T and B cells of cattle (Geysen *et al*., 1999).

2.3.2 Clinical signs

First usual sign of ECF is swelling of the lymph nodes (the parotid, the pre-escapular and the pre-femoral). Predilection site for the vector to feed is the ear, causing the parotid lymph nodes to swell (Radostits *et al*., 2006). This is followed by a fever of 40- 42ºC. The buccal and conjunctival cavities develop ecchymosis and petechial haemorrhages. Lacrimation, opacity of the cornea, dyspnoea and diarrhoea occur (OIE, 2014; Radostits *et al*., 2006). Anorexia leading to loss of condition ensues. Paralysis is sometimes reported (CFSPH, 2009b; Radostits *et al*., 2006). During the dry season, clinical manifestations are severe as a result of (i) The scarcity of food and water, (ii) Animals will have to cover longer distances in search of water and pastures. Temperature falls and sick animal becomes recumbent. Frothy nasal discharge due to pulmonary oedema causing severe dyspnoea precedes death (OIE, 2009). The degree of infected tick challenge and the strain of parasites define the time course and the severity of the infection as ECF is dose dependent (OIE, 2009; Iams *et al*., 1990). Subclinical cases are difficult to determine clinically because productivity of cattle is virtually not affected (OIE, 2009).

2.3.3 Life cycle of *Theileria parva*

The life cycle of *T. parva* has two developmental stages. After the tick ingests piroplasms in the erythrocytes of infected cattle they develop into the sexual stage in the guts of the tick host (Gonder, 1910) (Fig. 1). Immature stages of the tick detaches from the host mammal, leading to the final development of the parasite (Norval *et al*., 1992). A kinete which is formed in the zygote during moulting of the tick is liberated, which then invades the epithelial cells of the salivary glands and develops into a large syncitial sporoblast (Fig. 1). Thousands of elongated sporozoites develop from the sporoblast during the subsequent feeding stage of the tick (OIE, 2014; Radostits *et al*., 2006; Gonder, 1910). It is at this next feeding stage where multinucleated schizonts are formed from sporozoites liberated into saliva, penetrating and developing within lymphocytes after three days, and inoculated into parasitized animal (Lawrence *et al*., 1994; Norval *et al*., 1992) (Fig. 1).

Lymphocyte of parasitized animal (host) transforms into lymphoblast being a stimulation of the formation of schizonts which undergoes division in synchrony with the host cell as mitosis occur (Norval *et al*., 1992). At this time Schizont-infected cells are distributed in the lymphoid system after an exponential proliferation (Norval *et al*., 1992; Gonder, 1910). Metastases of infected cells to non-lymphoid tissues also occur. Schizonts have large chromatin particles initially and therefore called macroschizonts. This is called "Koch's body" and when stained with Romanowsky, it's called "Koch's blue body" (Afrivip, 2016; Vetbook, 2010; Lawrence *et al.,* 2005). The first line of diagnosis after clinical signs, involves microscopic examination of Koch's blue bodies in lymph node smears, which has limited chances of confirming the disease (CFSPH, 2009b).

Furhermore, with all the individual investigations carried out about Theileria, sporogony and piroplasm stages have been studied separately. No one has taken an overview of all

three stages. As *T. parva* undergoes the same or a very similar process three times during its life cycle it would seem reasonable to predict that the parasite might use the same mechanism to develop uninucleated cells containing a similar set of organelles from a syncytium. The only major difference between the three life cycle stages must be the appearance of different molecules on the cell surface which are related to the specificity of the subsequent host cell (Shaw and Tilney, 1992).
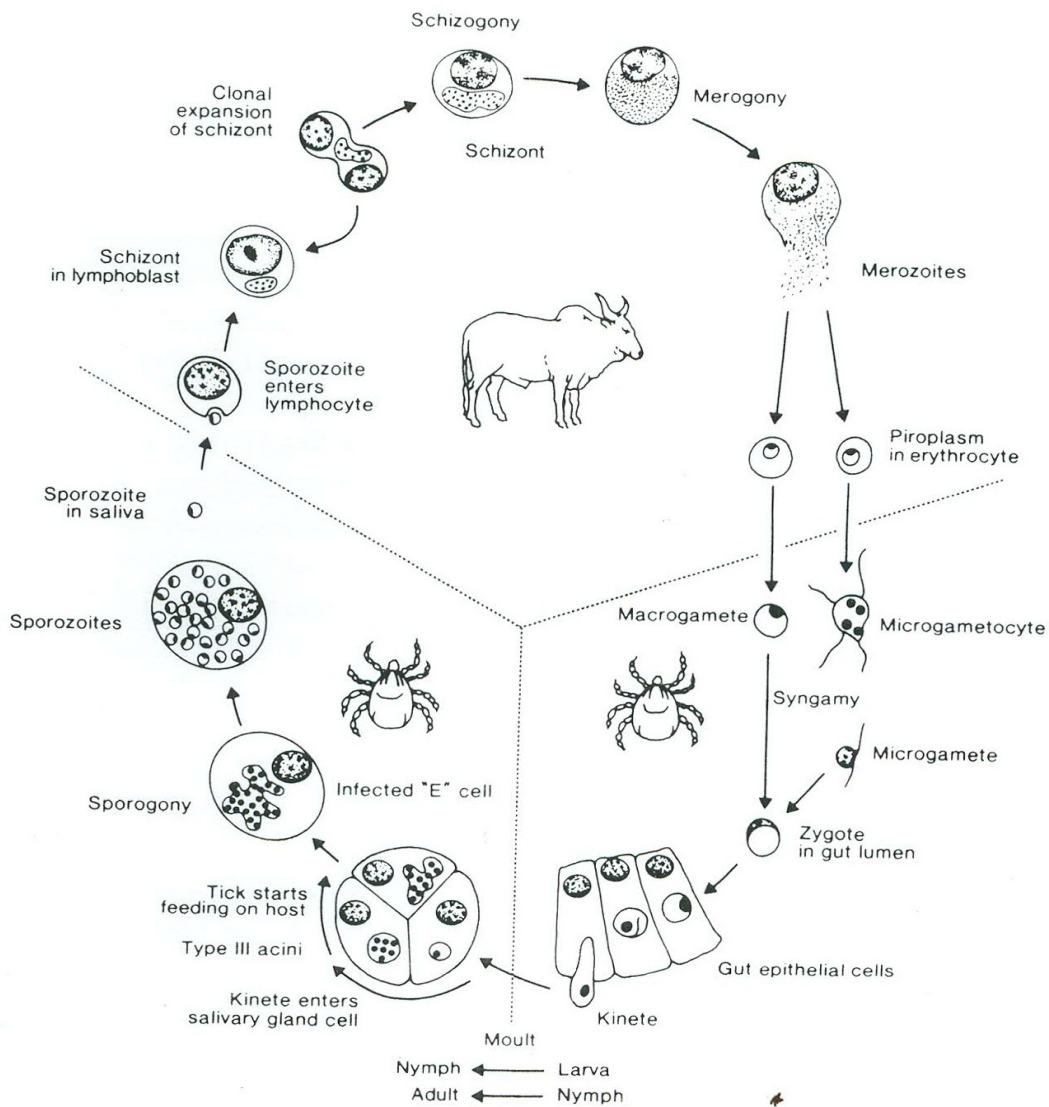
Figure 1: Life cycle of *T. parva* in the tick (*R. appendiculatus*) and Mammalian host. Source: Norval *et al.*, 1992.

2.3.4 The Vector *R. appendiculatus*

One of the few ticks in sub-saharan Africa noted for the transmission of very important vector diseases like ECF is *Rhipicephalus appendiculatus* (Madder *et al*., 2001). Hezron *et al*. (2012) report that *R. appendiculatus* is the most common tick affecting cattle production in Tanzania. It is a three host tick as it needs to feed on three host to complete its life cycle. A hard tick (Ixodid) and commonly called the brown ear tick, emanating from the fact that it is brown in colour and the adult forms are found in the ears of host animals (Madder *et al.,* 2001). It is the main vector for the transmission of *T. Parva* (Kazungu *et al.,* 2015b; Gachohi *et al.,* 2012; Bazarusanga, 2008; Mtambo, 2008; Olwoch *et al.,* 2008) which causes ECF or Corridor disease in cattle. Buffalo is the carrier of the parasite strain that causes Corridor disease in cattle (Grootenhuis, 1989). It is transmitted to cattle when there is interaction between cattle and buffalo when the latter stray out of wildlife reserves (Madder *et al*., 2001). Corridor disease occurs in Southern and East Africa especially areas where there is contact between cattle and infected buffalo with *R. appendiculatus*, *R.zambeziensis and R. duttoni* being the main vectors for the disease. According to Neitz *et al*. (1955), the disease was diagnosed in a corridor between Hluhluwe and Umfolozi Game Reserve in South Africa (Tarimo, 2013).

The cause of benign bovine theileriosis, *Theileria taurotragi*, bovine anaplasmosis, *Anaplasma marginale* and the Nairobi sheep disease caused by a virus (NSDV) are also transmitted by *R. Appendiculatus* (Morzaria, 1989). Some antelope species in wildlife parks near high rainfall areas are also affected with severe infestations. This tick also transmits *Rickettsia conorii to humans* (CFSPH, 2009b; Madder *et al*., 2001).

The trinomial system for the classification of *T. parva* parasites was proposed by Uilenberge (1976) and Lawrence (1979) as (i) *T. parva* for parasites causing classical

ECF, (ii) *T. parva* lawrencei for parasites causing Corridor disease and (iii) *T. parva* bovis for parasites causing January disease. However, this classification was abandoned as the molecular characterization and cross immunity data did not support the existence of these subspecies within the *T. parva* complex (Conrad *et al.,* 1987; Allsopp *et al.,* 1989).

2.3.5 Life cycle of *R. appendiculatus*

The life cycle of this Ixodid tick is completed on three hosts, warranting its classification as a three host tick (CFSPH, 2009b; Mtambo, 2008; Randolph, 1994). After hatching from the egg, the 3-legged larvae quest for the first host which are mostly small animals (hares, birds and rodents). They feed for a number of days and drop to the ground to digest the blood-meal for days to weeks, then moult into nymphs. The nymphs which are 4-legged repeat the process by questing for the second host. After feeding they drop to the ground before moulting into an adult tick. The adult (sexually mature males and females) quests again, get attached to the third host. They feed for several days and mate on the host. Males stay attached to host till it dies, while the females drops to the ground to lay a large batch of eggs and dies (CFSPH, 2009b; Radostits *et al.,* 2006; Randolph, 1994).

2.4 Vector Ecology

The ecology of vectors in this case ticks especially *R. appendiculatus,* is determined by abiotic and biotic factors (Latif and Walker, 2004). It depends on temperature variations, weather movements and geographical location (Mtambo, 2008). Abiotic factors like seasonally induced changes in habitat physiology and climatic indications, are all drivers of vector ecology (Mtambo, 2008). Furthermore, biotic factors such as the physiology and biology of the species, life cycle, host range and availability and competition within the species and with other species also shape the ecology of the tick (CFSPH, 2009b; Mtambo, 2008; Norval *et al.,* 1982). In Northern Tanzania where this study was carried

out, the ecological conditions for the distribution of *R. appendiculatus* exist (Madder *et al*., 2001). First of all biotic factors such as, birds, hares and small ruminants (sheep and goats) including antelopes serve as host in the life cycle of the tick. The area has a high cattle population and extensive farming system making animals accessible to questing ticks for parasitism exist. During the rainy season the required, temperature humidity and vegetation is available for the eclosion of eggs and moulting of larvae into the next stage of the life cycle paving way for the abundance of ticks (Olwoch *et al*., 2009).

2.5 Host of Theileria Parva

Species of animals infected by *T. parva* include *Bos indicus*, *Bos taurus* , African buffalo (*Syncerus caffer*), Egyptian buffalo (*Bubalus bubalis*) and Waterbuck (*Kobus defassa*) ((Mbassa *et al.,* 1998b OIE, 2008). Natural reservoir host of *T. parva* parasite is the African buffalo (*Syncerus caffer*) (Sibeko *et al*., 2011).Cattle may become infected in areas where the suitable tick species of *R. appendiculatus* and *R. zambeziensis* occur. However, the presence of the does not mean disease (Sibeko *et al*., 2011).Mortality can reach 100% in cattle that are copiously susceptible. Stunted growth in calves and low productivity in adult cattle as a result of chronic disease, are seen in recovered cattle (OIE, 2008).

Generally, resistant breeds to ECF are the Zebu (*Bos indicus*) or Sanga breeds. The Taurine breeds are susceptible (Kazungu *et al*., 2015). Cattle introduced into an endemic area whether Sanga, Taurine or zebu have a higher susceptibility in acquiring infections (Kazungu *et al*., 2015a). While the African buffalo and waterbuck are reservoirs of *T. parva* parasites (Githaka *et al* 2014), subclinical infections are only common in cattle and water buffalo (Kazungu *et al*., 2015a).

Largely wildlife species share a lot of multi-host pathogens with domestic animals (Walker *et al*., 2014). Various wildlife mammals and even reptiles play an important role in the life cycle of *R. appendiculatus* (Walker *et al*., 2014*)*. The other fact is, sharing of the ticks alone leading to high tick burden on cattle results in weak immune response to other infections (Anderson *et al*., 2013).

2.6 Techniques in the Diagnosis of *T. parva* infections

Diagnosis of *T. parva* and trypanosome infection are routinely done by observing clinical signs and symptoms presented by sick animals and confirmed with microscopy of blood and/or lymph node smears. However, most of the signs and symptoms are quiet similar in these two important vector infections that sometimes co-infect one host (Radostits *et al*., 2006). This may not lead to specific diagnosis for effective treatment and control measures (Cox *et al*., 2010; Thumbi *et al*., 2014, 2014b; Van Wyk *et al*., 2014; Woolhouse *et al*., 2015). Serological and molecular techniques have high specificity and sensitivity exist, but are normally used for epidemiological investigations or research purposes only (Young and *Groocock*, 2004; Altay *et al*., 2008; OIE, 2009; Mans *et al.*, 2015).

Indirect Fluorescent Antibody Test (IFAT) is recommended by the World Organization for Animal Health (OIE) as a Gold standard test to carry out Agent identification of most economically important parasites as stated in the OIE Terrestrial Manual (OIE, 1993; Mans *et al*., 2015).

2.7 Clinical Diagnosis of *T. parva*

Routine diagnosis of *T. parva* infections: Conventionally in endemic areas, animals with tick (*R. appendiculatus*) infestation, enlarged lymph nodes (especial parotid and pre-escapular) and a fever are suspected cases (OIE, 1993). Mortality is high amongst calves.

Average incubation period is 8 -12 days (OIE, 1993). Either thick or thin Giemsa stained blood is used to detect piroplasms or lymph node smears to detect schizonts (OIE, 1993). However, the presence of piroplasms alone should not be used to confirm a case as positive since piroplasms are present in healthy carrier animals (OIE, 1993). Detection of schizonts confirms a clinical positive case which is the pathogenic state of the disease as well (OIE, 1993). Conversely, this method is difficult to identify *T. parva* parasites specifically since there is morphological similarities of most *Theileria* parasites (Mans *et al.*, 2015).

Serological Diagnosis: Serological test are very important in *T. parva* infection diagnosis since infections are usually acute. It is also useful to detect the immune state of recovered animals to ascertain previous infection state and measure antibody levels (Young *et al.*, 2004). However, serological test in general are affected by false-positive and false negative results, caused by cross-reactions or weak specific immune responses (Altay *et al.*, 2008). Enzyme Linked Immunosorbent Assay (ELISA), IFAT, Immunodiffusion Test, Indirect Hemagglutination Assay, Complement Fixation, Capillary Tube Agglutination and Coagulation Test are some serological test that can be used for the diagnosis of *T. parva* infection in a herd of cattle (Tarimo, 2013; Mans *et al.*, 2015).

However, IFAT is the most used serological test in *T. parva* infection detection to measure antibody levels of natural infection and immunity of vaccinated animals (Young *et al.*, 2004). A setback for IFAT however, is the cross-reactivity of species that are closely related genetically (Mans *et al.*, 2015). ELISA test has > 99% sensitivity and specificity between 94-98% than IFAT. Other studies have found equally high sensitivity for both tests and only a higher specificity for IFAT (Muraguri *et al.*, 1999). Disadvantage of both test are, animals that are already free from the parasite will test positive. There is an

increase in the use of ELISAs in parasite-specific antibody detections, able to pick antibodies of longer period of time than IFAT (OIE, 1993). Yet another advantage is that, ELISA is fast and cheap sero-diagnostic method (workhorse) (Mans *et al*., 2015). Large numbers of samples can be screened in places especially where sophisticated techniques are absent (Mans *et al*., 2015).

Molecular Diagnosis: Polymerase Chain Reaction (PCR), Loop-mediated isothermal amplification (LAMP) assay, real-time polymerase chain reaction (RT-PCR), Luminex xMAP, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and restriction fragment length polymorphism (RFLP) are some molecular techniques used in the diagnosis and confirmation of infection of *T. parva*. (Hamburgo *et al*., 2011).

PCR has a sensitivity of about a 100% of gene specificity (Muhanguzi *et al*., 2014b; Odongo *et al*., 2010). It has the capacity to detect low parasitic levels in chronic cases (Muhanguzi *et al*., 2014b). Odongo *et al*. (2002) concluded that p104 PCR is more reliable and sensitive in current *T. parva* infections detections. For prevalence studies with a large-scale magnitude, p104 PCR combined with serology is essential for *T. parva* (Odongo *et al*., 2002).

The Loop-mediated isothermal amplification (LAMP) assay is an isothermal nucleic acid amplification technique which unlike PCR technology, the reaction is carried out with a series of alternating temperature steps or cycles and isothermal amplification at a constant temperature not require thermal cycler (Nagamine *et al*., 2002). Amplification products can be detected using photometry for turbidity caused by an increasing quantity of magnesium pyrophosphate precipitate solution as a by-product of amplification (Mori *et*

al., 2001). The biggest advantage of LAMP assays is that, it can function at isothermal conditions and its possible application under field conditions (Mans *et al*., 2015).

LAMP assays for the detection of *T. parva* have been developed which target the polymorphic immunodominant molecule (PIM) and p150 LAMP genes (Thekisoea *et al*., 2010). The primer set for each gene are highly specific for the detection of *T. parva* and can amplify DNA of *T. parva* isolates from cattle and buffalo from different countries including Tanzania (Thekisoea *et al*., 2010). This indicates their ability to detect *T. parva* from different countries. LAMP assays are good candidates for molecular epidemiology studies and for monitoring control programs in ECF-endemic because of their simplicity, rapidity and cost effectiveness (Thekisoea *et al*., 2010).

2.8 Control

2.8.1 Control and Treatment of *Theileria parva* infections

Several methods to control ECF and other tick borne diseases exist from; acaricide use to control vectors, immunization, fencing off animals from wildlife, pasture management, introduction and selection of tick resistant cattle and rotational grazing to minimize tick challenge level (Kasibule, 2007).

2.8.2 Immunization

Current trend is towards immunization of the cattle with the infection and treatment method (ITM) (Kasibule, 2007). ITM was developed from live *T. parva* sporozoites from spleens of infected animals. (Radley *et al*., 1975). Animals that recovered from natural infections developed immunity, so this method was applied based on the simultaneous use of sporozoite stabilate of the appropriate strain(s) of *Theileria* parasites derived from infected ticks and a single dose of long-acting Oxytetracycline injected into the animal

(D'Haese *et al*., 1999; Kasibule, 2007). A cocktail (Muguga Cocktail) of three parasite stocks (Kiambu 5, Muguga and Serengeti-transformed) is a trivalent vaccine stabilate extensively used in East Africa including Tanzania and other countries in the region (Musisi *et al*., 1992; GALVmed, 2015). A 50% cost reduction in the use of acaricide and a 2% decrease in annual mortality rate of calves amongst pastoralists is a benefit of this control method (Lynen *et al.,* 2006). However, most farmers see the vaccination method as disadvantageous because the risk of their animals still getting infected with ticks and suffering from other TBDs is high (Mukhebi *et al*., 1992). This is so because it is seen as expensive to vaccinate against ECF and still use acaricide (Kivaria *et al*., 2007).

Yet another factor that hinders the use of ITM is that, protection against other strains of *Theileria parva* parasites is limited with the use of one particular stock of *Theileria parva* stabilate for ITM (Irvin and Mwamachi, 1983; Radley *et al*., 1975). Steps are being taken to combine different strains of *Theileria parva* parasites to provide protection against different strains of *Theileria parva* parasites with one immunization (Odongo *et al*., 2006).

2.9 Vector Control of *Theileria parva* Infection

Controlling the vector of ECF is currently done with synthetic amidines and pyrethroids. These are used in dips, spray machines, as 'spot-on' or 'pour-on.' Most commonly used acaricides today belong to the synthetic pyrethroid group.and few organophosphorus (Stoltsz, 2013). It is reported that the frequent use of acaricide is becoming less popular due to the rapid development of resistance by ticks (Rajput *et al.*, 2006; Stoltsz, 2013; Vudriko *et al*., 2016). The frequency of using acaricide increases with a high challenge of ticks. However, its use on weekly basis is of common practice by farmers which leads to increases in cost of production, not sustainable by most pastoralist Consequentially, reduced application sets in, which is then seen as an advantage in the epidemiology of the

disease (Rajput *et al*., 2006). Epidemic instability leading to a high proportion of animals in the population at risk of infection is reduced. More so the high level of acaricide exposure results in residues in meat and milk, environmental pollution and very importantly, resistance of vectors.

One other important factor is the selection and keeping of tick resistant cattle (Tarimo, 2013). Biological tick control methods are also implemented by cattle owners. Physical hand picking and destroying ticks is as well a method (Tarimo, 2013). It is recommended to dip animals every 14 days in place of the weekly dipping because of the long residual activity of pyrethroids. (Mbassa *et al*., 2009a).

2.9.1 Grazing method

Another control method is preventing cattle from grazing close to wildlife sources of infection (infected buffalo), by separation of grazing areas (FAO, 2016). The practice of rotational grazing is also implemented to reduce cattle interaction with tick challenge (AfriVip, 2016) as well as reducing the stocking density to decrease number of host animals on pastures.

2.9.2 Chemotherapeutics

Treating *T. parva* infection to cure ECF is successful when treatment with Theilericidals is carried out in the very early stages of the infection (Radostits *et al*., 2006). When the infection advances to affect the hematopoietic and lymphoid tissues then treatment becomes less effective and consequentially, host animals develop carrier state (OIE, 2009). Gachohi *et al*. (2012) reported that tetracycline antibiotic was the first medication to be used as chemotherapy. More advanced compounds like Buparvaquone (Butalex by Coopers Animal Health, UK) which is a second-generation hydroxynaphthoquinone

related to parvaquone, but reported to last longer in plasma than the latter. These drugs ensure the survival of cattle with clinical *T. parva* or *T. annulata* infection. However, there is complete eradication of theilerial infections with these drugs which leads to the development of carrier states in their hosts. If these drugs are used in the early stages of clinical disease, they are highly effective but less effective in advanced stages of clinical disease due to fact that lymphoid and hematopoietic tissues are extensively destroyed (Sibeko, 2009).

2.9.3 Integrated control method

An effective and rational control method such as selection of animals resistant to ticks, the strategic application of acaricide, adoption of new vaccination methods, rotational grazing and effective pasture management are methods to control ECF (Young *et al*., 1988). They further argued that any single one of these methods may not be efficient enough to control ECF or other TBDs. It is stated by Radostits (2006) that elsewhere in Zimbabwe the sole reliance on acaricide use led to high mortality and morbidity in calves. For example Lawrence *et al.* (1996) recommended an integrated control method to ECF in especially crossbred cattle in endemic areas.

2.10 Endemic Stability and Carrier State

When there is ecological balance between the hosts, the parasite, the vector and the environment coexisting without clinical disease. It is termed as endemic stability (Norval *et al*., 1992). Coleman *et al.* (2001) described it as an epidemiological state with low clinical disease despite high infection levels in the population. For this to happen a high number of infected ticks are required to infest the animals at a young age (before 9 months) to ensure exposure to parasites before colostral and innate immunity wanes off (Kivaria *et al.*, 2004). This high levels of immunity consequently reduces the prevalence

of the disease in older animals. In place of this, endemic instability occurs causing high disease incidence in the population (Kivaria *et al.*, 2004). On the other hand, Hay (2001) reports that the requirements for endemic stability are, the probability of severity of clinical disease resulting from infection increases with age; and that after first infection, the chance that subsequent infections result in clinical disease is reduced (Kivaria *et al.*, 2004).

Recovered cattle from infection remain carriers of the parasite (Mbassa *et al.*, 2009b). A persistent carrier state in cattle is maintained after a recovery from primary infection *of T. parva* (Odongo *et al.*, 2002). The carrier state is important in the maintenance of the life cycle by alternate tick/cattle challenge. Maintenance of immunity is contributed to by both the host and the vector (Odongo *et al.*, 2002).

2.11 Population, Distribution and Seasonal Dynamics

Population dynamics of the vector for ECF is considered to vary considerably in different locations (Young *et al.*, 1994). Three generations of the vector can occur in a year with infestation of host throughout (Young *et al.*, 1994). On another part, only one generation of *R. appendiculatus* occurs due to prolonged dry seasons in the drier parts of East Africa. Activity of the tick is truncated hence. Consequently, *T. parva* transmission can be seasonal in its dynamics (Young *et al.*, 1994). This indicates that the seasonal dynamics and distribution of *R. appendiculatus* is controlled by climate variability which also influences the transmission of *T. parva* in its seasonal dynamics (Young *et al.*, 1994).

Distribution of ECF and *T. parva* infections are directly related to the distribution of the tick (Olwoch *et al.*, 2008). The distribution range encompasses the south from southern Sudan to eastern South Africa throughout to the Democratic Republic of Congo (DRC)

(Olwoch *et al*., 2008). Some factors known to determine the distribution of the vector, culminating in determining the distribution of the parasite itself, are climatic conditions, vegetation and host availability (Lawrence, 1991). Ticks are abundant in tropical areas as well as East Africa, where the problem is attributed to sharing of pastures between domestic and wild animals including communal pastoral grazing of livestock (Kadie *et al*., 2013).

The distribution of ECF is same as the vector that transmits the causative agent. Local cattle breeds in endemic areas are less susceptible than exotic breeds (Kazungu *et al*., 2015a).

2.12 Animal African Trypanosomosis

AAT is caused by several species of *Trypanosoma,* namely *T. vivax, T. congolense, T. brucei, T. simiae,* and *T. evansi* (Radostits *et al*., 2006) which are protozoan parasites. These parasites are transmitted is by the vector, tsetse fly causing heavy losses to the livestock sector (Nakayima *et al*., 2012; OIE, 2013; Radostits *et al*., 2006). However, *T. evansi* is transmitted mechanically by haematophagous flies such as *Tabanus spp*. and *Musca spp.* Vectors such as *Lyperosia*, *Stomoxys* and *Atylotus genera* with Tabanids (Horse flies) being the most important vectors (Nakayima *et al*., 2012; OIE, 2013). Species of *Trypanosoma* such as *T. brucei gambiense* and *T. brucei rhodesiense* have of public health importance as they cause human African trypanosomosis (HAT) (Madsen *et al*., 2013; Nakayima *et al*., 2012; OIE, 2013). Cattle are affected by *T. vivax, T. congolense and T. brucei* (Radostits *et al*., 2006). Nagana, dourine (Equidae) and surra (Camels and Horses) are names associated with the diseases caused in different animal species. Nagana is unquestionably the most important economically as it causes reduced meat and milk production and draught power for agricultural production (WHO, 2016b).

2.12.1 The Trypanosoma parasite

Trypanosome species are flagellate and belong to the subkingdom protozoa, genus Trypanosoma from the order Kinetoplastida, and family Trypanosomatidae (OIE, 2013). Furthermore, trypanosomes are divided into the salivarian, transmitted by the tsetse fly by invading blood plasma, lymph and other tissues of an infected host (OIE, 2013), and the stercoraria, that are transmitted by contamination of mucous membranes, wounds and faeces of infected vector.

Vertebrates including man and animals are affected by different species of trypanosomes. Cattle rearing and agricultural development as well as human welfare are affected by a number of *Trypanosoma* species and subspecies on the African continent with high negative economic impact and significance (WHO, 2016a). Insect vectors transmit most of these species.

Sub-species such as *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, cause human African trypanosomosis (HAT) (WHO, 2016b). Domestic and wild animals are possible host of these trypanosomes and could pass on infection to tsetse flies. *Trypanosoma cruzi* is not found on the African continent but causes Chagas disease, a devastating human disease in The Americas (WHO, 2016b).

2.12.2 Life cycle of *Trypanosoma spp*

An infected tsetse fly of genus Glossina, injects metacyclic trypomastigotes into the skin of a mammalian host during a blood meal. A chancre is formed and parasites invade the lymphatic system, eventually into the blood stream. Once inside the host, they transform into blood stream trypomastigotes, invade body lymphatic and spinal fluids. Binary division occurs as a form replication. Procyclic trypomastigotes are formed by binary

fission in the fly's midgut from where they exit and transform into epimastigotes. Furthermore, multiplication by binary fission continues in the salivary glands. In the fly, the cycle takes 3 weeks.

Life Cycle of Trypanosoma rhodesiense
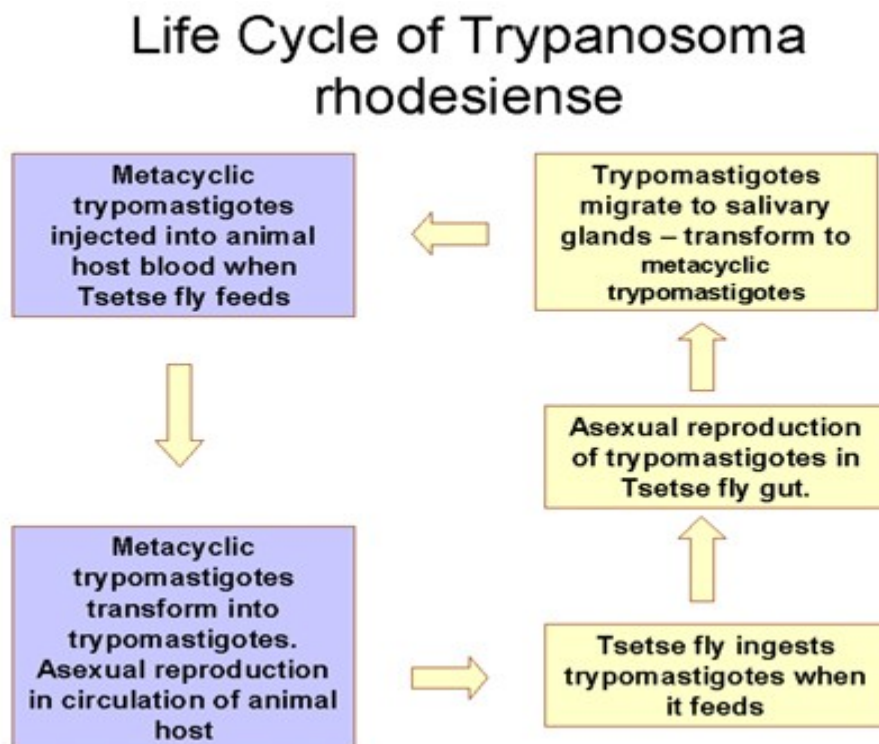
Figure 2: Life cycle of *Trypanosoma brucei rhodesiense*

Source: www.microbiologybook.org/lecture/images/rhodesiense.jpg
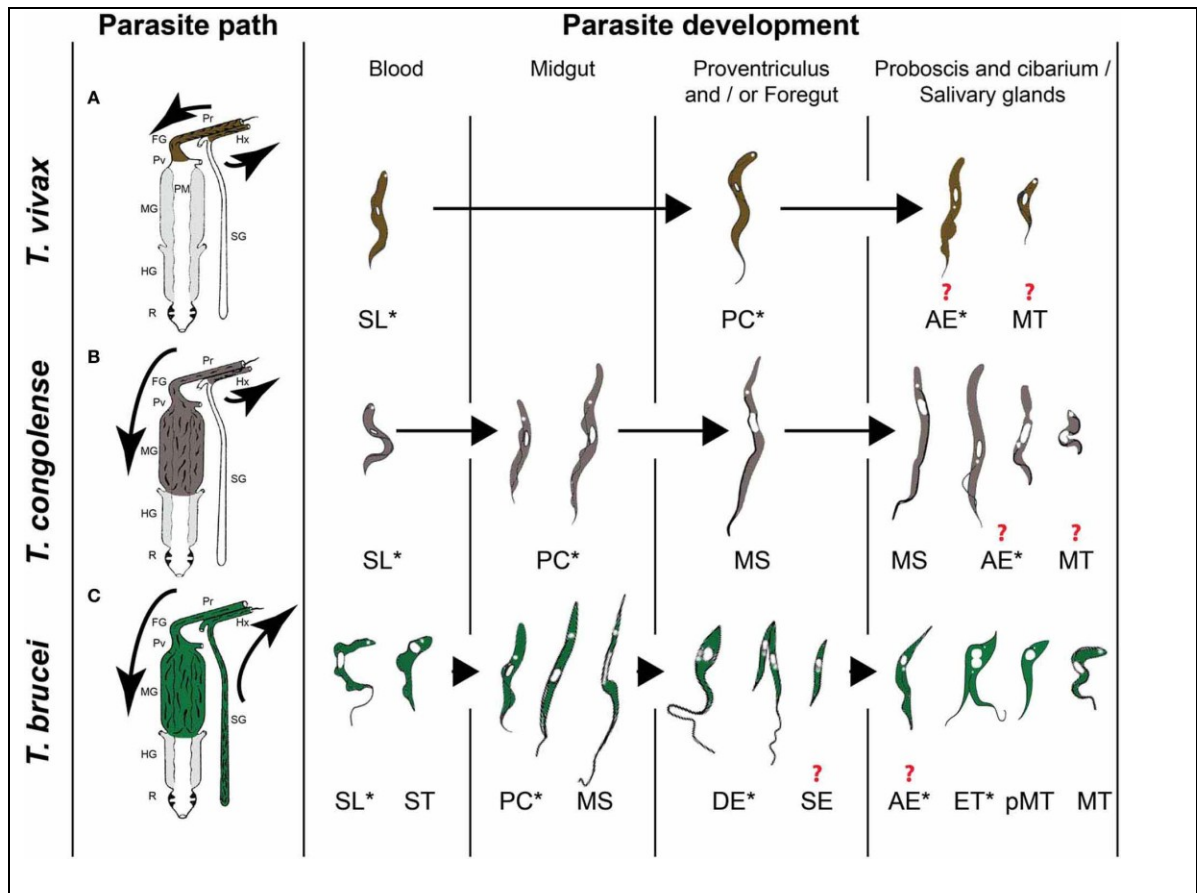
Figure 3: The three types of African trypanosomes development in the tsetse fly.

Source:*www.journal.frontiersin.org/article/10.3389/fcimb.2013.00053/full*

Note: (A) *T. vivax* group. (B) *T.congolense* group. (C) *T.brucei* group. Parasite paths in the tsetse digestive tract are schematically presented in the left panel [adapted from (Hoare, 1972)]. Successive parasite stages found in the different organs are presented in a chronological order in the right panel [adapted from (Hoare, 1972; Peacock *et al.,* 2007, 2012; Rotureauetal., 2012)]. ∗ indicate proliferating stages and? Indicate an uncertainty with respect to the type of division and/or the transitional forms involved at this stage of development. Pr: proboscis, FG: foregut, Pv: proventriculus, PM: peritrophic Matrix, MG: midgut, HG: hindgut, R: rectum, Hx: hypopharynx, SG: salivary glands, SL: slender trypomastigote, ST: stumpy trypomastigote, PC: procyclic trypomastigote, MS: mesocyclic trypomastigote, DE: long dividing epimastigote, SE: short epimastigote, AE: attached epimastigote, ET: epi-trypo dividing epimastigote, pMT: pre-metacyclic trypomastigote, MT: metacyclic trypomastigote

2.13 Clinical signs

A relapsing fever is noticed as a sign of trypanosomosis which appears 11-21 days after an infective bite (tsetse.org, 2012). The increase in the number of trypanosomes circulating in blood causes temperatures to peak (tsetse.org, 2012). This is preceded by the destruction of large numbers of the parasites and a reoccurrence of a normal temperature. Crisis occurs at the end of the time of parasite destruction when antibodies are being produced and large quantities of trypanosome protein are liberated into the bloodstream (tsetse.org, 2012). Death coincides with this period this crisis period. It occurs in between one to three months in locations commonly affected by frequent reinfection, if animals are not treated with trypanocides (Radostits *et al.*, 2006) (tsetse.org, 2012). Other signs apart from the intermittent fever are; swollen lymph nodes, diarrhoea, lacrimation and sometimes neurological signs (Acha and Szyfres 2006; CFSPH, 2009a). This leads to abortions and sometimes premature births. Reduced growth rate that affects beef and milk production are observed. But most cases are chronic, acute cases are fatal within 7days (CFSPH, 2009a; OIE, 2013).

2.14 The Vector Tsetse fly

There are three groups (Morsitans, Palpalis and Fusca) of tsetse flies with distinct ecological and behavioural characteristics, comprising 29 species and subspecies of tsetse flies are the main vectors of trypanosome transmission(De Denken, 2009; Phelps and Lovemore, 2004). The Morsitans group occurs in savannah woodlands across sub-Saharan Africa (De Denken, 2009; Phelps and Lovemore, 2004). They employ a combination of visual and olfactory stimuli to locate their hosts and are extremely mobile. The species in this group are the more important vectors of animal trypanosomosis comprising, *G. pallidipes*, *G. morsitans* subspp, *G. longipennis* and *G. austeni*. The Palpalis group occurs

largely in riverine woodland of west and central Africa (De Denken, 2009; Phelps and Lovemore, 2004). They are less mobile, so are confined to riverine habitats. Visual cues are used to locate their mammal hosts. Vectors of human sleeping sickness such as *G. fuscipes* and *G. palpalis* subspp are in this group. Generally considered as less important as vectors, is the Fusca group found in humid forests which are minimally used by livestock (De Denken, 2009; Phelps and Lovemore, 2004). However, *G. brevipalpis* has been reported as a vector of animal trypanosomosis.

2.15 Techniques in the Diagnosis of *Trypanosoma spp.* Infections

2.15.1 Clinical diagnosis

Clinical diagnosis is not straight forward since the disease has no pathognomonic signs. Moreover, acute incidences occur for a few days then become chronic after some few weeks, or become subacute or die (Radostits *et al*., 2006). Generally, the clinical picture is determined by the level of tsetse challenge, the species and strain of the trypanosomes, the breed and management of the host (Radostits *et al*., 2006). Trypanosomiasis should be a consideration in endemic areas when an animal is anaemic and in poor condition. Animals imported from these areas can be subclinical carriers and may become ill when they are stressed (CFSPH, 2009a).

Chronic cases may run a steady course, may be interrupted by periodic incidents of severe illness, or undergo spontaneous recovery (Radostits *et al*., 2006). After an incubation period of 8-20 days then basic clinical syndrome manifests with a long lasting intermittent fever. Animals become severely emaciated and cachectic, and death may occur with 2-4 months (Radostits *et al*., 2006). Dullness, anorexia, apathy, watery ocular discharge and lose of condition are observed (Radostits *et al*., 2006). Clinically useful are also swollen superficial lymph nodes, pale mucous membranes and oedema of throat. Oestrus cycles

become irregular, pregnant animals may abort (Radostits *et al*., 2006). However, other parasitic, viral and bacterial infections could mask or complicate the clinical syndrome of trypanosome infection (Radostits *et al*., 2006).

Commonly, *T. congolense* is more pathogenic to cattle in eastern and southern Africa, whereas *T. vivax* causes a more serious disease in West Africa. However, exotic dairy animals in East Africa have suffered severe outbreaks of *T. vivax* with signs like mucosal petechiation, rhinorrhagia, dysentery, and death after a few weeks. Mixed infections are common and are usually more severe (Radostits *et al*., 2006).

2.15.2 Routine diagnosis using direct examination technique

Examination of wet, thick or thin Giemsa stained fresh blood from either the jugular vein, the tail or from the ear vein are the simplest techniques (OIE, 2013). The stained thin blood films are noted as more specific but are less sensitive than the rest. Experience and skills of the Microscopist, the volume of blood for examination have a bearing on the actual specificity and sensitivity of these techniques (OIE, 2013). Diagnostic sensitivity can be increased significantly by concentrating the parasites prior to examination in combination with a phase-contrast or dark-ground microscope. The centrifugation parasite concentration techniques (Haematocrit Centrifuge Technique, HCT) have the added advantage that the packed cell volume, and hence the level of anaemia, can be determined at the individual animal and/or herd level (OIE, 2013).

2.15.3 Serological techniques

Serological test mostly used are the indirect immune fluorescent antibody test (IFAT), the capillary agglutination test (CAT), and the ELISA to detect anti-trypanosome antibodies in either sera or body fluids (OIE, 2013). A disadvantage is that they indicate past as well as

present infections, apart from being difficult to standardize for different laboratories, and are not species specific (OIE, 2013). The ELISA technique has been modified to detect circulating trypanosomes antigens (antigen-ELISA) using monoclonal antibodies that would differentiate *T. vivax, T. congolense*, and *T. brucei*, and also detect only current or very recent infections (OIE, 2013). Other parasitological techniques are required in order to get reliable results. An observation based field trials in Africa and the Caribbean indicated that the test is not sufficiently sensitive or specific (OIE, 2013).

2.15.4 Molecular techniques

Molecular techniques involving PCR technique can be used to detect trypanosomes DNA in tsetse tissues and in host animals' blood (OIE, 2013). It can be used for surveillance and monitoring of therapy experimentally due to the fact that treated animals become negative to PCR, 1 to 2 days after treatment (OIE, 2013). The test is sensitive and species specific and can be combined with ELISA (Odongo *et al.,* 2010; OIE, 2013) . It is also possible to do retrospective epidemiological survey using serum banks (OIE, 2013). Dried blood spots on filter papers are also a useful source of DNA for the detection of   *T. congolense* and *T. brucei* by PCR. But the test is expensive and can only be done in specialized laboratories (OIE, 2013).

The LAMP technique has been successfully used to develop assays for both human and animal trypanosomiasis, for example to test for *T. evansi* type B. The assay is highly sensitive and specific in addition to being rapid with results being obtained within 20–25 min using a real time PCR machine (Njiru *et al*., 2010).

2.16 Control of Trypanosomosis

In enzootic countries, trypanosomosis is controlled through, the control of tsetse fly population, prophylactic treatment, good husbandry of animals at risk and the use of

trypanotolerant animals (Radostits *et al*., 2006). Vaccination is not an option, at least not now as no vaccine has been found against this devastating disease despite exhaustive research (Radostits *et al*., 2006). This is so because of the ability of trypanosomes to readily change their glycoprotein surface coat through a process called antigenic variation (Radostits *et al*., 2006).

In Tanzania, tsetse fly control commenced before independence (Malele, 2011). They included total or partial clearing of vegetation (habitat for tsetse flies), environmental degradation such as drought and soil erosion were some challenges that affected the control efforts with Shinyanga and Mwanza being severely affected (Malele, 2011). Destruction of Host of trypanosomes especially wild animals (reservoir for trypanosomes) led to the control of passing infection from wild animal host to domestic animals and man. This method which was widely used in East and central Africa is no more practiced due to animal welfare issues (Malele, 2011).

2.16.1 Tsetse control

In many African countries, tsetse control has been successful except for reinvasion due to lack of improper utilization of the land in areas that have been initially cleared of infestation (Malele, 2011; Radostits *et al.,* 2006). More recently, aerial and ground spraying over large stretches of land using insecticides such as dichloro-diphenyl-trichloroethane (DDT) and endosulfan have been done elsewhere (CFSPH, 2009a; Malele, 2011; Radostits *et al*., 2006). Tsetses are sensitive to insecticides and resistance has been reported. Conversely, spraying insecticides is costly and harmful to the environment (Radostits *et al*., 2006). These harmful effects are considerably reduced if the insecticides (synthetic pyrethroids) are used topically as spray or pour-on (Bradberry *et al*., 2005). It has the advantage of also reducing tick infestation on treated animals thereby helping

combat other vector borne infections. The use of insecticide impregnated targets and traps which are easily constructed and maintained at village level, is also aimed at catching tsetse (Malele, 2011; Radostits *et al*., 2006). Additionally, they do not pollute the environment and are suitable for both small and large scale use. These control measures have been used elsewhere in west Africa with success at reducing tsetse population (Radostits *et al*., 2006).

There is the use of cheaper and very simple devices for tsetse control such as the suspended blue and black cloth screens (tsetse target), impregnated with biodegradable pyrethroid insecticide (deltamethrin) (Malele, 2011). The blue colour attracts the tsetse to the screen with addition of octanol and acetone odours (Malele, 2011).

2.16.2 Biological method

Sterile Insect Technique (SIT): Sterile tsetse males are released in a systematic and sustained manner to mate with target population (indigenous) in a specific area where other methods have been used to reduce the density of tsetse fly (Malele, 2011; Radostits *et al.*, 2006). The female tsetse fly mates once in a lifetime, therefore this technique theoretically eradicates targeted tsetse species (Radostits *et al*., 2006). Apart from *G. f. fuscipes,* the other females species remain infertile throughout their life span after mating with sterile male insects (Malele, 2011).

Animal breed: The use of trypanotolerant animals to establish farms in locations where there is high challenge of tsetse is available, for example the west African Shorthorn and the N'Dama from central Africa (FAO, 1998). However, their small size and low milk production as compared to other indigenous breeds and crosses with exotic breeds makes them not the best choice in some countries (FAO, 1998; Radostits *et al*., 2006).

2.16.3 Prophylaxis

In areas where there is a heavy tsetse challenge, prophylaxis is used along with other methods (Radostits *et al*., 2006). Chemotherapy with isometamidium (samorin) suramin and prothridium are used in treatment and prevention (Delespaux, *et al*., 2005). The development of antibodies supplements the prophylactic effect of chemotherapy with a period of protection which could last for 5 months (Radostits *et al*., 2006). Conversely, four or five treatments per year are customarily given which is well responded to if done in consonance with good husbandry practices (Radostits *et al*., 2006). This approach however led to drug resistance in many countries (Van den Bossche and Rowlands (2001; Van de Bossche *et al*., 2006).

2.16.4 Integrated approach

An integrated approach is the most likely method of tackling the control of trypanosome infection in Africa, central and south America (Radostits *et al*., 2006) Since vaccines are not available, the combination of factors such as, reducing exposure to the vectors, for example the use of large scale tsetse trapping and pour-on applications. Strategic treatment of exposed animals with chemotherapy and chemoprophylaxis and the use of trypanotolerant animals are some of the control methods (Radostits *et al*., 2006). Malele, (2011), indicated that tsetse control and eradication is best approached with integrated technologies and the inclusive participation of stakeholders. Furthermore, Malele (2011) stated that in Tanzania, the strategic key stakeholders are the Ministries responsible for Livestock, Agriculture, Natural resources, Local Government, Gender and Children, Lands and Planning, Education, Research Institutes, private sectors and community based organisations (CBOs) and non-governmental organisations (NGOs) inclusive. The tsetse belt is described as Trans-boundary and it is therefore important that all countries cave out control and eradication programmes in consonance with the ones stipulated (Area-Wide

and Sustainable Approach) in the Pan African Tsetse and trypanosomiasis Eradication Campaign (PATTEC) Initiative (Malele, 2011). Tanzania ratified the declaration number AGH/Dec.156 of the 36[th]Summit of AU Heads of States and Government which took place in Lome Togo in July 2000 to implement the PATTEC objectives (Malele, 2011).

2.17 Ecology and Seasonal Dynamics

The Equatorial regions of Africa is where animal trypanosomosis causes serious production and economic loses (CFSPH, 2009a; Malele, 2011; Radostits *et al*., 2006). It occurs in most parts of the tropical and subtropical regions of the continent (the tsetse belt) between latitude 15º North and 29º South, where it spreads from the southern edge of the Sahara desert to Zimbabwe, Mozambique and Angola (Franco *et al*., 2014). However, some *Trypanosoma spp* which are transmitted by mechanical vectors can be found outside the "tsetse fly belt". For instance *T. vivax* occurs in the Caribbean, Central and South America through mechanical transmission (CFSPH, 2009a; Luiza *et al.,* 2008). This distribution determines the epidemiology of the disease and its importance (Radostits *et al*., 2006).
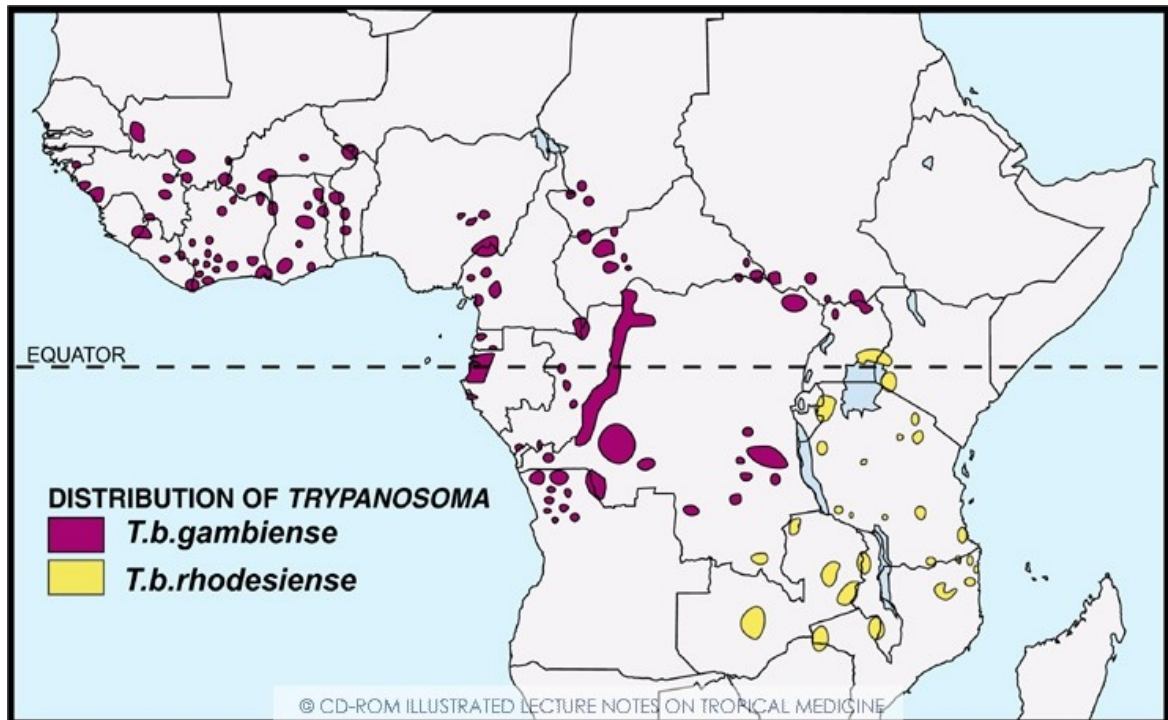
Figure 4: A map of Africa showing the distribution and African trypanosomosis in West and East Africa

Source: http://www.med-chem.com/para-site.php?url=org/ass

2.18 Environment, Climate Change and the Epidemiology of Vector borne Infections

Fossil fuel and land use by man are drivers of climate change (Githeko *et al*., 2000), leading to increased atmospheric concentrations of greenhouse gases- carbon dioxide, methane and nitrous oxide increase (Dhiman, 2000; Githeko *et al.,* 2000). Consequently, earth's surface and atmospheric surface become warm (Githeko *et al*., 2000). Climate change affects the epidemiology of vector-borne infections as a result of changes in the life cycle and distribution of the vector and the pathogen that it transmits (Tabachnick, 2010).

Interaction between pathogens and vectors as well as hosts, is also affected (Tabachnick, 2010). For instance the biology and ecology of vectors and their intermediate host is influenced by temperature, humidity and precipitations (Githeko *et al*., 2000; Patz *et al*.,

2008). It is estimated that by 2100, global temperatures will rise as a result of climate change by 1.0- 3.5°c (Githeko *et al*., 2000). Humidity and precipitations will also rise, increasing the transmission of vector-borne infections (Githeko *et al*., 2000).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

The study was conducted in 10 villages in the Monduli district (3º20'S, 36º15'E) of Arusha region in Northern Tanzania (Table1). Monduli district is a very important wildlife-domestic animal-human interface area (Haji *et al*., 2014). The arid and semiarid areas of the district which covers its larger part was covered for the study. Predominantly, pastoralism and extensive farming systems (communal grazing) are practiced in the district.

Table 1: Selected 10 Villages of Monduli district for the study of the Prevalence of *T. parva* and trypanosome infection during the dry season of 2015

S/N	Village Name	No of Selected Farmers	Sample size
1	Meserani Bwawani	12	48
2	Lepurko	12	48
3	Esilalei	12	48
4	Naiti	12	48
5	Mbaash	12	48
6	Eluwai	12	48
7	Oltukai	12	48
8	Lossimingori	12	48
9	Idonyonado	12	48
10	Engaruka Chini	12	48
	Total	**120**	**480**

3.1.1 Selection of Study Area

Monduli district (Fig. 5) (Arc GIS 10.3 version, 2015) was selected for: being homeland to many pastoralist and agro-pastoralist, whose livelihood depends on livestock keeping. The district is also in an area that experiences relatively high levels of vector-borne diseases such as ECF and nagana, and experiences effects of climate change and repetitive periods of erratic rainfall, increased temperatures, and in recent years it has been affected by droughts and floods.
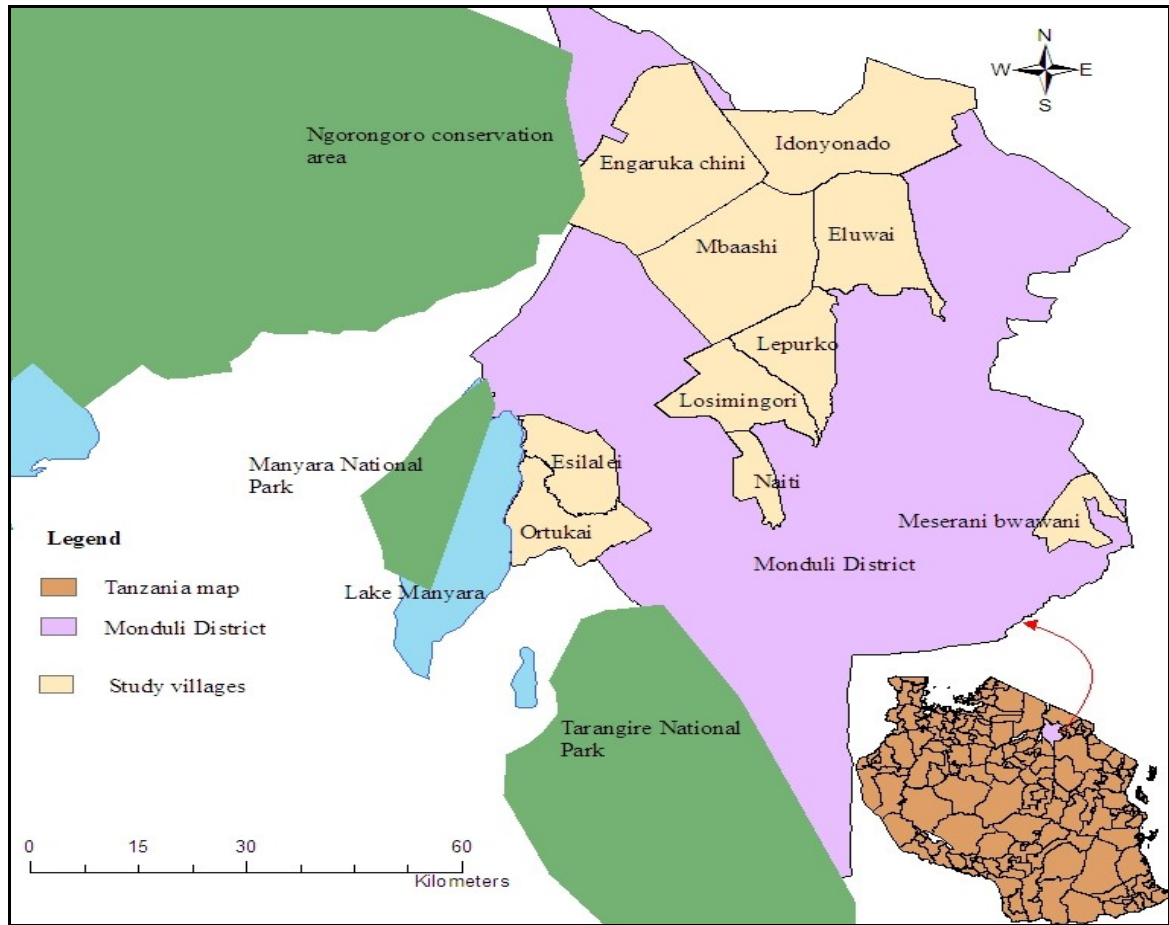
Figure 5: Map of Monduli showing study villages

Source : Arc GIS 10.3 version (2015)

The district is in an area that has a Savannah climate, modified by altitude and experiences two distinct wet periods: (1) the short wet season (referred to as Vuli in Kiswahili) happening from November to December and the long wet season (referred to as Masika in Kiswalhili) from March to May (Sindato *et al.*, 2013; Kabanda and Jury, 1999). In between June and October is the dry season (season of importance for this study).

The amount of rainfall in these seasons is usually 50-20mm per month but varies greatly between regions and can be as much as 300mm per month in the wettest regions and seasons. The climate ranges from semi-arid to sub-humid, with an annual rainfall of 400mm-1000mm. The most well documented oceanic influences on rainfall in this zone is the El Niño Southern Oscillation (ENSO).

3.1.2 Selection of villages

Ten villages located in the previously described Agro-ecological Zone (AEZ) were randomly selected (Table 1). A list of Pastoralists villages were identified in collaboration with Monduli District Veterinary Officers, where all villages were assigned numbers. Small pieces of paper with numbers folded and placed into a closed box and mixed up by shaking for 3-5 minutes. Four participants picked the pieces of paper till all ten villages were selected.

3.1.3 Cattle survey

Sampling frames of cattle owners for each village were obtained from the village executive officers in collaboration of livestock officers. The sampling frames structure was determined according to the number of sub-villages for the selected villages.

Microsoft Excel 2010 was used to randomly select 12 cattle owners of each village. Leaders of selected villages were informed of the selection and invitation letters sent to all 12 cattle owners in each village with participant's information.

3.2 Data Collection

3.2.1 Sampling procedures

This study involved a cross sectional study design carried out to collect data during the dry season period of August to October 2015. A cross sectional study allows collecting data at one point in time Ben-Shlomo *et al*. (2013). Blood samples from cattle were collected to estimate levels of infection of *T. parva* and trypanosomes at different agro-ecological zones. The investigation of two-stage sampling was used to select animals for blood collection. Initially, villages were stratified according to agro-ecological zone (altitude, climate and agricultural activities). This included semi-arid/arid and sub-humid highland.

Stratification of the study area increases precision for the investigation of disease variations according to locations for cattle vector-borne diseases, and this methodology has been reported elsewhere (Gitau *et al.*, 1997). Furthermore, stratification of villages improved precision and made the survey easier to accomplish. Proportional sampling was implemented in relation to number of villages in each agro-ecological zone and livestock production systems. Leaders and community based animal health workers of the 10 selected villages provided a full list of herds and livestock farmers from each village.

3.2.2 Sample size

The sample size was determined using the following following (Thrusfield, 2005).

$$n = \frac{1.96^2 \times p \times (1-p)}{L^2}$$

where 1.96 is the z Value for the desired confidence level (95%) in the normal distribution, p is the estimated probable prevalence and L is the level of precision (tolerable error) . The antibody prevalence of ECF and nagana in the district is unknown, so 50% prevalence and a 5% tolerable error have been assumed. Using this formula, a sample size of 384 cattle from the district was determined and adjusted to 480 to cater for possible accidental loses both in the field and in the laboratory. In order to ensure that each animal in a village had the same probability of being selected, 64 animals were randomly selected from each village sampling frame. Then individual animals for blood collection from the selected farmer/ herds also randomly selected using a simple random approach.

3.2.3 Blood sample collection

An amount of 10ml of blood was collected from cattle of all age groups, ranging from eight-week calves to adults (Kivaria *et al.,* 2004). Age of the animals was determined by

animal dentition and farmer-derived information (Deem *et al*., 1993; Kivaria *et al*., 2012). Due to the limited effectiveness of parasitological examination for disease diagnosis, molecular diagnostic techniques were carried out for accurate detection of parasite prevalence. However, lymph node biopsy was performed on animals that were examined and confirmed sick with swollen inflamed lymph node.

Blood samples were collected in vacutainer EDTA tubes, labelled, stored and transported in ice boxes and then finally stored in freezers (-20ºC) in the laboratory until day of analysis. Apart from the technique having the capacity to detect low parasitic levels in chronic cases, it is considered best in sensitivity compared to other parasitological techniques.

3.2.4 Laboratory analysis

The genomic DNA was extracted from 200µl whole blood using the protocol as described in Thermo Scientific Gene JET Genomic DNA Purification Kit (#0721). Initially 400µl of Lysis Solution and 20µl of Proteinase K Solution was added to 200µl of whole blood and mixed thoroughly by vortexing for uniform suspension. The sample was then incubated at 56°C with occasional vortexing for 10 min to achieve complete lyses of cells. A volume of 200µl of ethanol (96-100%) was added and mixed by vortexing. The lysate was then transferred to a GeneJET Genomic DNA Purification Column placed in in a collection tube. The column was centrifuged for 1 min at 6000 x g. The collection tube containing the flow-through solution was discarded. The GeneJET Genomic DNA Purification Column was then placed into a new 2 ml collection tube and 500µl of Wash Buffer I added (with ethanol added). It was centrifuged for 1 min at 8000 x g and the flow-through discarded and purification column placed back the into the collection tube. At this point, 500µl of Wash Buffer II (with ethanol added) was put added to the GeneJET Genomic

DNA Purification Column and centrifuged for 3 min at maximum speed (≥12000 x g). An elution Buffer of 200µl was added to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. It was then incubated for 2 min at room temperature and centrifuged for 1 min at 8000 x g. Extracted DNA was diluted in 100µl dilution buffer and then stored at −20∘C until further analysis was carried out.

3.2.5 Polymerase Chain Reaction

The nested p104 PCR was implemented to screen cattle DNA samples for the presence of *T. parva*. Primers derived from the *T. parva*-specific104-kDa antigen (p104) gene were used in the PCR amplification as previously described by Odongo *et al*. (2010) and Iams *et al*. (1990). Kazungu *et al*. (2015a) also used the same method to detect carriers of *T. parva* parasites.

For trypanosomes PCR amplifications primers targeting Internal Transcribed Spacer (ITS1) gene were performed (Silbermayr *et al*., 2013).

Polymerase chain reaction to detect *Theileria parva*: The sequences of the forward and reverse primers used were as seen in Table 2.

Table 2: Forward and reverse primers used in p104 PCR for *T. parva* detection

Type	Primer	Type	Amplicon size
Forward1	5´ATT TAAGGA ACC TGA CGT GAC TGC 3´	Outer	277
Reverse1	5´TAA GAT GCC GAC TAT TAAT-GACAC C 3´	Outer	
Forward2	5´GGC CAA GGT CTCCTT CAG AAT ACG3´	Inner	
Reverse2	5´TGG GTG TGT TTC CTC GTC ATC TGC3	Inner	

The nested polymerase chain reaction (nPCR) amplifications was performed in a total volume of 20µl containing 14µl nuclease-free water, 0.5µl (10 pmol) of forward and reverse primers and 5µl of genomic DNA (20ng/µl) template added into the lyophilized pellet (Bioneer PCR Premix –Korea), followed by vortexing and brief spin down to

dissolve the pellet. For the second round, the amount of water used was 18.5µl, and 0.5µl of the primary PCR product was used as a template. Reaction conditions for the primary PCR included initial denaturation at 94◦C for 5 min, denaturation at 94◦C for 60s, annealing at 60◦C for 60s and extension at 72◦C for 60s, and the amplification done in 30 cycles. The cycling profile condition for the second PCR was the same as the primary amplification, except for the annealing temperature which was 50◦C. The nPCR reactions then carried out in a thermocycler (VeritiTM, Applied Biosystems, USA). The nPCR products were separated on 1.5% agarose gel and images visualized and documented on a Gel DocTMb (Bio Rad, USA). Positive nPCR products were identified as 277 bp DNA fragments.

Polymerase chain reaction to detect trypanosomes: PCR amplification was performed in a total volume of 12.5 µl containing 6.25µl Dream Taq master mix (dATP, dCTP, dGTP and dTTP, 0.4 mM each, and 4 mM MgCl2), 0.24µM of forward and reverse primers and 3.15µl of nuclease free water.

Sequences of the forward and reverse primers used were as seen on Table 3. and conditions for PCR cycle conditions were preliminary denaturation at 94ºC for 3min, 30 cycles of 94ºC for 30sec, 55ºC for 30sec, 72 ºC for 30sec and a final extension at 72ºC for10 min. The amplified products were separated by electrophoresis on a 1.5% agarose gel (peqGOLD, PeqLab, Erlangen, Germany) stained with Gel red. Positive results were identified based on PCR products sizes corresponding to 300bp for *T. vivax*, 400bp for *T. brucei* and 700bp for *T. congolense savannah*.

Table 3: Primers for ITS1

Type	Primers	Amplicon size
Forward	CF 5'-CCG GAA GTT CAC CGA TAT TG-3'	Various
Reverse	BR 5'-TTG CTG CGT TCT TCA ACG AA-3'	

3.3 Data Analysis

Data was collected, checked, cleaned and stored in Microsoft Office Excel 2013. Graphs and tables were also drawn in Excel 2013. Biodata and Laboratory data were analysed using Epidemiological Package for Information (EPI Info) Version 7 statistical software (CDC, Atlanta, GA, USA).

Descriptive statistics were computed at 95% Confidence Interval (CI). Chi-square test was used to determine association between outcome variables (*T. parva* trypanosome infection) and categorical variables such as sex, villages, vaccination status, tick infestation, use oxytetracycline and use of Berenil. Association between age, breed and source of animals was determined using Chi-square for trend in Epi info as well. Statistical significance was determined at a $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Descriptive Statistics and Distribution Factors of *T. parva* and trypanosome prevalence in Monduli in the Dry Season

The results of this study represent findings for the study area since the study design used a random sampling technique involving a sample frame of cattle population of the whole study area. Limitations of the study design used was the inability to measure incidence of the two infections. The PCR techniques (p104 and ITS1) were highly sensitive and specific to capture all trypanosome species which may potentially occur in the study area.

4.1.1 Biological factors – animal level

Detection of p104 genes of *T. parva* parasites and ITS1 of *trypanosomes* parasites in blood samples of 480 cattle in the study area was quantified. Various factors contributing to the prevalence of both infections were determined. In biological factors, characteristics of animal-level variables were studied based on age categories (Fig. 6). There was an even proportions of adults and young animals sampled at 480(50.0%) with a (95%CI: 45.44, 54.56) each. Young animals were further categorized into calves and weaners at 16.0 % at (95%CI: 12.94, 19.70) and 34.0%, (95%CI: 29.76, 38.41) respectively.
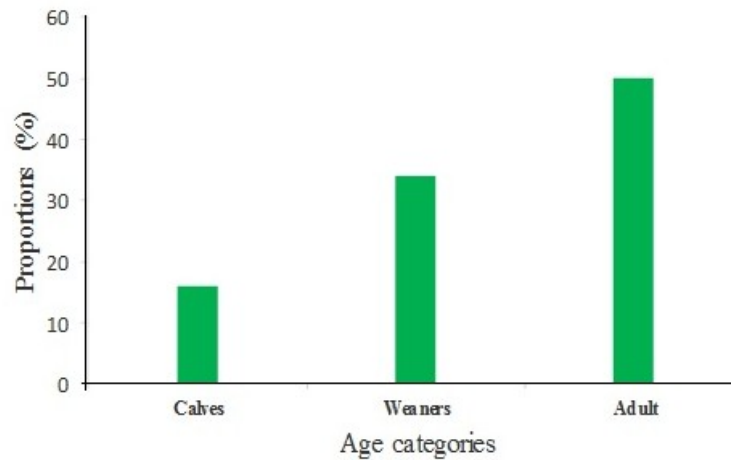
Figure 6: Proportions of Age categories of sampled cattle in Monduli District during the dry season

Female animals constituted 67.7% (95% CI: 63.29 - 71.84) of the overall cattle sampled (Table 4). The indigenous Tanzanian Short Horn Zebu (TSHZ) breed represented 47.1% (95% CI: 42.56 - 51.66), being the common breed in the district. The Boran breed which is not endemic in the area, was 7.5% at (95%CI: 5.38, 10.33), being the less common. Cross breeds of mostly TSHZ and Boran was 45.4% at (95% CI: 40.91, 49.99).

Table 4:   Proportions of animal-level biological factors in Monduli District during the dry season

Variable	Categories	Proportions (%)	95%CI
Age	Calves	16	12.94 - 19.70
	Weaners	34	29.76 - 38.41
	Adult	50	45.44 - 54.56
Sex	Male	32.3	28.16 - 36.71
	Female	67.7	63.29 - 71.84
Breed	Boran	7.5	5.38 - 10.33
	Cross breed	45.4	40.91 - 49.99
	TSHZ	47.1	42.56 - 51.66

4.1.2 Disease control factors for *T. parva* prevalence

Table 5 indicates that only 8.1% of animals were vaccinated against ECF whiles different concentrations of Oxytetracycline was used at a proportion of 28.3%.

Disease control factors for *trypanosomes prevalence*

The use of Berenil (Diminazene) against trypanosome infection was recorded at a proportion of 7.5% across the villages during the study (Table 5).

Table 5: Proportions of disease control factors in Monduli District during the dry season

Variable	Categories	Proportions (%)	95%CI
Vaccination	Yes	8.1	5.91 - 11.03
	No	91.9	88.97 - 94.09
OTC use	Yes	28.3	24.39 - 32.63
	No	71.7	67.37 - 75.61
Berenil use	Yes	7.5	5.38 - 10.33
	No	92.5	89.67 - 94.62

OTC = Oxytetracycline

4.1.3 Other inherent factors

Most animals, 95.8% (95%CI: 93.53 - 97.37), were born in the herd (bomas) whiles 2.9% (95%CI: 1.67- 4.96), were purchased from other areas. Animals transferred from other bomas within the district were 1.3% (95%CI: 0.51 - 2.84) of the 480 studied (Table 6).

Table 6: **Proportions of other inherent factors in the prevalence of *T. parva* infection in Monduli District during the dry season**

Variable	Categories	Proportions (%)	95%CI
Source	Born in the Herd	95.8	93.53 - 97.37
	Purchased	2.9	1.67- 4.96
	Transferred	1.3	0.51 - 2.84
Disease State	Sick	2.7	1.51 - 4.71
	Not sick	97.3	95.29 - 98.49
Swollen L.N.	Yes	32.1	27.96 - 36.49
	No	67.9	63.51 - 72.04
Ticks	Present	2.7	1.51 - 4.71
	Absent	97.3	95.29 - 98.49

LN = Lymph Node

A proportion of 2.7% (95%CI: 1.51 - 4.71) animals were clinically sick at the time of sample collection. Sick animals were determined by history provided by cattle owners, inspection and examination of studied animals before blood sampling. Biopsy was performed on animals that had swollen lymph nodes (32.1%) and in microscopy examination, presence of KBBs was found in only 2.

4.2 Prevalence of *T. parva* and Trypanosome Infection in Monduli District During the Dry Season

4.2.1 Prevalence of *T. parva*

Of the 480 samples analysed *T. parva* infections were 152 with an overall prevalence of 31.7% (95%CI: 27.56, 36.07). (Fig. 7). Prevalence of *T. parva* varied highly ranging from 6.3% to 60.4% between villages (Table 7). Lepurko village recorded the highest *T. parva* prevalence of 60.4% (95%CI: 45.27, 74.23) as compared to Engaruka Chini with 6.3% (95%CI: 1.31, 17.20).

4.2.2 Prevalence of trypanosome infection

In Fig. 7 indicates an overall prevalence of 4.2% (95%CI: 2.63, 6.47). For the 20 trypanosome infections of the 480 samples. There was a variation between villages of 2.1% to 14.6% as seen in (Table 8). For trypanosome infections, Meserani Bwawani recorded the highest of 14.6% at (95%CI: 6.07, 27.76) as against 3 villages (Naiti, Mbaash and Idonyonado) with no infections at all (Table 8).
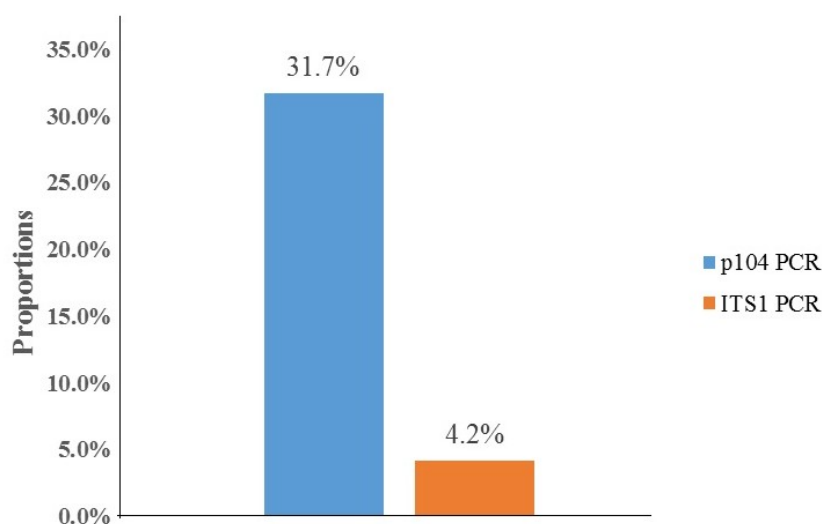
Figure 7: **Overall Prevalence of *T. parva* and trypanosome infection in Monduli District during the dry season of 2015**

Table 7: **Prevalence of *T. parva* infection in Monduli District during the dry season of 2015**

Village Name	No sampled	*T. parva* prevalence (%)	95% CI
Meserani Bwawani	48	20.8	10.47 34.99
Lepurko	48	60.4	45.27, 74.23
Esilalei	48	43.8	29.48, 58.82
Naiti	48	27.1	15.28, 41.85
Mbaash	48	47.9	33.29, 62.81
Eluwai	48	54.2	39.17, 68.63
Oltukai	48	31.3	18.66, 46.25
Lossimingori	48	16.7	7.48, 30.22
Idonyonado	48	8.3	2.32, 19.98,
Engaruka Chini	48	6.3	1.31, 17.20
Overall	**480**	**31.7**	27.56, 36.07

Table 8: Prevalence of trypanosome infection in Monduli District during the dry season of 2015

Village Name	No sampled	Trypanosomes prevalence (%)	95% CI
Meserani Bwawani	48	14.6	6.07, 27.76
Lepurko	48	4.2	0.51, 14.25
Esilalei	48	4.2	0.51, 14.25
Naiti	48	0	100.00, 100.00
Mbaash	48	0	100.00, 100.00
Eluwai	48	4.2	0.51, 14.25
Oltukai	48	6.3	1.31, 17.20
Lossimingori	48	2.1	0.05, 11.07
Idonyonado	48	0	100.00, 100.00
Engaruka Chini	48	6.3	1.31, 17.20
Total	**480**	**4.2**	**2.63, 6.47**

Out of the 20 positives of trypanosome infection, *T. vivax* was highest at 95% and *T. congolense* the lowest 5%. No *T. brucei* species were detected. Mixed infection *of T. vivax* and *T. congolense* occurred in only Oltukai village (Table 9).

Table 9: Distribution of trypanosome species infection in Monduli District during the dry season of 2015

Village name	*T.vivax* (%)	*T.congolense* (%)	Mixed (%)	Total (%)
Meserani Bwawani	14.6	0.0	0.0	14.6
Lepurko	4.2	0.0	0.0	4.2
Esilalei	4.2	0.0	0.0	4.2
Naiti	0.0	0.0	0.0	0.0
Mbaash	0.0	0.0	0.0	0.0
Eluwai	4.2	0.0	0.0	4.2
Oltukai	4.2	2.1	2.1	6.3
Lossimingori	2.1	0.0	0.0	2.1
Idonyonado	0.0	0.0	0.0	0.0
Engaruka Chini	6.3	0.0	0.0	6.3
Total	**4.0**	**0.2**	**0.2**	**4.2**

4.3 Co-infections of *T. parva* and trypanosomes in Monduli District during the Dry Season of 2015

The occurrence of co-infections of both *T. parva* and trypanosomes in the same host in the study are 4.1% of the 152 and 20 positive infections, respectively. Engaruka Chini village had 2 cases of co-infections being the highest (33.3%), followed by Oltukai village also with 2 cases but with a prevalence of 11.1% and Lepurko being the least with 3.2% (Table 10).

Table 10: Distribution *of T. parva* and trypanosome co-infections in Monduli District during the dry season 2015

Villages	*T. parva*	Trypanosomes	Co-infection	Prop (%)	95%CI
Meserani Bwawani	10	7	0	0.0	100, 100
Lepurko	29	2	1	3.2	0.05, 11.07
Esilalei	21	2	0	0.0	100, 100
Naiti	13	0	0	0.0	100, 100
Eluwai	26	2	2	7.1	0.51, 14.25
Mbaash	23	0	0	0.0	100, 100
Oltukai	15	3	2	11.1	0.51, 14.25
Lossimingori	8	1	0	0.0	100, 100
Idonyonado	4	0	0	0.0	100, 100
Engaruka Chini	3	3	2	33.3	0.51, 14.25
Total	**152**	**20**	**7**	**4.1**	**0.64, 3.12**

4.4 Factors Related to *T. parva* and Trypanosome Prevalence in Monduli in the Dry Season

4.4.1 Biological factors

4.4.1.1 Related to *T. parva*

The total prevalence of *T. parva* infection for males and females during the study period was 34.2% and 30.5% respectively. There was no statistical difference between sex and *T.*

parva infection as stated in (Table 11) with a (P = 0.413). Calves of less than 6 months had the highest prevalence of 33.8% from a total of 77 for *T. parva.* Statistically, between age categories and *T. parva* infections, there was no statistical difference. With the different breeds, Boran breed had the highest *T. parva* infection prevalence at 38.9% (Table 11), whiles prevalence for the indigenous TSHZ stood at 29.2%, being the breed widely owned by the pastoralist. However, there was no statistical difference between *T. parva* infection and breeds (P=0.43) (Table 11).

4.4.1.2 Related to trypanosome

For trypanosomes, of the 155 males 3.9% positives were recorded whiles 4.3% was the prevalence for the 325 females. There was equally no statistical difference between sex and trypanosome infection as (P=0.847). Adult animals recorded the highest prevalence for trypanosomes across the study villages, at 5.1% (Table 12). There was statistical difference (P=0.019) between trypanosome infection and breeds.

Table 11: Animal related factors of the Prevalence of _T. parva_ in Monduli District during the dry season of 2015

Variable	_T. parva_ prevalence (%)	χ^2	p. Value
Age Categories			
Calves	33.8	0.171	0.773
Weaners	31.3		-
Adults	31.3		-
Sex			
Male	34.2	0.674	0.412
Female	30.5		-
Breed			
Boran	38.9	1.854	0.43
Cross Bred	33.0		
Short Horn Zebu	29.2		

Table 12: Animal related factors of the Prevalence of trypanosomesin Monduli district during the dry season of 2015

Variable	Trypanosomes prevalence (%)	χ^2	p. Value
Age Categories			
Calves	1.3	1.314	0.253
Weaners	4.3		-
Adults	5.0		-
Sex			
Male	3.9	0.050	0.823
Female	4.3		-
Breed			
Boran	8.3	5.509	0.019
Cross Bred	5.5		-
Short Horn Zebu	2.2		-

4.5 Ecological and Environmental related Factors

T. parva related

Prevalence for _T. parva_ positive animals that were infested by ticks was 7.7% and 32.3% for cattle that had no tick infestation. There was no statistical difference between tick infestation and _T. parva_ infection (P>0.05) (Table 17). The location of villages showed

statistical difference with *T. parva* infections (P=0.010) (Table 13). Conversely, there was statistical insignificance between trypanosome infection and Village location (P=0.074) (Table 14).

Table 13: Studied villages and measures of association with *T. parva* in Monduli District during the dry season of 2015

Variable Village	*T. parva* Pos.	Neg.	χ^2	p. Value
Meserani Bwawani	10	38	25.337	0.010
Lepurko	29	19		
Esilalei	21	27		
Naiti	13	35		
Mbaash	23	25		
Eluwai	26	22		
Oltukai	15	33		
Lossimingori	8	40		
Idonyonado	4	44		
Engaruka Chini	3	45		
Total	**152**	**328**		

Table 14: Studied villages and measures of association with trypanosome infection in Monduli District during the dry season of 2015

Variable Village	trypanosomes Pos.	Neg.	χ^2	p. Value
Meserani Bwawani	7	41	3.19491	0.0738
Lepurko	2	46		
Esilalei	2	46		
Naiti	0	48		
Mbaash	0	48		
Eluwai	2	46		
Oltukai	3	45		
Lossimingori	1	47		
Idonyonado	0	48		
Engaruka Chini	3	45		
Total	**20**	**460**		

4.6 Husbandry Practices and Disease Control Measures

4.6.1 Measures to control *T. parva* infections

Table 15 shows a *T. parva* prevalence among animals vaccinated against ECF at a proportion of 28.2%. There was no statistical association between the two variables. The use of different concentrations of Oxytetracycline (OTC) was as seen in Figure 8. The use of OTC10% was higher at 23.3%.with a statistical association (P=0.024) between the use of OTC and *T. parva* infection prevalence in animals that previously had OTC injections were low at 25.0%. These might be animals that were sick and treated or were injected for prophylaxis against other diseases. Animals that reportedly never had OTC injections had a prevalence of 34.3%. (Table 15).

4.6.2 Measures to control trypanosomes infections

Conversely, the use of Berenil to treat suspected and confirmed cases of trypanosome infections were equally not statistically associated across the study area (P=0.317) (Table 16).
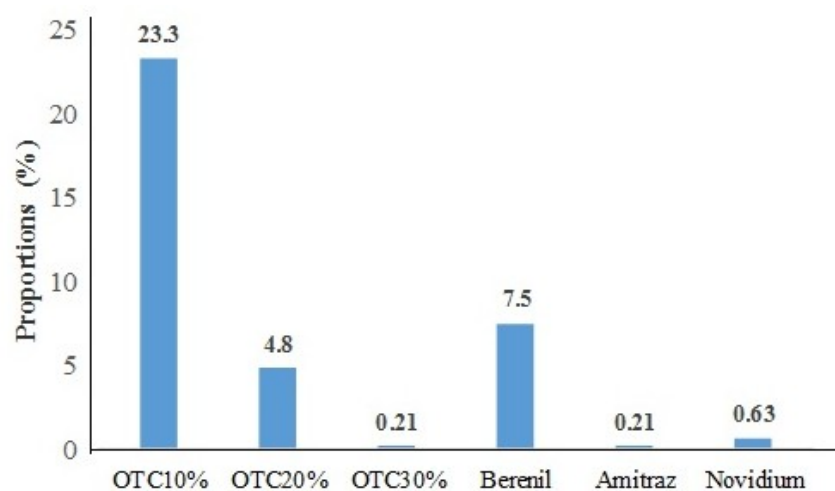
Figure 8: Treatment with different concentrations of oxytetracycline

preparations in Monduli district during the dry season of 2015.

Table 15: Disease control measures related to Prevalence and *T. parva* infection

in Monduli district during the dry season of 2015

Variable	n	*T. parva* prevalence (%)	95%CI	p. Value
Vaccination Status				
Vaccinated	39	28.2	0.390, 1.704	0.644
Not Vaccinated	441	32.0		-
OTC				
Used	136	25.0	0.405, 0.995	0.024
Not used	344	34.3		

Table 16 Disease control measures related to Prevalence and trypanosome

infection in Monduli district during the dry season of 2015

Variable	n	Trypanosomes prevalence (%)	95%CI	p. Value
Berenil				
Used	36	5.6	0.210, 5.488	0.317
Not used	444	4.1		

4.4.4 Host related factors

Most of the animals 95.8% were borne in the herd and 2.9% were purchased from elsewhere, whiles 1.3% transferred within Bomas across the study villages.

For *T. parva*: There was no statistical association between source of animals and *T. parva* infection (P=0.538) as indicated in Table 17. The presence of ticks on animals as well as their disease state had no statistical association with the prevalence of *T. parva*.

Trypanosomes: Source of animals and trypanosome infection had a statistical association with a (P=0.020). However, there was no statistical association (P=0.111) between disease state of animals and trypanosomes infection (Table 18).

Table 17: Other inherent factors related to T. parvain Monduli district during the Dry season of 2015

Variable	n	T. parva prevalence %	χ^2	pValue	95%CI (%)
Source					
Born in Herd	460	32.0	0.379	0.538	27.76, 36.46
Purchased	14	21.4			
Transferred	6	33.3			
Disease state					
Sick	13	30.8	0.054	0.972	0.2528, 3.116
Not sick	467	31.7			
Tick infestation					
Present	13	7.7	3.541	0.054	0.008033, 1.024
Absent	467	32.3			

Table 18: Other inherent factors related to trypanosome infection in Monduli district during the Dry season of 2015

Variable	n	trypanosomes prevalence %	χ^2	p. Value	95%CI
Source					
Born in Herd	460	3.7	5.415	0.020	2.24, 5.97
Purchased	14	14.3			
Transferred	6	16.7			
Disease state					
Sick	13	15.4	0.055	0.111	0.6375, 19.91
Not sick	467	3.9			

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence of *T. parva* and Trypanosome Infection During the Dry Season

This study was conducted with the purpose of determining the prevalence of *T. parva* and trypanosome infection during the dry season in Monduli District. The district is severely affected by extreme weather conditions. These weather conditions contribute to food and water shortages leading to increased interaction of livestock and wildlife, and to ascertain the factors associated with the prevalence.

Overall prevalence of *T. parva* infection was 31.7%, as determined using the p104 based PCR and it varied widely across the villages from 6.3% to 60.4%. The prevalence of trypanosome infection was 4.2% as determined by the ITS1 based PCR and also ranging from 0 to14.6% among the villages.

The overall prevalence of *T. parva* agrees with that reported by Kazungu *et al*. (2015a), who found a prevalence of 37.1% in Simanjiro District very close to Monduli District during the rainy season. Lepurko village had the highest prevalence of *T. parva*. This is a village that was sampled during the peak of the dry season in September, when there was severe scarcity of food and water. Livestock were moved in and out daily to other areas in search of food and water leading to interaction with other animals and the possible infestation with infected ticks and other parasites. This movement of cattle by Maasai pastoralist in Monduli District was also reported by (Conroy, 1999; Yurco, 2011). There was no observed tick infestation of cattle in Lepurko, as well as no use of acaricide was recorded during that period due to the scarcity of water and probably low tick challenge.

Engaruka Chini, a village bordering the Ngorongoro Conservation Area (NCA) had the lowest *T. parva* prevalence. This does not agree with what was reported by Norval *et al*. (1992) that there is increased interaction between cattle and buffaloes in areas closer to National parks and therefore increased diseases transmission and its dynamics. There was no recorded acaricide use by dipping or spraying of animals against ectoparasites, however, there was no tick infestation. A very low proportion of animals in this village (Engaruka Chini) were vaccinated against ECF. The use of OTC was high (58.3%, 95%CI: 43.21, 72.39). It is very possible that the use of OTC significantly contributed to the low prevalence of *T. parva* Engaruka chini village. Oxytetracycline is a drug of choice for most Maasai which is used in treating most sick animals as first line measure (Chengula *et al*., 2013; Jacob *et al*., 2004). This factor is likely to have influenced the low parasite prevalence of *T. parva* in the village together with some ecological and environmental characteristics (signs of environmental degradation and overgrazed land cover), as described by Norval *et al*. (1992) and Gachohi *et al*. (2012) who stated that overgrazed and environmentally degraded pastures were unsuitable for vectors. Furthermore, Gachohi *et al*. (2012) also stated that dry areas, open grasslands and sparse vegetation were equally not suitable for vectors.

The variation of prevalence between the villages have been due to different ecological suitability for vectors across each area (Gachohi *et al*. 2012). Additionally, environmental factors such as precipitation, temperature, soil type and land use can influence the distribution of vectors and the host of a particular disease (Ostfeld *et al*., 2005). The risk of contact with disease causing pathogens such as *T. parva* parasites is highly influenced by that distribution (Ostfeld *et al*., 2005).

Three villages (Naiti, Mbaash and Idonyonado) did not record trypanosome infection during the study period. Increased human activities such as crop production and other infrastructural developments have destroyed the hither to habitats of tsetse in the previously tsetse infested areas of Northern Tanzania and Tanzania in general (Malele *et al.,* 2011). In comparison with the current prevalence of 4.2%, Swai and Kaaya (2012) had a prevalence of 5% at 95% CI:2.6, 8.6 with a less sensitive method (microscopy) in the same study area but during the beginning of the rainy season in November whiles Haji *et al*. (2015) obtained a higher prevalence of 27.8% at 95% CI:22.3–32.5, with the more sensitive LAMP technique in the same area and also at the end of the dry season. This difference might be due to the sensitivity of the method used as indicated by Haji *et al*. (2015).

Ninety five percent of the *Trypanosoma* spp that infected the cattle in Monduli during the dry season were *T. vivax* and 5% were *T. congolense.* This agrees with the findings of Haji *et al*. (2015) and Swai and Kaaya, (2012) who also found similar results of high prevalence of *T. vivax* in Monduli district. However, the negative results of *T. brucei* in this study may likely be as explained by findings of Karimuribo *et al.* (2011) that cattle may be resistant or susceptible to a particular species and not to the other. It is therefore possible that the TSHZ and Boran crosses may be resistant to *T. brucei* infection. In cattle, *T. brucei* causes a mild to chronic disease where as *T. vivax* causes a more acute disease and *T. congolense* causes chronic disease (Radostits *et al*., 2006) Other studies done by Haji *et al.* (2015) and by Swai and Kaaya (2012) found *T. brucei* in the study area. The two main trypanosomes causing disease in cattle and small ruminants in SSA and Tanzania for that matter are *T. vivax* and *T. congolense* (Haji *et al*., 2015; Majekodunmi *et al*., 2013). The reduced presence of the biological tsetse vector for *T.*

congolense may be associated with its low prevalence (Haji *et al*., 2015). This *Trypanosom*a spp, apart from the tsetse fly as a vector, can also be transmitted mechanically by other biting flies such as Tabanids and *Stomoxys* (Desquesnes and Dia, 2004; Jones and Dávila, 2001; Osório *et al*., 2008). According to Van de Bossche cited by Haji *et al*. (2015), the incidence of wild animal reservoirs and that of the mechanical haematophagous arthropod vectors are accountable for the higher prevalence of *T. vivax*. However, the mechanical transmission of *T. vivax* in Africa is a controversy (CFSPH, 2009a; Desquesnes and Dia, 2003), where it is presumed to only occur in areas free from tsetse flies (Luiza *et al.,* 2008; Nimpaye *et al*., 2011). Mixed species infection is also reported by Radostits *et al*. (2006).

There is low prevalence of trypanosome infection in Monduli during the dry season as seen in this study. It is attributed to inceased human activities such as land cultivation and the use of traps in ares suspected to be infested with tsetse. Malele *et al*. (2011) reported that, most areas which were once tsetse infested are currently not infested. Increased land use, especially agricultural activities (shifting cultivation) and infrastructural development due to increased human population have contributed to modifying the habitat of tsetse leading to their disappearance (Malele *et al*., 2011). Other related reasons could be low vector activities and distribution during the dry season (Madsen *et al*., 2013). Contrary to Madsen *et al*. (2013), Swai *et al*. (2006) reported that there is tick challenge all year round with a low abundance during the cool dry season. This assertion was also stated by a Maasai pastoralist (Kisoi LojiLoji of Meserani village, 10-06-2015 personal communication) during interaction in the study area. Another reason could be attributed to the use of chemotherapeutics such as diminazene (Berenil®) and homidium chloride (Novidium). These chemotherapeutics are known and used by the Pastoralist for the

treatment of suspected trypanosome infection or AAT as was reported by Muhanguzi *et al.* (2004). Its use was however very minimal during the dry season possibly due to perceived low infections of trypanosomes .Another reason is, the use of acaricide for the control of tick borne diseases (TBDs) and other biting flies, which also have a collateral effect on the tsetse fly vector. Conversely the use of acaricide was not observed during the dry season. This is also most likely related to the scarcity of water during the dry season and the reduced tick numbers and other insect vectors. In contrast to the seemingly low prevalence of trypanosome infection in Monduli during the dry season, Van den Bossche and Rowlands (2001) reported that in Zambia, the resistance of trypanosome infection during the dry season is less leading to a high prevalence. Normally during the dry season, especially in the Savannah ecosystems, the environment is not suitable for the tsetse fly due to the absence of a suitable vegetation cover. However, as a result of the scarcity of food and water animals move from their normal grazing areas to places closer to wildlife grazing areas around National parks and forest reserves. In these areas the vegetation in serves as a habitat for tsetse fly, parasites are therefore transmitted from wild animal reservoirs to livestock. Majekodunmi *et al.* (2013) also found that social factors such as land use for agriculture and the competition for natural resources are factors that contributed to high prevalence of trypanosome infection in the Jos plateau of Nigeria. This contradicts what Malele *et al.* (2011) stated about the use of land for agricultural cultivation and the distribution of the vector, tsetse fly in Tanzania.

5.2 Co-infections of *T. parva* and Trypanosome in Monduli District during the Dry Season

There was concurrent infection of *T. parva* and trypanosomes during the study with an overall prevalence of coinfections of 4.1%. Haji *et al.* (2014) also found co-infections of

multiple species including *T. parva* and trypanosomes in Monduli District and concluded that the presence of both ticks and tsetse fly could have been the cause of transmission of these haemoparasites. The signs and symptoms of diseases caused by these two important infection are similar and require confirmation so that proper treatment is carried out (Radostits *et al.*, 2006).

5.3 Factors Related to Prevalence of *T. parva* and Trypanosome Infection

Ecologically, tick infestation as a factor influencing the prevalence of *T. parva* in Monduli during the dry season was insignificant statistically ($p>0.05$). There was very low proportion of animals infested with ticks at 2.7% of which 7.7% of 480 were *T. parva* positive. It is possible that since the animals were adapted to the environment and its ecological conditions, and the period during which the study was carried out are factors that accounted for the low tick infestation. Wambura *et al.* (1998) stated that animals adapted to a particular environment are usually burdened with less ticks. Norval *et al.* (1992) and Gachohi *et al.* (2012) also reported that overgrazed and environmentally degraded areas are not suitable for vector proliferation and distribution. Furthermore, sparse vegetation, dry and open grasslands are equally not suitable for vectors (Gachohi *et al.*, 2012).

This is in agreement with Swai *et al.* (2006) who also found a low tick challenge during the cool dry season in a study elsewhere in an ECF endemic zone in Tanzania. Billiouw (2005), inferred that the size of the tick population and the proportion of infected ticks determine the overall success of transmission.

Environmentally, village location was statistically significant to the prevalence of *T. parva* ($p<0.05$) and not insignificant ($p>0.05$) to the prevalence of trypanosomes in

Monduli during the dry season. The variation in prevalence and factors associated in the villages with high low *T. parva* infections have already been discussed earlier in this work. Pastoralist in the study villages practice extensive grazing as a management system making their animals vulnerable to interactions with sources of vectors and parasites, other factors such as closeness to game reserves and national parks, and whether they are pastoralist "par excellence" or are agro- pastoralist have an influence in the epidemiology of the infection. Muhanguzi *et al*. (2014b) in a study Uganda, found out that microclimate in various villages, the tick control measures by handpicking and even the presence of some known anti tick plants like *Lantana camara* and *Ocimum suave* were factors that influenced in the variation of *T. parva* prevalence.

In husbandry practices, disease control measures such as vaccination against ECF was insignificant statistically to the prevalence of *T. parva* infection during the dry season. The number of vaccinated animals was really considered to be very small at 8.1%. This is most likely because of the high cost of the ITM vaccine which is the one currently used. It cost up to US10$ per animal and the requirement of a cold chain to deliver vaccines to remote areas which is not affordable by smallholder cattle owners (Laisser *et al*., 2014). Moreover, the fact that farmers will still have to use Acaricide to control other TBDs adds up to the cost (Laisser *et al*., 2014). There is a general believe that the TSHZ and Boran crosses which are the common breeds are resistant to ECF(Muhanguzi *et al*., 2014b). Other disease control measures such as the use of oxytetracycline and Berenil have been discussed previously in this chapter.

Biological factors in terms of animal-level factors were analysed. The age of animals was not significantly associated to *T. parva* and trypanosome infection during dry season in

this study. Though the age category of calves were of the highest proportion at 33.8%, it was not significantly associated. Calve mortality amongst indigenous zebu breed associated to ECF is high. For instance, Thumbi *et al*. (2014) reported 80% of calves deaths was due to ECF especially in those less than 6 months of age. Sex of animals was neither significantly associated with the prevalence of *T. parva* and trypanosome infection. Nonga and Kambarage (2009) found similar results in their study of prevalence of Bovine trypanosomosi*s* elsewhere in Morogoro. It is believed that females are mostly affected because most cattle owners keep more females in their herd for their economic and productive importance. As found in this study, females had a higher proportion and therefore were most affected. However, breed was significantly associated with prevalence of trypanosome infection and not with prevalence of *T. parva.* Local cattle breeds in endemic areas are less susceptible than exotic breeds (Kazungu *et al*., 2015b).

CHAPTER SIX

6.0 CONCLUSSIONS AND RECOMENDATIONS

6.1 Conclusions

This study was conducted during the dry season, which is characterised by shortage of food and water and further exacerbated by the effects of climate change with climatic variations leading the spread of vectors. There is increased interaction between livestock and wild animals in search of food and water paving the way for increased vector sharing and disease transmission.

The study therefore revealed that the prevalence of *T. parva* during the dry season in Monduli district of the Maasai steppe remains a significant constraint to the extensive livestock system as this could lead to increased ECF cases and serve as a constraint to the introduction of exotic improved breeds to boost production. This is because the indigenous cattle will serve as carriers of *T. parva* which will subsequently, be transmitted to the introduced improved more productive breeds.

Though the prevalence of trypanosomes was not high, it still revealed that animals were carriers of the parasites, which could also lead to increased cases of AAT with the presence of the tsetse fly vector.

Clinical cases of *T. parva* and trypanosome infection were very low despite the prevalence revealed by the PCR techniques with low tick and tsetse fly challenge including the increased interaction between cattle and wildlife in search of scarce food and water during the dry season.

6.2 Recommendations

i.   It is necessary to conduct a longitudinal study to establish an all year round state of endemicity of *T. parva* infection in Monduli District. Since there is an established endemic stability, it is recommended to apply vector control methods like an increase interval in dipping of animals with acaricide as this will partially reduce the force of infection and consequently the development and maintenance of immunity. In addition, it is highly recommended to make vaccination against ECF more accessible and affordable to smallholder cattle owners especially, as they have the quest to improve their cattle breeds to the more productive exotic breeds. The implementation of integrated vector control methods that will not only control ticks and tsetse fly, but also target other biological vectors is more than imperative for all stakeholders.

ii. Proper land planning, demarcation and management strategies are necessary especially with stakeholders such as conservationist, wildlife and national park managers including agriculturalist. This approach will help to plan sustainable control methods and pave way for pastoralist to share valuable natural resources such as grazing lands with other landers users, and also help in disease control strategies.

iii. It is equally important that, the Area-Wide and Sustainable Approach be implemented systematically in the study area to help eradicate AAT and HAT as well to increase productivity and decrease poverty in line with the development goals of Tanzania indicated in (NSGRP), MKUKUTA of Vision 2025. (URT, 2010).

iv. The provision of charcos, dams and boreholes and other environmentally and economically sustainable watering systems in the study area and the Maasai steppe of Northern Tanzania cannot be overemphasized. This will not only help revive the communal and regulated dipping systems which will be cheaper and affordable for all cattle owners throughout the season especially during high tick challenge in the rainy season, but it will also be a source of drinking water for livestock and community use.

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APPENDIX

Appendix 1: Sample collection form

Name of village: village ID
Famer ID_____   DATE OF BLOOD COLLECTION: /   /2015
CATTLE ID/NAME:

1. Breed: Shorthorn Zebu Sahiwal Boran Cross breed other	2. Gender: M F	3. Age……………Years Or If<1year _____-months

4. How did you get this animal?	Born in your herd	Purchased	Gift
	Other-Specify _____		

5.Body condition score	_____/5	6.Pre-scapular lymph node other:	Enlarged (Size) Not enlarged
		IF ENLARGE – Was sample collected	Yes no

7.Vaccinated ECF	Yes No	If yes When was animal vaccinated	_____Year?
		ECF Vaccination Ear Tag Colour? Year? Number?	

8. Has this animal been sick in the last 3 months	Yes No	If yes Clinical signs	
What was the disease?			

Treatment given?	Yes No	If Yes	Details	
Animal sick on day of blood collection	Yes No	If Yes	Details	

9. Tick collection	Yes No	If Yes Number of ticks	Which part of the body collected Half body count	

10. Sample collected	<= 12monsths Months	Yes No	How many	
	>=12 months	Yes No	How many	